

# ICPD 2019

June 27 - 29

17th International Conference  
on Production Diseases  
in Farm Animals

**Bern**  
**Switzerland**

$u^b$

<sup>b</sup>  
UNIVERSITÄT  
BERN



# Proceedings

### Impressum

ISBN: 978-3-906813-93-6

DOI: 10.7892/boris.131406

Editors: Rupert M. Bruckmaier, Josef J. Gross  
Veterinary Physiology, Vetsuisse Faculty  
University of Bern  
Bremgartenstrasse 109a  
CH-3012 Bern, Switzerland

This work is licensed under a Creative Commons  
Attribution 4.0 International Licence  
<https://creativecommons.org/licenses/by/4.0/>

Text © The Authors

Cover Image by Bern Tourism



### Welcome to the 17th International Conference on Production Diseases in Farm Animals (ICPD)

Breeding and husbandry of livestock is often accompanied by a conflict of interest between biological requirements of the animal and economic needs of the producer. This conflict is increasingly gaining attention not only by producers, animal scientists, and veterinarians, but also by the public. An inadequate breeding strategy or animal housing and feeding may lead to production diseases, coming along impaired animal welfare, but also reduced income for the producer, and reduced product quality for the consumer.

The ICPD is a traditional conference, held for the first time in 1968 in Urbana, Illinois, and from then on mostly in 3-year intervals either in Europe or in the US. In contrast to most conferences related to farm animals, the ICPD focusses mainly on production diseases and their prevention in ruminants, swine, and poultry.

From the very beginning the ICPD has been commonly organized by farm animal and veterinary scientists. It is the goal to gather the different fields of science to exchange and discuss results and developments, and to create novel ideas to prevent production diseases and to increase animal welfare and product quality in farm animal production.

The ICPD always attracted international scientists from various disciplines. In this unique interdisciplinary environment, latest and groundbreaking research from scientists meets the questions, experiences and approaches of practitioners, industry, and consumers.

Although a traditional conference, each ICPD addressed the most current issues of farm animal production. The main topics of the 17th ICPD are the use of antimicrobials with emphasis on security and safety for producers and consumers, the impact of locomotion disorders on performance and welfare of farm animals, the interactions of gut microbiome, climate change, microclimate, genetics, metabolic status and mineral homeostasis with reproduction, performance, animal health and welfare. Behavioral signs indicating an impaired animal welfare are the basis for precision livestock farming technology and development of new management strategies. Aspects of infectious diseases, raising of neonates and their development are completing the program. Progress in analytical procedures, new regulations, the problem of antimicrobial resistance, and the implementation of sensor and precision livestock farming technology do not only trigger new management strategies on the farms. We are facing significant challenges: Big data and data security, consumer perceptions, and a growing demand for animal-derived food products in the developing countries.

It is our pleasure to welcome all people engaged in farm animal production in Bern to contribute in the 17th ICPD! Enjoy the conference!

Rupert M. Bruckmaier & Josef J. Gross

## Committees

---

### **Local organizing committee:**

Rupert Bruckmaier  
Josef Gross  
Olga Wellnitz  
Hélène Meier  
Jürg Danuser  
Cord Drögemüller  
Samuel Kohler  
Heiko Nathues  
Adrian Steiner  
Mike Toscano  
Heiner Bollwein  
Xaver Sidler  
Roger Stephan

### **International scientific committee:**

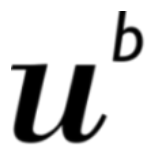
Sigrid Agenäs (Sweden)  
Alex Bach (Spain)  
Adam Geiger (USA)  
Harald Hammon (Germany)  
Laura Hernandez (USA)  
Akio Miyamoto (Japan)  
Geert Opsomer (Belgium)  
Helga Sauerwein (Germany)  
Erminio Trevisi (Italy)  
Ariette van Kneysel (the Netherlands)  
Marina von Keyserlingk (Canada)  
Peter Zieger (Germany)



## List of previous ICPDs

---

- I. Urbana, Illinois, USA, 1968
- II. Reading University, England, 1972
- III. Wageningen, the Netherlands, 1976
- IV. Munich, Germany, 1980
- V. Uppsala, Sweden, 1983
- VI. Belfast, Northern Ireland, 1986
- VII. Ithaca, New York, 1989
- VIII. Bern, Switzerland, 1992
- IX. Berlin, Germany, 1995
- X. Utrecht, the Netherlands, 1998
- XI. Copenhagen, Denmark, 2001
- XII. East Lansing, Michigan, USA, 2004
- XIII. Leipzig, Germany, 2007
- XIV. Ghent, Belgium, 2010
- XV. Uppsala, Sweden, 2013
- XVI. Wageningen, the Netherlands, 2016



<sup>b</sup>  
**UNIVERSITÄT  
BERN**



AMERICAN SOCIETY OF **ANIMAL SCIENCE**



We create chemistry



**Burgergemeinde  
Bern**



Schweizerische Eidgenossenschaft  
Confédération suisse  
Confederazione Svizzera  
Confederaziun svizra

Eidgenössisches Volkswirtschaftsdepartement EVD  
Bundesamt für Landwirtschaft BLW



**SWISS NATIONAL SCIENCE FOUNDATION**



**PERFORMANCE MINERALS®**



Global Agenda for  
Sustainable Livestock



## Table of contents

---

<b>IMPRESSUM</b>	<b>2</b>
<b>INTRODUCTION</b>	<b>3</b>
<b>COMMITTEES</b>	<b>4</b>
<b>LIST OF PREVIOUS ICPDS</b>	<b>5</b>
<b>MAIN SPONSORS</b>	<b>6</b>
<b>SPONSORS</b>	<b>7</b>
<b>TABLE OF CONTENTS</b>	<b>8</b>
<b>SESSION 01:</b>	
<b>USE OF ANTIMICROBIALS: SECURITY AND SAFETY ASPECTS FOR PRODUCERS AND CONSUMERS</b>	<b>20</b>
PRUDENT AND EFFECTIVE ANTIMICROBIAL USE IN A DIVERSE LIVESTOCK AND CONSUMER'S WORLD	20
<i>U. Magnusson</i>	
ACTUAL RESISTANCE SITUATION AMONG BACTERIAL PATHOGENS OF FOOD-PRODUCING ANIMALS	21
<i>H. Kaspar and S. Schwarz</i>	
ANTIMICROBIAL USE AND ANTIMICROBIAL RESISTANCE IN DAIRY CATTLE	22
<i>T. J. G. M. Lam, A. E. Heuvelink, R. G. M. Olde Riekerink and I. M. G. A. Santman-Berends</i>	
THE FUTURE-PROOF BOVINE PRACTITIONER UNDERSTANDS THAT IMPROVED MASTITIS MANAGEMENT IS KEY TO PRUDENT USE OF ANTIMICROBIALS ON DAIRY FARMS	23
<i>S. De Vliegheer, M. Stevens, Z. Lipkens and S. Piepers</i>	
EFFECTS OF ANTIBIOTICS ON SESSILE BACTERIA IN DRINKING WATER SYSTEMS IN POULTRY FARMS: A MODEL APPROACH	24
<i>R. H. Mateus-Vargas, F. Hahne, S. Jensch, N. Kemper, G. Hamscher and J. Schulz</i>	
EFFECTS OF A BACTERIAL BIOFILM ON VARIOUS ANTIBIOTICS	25
<i>F. Hahne, S. Jensch, R. H. Mateus-Vargas, J. Schulz and G. Hamscher</i>	
EVALUATION OF ANTIBIOTIC SENSITIVITY OF BACTERIA ISOLATED FROM MASTITIS CASES IN DAIRY COWS IN THE 4-YEAR PERIOD	26
<i>K. Wódz, T. Nowak and P. Kwieciński</i>	
DOES THE FEEDING OF DISCARD MILK TO DAIRY CALVES LEAD TO ANTIMICROBIAL RESISTANCE ON FARM?	27
<i>C. L. Firth, A. Käsböhrer, P. Pless, B. Springer, C. Egger-Danner and W. Obritzhauser</i>	
ANTIBIOTIC USE AND RESISTANCE IN <i>E. COLI</i> FROM POULTRY – A GLOBAL OVERVIEW	28
<i>N. Roth, A. Kaesböhrer, S. Mayrhofer, U. Zitz, C. Hofacre, J. Wirges and K. J. Domig</i>	
ANTIMICROBIAL RESISTANCE IN COMMENSAL <i>ESCHERICHIA COLI</i> FROM FECAL SAMPLES ON BAVARIAN DAIRY FARMS	29
<i>I. Lorenz</i>	
ASSOCIATIONS BETWEEN ANTIMICROBIAL TREATMENT MODALITIES AND ANTIMICROBIAL SUSCEPTIBILITY IN <i>PASTEURELLACEAE</i> AND <i>E. COLI</i> ISOLATED FROM VEAL CALVES UNDER FIELD CONDITIONS	30
<i>L. Schönecker, P. Schnyder, G. Overesch, G. Schüpbach-Regula and M. Meylan</i>	
EFFECTS OF GARLIC ( <i>ALLIUM SATIVUM</i> L.) IN POSTWEANING PIGS – A PLACEBO CONTROLLED STUDY	31
<i>H. Ayrle, H. Nathues, A. Bieber, M. Durrer, N. Quander, M. Mevissen and M. Walkenhorst</i>	
THE RESPIRATORY TRACT MICROFLORA SENSITIVITY TO ANTIMICROBIAL DRUGS IN NEWBORN CALVES WITH DIFFERENT LEVELS OF VITALITY	32
<i>A. Chernitskiy, S. Shabunin, O. Manzhurina and V. Safonov</i>	
ANTIMICROBIAL USE INDICATORS AND THEIR EFFECT ON RELATIVE RANKING OF GROWER-FINISHER HERDS	33
<i>A. L. Bosman, C. A. Carson, A. Deckert, R. J. Reid-Smith, Z. Poljak, S. Gow, D. F. Léger and S. A. McEwen</i>	



<b>SESSION 02:</b>	
<b>LOCOMOTION DISORDERS: IMPACT ON PERFORMANCE AND WELFARE</b>	<b>34</b>
ETIOPATHOGENESIS OF NON-INFECTIOUS CLAW LESIONS IN CATTLE AND PIGS – IS IT ALL ABOUT BIOMECHANICS? <i>C. K. W. Mülling</i>	34
INFLAMMATION, PART OF THE FOOT PRINT OF SOW LAMENESS <i>M. Wilson, W. Schweer, A. Tinkle, C. R. Dove, A. Cornelison, J. Torrison and Z. Rambo</i>	35
LAMENESS IN DAIRY COWS; WHERE HAVE WE COME FROM AND WHERE ARE WE GOING? <i>L. V. Randall</i>	36
DIGITAL DERMATITIS UPDATE – WHAT SHOULD ARTIFICIAL INTELLIGENCE DO FOR YOU IN THE NEAR FUTURE? <i>D. Döpfer</i>	37
HOOF CARE AND LAMENESS: LEARNING FROM COMMERCIAL DAIRY GOAT FARMS <i>G. Zobel and J. Webster</i>	38
EVALUATION OF INTRA- AND INTERRATER AGREEMENT AND RELIABILITY OF VISUAL LOCOMOTION SCORING DURING THE DAILY ROUTINE OF DAIRY COWS <i>A. Hund and M. Dolezal</i>	39
ADVANCES IN TURKEY LAMENESS AND GAIT ANALYSIS <i>M. Erasmus</i>	40
THE PREVALENCE OF CARPAL, HOCK, AND STIFLE LESIONS AND POTENTIAL RISK FACTORS IN SWISS DAIRY COWS KEPT IN TIE STALLS <i>J. Bernhard, B. Vidondo and A. Steiner</i>	41
USE OF AUTOMATED METHODS OF LAMENESS DETECTION FOR EVALUATING THE EFFICACY OF KETOPROFEN IN LAME COWS <i>M. Alsaad, M. Fadul, R. Deiss, E. Bucher and A. Steiner</i>	42
DETECTION OF DIGITAL DERMATITIS TREPONEMES AND <i>DICHELOBACTER NODOSUS</i> FROM MORTELLARO LESIONS IN SWISS CATTLE <i>M. Alsaad, I. Locher, J. Jores, P. Grimm, I. Brodard, A. Steiner and P. Kuhnert</i>	43
SUPPORTING GERMAN DAIRY FARMERS: DEVELOPMENT OF AN E-LEARNING TOOL TO IMPROVE HOOF HEALTH IN DAIRY COWS <i>M. Au, B. Behr, S. Hachenberg, M. Stumpf and A. Fiedler</i>	44
RISK FACTORS FOR PODODERMATITIS (PD) IN GROUP HOUSED DOES <i>M. Braconnier, S. G. Gebhardt-Henrich, S. Ruchti, A. Meier, H. Würbel, R. Furrer, G. Kratzer and S. Hartnack</i>	45
IMPACT OF FARM INDIVIDUAL ACTION PLANS ON LAMENESS PREVALENCE, PRODUCTIVITY AND WELFARE OF DAIRY CATTLE <i>F. Ebert, A. Cerna, M. Schären, R. Weck, A. Wöckel, W. Wippermann, P. Hufe, E. Ullrich and A. Starke</i>	46
VALIDATION OF A VISUAL SCORING SYSTEM FOR FOOTPAD DERMATITIS IN BROILER CHICKENS ON FARM LEVEL <i>S. Heitmann, J. Stracke, H. Petersen, N. Kemper and B. Spindler</i>	47
<b>SESSION 03:</b>	
<b>PRODUCTION DISEASES AND WELFARE IN LAYING HENS</b>	<b>48</b>
THE CAUSES OF KEEL BONE FRACTURES IN LAYING HENS <i>M. J. Toscano</i>	48
ENVIRONMENTAL FACTORS CONTRIBUTING TO KEEL BONE DAMAGE SUSTAINED BY LAYING HENS <i>M. Makagon</i>	49
QUALITY OF THE LAYING HEN SKELETON; INSIGHTS AND SOLUTIONS FROM GENETICS <i>I. C. Dunn, B. Andersson, R. H. Fleming, D. J. De Koning, H. M. McCormack, A. B. Rodriguez-Navarro, M. Schmutz and P. W. Wilson</i>	50
IDENTIFYING GENETIC COMPONENTS CONTRIBUTING TO BONE STRENGTH IN LAYING HENS <i>D. J. de Koning, F. Lopes Pinto, H. A. McCormack, R. H. Fleming, I. C. Dunn, A. Kindmark, A. B. Rodriguez-Navarro and H. Wall</i>	51

COMPARING BONE STABILITY OF LAYING HENS DIFFERING IN PHYLOGENY AND PERFORMANCE LEVEL IN TWO HUSBANDRY SYSTEMS	52
<i>S. Jansen, U. Baulain, A. Weigend, I. Halle, A. R. Sharifi, H. Simianer and S. Weigend</i>	
EFFECT OF EGG PRODUCTION ON BONE QUALITY IN LAYING HENS	53
<i>S. Petow, B. K. Eusemann, A. B. Rodriguez-Navarro, E. Sanchez-Rodriguez, A. Patt, C. Benavides-Reyes, N. Dominguez-Gasca, L. Schrader and R. Ulrich</i>	
IMPROVING INTRA- AND INTER-OBSERVER REPEATABILITY AND ACCURACY OF KEEL BONE ASSESSMENT IN LAYING HENS BY TRAINING WITH RADIOGRAPHS	54
<i>S. G. Gebhardt-Henrich, C. Rufener and A. Stratmann</i>	
BLACK FOOD FOR FOWL – EFFECTS OF ENRICHED CHARCOAL IN A TEMPORARY CRUDE PROTEIN REDUCED DIET ON MALE TURKEYS FATTENED UNDER ON-FARM CONDITIONS	55
<i>K. Hinz, J. Stracke, N. Kemper and B. Spindler</i>	
FEATHER PECKING IN LAYING HENS IN RELATION TO MOTOR IMPULSIVITY	56
<i>J. Heinsius, N. van Staaveren, I. Y. Kwon, A. Li, J. B. Kjaer and A. Harlander</i>	
IDENTIFYING WELFARE ISSUES IN TURKEY HEN AND TOM FLOCKS APPLYING THE TRANSECT WALK METHOD	57
<i>J. Marchewka, G. Vasdal and R. Oppermann Moe</i>	
DECISION MAKING OF HIGH AND LOW FEATHER PECKING LINE OF LAYING HENS UNDER AMBIGUITY	58
<i>K. Pichova, J. van der Eijk, L. Kostal, B. Bilcik and T. B. Rodenburg</i>	
THE INFLUENCE OF TACTILE STIMULATION ON AFFECTIVE STATES IN LAYING HENS	59
<i>Z. Skalná, K. Pichová and L. Košťál</i>	
EFFICACY OF AMMONISATION TO ELIMINATE AFLATOXIN, OCHRATOXIN, AND ZEARALENONE	60
<i>V. Dogan and A. Hayirli</i>	
<b>SESSION 04:</b>	
<b>GUT MICROBIOME AND ANIMAL HEALTH</b>	<b>61</b>
MICROBIAL COLONISATION IS ESSENTIAL FOR THE DEVELOPMENT OF THE GUT IMMUNE SYSTEM IN CHICKENS	61
<i>B. Kaspers, S. Lettmann, S. Härtele, S. Röhl, B. Schusser, P. Velge, and C. Schouler</i>	
INFECTIOUS BURSAL DISEASE VIRUS INFECTION LEADS TO MODIFICATIONS OF GUT-ASSOCIATED IMMUNE PARAMETERS AND MICROBIOTA COMPOSITION	62
<i>S. Rautenschlein, L. Li, T. Kubasová, I. Rychlik and F. J. Hoerr</i>	
SUPPORTING GUT HEALTH: PRINCIPLES UNDERLYING ALTERNATIVES TO ANTIBIOTICS IN FOOD ANIMAL MANAGEMENT	63
<i>T. H. Elsasser and A. Cross</i>	
HOW TO SELECT GUT MICROBIOTA MEMBERS WITH INCREASING CHICKEN RESISTANCE TO ENTERIC DISEASES	64
<i>I. Rychlik</i>	
COMPETITIVE EXCLUSION AND COMPARTMENTALIZATION TO INTERVENE IN COLONIZATION AND TRANSMISSION OF ESBL- <i>E. COLI</i> IN BROILER FLOCKS	65
<i>M. A. Dame-Korevaar, E. A. J. Fischer, J. A. van der Goot, F. C. Velkers, D. Ceccarelli, D. J. Mevius and J. A. Stegeman</i>	
IMPACT OF A MIXTURE OF MONO-, DI- AND TRIGLYCERIDES OF BUTYRIC, CAPRIC, AND CAPRYLIC ACID, FORMIC ACID AND A PROBIOTIC <i>CLOSTRIDIUM BUTYRICUM</i> (CBM588) ON PERFORMANCE AND GUT HEALTH IN BROILER CHICKENS	66
<i>J. Zentek, W. Vahjen, K. Männer and P. Ader</i>	
CHANGES IN RUMEN pH, VOLATILE FATTY ACIDS, LIPOPOLYSACCHARIDE, AND THE BACTERIAL COMMUNITY OF JAPANESE BLACK BEEF CATTLE AT DIFFERENT FATTENING STAGES	67
<i>S. Sato, H. Makino, N. Ishizuka, Y.-H. Kim, T. Masaki, E. Iwamoto, S. Kushibiki and H. Mizuguchi</i>	
EFFECT OF DIETARY PHYTASE AND LACTIC ACID TREATMENT OF CEREALS ON METABOLIC ACTIVE BACTERIA IN ILEAL DIGESTA, MUCOSA AND ILEO-CECAL LYMPH NODES AND EXPRESSION OF INNATE IMMUNE GENES IN PIGS	68
<i>J. Klinsoda, J. C. Vötterl, Q. Zebeli and B. U. Metzler-Zebeli</i>	

COMPARISON OF PH, VOLATILE FATTY ACIDS, LIPOPOLYSACCHARIDE, AND THE BACTERIAL COMMUNITY IN THE RUMEN AND RETICULUM OF JAPANESE BLACK BEEF CATTLE <i>S. Sato, H. Makino, Y.-H. Kim, T. Masaki, E. Iwamoto, S. Kushibiki, T. Ichijo and H. Mizuguchi</i>	69
<b>SESSION 05:</b>	
<b>IMPACT OF CLIMATE CHANGE AND MICROCLIMATE ON ANIMAL HEALTH</b>	<b>70</b>
PERMANENT LONG-TERM SURVEILLANCE OF AMMONIA IN LIVESTOCK – POSSIBILITIES OF A NOVEL SENSOR TECHNOLOGY <i>S. Dennier</i>	70
THE IMPACT OF HEAT STRESS ON REPRODUCTION AND FERTILITY IN DAIRY COWS <i>Z. Roth</i>	71
EFFECTS OF VET.SMART.TUBES VENTILATION SYSTEMS ON CALF RESPIRATORY HEALTH <i>A.-C. Endres, J. John-Wiedmer, J. Neumayer and W. Hasseler</i>	72
PHYSIOLOGICAL INDICATORS OF BEGINNING HEAT STRESS IN GRAZING HOLSTEIN DAIRY COWS <i>A. Pontiggia, A. Münger, F. Dohme-Meier, L. Eggerschwiler, W. Heuwieser, L. Schüller, S. Ammer, R. Bruckmaier and N. Keil</i>	73
<b>SESSION 06:</b>	
<b>PRECISION LIVESTOCK FARMING METHODS TO CONTROL ANIMAL HEALTH AND WELFARE</b>	<b>74</b>
SMART DATA IN HEALTH MANAGEMENT FROM PRECISION LIVESTOCK FARMING <i>D. Berckmans</i>	74
ANIMAL HEALTH SURVEILLANCE AND SMART FARMING: CYBER SECURITY IN TIMES WITH ROBOTS, BIG DATA, AI AND SOCIAL MEDIA <i>A. Dörig</i>	75
PRECISION DAIRY OPPORTUNITIES AND CHALLENGES <i>J. M. Bewley</i>	76
INTENSIVE PIG PRODUCTION SYSTEMS: GOOD OR BAD FOR ANIMAL HEALTH AND WELFARE? <i>D. Maes</i>	77
ACOUSTIC OF DAIRY COWS' GAIT: DETECTING CLAW LESIONS BY ANALYZING THE FOOTFALL SOUND <i>N. Volkmann, B. Kulig, S. Hoppe, O. Hensel and N. Kemper</i>	78
MONITORING CALVES AT RISK FOR DIARRHEA BY USE OF AN EAR-ATTACHED ACCELEROMETER SYSTEM <i>M. Goharshahi, L. Lidauer, A. Berger, F. Kicking, M. Öhlschuster, W. Auer, D. Klein-Jöbstl, M. Drillich and M. Iwersen</i>	79
ZELLDiX – A NEW APPROACH TO PREDICT UDDER HEALTH BY USING DHI RESULTS AND CELL DIFFERENTIATION <i>A. Bartel, F. Querengässer, E. Gass, F. Onken, C. Baumgartner and M. Doherr</i>	80
USING COGNITIVE BIAS AS A WELFARE TOOL IN POULTRY <i>L. Košťál, Z. Skalná and K. Pichová</i>	81
AUTOMATED ASSESSMENT OF WELFARE IN CHICKENS <i>M. S. Dawkins</i>	82
ASSOCIATION OF RUMINATION TIME WITH SOME BIOMARKERS FROM AUTOMATIC MILKING SYSTEM <i>D. Malašauskienė, M. Televičius, V. Juozaitienė, Ž. Prokopavičiūtė, I. Navalinskaitė, E. Pocevičienė and R. Antanaitis</i>	83
ASSOCIATION BETWEEN SOCIAL PREFERENCE AND DEVELOPMENT OF TAIL BITING IN GROWING-FINISHING PIGS <i>Y. Z. Li, K. St Charles and L. J. Johnston</i>	84
STAKEHOLDER GROUPS' PERCEPTIONS OF THE PRIMARY CAUSES OF POOR DAIRY COW WELFARE AND DAIRY COW CULLING <i>J. F. Mee, J. Marchewka and L. Boyle</i>	85
PROBLEMS AND SOLUTIONS ASSOCIATED WITH TESTING A NOVEL TAIL-AFFIXED CALVING BIOSENSOR IN DAIRY COWS <i>J. F. Mee, L. English and J. P. Murphy</i>	86
TAIL-BITING IN PIGS: CHANGE IN FEEDING BEHAVIOURS DURING A TAIL-BITING OUTBREAK <i>C. Ollagnier</i>	87

<b>SESSION 07:</b>	
<b>METABOLIC STATUS AND RISK OF DISEASE</b>	<b>88</b>
TOWARD A HOMEOSTATIC VIEW OF INFLAMMATION: THE TRANSITION DAIRY COW EXAMPLE <i>B. Bradford and T. Swartz</i>	88
INNOVATIVE INSIGHTS IN THE INTERACTION BETWEEN METABOLIC STRESS AND REPRODUCTION IN HIGH YIELDING DAIRY COWS <i>G. Opsomer</i>	89
MECHANISMS AND IMPACT OF INFLAMMATORY DISEASES ON REPRODUCTION IN DAIRY COWS <i>J. E. P. Santos and E. S. Ribeiro</i>	90
INTERACTION BETWEEN INFLAMMATION AND METABOLISM IN PERIPARTURIENT DAIRY COWS <i>E. Trevisi, A. Minuti and M. Mezzetti</i>	91
AMINO ACIDS AND THE REGULATION OF OXIDATIVE STRESS AND IMMUNE FUNCTION IN DAIRY COWS <i>D. N. Coleman, V. Lopreiato and J. J. Looor</i>	92
KETOSIS IN DAIRY COWS: PREVENTION AND THERAPY <i>S. Dänicke</i>	93
THE EFFECT OF MONENSIN ON DAIRY COWS IN RELATION TO FARM OR BREED <i>P. Karis, K. Ling, H. Jaakson, L. Post and M. Ots</i>	94
KETONE BODIES IN MILK OF POLISH HOLSTEIN-FRIESIAN COWS IN EARLY LACTATION – A BIG DATA STUDY <i>Z. M. Kowalski, M. Sabatowicz, W. Jagusiak, P. Gorka and A. Otwinowska-Mindur</i>	95
DO <i>FUSARIUM</i> TOXIN CONTAMINATED DIETS HAVE AN IMPACT ON DAIRY COW HEALTH AND PERFORMANCE? <i>S. Carp - van Dijken, G. Hop, G. Counotte, K. van den Broek, K. Junker and A. Velthuis</i>	96
EFFECTS OF PRE-TRANSPORT DIET, TRANSPORT DURATION AND TYPE OF VEHICLE ON METABOLISM AND IMMUNITY OF YOUNG VEAL CALVES <i>F. Marcato, H. van den Brand, B. Kemp, B. Engel, M. Wolthuis-Fillerup and K. van Reenen</i>	97
EFFECT OF ENERGY RESTRICTION DURING A ONE-PHASE DRY PERIOD ON THE PRODUCTIVE, METABOLIC AND ENDOCRINE STATUS OF DAIRY COWS <i>M. Elsabagh, T. Hasunuma, K. Kawashima, S. Sato, S. Kushibiki, T. Obitsu and T. Sugino</i>	98
EFFECT OF OVER-CONDITIONING AROUND CALVING ON THE mRNA ABUNDANCE OF GENES RELATED TO THE MITOCHONDRIAL PROTEIN IMPORT MACHINERY IN THE LIVER OF DAIRY COWS <i>M. H. Ghaffari, M. T. Alaedin, K. Schuh, H. Sadri, C. Koch, D. Fieten and H. Sauerwein</i>	99
EFFECT OF OVER-CONDITIONING AROUND CALVING ON CARNITINE PALMITOYLTRANSFERASE GENE EXPRESSION IN THE LIVER AND SKELETAL MUSCLE OF DAIRY COWS <i>M. H. Ghaffari, M. T. Alaedin, K. Schuh, I. Hofs, D. Fieten, C. Koch, H. Sadri and H. Sauerwein</i>	100
IMPACT OF ESSENTIAL FATTY ACIDS AND CONJUGATED LINOLEIC ACID ON ACUTE PHASE RESPONSE AND ANTIOXIDATIVE STATUS OF TRANSITION DAIRY COWS FED AN N-3 FATTY ACID REDUCED DIET <i>M. Gnott, L. Vogel, A. Starke, A. Tröschner, E. Trevisi, U. Bernabucci, T. Stefaniak and H. M. Hammon</i>	101
DYNAMICS OF METABOLIC OXIDATION IN LATE-PREGNANT AND EARLY LACTATING DAIRY COWS SUPPLEMENTED WITH CONJUGATED LINOLEIC ACID OR A-LINOLENIC ACID USING MEASUREMENTS OBTAINED IN RESPIRATION CHAMBERS <i>L. Vogel, M. Gnott, A. Tröschner, M. Derno, B. Kuhla, A. Starke and H. M. Hammon</i>	102
RISK FACTORS FOR DAIRY COW HERDS TO BE CLASSIFIED AS “KETOTIC” <i>M. Sabatowicz, Z. M. Kowalski, W. Jagusiak and P. Gorka</i>	103
THE ROLE OF ALTERED IMMUNE FUNCTION DURING THE DRY PERIOD IN PROMOTING THE DEVELOPMENT OF KETOSIS IN EARLY LACTATION <i>M. Mezzetti, A. Minuti, F. Piccioli-Cappelli, M. Amadori, M. Bionaz and E. Trevisi</i>	104



TESTING DRY COWS FOR RISK OF POSTPARTUM DISEASES: TWO-YEAR RESULTS FROM A ROUTINE LABORATORY <i>S. van der Drift and S. Carp – van Dijken</i>	105
FEED RESTRICTION IN LATE GESTATION AND EARLY LACTATION AFFECTS BODY WEIGHT AND COLOSTRUM AND MILK PRODUCTION IN IRANIAN FAT-TAILED EWES <i>M. Zarrin, M. Sanginabadi, M. Nouri, A. Ahmadpour and R. M. Bruckmaier</i>	106
REGULATION OF THE ENDOCANNABINOID SYSTEM IN THE HYPOTHALAMUS OF LATE AND EARLY LACTATING DAIRY COWS <i>B. Kuhla and A. Kuhla</i>	107
EVALUATION AND REDUCTION OF INFLUENCE OF STRESS IN FRESH DAIRY COWS <i>G. Lošytė, M. Televičius, D. Malašauskienė and R. Antanaitis</i>	108
INTENSIVE FEEDING ALTERS SPHINGOLIPID METABOLISM IN HOLSTEIN BULLS <i>Á. Kenéz, S. Bäßler, C. Koch, T. Scheu and K. Huber</i>	109
EFFECTS OF PHYSIOLOGICAL STRESS DURING PREGNANCY ON ADAPTATION PROCESSES IN IMPORTED COWS DURING POSTPARTUM AND DRY PERIODS <i>I. Ventsova, A. Vostroilov and V. Safonov</i>	110
EFFECTS OF AN INTRAMAMMARY LPS CHALLENGE ON INFLAMMATORY AND METABOLIC RESPONSES IN EARLY LACTATING COWS SUPPLEMENTED WITH CONJUGATED LINOLEIC ACID <i>J. J. Gross, L. Grossen-Rösti, R. Héritier, A. Tröschler and R. M. Bruckmaier</i>	111
RECOVERY OF MILK SOMATIC CELL COUNT AND PERFORMANCE AFTER AN INTRAMAMMARY LPS CHALLENGE IS DEPENDENT ON THE METABOLIC STATUS OF DAIRY COWS <i>J. J. Gross, L. Grossen-Rösti, S. K. Wall, O. Wellnitz and R. M. Bruckmaier</i>	112
PREVALENCE OF SUBCLINICAL KETOSIS AND PRODUCTION DISEASES DURING EARLY LACTATION IN DAIRY COWS IN CENTRAL AND SOUTH AMERICA, AFRICA, ASIA, AUSTRALIA, NEW ZEALAND AND EASTERN EUROPE <i>N. Brunner, S. Groeger, J. Canelas Raposo, R. M. Bruckmaier and J. J. Gross</i>	113
IS THERE AN ASSOCIATION BETWEEN METHANE EMISSION AND LYMPHOCYTE RESPONSE IN PERIPARTAL COWS? <i>S. Meese, S. E. Ulbrich, H. Bollwein, R. Bruckmaier, O. Wellnitz, M. Kreuzer, M. Röntgen, U. Gimsa and A. Schwarm</i>	114
<b>SESSION 08:</b>	
<b>IMPACT OF ANIMAL GENETICS ON ANIMAL HEALTH AND DISEASE</b>	<b>115</b>
BREEDING FOR HEALTH, WELFARE AND EFFICIENCY: HELP WANTED <i>C. F. Baes, G. de Oliveira, H. Emamgholi-Begli, E. Abdalla and B. J. Wood</i>	115
COMBINING MULTIPLE OMICS DATA TYPES TO BREED HEALTHIER ANIMALS <i>H. D. Daetwyler, R. Xiang, I. M. MacLeod, T. D. W. Luke, P. N. Ho, T. T. T. Nguyen, M. Wang, C. P. Prowse-Wilkins, C. J. Van der Jagt, Z. Liu, B. Sunduimijid, A. Benedet, C. Phyn, B. J. Hayes, S. J. Rochfort, V. Bonfatti, A. J. Chamberlain, M. E. Goddard and J. E. Pryce</i>	116
WHY DOES THE HEN PECK? – EMPLOYING OMICS-APPROACHES TO UNDERSTAND THE MOTIVATION FOR AN UNWANTED BEHAVIOUR <i>J. Beier, C. Falker-Gieske, H. Iffland, S. Preuß, W. Bessei J. Bennewitz and J. Tetens</i>	117
BREEDING FOR RESILIENCE: NEW OPPORTUNITIES IN MODERN PIG BREEDING PROGRAMS <i>B. Harlizius, P. Mathur and E. F. Knol</i>	118
FROM LAB TO FARMYARD: GENOME EDITING OUR LIVESTOCK <i>C. Tait-Burkard</i>	119
DIFFERENT GENETIC REACTIONS OF SIMMENTAL AND HOLSTEIN DAIRY CATTLE CONCERNING UDDER HEALTH ALONG A CONTINUOUS CLIMATE SCALE <i>B. Bapst, M. Bohlouli, S. König and K. Brügemann</i>	120
EXPLOITING MASSIVE GENOMIC DATA TO IMPROVE FERTILITY AND REARING SUCCESS IN SWISS DAIRY CATTLE <i>I. M. Häfliger, F. R. Seefried and C. Drögemüller</i>	121
CHROMOSOMAL IMBALANCE IN A BOAR CAUSING CLEFT PALATE IN THE OFFSPRING AND AFFECTING THE LITTER SIZE IN SOWS <i>A. Grahofner, A. Letko, I. M. Häfliger, V. Jagannathan, A. Ducos, O. Richard, V. Peter, H. Nathues and C. Drögemüller</i>	122

ENTROPION IN SWISS WHITE ALPINE SHEEP IS ASSOCIATED WITH THE <i>CTNND1</i> GENE REGION ON CHROMOSOME 15 <i>N. Hirter, A. Letko, I. Häfliger, D. Greber and C. Drögemüller</i>	123
HERITABILITY AND PHENOTYPIC CORRELATIONS OF PROTEIN EFFICIENCY IN A SWISS LARGE WHITE PIG POPULATION <i>C. Kasper, I. Ruiz-Ascacibar, P. Stoll and G. Bee</i>	124
THE <i>APOB</i> LOSS-OF-FUNCTION MUTATION OF HOLSTEIN DAIRY CATTLE REDUCES THE CAPACITIES FOR CHOLESTEROL TRANSPORT IN CIRCULATION BUT DOES NOT IMPLY A CHOLESTEROL DEFICIENCY <i>J. J. Gross, A.-C. Schwinn, F. Schmitz-Hsu, A. Barenco, T. F.-O. Neuenschwander, C. Drögemüller and R. M. Bruckmaier</i>	125
<b>SESSION 09:</b>	
<b>NEONATE HEALTH: IMPACT OF FEEDING MANAGEMENT AND COLOSTRUM</b>	<b>126</b>
COLOSTRUM IN SWINE: ESSENTIAL ROLES AND FACTORS OF VARIATION <i>C. Farmer</i>	126
COLOSTRUM: BACK TO THE BASICS WITH IMMUNOGLOBULINS <i>A. J. Geiger</i>	127
BEYOND IMMUNOGLOBULINS: THE IMMUNOREGULATORY ROLE OF COLOSTRUM <i>H.-J. Schuberth</i>	128
UPDATE ON POSTPARTUM DYSGALACTIA SYNDROME IN SOWS <i>N. Kemper</i>	129
RELATIONSHIPS BETWEEN COLOSTRUM CONSUMPTION AND KID IGG STATUS <i>E. V. St. Clair, R. W. Russell and R. J. Van Saun</i>	130
EVALUATION OF TWO DIFFERENT TREATMENT PROCEDURES AFTER CALVING TO IMPROVE HARVESTING OF HIGH QUANTITY AND QUALITY COLOSTRUM <i>F. Sutter, S. Borchardt, G. M. Schuenemann, E. Rauch, M. Erhard and W. Heuwieser</i>	131
PRACTICE REPORT: AUTOMATIC CALF FEEDING - SCIENTIFIC RESULTS SUCCESSFUL IMPLEMENTED IN PRACTICE <i>J. Plesse</i>	132
ASSESSING THE UTILITY OF LEUKOCYTE DIFFERENTIAL CELL COUNTS FOR PREDICTING MORTALITY RISK IN NEONATAL HOLSTEIN CALVES UPON ARRIVAL AND 72 HOURS POST-ARRIVAL AT CALF REARING FACILITIES <i>T. E. von Königslow, D. L. Renaud, T. F. Duffield, C. B. Winder, V. Higginson and D. F. Kelton</i>	133
USE OF <i>SACCHAROMYCES CEREVISIAE</i> FERMENTATION PRODUCTS IN COMPARISON TO HALOFUGINONE IN <i>CRYPTOSPORIDIUM</i> INFECTED NEWBORN CALVES <i>J. Vélez, M. K. Lange, P. Zieger, I. Yoon, K. Failing and C. Bauer</i>	134
HYPERNATREMIA IN DIARRHEIC CALVES FED ELECTROLYTES MIXED WITH MILK REPLACER WITHOUT ACCESS TO WATER <i>J. Wilms, L. Leal and J. Martín-Tereso</i>	135
IMMUNE MODULATING EFFECT OF A SEAWEED EXTRACT ON SPECIFIC IGG AND TOTAL IGA TITERS IN THE COLOSTRUM AND MILK <i>F. Bussy, M. Le Goff and P. Collen</i>	136
EFFECT OF THE MATERNAL SUPPLY WITH ESSENTIAL FATTY ACIDS AND CONJUGATED LINOLEIC ACID ON THE IMMUNE AND OXIDATIVE STATUS IN NEONATAL CALVES <i>K. L. Uken, L. Vogel, T. Gnott, A. Tuchscherer, A. Tröscher, E. Trevisi, T. Stefaniak, H. Sauerwein and H. M. Hammon</i>	137
EFFECT OF DIFFERENT DIETARY SELENIUM CONCENTRATIONS IN DRY COWS ON FIRST COLOSTRUM IGG LEVELS <i>J. Illek, K. Mikulková, R. Kadek, V. Sujová and D. Kumprechtová</i>	138
THE IMPACT OF WARMING OF NEWBORN HOLSTEIN CALVES ON COLOSTRUM INTAKE, BLOOD PARAMETERS AND VITALITY <i>L. Jürgensen, J. Sohrt and M. Kaske</i>	139
CONCENTRATIONS AND REFERENCE INTERVALS OF TRACE ELEMENTS, VITAMIN E, AND OTHER CLINICAL-CHEMICAL PARAMETERS IN SERUM OF 1 TO 6 DAY OLD CALVES <i>J. Muskens, S. van der Drift, R. Grotentraast and G. Counotte</i>	140

CAN BOVINE COLOSTRUM MICROBIOME BE LINKED TO PASSIVE TRANSFER OF IMMUNITY? <i>I. Van Hese, K. Goossens, L. Vandaele and G. Opsomer</i>	141
USE OF BRIX REFRACTOMETER IN ASSESSING GOAT COLOSTRUM <i>E. V. St. Clair, R. W. Russell and R. J. Van Saun</i>	142
DETERMINATION OF THE NUTRITIONAL BOUNDARIES FOR LACTOSE REPLACEMENT WITH DEXTROSE IN MILK REPLACERS FOR REARING CALVES FED TWICE DAILY <i>J. Wilms, H. Berends, L. Leal, and J. Martín-Tereso</i>	143
VARIATION OF COLOSTRUM COMPOSITION AND IGG CONTENT IN GOAT AND SHEEP BREEDS <i>E. C. Kessler, R. M. Bruckmaier and J. J. Gross</i>	144
ASSESSMENT OF COLOSTRUM QUALITY IN DAIRY COWS BY COLOR MEASUREMENT, BRIX REFRACTOMETRY, AND SPECIFIC GRAVITY <i>J. J. Gross, E. C. Kessler and R. M. Bruckmaier</i>	145
<b>SESSION 10: NOVEL STRATEGIES IN HEALTH AND DISEASE MANAGEMENT</b>	<b>146</b>
EFFECTS OF THE NOVEL CONCEPT "OUTDOOR VEAL CALF" ON ANTIMICROBIAL USE, CALF GROWTH AND MORTALITY IN SWITZERLAND <i>J. Becker, A. Steiner, G. Schüpbach, V. Perreten and M. Meylan</i>	146
CUSTOMISING DRY PERIOD MANAGEMENT: CONSEQUENCES FOR MILK YIELD, BODY CONDITION AND DISEASE INCIDENCE <i>A. T. M. van Knegsel, R. J. van Hoeij, B. Kemp and A. Kok</i>	147
CUSTOMISING LACTATION LENGTH: IMPACT OF CALVING INTERVAL, PARITY, AND LACTATION PERSISTENCY ON MILK PRODUCTION OF DAIRY COWS <i>E. E. A. Burgers, A. Kok, R. M. A. Goselink and A. T. M. van Knegsel</i>	148
DAIRY VETERINARIANS' SKILL IN MOTIVATIONAL INTERVIEWING IS LINKED TO ENHANCED VETERINARY HERD HEALTH MANAGEMENT CONSULTATIONS <i>C. Svensson, H. Wickström, U. Emanuelson, C. von Brömssen, S. Betnér and L. Forsberg</i>	149
THE POTENTIAL OF LOCAL ADMINISTRATION OF NSAID IN MASTITIS TREATMENT <i>O. Wellnitz and R. M. Bruckmaier</i>	150
A MODEL FOR ASSESSING MANAGEMENT-DRIVEN ALTERNATIVES FOR DISEASE CONTROL: A CASE-STUDY ON STREPTOCOCCUS SUI DISEASE IN NURSERY PIGS <i>E. Giang, J. M. Sargeant, Z. Poljak and A. L. Greer</i>	151
ASSOCIATIONS BETWEEN PIG HEALTH, WELFARE, PERFORMANCE AND ANTIBIOTIC USE ON FARM AND POST MORTEM MEAT INSPECTION OUTCOMES <i>J. C. Pessoa, M. R. Costa, E. G. Manzanilla, T. Norton, O. Clear, C. McAloon and L. A. Boyle</i>	152
MANAGEMENT PRACTICES ASSOCIATED WITH LAMB MORTALITY ON IRISH SHEEP FARMS: A NATIONAL SURVEY <i>D. Shiels, J. F. Mee, C. Dwyer and T. Keady</i>	153
DEMOGRAPHY AND POTENTIAL BIOSECURITY THREATS ASSOCIATED WITH CONTRACT DAIRY HEIFER REARING <i>M. C. McCarthy, J. Kennelly, N. Byrne, L. O'Grady and J. F. Mee</i>	154
THE EFFECT OF PEER GROUP EDUCATION ON VETERINARY PRESCRIPTION PRACTICES <i>V.-B. Pucken, M. Bodmer, S. Schouwey, F. Nägele and G. Schüpbach-Regula</i>	155
BIOSECURITY ON FINNISH SWINE AND CATTLE FARMS, WITH SPECIAL EMPHASIS ON RODENT CONTROL <i>L. Seppä-Lassila, H. Rossow, M. Simola, J. Niemimaa, O. Huitu and P. Tuominen</i>	156
LABOUR PROCESS DURING MACHINE MILKING OF EWES WITH REGARDS TO THEIR BIOLOGICAL NEEDS AND WELFARE <i>L. Mačuhová, V. Tančin, M. Uhrinčat' and J. Mačuhová</i>	157
LOCOMOTION, METABOLIC AND ALIMENTARY DISORDERS IN HOLSTEIN AND CZECH FLECKVIEH DAIRY COWS – COMPARISON OF OCCURRENCE IN THE CZECH REPUBLIC <i>S. Šlosárková, E. Kašná, P. Fleischer, A. Pechová and L. Zavadilová</i>	158

RISK FACTOR ANALYSIS FOR DAIRY COW PRODUCTION DISEASES BY A SYSTEM ANALYSIS - FIRST RESULTS FROM THE EIP-PROJECT "DIE ENTWICKLUNG DES KUH-MEHR-WERT NAVIGATORS"	159
<i>M. Schären, B. Waurich, F. Ebert, A. Wöckel, W. Wippermann, A. Özcan, G. Hermenau, J. Wittich, S. Dänicke, H. Swalve, P. Hufe, D. May and A. Starke</i>	
METABOLIC AND BIOCHEMICAL CHANGES IN SOWS AROUND PARTURITION IN RELATION TO FARROWING PROGRESS, PIGLET VITALITY AND HOUSING CONDITION	160
<i>S. Blim, D. Schupp, T. Scheu, C. Koch, G. Thaller, K. Failing and H. Bostedt</i>	
EFFECTS ON MILK QUALITY WHEN REPLACING SOYBEAN MEAL WITH SPIRULINA IN A HAY-BASED DIET FOR DAIRY COWS	161
<i>E. Manzocchi, B. Guggenbühl, M. Kreuzer and K. Giller</i>	
MICROBIOLOGICAL QUALITY OF RAW SHEEP'S BULK MILK SAMPLES ON SELECTED SLOVAK FARMS	162
<i>M. Vršková, V. Tančín, L. Mačuhová and M. Uhrinčat'</i>	
<b>SESSION 11:</b>	
<b>BEHAVIORAL SIGNS OF IMPAIRED ANIMAL HEALTH AND WELFARE</b>	<b>163</b>
RETHINKING INTENSIVE ANIMAL AGRICULTURE AND MEETING PUBLIC EXPECTATIONS FOR OUR FUTURE FARMS	163
<i>M. A. G. von Keyserlingk, M. J. Hötzel and D. M. Weary</i>	
REFINING THE UTILITY OF PRECISION BEHAVIOR MONITORING OF DAIRY CATTLE	164
<i>T. J. DeVries</i>	
POTENTIAL UNDERLYING FACTORS OF SUBACUTE RUMEN ACIDOSIS: EFFECTS OF FREQUENCY AND DURATION OF FEED AVAILABILITY ON PERFORMANCE AND MILK FATTY ACID PROFILE IN DAIRY COWS	165
<i>S. Abd Rahim, H. van Laar, J. Martín-Tereso, W. H. Hendriks and J. Dijkstra</i>	
STRONG DIFFERENCES BETWEEN EARLY LACTATING PRIMIPAROUS AND MULTIPAROUS COWS IN FEED SORTING BEHAVIOR, CHEWING ACTIVITY, AND RUMEN ACIDOSIS INDEX	166
<i>A. Stauder, E. Humer, V. Neubauer, N. Reisinger, A. Kaltenegger and Q. Zebeli</i>	
FEEDING TIME, BODY CONDITION AND LAMENESS DURING THE DRY PERIOD: ASSOCIATIONS WITH TRANSITION PERIOD DISEASE IN DAIRY COWS	167
<i>R. R. Daros, H. K. Eriksson, D. M. Weary and M. A. G. von Keyserlingk</i>	
ANALYSIS OF BEHAVIORAL CHANGES FOR EARLY DETECTION OF LAMENESS IN DAIRY COWS	168
<i>J.-B. Burla, H. Weigele, L. Gyax, A. Steiner and B. Wechsler</i>	
EFFECTS OF SOCIAL DEPRIVATION IN PIGS ON THEIR BEHAVIOURAL, NEUROENDOCRINE AND IMMUNOLOGICAL RESPONSES TO ACUTE ENDOTOXEMIA	169
<i>R. Brückmann, M. Tuchscherer, A. Tuchscherer, U. Gimsa and E. Kanitz</i>	
<b>SESSION 12:</b>	
<b>DISEASE ASPECTS IN REPRODUCTION</b>	<b>170</b>
MANAGEMENT OF PARTURITION IN THE HYPER PROLIFIC SOW	170
<i>O. A. T. Peltoniemi, S. Björkman and C. Oliviero</i>	
DIAGNOSIS OF ENDOMETRITIS AND CYSTITIS IN SOWS: USE OF BIOMARKERS	171
<i>A. Grahofer, S. Sipos and W. Sipos</i>	
METABOLISM AND HOST-PATHOGEN INTERACTIONS SHAPE THE DEVELOPMENT OF UTERINE DISEASE IN DAIRY COWS	172
<i>I. M. Sheldon</i>	
REPEAT BREEDING: THE CAUSE AND THE POSSIBLE TREATMENT TO RESTORE FERTILITY	173
<i>S. Katagiri, Y. Yanagawa and M. Nagano</i>	
FACTORS AFFECTING REPRODUCTIVE WASTAGE IN BEEF CATTLE HERDS	174
<i>D. A. Kenny, M. G. Diskin and P. Lonergan</i>	
SENSING SPERM BY MATERNAL IMMUNE CROSSTALK: A POTENTIAL MECHANISM FOR INTERFERING WITH FERTILITY IN THE COW	175
<i>A. Miyamoto, M. A. Ezz, I. Akthar, M. S. Yousef, K. Imakawa, M. Shimada and M. A. Marey</i>	



RELATIONSHIP BETWEEN RESUMPTION OF OVARIAN ACTIVITY, DAYS OPEN, ENERGY BALANCE AND METABOLIC STATUS IN DAIRY COWS WITH DIFFERENT DRY PERIOD LENGTHS IN EARLY LACTATION <i>J. Ma, R. J. van Hoeij, T. J. G. M. Lam, B. Kemp and A. T. M. van Kneegsel</i>	176
EFFECT OF LECIRELIN ACETATE INTRAVAGINAL ADMINISTRATION ON RABBIT REPRODUCTIVE PARAMETERS AND WELFARE <i>C. Munari, P. Ponzio, A. R. Alkhawagah, A. Schiavone and C. Mugnai</i>	177
EXTENDED LACTATION IN HIGH-YIELDING DAIRY COWS: EFFECTS ON REPRODUCTIVE PERFORMANCE AND MILK PRODUCTION <i>G. Niozas, G. Tsousis, I. Steinhöfel, A. Römer, S. Wiedemann, H. Bollwein and M. Kaske</i>	178
ASSOCIATION BETWEEN UTERINE INVOLUTION IN SOWS AND REPRODUCTIVE PERFORMANCE IN THEIR NEXT GESTATION <i>A. Meile, H. Nathues and A. Grahofner</i>	179
TIMING OF ARTIFICIAL INSEMINATION USING FRESH OR FROZEN SEMEN AFTER AUTOMATED ACTIVITY MONITORING OF ESTRUS IN LACTATING DAIRY COWS <i>C. M. Tippenhauer, J. L. Plenio, A. M. Madueira, R. L. Cerri, W. Heuwieser and S. Borchardt</i>	180
INTRAUTERINE ANTIBIOTIC TREATMENT AND BACTERIAL GROWTH IN DAIRY COWS WITH MILD ENDOMETRITIS AT THE TIME OF INSEMINATION <i>K. Wagener, P. Panagiotis, U. Reinländer, R. Schlegl, M. Ehling-Schulz and M. Drillich</i>	181
MASTITIS AND REPRODUCTION DISORDERS IN HOLSTEIN AND CZECH FLECKVIEH DAIRY COWS – COMPARISON OF OCCURRENCE IN CZECHIA <i>P. Fleischer, E. Kašná, S. Šlosárková, A. Pechová and L. Zavadilová</i>	182
PRE-FARROWING SUPPLEMENT ADDED TO DRINKING WATER REDUCES STILLBIRTH IN SOWS <i>P. Langendijk, J. Bergman and M. Fleuren</i>	183
RELATIONS BETWEEN NEGATIVE ENERGY BALANCE AND EMBRYO HARVEST IN DONORS OF LATVIAN NATIVE COW BREEDS <i>I. Sematovica, G. Ringa-Karahona, V. Antane and I. Kanska</i>	184
<b>SESSION 13:</b>	
<b>MINERAL HOMEOSTASIS AND ASSOCIATED DISEASES</b>	<b>185</b>
THE ROLE OF MAMMARY GLAND CONTROL OF CALCIUM HOMEOSTASIS DURING THE PERIPARTURIENT PERIOD <i>L. L. Hernandez</i>	185
MINERAL METABOLISM IN THE PERIPARTURIENT PERIOD: IMPLICATIONS TO HEALTH AND PRODUCTIVITY <i>J. E. P. Santos and A. Vieira-Neto</i>	186
SKELETON HEALTH: NUTRITIONAL AND MANAGEMENT INTERVENTIONS <i>D. M. Karcher, P. Regmi, C. I. Robison and W. K. Kim</i>	187
LESSONS LEARNED FROM THE HYPOVITAMINOSIS D KYPHOTIC PIG MODEL <i>L. A. Amundson and T. D. Crenshaw</i>	188
EFFECTS OF DIETS HIGH OR LOW IN DIETARY CATION-ANION DIFFERENCE COMBINED WITH ADEQUATE OR RESTRICTED CALCIUM SUPPLY ON RENAL CALCIUM HOMEOSTASIS IN SHEEP <i>J. Freitag, M. R. Wilkens, A. S. Muscher-Banse, N. Schnepel and A. Liesegang</i>	189
ALTERATION IN RESPONSE OF NEGATIVE FEEDBACK TO SUBCLINICAL HYPOCALCEMIA INDUCTION IN DIFFERENT LACTATIONAL STAGES OF HOLSTEIN DAIRY COWS <i>M. K. Connelly and L. L. Hernandez</i>	190
EFFECT OF PHOSPHORUS INTAKE DURING THE TRANSITION PERIOD ON PLASMA PHOSPHORUS CONTENT AND HYPOCALCEMIA IN DAIRY COWS <i>P. Keanthao, R. M. A. Goselink, J. T. Schonewille, J. Dijkstra and W. H. Hendriks</i>	191
DIETARY PHOSPHORUS RESTRICTION INDUCES BONE MOBILIZATION, AFFECTS ACID-BASE BALANCE AND DOWNREGULATES SYNTHESIS OF FIBROBLAST GROWTH FACTOR 23 IN BONE <i>W. Grünberg, A. S. Muscher-Banse, O. Köhler, A. Rajaeerad, N. Schnepel, K. Hansen and M. R. Wilkens</i>	192
EFFECTS OF GREEN LED LIGHT DURING INCUBATION AND DIETARY MINERAL VARIETY DURING REARING ON TIBIA CHARACTERISTICS OF BROILER CHICKENS AT SLAUGHTER AGE <i>B. C. Güz, R. Molenaar, I. C. de Jong, M. van Krimpen and H. van den Brand</i>	193

CALCIUM AND NITROGEN METABOLISM IN GROWING SHEEP ARE AFFECTED BY A MENTHOL-BASED FEED ADDITIVE <i>A. K. Patra, S. Geiger, A. Cieslak, M. Szumacher-Strabel, H.-S. Braun, K. T. Schrapers and J. R. Aschenbach</i>	194
CONCENTRATIONS OF TRACE ELEMENTS IN SERUM OF DRY COWS AND CORRESPONDING REFERENCE INTERVALS FOR THE DRY PERIOD <i>S. van der Drift, R. Grotentraast, S. Carp – Van Dijken, J. Muskens and G. Counotte</i>	195
APPLICATION OF ALTERNATIVE LIME AMENDMENT TO SOIL INCREASES FORAGE MOLYBDENUM INDUCING COPPER DEFICIENCY <i>R. J. Van Saun, M. Hall and J. T. Spargo</i>	196
MATERNAL-FETAL HEPATIC MINERAL INTERACTIONS <i>R. J. Van Saun</i>	197
EFFECT OF DIETARY PHYTASE AND LACTIC ACID TREATMENT OF CEREALS ON GENE EXPRESSION RELATED TO CA AND P HOMEOSTASIS ALONG THE INTESTINAL TRACT, BONES, AND KIDNEY IN PIGS <i>J. C. Vötterl, J. Klinzoda, Q. Zebeli, I. Hennig-Pauka and B. U. Metzler-Zebeli</i>	198
EFFECT OF VOLUNTARY CALCIUM SUPPLEMENTATION AT CALVING ON SURVIVAL TO THE NEXT LACTATION, MILK YIELD AND MILK COMPOSITION OF SUBSEQUENT LACTATION <i>J. B. Daniel, J. Wilms and J. Martín-Tereso</i>	199
EFFECT OF SUPPLEMENTING ZN AND SE IN DIFFERENT RATION AROUND PARTURITION ON MAMMARY HEALTH AND NATURAL ANTIBODIES IN DAIRY COWS <i>N. Mayasari, E. Y. Setyowati, A. Arfiana and L. B. Salman</i>	200
DISELEMENTOSIS AS A RISK FACTOR OF PREECLAMPSIA IN DAIRY COWS <i>V. Safonov and A. Chernitskiy</i>	201
CHANGE IN PLASMA pH IN COWS INDUCED HYPOCALCEMIA WITH Na <sub>2</sub> EDTA <i>Y. Ro, J. Park, W. Choi, E. Choe and D. Kim</i>	202
<b>SESSION 14:</b>	
<b>INFECTIOUS DISEASES AND PATHOBIOLOGY</b>	<b>203</b>
DIAGNOSIS AND CONTROL OF VIRAL DISEASES OF REPRODUCTIVE IMPORTANCE IN CATTLE AND PIGS <i>M. D. Givens</i>	203
INFECTIOUS LARYNGOTRACHEITIS - AN UPDATE ON CURRENT APPROACHES FOR THE PREVENTION OF AN OLD DISEASE <i>N. Wolfrum</i>	204
IMPACT OF THE POULTRY RED MITE ( <i>DERMANYSSUS GALLINAE</i> ) ON POULTRY PRODUCTION SYSTEMS <i>O. A. E. Sparagano</i>	205
PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS: PHYLOGENETIC ANALYSIS OF CIRCULATING STRAINS IN THE REPUBLIC OF IRELAND FROM NOVEMBER 2016 TO JUNE 2017. <i>R. Fitzgerald, P. J. Collins, M. J. McMenamy, J. A. Calderón Díaz, J. Moriarty, E. G. Manzanilla, F. C. Leonard, H. McGlynn and H. O'Shea</i>	206
NORMALIZATION OF DEVIANCE IN BRD DIAGNOSIS: PART 1. KNOWING YOUR WHY <i>S. Barringer</i>	207
NORMALIZATION OF DEVIANCE IN BRD DIAGNOSIS PART 2. LUNG ULTRA SONOGRAPHY - NEW TOOL FOR INVIVO DIAGNOSTICS <i>S. Barringer</i>	208
SWISS FARMERS ETHNOVETERINARY KNOWLEDGE ABOUT THE USE OF MEDICINAL PLANTS TO TREAT AND PREVENT LIVESTOCK DISEASES IN 19 GERMAN SPEAKING CANTONS <i>M. Walkenhorst, A. Maeschli, B. Meier and M. Hamburger</i>	209
VACCINATION AND CHALLENGE AGAINST <i>STAPHYLOCOCCUS AUREUS</i> IN DAIRY COWS USING ANTIGENS EXPRESSED DURING BOVINE INTRAMAMMARY INFECTIONS <i>C. Ster, M. Allard, J. Côté-Gravel, S. Boulanger, P. Lacasse and F. Malouin</i>	210

STAPHYLOCOCCUS AUREUS RELATED TO BOVINE MASTITIS IN SWITZERLAND: CLONAL DIVERSITY, VIRULENCE GENE PROFILES, AND ANTIMICROBIAL RESISTANCE <i>N. Käppeli, M. Morach, S. Corti, C. Eicher, R. Stephan and S. Johler</i>	211
ERADICATION PROGRAM OF STAPHYLOCOCCUS AUREUS GENOTYPE B IN DAIRY COWS IN CANTON TICINO (SWITZERLAND) <i>L. Sesso, M. Vaccani, L. Bacciarini, A. Steiner, M. Bodmer and H. U. Graber</i>	212
OCCURRENCE AND SIGNIFICANCE OF DICHELOBACTER NODOSUS IN SHEEP, CATTLE, GOATS AND SOUTH AMERICAN CAMELIDS IN SWITZERLAND <i>F. Ardüser, G. Moore-Jones, S. Gobeli Brawand, S. Dürr, A. Steiner, M.-P. Ryser-Degiorgis and P. Zanolari</i>	213
DEVELOPMENT OF A MULTIVALENT PEPTIDE VACCINE FOR PREVENTION OF IMPORTANT INFECTIONS IN DAIRY COWS <i>D. V. Bran-Barrera, E. Brouillette, C. Ster and F. Malouin</i>	214
ULVAN ACTIVATES CHICKEN HETEROPHILS AND MONOCYTES THROUGH TOLL-LIKE RECEPTOR 2 AND TOLL-LIKE RECEPTOR 4 <i>N. Guriec, F. Bussy, C. Gouin, O. Mathiaud, B. Quero, M. Le Goff and P. Nyvall Collén</i>	215
COMPARISON OF LUNG LESIONS AT SLAUGHTER, THEIR ASSOCIATED PATHOGENS AND SEROLOGY IN 9 FARROW-TO FINISH IRISH COMMERCIAL PIG HERDS <i>L. O'Brien, M. Costa da Rodrigues, R. Fitzgerald, J. Moriarty, H. O'Shea, F. Leonard, J. Calderon Diaz, E. Garcia Manzanilla and M. McElroy</i>	216
SOMATIC CELL COUNT IN MILK OF EWES AND POSSIBLE EFFECT ON MILK YIELD AND COMPOSITION <i>V. Tančin, K. Tvarožková, L. Mačuhová, M. Uhrinčat' and M. Vršková</i>	217
FREQUENCY OF OCCURRENCE COAGULASE NEGATIVE STAPHYLOCOCCI AND THEIR ANTIBIOTICS RESISTANCE IN LACAUNE BREED <i>K. Tvarožková, V. Tančin, I. Holko, L. Mačuhová, M. Uhrinča and M. Vršková</i>	218
THE EFFECT OF VACCINATION ON CALF GROWTH, MORTALITY AND FARM ECONOMY ON A FINNISH BEEF FARM – A CASE STUDY <i>A. Wainio</i>	219
BOVINE MAMMARY CHEMERIN: MRNA EXPRESSION, REGULATION, AND EFFECT ON PRIMARY BOVINE MAMMARY EPITHELIAL CELLS <i>C. R. Baumrucker, A. L. Macrina, O. Wellnitz and R. M. Bruckmaier</i>	220
EXAMINING THE MICROBIAL COMPOSITION OF ANIMALS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS <i>C. Matthews, A. M. Walsh, P. D. Cotter and J. M. O'Mahony</i>	221
<b>SESSION 15:</b>	
<b>PERSISTENT HEALTH EFFECTS OF JUVENILE DEVELOPMENT</b>	<b>222</b>
NUTRITION IN EARLY LIFE IMPRINTS METABOLIC HEALTH <i>K. Huber</i>	222
DENORMALISING POOR DAIRY YOUNGSTOCK MANAGEMENT – DEALING WITH 'FARM-BLINDNESS' <i>J. F. Mee</i>	223
EFFECTS OF MILK REPLACER COMPOSITION ON GUT AND LUNG HEALTH AND PERFORMANCE IN VEAL CALVES <i>A. de Greeff, A. J. M. Jansman, L. Ruuls, S. A. Vastenhouw, D. Schokker, M. Smits, E. van der Wal, P. Mölder and N. Stockhofe</i>	224
INFLUENCE OF SELECTION FOR GROWTH AND IN OVO FEEDING ON CHICKEN BODY WEIGHT AND IMMUNE ORGAN SIZE IN THE FIRST TWO WEEKS OF LIFE <i>H. Abdel Aziz, S. E. Ulbrich, S. Mueller and S. Thanner</i>	225
THE EFFECT OF SPLIT-SUCKLING IN THE POSTNATAL PERIOD ON PIGLET PERFORMANCES <i>M. Vandaele, C. Van Kerschaver, J. Degroote and J. Michiels</i>	226
<b>AUTHORS</b>	<b>227</b>

### Prudent and effective antimicrobial use in a diverse livestock and consumer's world

U. Magnusson

Department of Clinical Sciences, SLU, Uppsala, Sweden; Livestock Antimicrobial Partnership (LAMP) at the Global Agenda for Sustainable Livestock hosted by FAO, Rome, Italy

Email: [Ulf.Magnusson@slu.se](mailto:Ulf.Magnusson@slu.se)

**Anti-microbial resistance and livestock and human health** The emergence of antimicrobial resistance is regarded as one of the major threats to modern medicine and human health. Similarly, in the livestock sector substantial economic losses are foreseen because of therapeutic failures of infections. The interrelatedness between resistance in microbes in humans and in those in livestock, is, with a few exceptions, not that well established. However, given that the use of antimicrobials drives the development of resistance and that the livestock sector is estimated to be the largest user of antimicrobials world-wide, it is reasonable, both for the sake of animal health and productivity as well for the risk of increasing the resistance in human pathogens, to take a precautionary position and work for a reduced use in the sector. Notably, the risk for transfer of resistance from the livestock sector to the human health sector relates mainly to resistance in bacteria, as similar antibiotics are used for humans and livestock, and the same resistance mechanisms may thus be at hand.

**Access to antibiotics in the livestock sector** The access to antibiotics varies a lot for farmers around the world and sometimes within a country. In several settings, it is the access to antibiotics, especially of good quality, that is the challenge. Rough estimates suggest that more than 50% of the antibiotics in low-income countries are substandard or falsified. The distribution channels of antibiotics do also vary around the world; in many low and middle-income countries one may buy antibiotics over the counter without prescription. Also, in several countries a large, sometimes the major, proportion of the income for physicians and veterinarians comes from sales of antibiotics and other pharmaceuticals. Thus, there is an incentive for these professionals to use antibiotics in their practice. In addition, the increasing trade over internet is a new challenging and unregulated source of antibiotics. Remarkably, within the relatively culturally and economically homogenous Europe, there are immense differences between countries in the use of antibiotics in the livestock sector.

**Use of antibiotics in the livestock sector** One of the large differences between use of antibiotics in the livestock sector and human health sector is that in the former there is a large prophylactic use and use antibiotics as “growth promoters”. These kinds of uses are most prevalent in pig and poultry rearing but may be avoided by instead applying adequate preventive measures. For a medically rational use of antibiotics in livestock the following deliberations are generally recommended: use only quality-controlled drugs and avoid antibiotics critical for humans, treat only for therapeutic purposes and strive for treating animals individually (except for poultry), treat only after a proper clinical diagnosis (if needed after microbiological diagnosis), use only antibiotics adequate for the diagnosis and in correct dosage and duration, and finally, respect withdrawal periods.

**Reduction of antibiotic use and productivity** Northern European countries have the lowest use of antibiotics per livestock biomass among the OECD-countries. However, to reduce the use of antibiotics to those levels in other settings without other measures would be detrimental for animal health and productivity. Actually, in Sweden, that banned antibiotics as feed additives in 1985, the use of antibiotics initially increased due to more diseases before appropriate preventive measures were in place. However, nowadays the productivity per dairy cow and sow is among the highest in the world. Of course, these measures can be difficult to be put in place everywhere in the world due to resource and cultural constraints. Even so, by applying the following basic principles – or parts of them – would reduce the unnecessary use of antibiotics and often increase animal health and productivity: the basis is the rearing of robust animals by providing sufficient and good quality feed and water in adequate facilities, second to apply good external and internal biosecurity measures to prevent infections from entering the farm and spread within the farm and finally applying relevant vaccination schemes for specific diseases.

**Conclusions** The role of the resistance in bacteria in livestock herds for the over-all resistance emergence in the human population is not fully elucidated. Still it is wise to try to mitigate the resistance in the livestock sector. This is best done by applying a prudent and medically rational use of antibiotics in the sector. This approach requires a matching by good disease preventive measures, based on good animal management that generates robust and disease resistant animals, high biosecurity and effective vaccination programs. Hereby, a high productivity may be maintained or achieved. However, the approach does require, in several settings, new skills and mindsets among producers, animal health professionals and other extension personnel. Then, the livestock sector has done a major contribution to the antimicrobial resistance issue.

### References

SLU/Future Learn, 2019: “Effective Livestock production with low use of antibiotics” Massive Open On-line course <https://www.futurelearn.com/courses/effective-livestock-production>

Livestock antimicrobial partnership (LAMP), Global Agenda for Sustainable Livestock, <https://www.slu.se/lamp>



### Actual resistance situation among bacterial pathogens of food-producing animals

H. Kaspar<sup>1</sup> and S. Schwarz<sup>2</sup>

<sup>1</sup>Federal Office of Consumer Protection and Food Safety (BVL), Referat 505, Berlin, Germany; <sup>2</sup>Institute of Microbiology, Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Germany

Email: [Stefan.Schwarz@fu-berlin.de](mailto:Stefan.Schwarz@fu-berlin.de)

**Introduction** There are numerous programmes in different countries that aim at monitoring the antimicrobial susceptibility of bacteria from food-producing animals. In Germany, the national resistance monitoring programme GERM-Vet, conducted by the Federal Office of Consumer Protection and Food Safety (BVL), fulfils this task since 2001 on a yearly basis. The results obtained in this programme are freely available via the BVL website. The bacteria monitored each year are collected by at least 25 laboratories from at least 12 federal states in Germany according to a structured sampling plan. All bacteria originate from acutely diseased animals which have not been treated with antimicrobial agents. The majority of bacteria are from defined disease conditions of cattle, pigs and poultry. However, also bacteria originating from defined disease conditions of sheep and goats, dogs and cats, horses and commercially reared fish are included. The data determined in the GERM-Vet programme allow to see whether certain resistance properties in defined bacteria of animal origin emerge. Moreover, the data from this programme are an excellent source to guide empirical therapy.

**Materials and Methods** Concerning the methodology of susceptibility testing, the GERM-Vet programme follows the recommendations for broth microdilution of the Clinical and Laboratory Standards Institute (CLSI) as laid down in the VET01 document [1]. All bacteria are tested for their susceptibility to a set of 24 antimicrobial agents/combinations of antimicrobial agents. For the evaluation of the results and the categorization of the minimal inhibitory concentrations (MIC values) as ‘susceptible’, ‘intermediate’ (if this category is available) or ‘resistant’, clinical veterinary-specific breakpoints as laid down in the CLSI document VET08 [2] are used. Moreover, MIC<sub>50</sub> and MIC<sub>90</sub> values are calculated [3]. For specific bacteria and resistance properties, e.g. ESBL-producing *Escherichia coli*, in depth studies with regard to the ESBL gene present, its location on plasmids, co-located resistance properties, or the phylogroup and the MLST type of the respective isolates are conducted.

**Results** In this overview, the most recent MIC distributions are presented for the following combinations of animal host/disease condition/bacterial pathogen as shown in Table 1:

**Table 1** Combinations of animal host/disease condition/bacterial pathogen as monitored in the GERM-Vet programme 2016 [4] and 2017.

Animal host	Disease condition	Bacterial pathogen
Cattle	Mastitis	<i>Staphylococcus aureus</i>
		<i>Escherichia coli</i>
		<i>Streptococcus agalactiae</i>
		<i>Streptococcus dysgalactiae</i>
		<i>Streptococcus uberis</i>
	Infection of the intestinal tract	<i>Escherichia coli</i>
	Infection of the respiratory tract	<i>Mannheimia</i> spp.
<i>Pasteurella multocida</i>		
Pigs	Infection of the intestinal tract	<i>Escherichia coli</i>
	Infection of the respiratory tract	<i>Bordetella bronchiseptica</i>
		<i>Pasteurella multocida</i>
		<i>Streptococcus suis</i>
Poultry	Various disease conditions	<i>Escherichia coli</i>
		<i>Staphylococcus aureus</i>

**Conclusions** There is no general trend towards increasing resistance over the years in Germany. Isolates from cases of bovine mastitis are usually less resistant than the corresponding isolates from other animals and/or disease conditions.

### References

- [1] Clinical and Laboratory Standards Institute (CLSI) (2018): CLSI document VET01-S. 5<sup>th</sup> ed.
- [2] Clinical and Laboratory Standards Institute (CLSI) (2018): CLSI document VET08-S. 4<sup>th</sup> ed.
- [3] Clinical and Laboratory Standards Institute (CLSI) (2011): CLSI document VET05-R. 1<sup>st</sup> ed.
- [4] BVL-Report 12.5 (2018); Bericht zur Resistenzmonitoringstudie 2016. Online available at: [https://www.bvl.bund.de/DE/09\\_Untersuchungen/01\\_Aufgaben/03\\_Nationales%20Resistenz-Monitoring/Archiv\\_Berichte/Archiv\\_Resistenzmonitoring\\_node.html](https://www.bvl.bund.de/DE/09_Untersuchungen/01_Aufgaben/03_Nationales%20Resistenz-Monitoring/Archiv_Berichte/Archiv_Resistenzmonitoring_node.html)

### Antimicrobial use and antimicrobial resistance in dairy cattle

T. J. G. M. Lam<sup>1,2</sup>, A. E. Heuvelink<sup>1</sup>, R. G. M. Olde Riekerink<sup>3</sup> and I. M. G. A. Santman-Berends<sup>1</sup>

<sup>1</sup>GD Animal Health, Deventer, the Netherlands; <sup>2</sup>Department of Farm Animal Health, Faculty Veterinary Medicine, Utrecht University, the Netherlands; <sup>3</sup>Current Address: Bayer Dairy Innovation and Scouting and External Collaborations, Auckland, New Zealand

Email: [t.lam@gddiergezondheid.nl](mailto:t.lam@gddiergezondheid.nl)

**Introduction** Since the beginning of this century antimicrobial use (AMU) in farm animals became an important issue on the political agenda of several European countries. Where Scandinavian countries traditionally had a restricted approach on the usage of antimicrobials, the issue got full attention in other countries due to several incidents on the supposed relation between AMU in farm animals and antimicrobial resistance (AMR) in humans. In this presentation several aspects will be discussed on AMU and AMR in dairy cattle, based on experiences in the Netherlands during a period in which the political approach towards AMU in animal husbandry changed from indifferent to very demanding. General aspects of this changing approach will be discussed, while two types of pathogens, methicillin resistant *Staphylococcus aureus* (MRSA) and Extended spectrum  $\beta$ -lactamase producing bacteria (ESBL) will be discussed in more detail. Additionally possible approaches to change behavior towards AMU will be discussed as will be the added value of prudent AMU.

**A changed approach towards AMU in the Netherlands** Based on overusage of antimicrobials in veterinary medicine, the high prevalence of antimicrobial resistance in animal pathogens, the fact that the same types of antimicrobials are used in animals and in humans and that humans can be exposed to possibly antimicrobial resistant pathogens from animals raised the attention on AMU in animal husbandry in the Netherlands. Several incidents, specifically on MRSA and ESBL further increased the political pressure and made the parliament decide to decrease the usage of antimicrobials stepwise to ultimately 70% in 2015 as compared to 2009. In cattle that was realized through an approach in which transparency of antimicrobial usage at the herd level, a ban on preventive AMU and on the use of critically important antimicrobials were crucial parts<sup>1</sup>. Apart from that, much collateral research was executed in the country in order to understand the background of the process and to smoothen the reduction in AMU.

**MRSA** One of the first pathogens of which the association between AMU in animals and AMR in humans was supposed was MRSA. Specifically in pigs MRSA prevalences were high, as was the prevalence of MRSA in pig farmers. Over the years MRSA was also cultured from milk samples from dairy cattle. An important risk factor for the prevalence of MRSA in dairy herds was the presence of pigs on the dairy herd and the density of pigs in the area<sup>2</sup>. Ultimately all MRSA isolates in both, pigs and dairy were found to be MLST 398, a livestock associated strain, which is a different type of MRSA as compared to the hospital associated strain that is found in hospitalized people.

**ESBL** Due to their multi-resistant character, their capacity to inactivate  $\beta$ -lactam antimicrobials including 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins, and the potential horizontal transmission within and between bacterial species, ESBL producing bacteria are a serious threat to human and animal health. Studies were done on the prevalence and risk factors of ESBL in both conventional and organic dairy herds, in which the relation between AMU and AMR in animals was evaluated as well as risk factors for the occurrence of ESBL<sup>3,4</sup>. It was found that the prevalence of ESBL was higher in conventional than in organic herds. In both type of herds, no relation was found between the total usage of antimicrobials and the prevalence of ESBL, whereas a significant association was found with the usage of critically important antimicrobials in these herds. Studies on the dynamics of ESBL in dairy herds learned that the ESBL prevalence in calves up to 21 days of age is much higher than in older animals, which was found not to be related to possible residues of dry cow antimicrobials in colostrum that was consumed by the calves.

**Prudent AMU in farm animals** Recent studies in which the relatedness of human, animal, food and environmental ESBL isolates were compared, could not find a linkage between AMU in animals and AMR in humans with respect to ESBL<sup>5</sup>. Nevertheless, from a precautionary perspective as well as from the knowledge that there is a relation between AMU and AMR in animals, prudent AMU in animals is of utmost importance. To realize this, the mindset of people involved is crucial, which can be influenced by using several approaches at the same time, reaching different types of people with the ultimate goal to realize a tipping point in social pressure<sup>1</sup>. This ultimately led to a significant decrease in the usage of antimicrobials in dairy cattle in the Netherlands. This was realized without negative consequences for animal health and production and therefore seem attractive to be implemented in other countries.

### References

- 1) Lam TJGM et al. The RESET Mindset Model applied on decreasing antibiotic usage in dairy cattle in the Netherlands. *It. Vet. J.* (2017) 70:5
- 2) Olde Riekerink RGM et al. Prevalence of MRSA intramammary infections and riskfactors in Dutch Dairy herds. *Proceedings ISVEE XII, Durban South Africa*, August 2009.
- 3) Gongrijp et al. Prevalence and risk factors for extended-spectrum  $\beta$ -lactamase and AmpC-producing *Escherichia coli* in dairy farms. *J. Dairy Sci.* (2016) 99:9001.
- 4) Santman-Berends IMGA et al. Prevalence and risk factors for extended-spectrum  $\beta$ -lactamase or AmpC-producing *Escherichia coli* in organic dairy herds in the Netherlands. *J. Dairy Sci.* (2017) 100:562.
- 5) Dorado-Garcia A et al. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: A pooled analysis. *J. Antimicrob. Chemother.* (2017) doi:10.1093/jac/dkx397

### The future-proof bovine practitioner understands that improved mastitis management is key to prudent use of antimicrobials on dairy farms

S. De Vliegher, M. Stevens, Z. Lipkens and S. Piepers

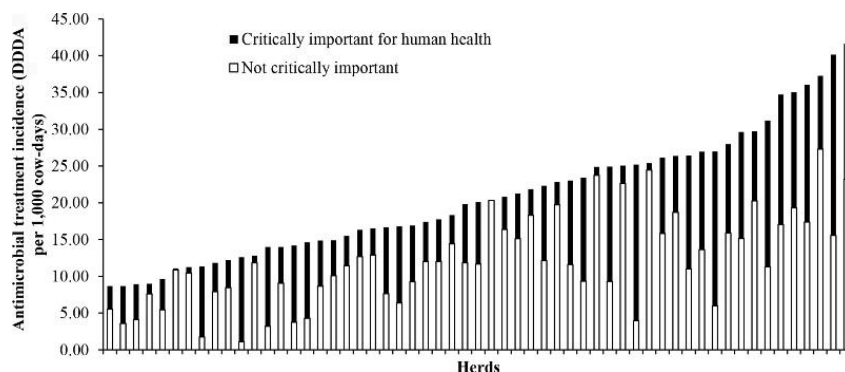
M-team & Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Email: [SamDeVliegher@UGent.be](mailto:SamDeVliegher@UGent.be)

**Introduction** Emerging antimicrobial resistance in human pathogens threatens public health. The potential link with antimicrobial abuse and overuse in animal husbandry has resulted in a strong societal demand for more responsible and lower use of antimicrobials in animal production, also in dairy cattle. This has initiated the implementation of restrictive legislation in a number of countries. As bovine practitioners are co-responsible for the amount and type of antibiotics used on the dairy herds they serve, they might feel threatened by this phenomenon. However, there are plenty of opportunities for bovine practitioners to turn their business model around this threat through shifting from a curative to a preventive approach. In that respect, it is essential to understand that improved mastitis management is not only key for judicious antimicrobial use but also for the practitioner's future income.

**Materials and Methods** In a first project (Stevens, 2018), we quantified antimicrobial use on a good number of commercial dairy herds, reported what type of products were used and for what reasons, and we identified associated factors. As well, we studied whether mastitis management input helps to improve udder health and milk quality at the same time as lowering or shifting antimicrobial consumption. In a second project (Lipkens, 2019), we studied whether Dairy Herd Improvement (DHI) data are useful in identifying non-infected cows at dry-off and what the consequences are of implementing selective dry cow therapy (SDCT) in commercial dairy herds using an algorithm based on DHI data and clinical mastitis records.

**Results** The average antimicrobial treatment incidence in adult dairy cattle was 20.8 defined daily doses animal (DDDA) per 1,000 cow-days. Large variation existed between herds, ranging from 8.7 to 41.6 DDDA (Fig. 1). The average ATI of the critically important antimicrobials for human health (3<sup>rd</sup>- and 4<sup>th</sup>-generation cephalosporins and fluoroquinolones according to the WHO classification) was only a bit lower than the average ATI of the other antimicrobials. Herds participating in a veterinary herd health management program and herds selectively drying off cows used fewer antimicrobials compared with herds not participating in such a program or applying blanket dry-cow therapy (BDCT). Large variation was observed in mastitis management and mastitis management implementation between herds that actively received mastitis management input on a regular basis (intervention herds) and the control herds that did not receive this form of coaching. Also, intervention herds in which the herd veterinarian attended each herd visit when mastitis management input was provided, had a better mastitis management and implemented mastitis management changes better than herds in which the veterinarian did not. Further, an acceptable or improved mastitis management was associated with a decrease in the use of for human health critically important antimicrobials (Stevens, 2018).



**Figure 1** The herd-level antimicrobial treatment incidence per 1,000 cow-days per herd divided into critically important antimicrobials for human health and not-critically-important antimicrobials (Stevens, 2018).

**Conclusions** We confirmed that somatic cell count (SCC) information derived from milk recording (DHI) data is capable of predicting the absence of intramammary infection with major pathogens at dry-off in cows, yet (an estimate of) the herd prevalence of subclinical mastitis, the cow's milk yield and parity impact the estimates of the sensitivity, specificity and predictive values to some extent. Using this information, we developed and applied a selective dry cow algorithm, which included the cow's test-day information and clinical mastitis history prior to drying off on commercial dairy herds with a general good udder health and previously applying BDCT. The clinical mastitis risk and culling hazard were not significantly higher in the SDCT groups within herds when compared to the BDCT groups. Test-day milk yield and SCC in the subsequent lactation did not significantly differ between treatment groups and were even numerically favorable in the SDCT group. The antimicrobial use for udder health, monitored from drying off until 100 days in milk, decreased significantly through SDCT implementation (Lipkens, 2019).

#### References

- Stevens, M. (2018). Antimicrobial consumption on Flemish dairy herds: quantification, associated factors and mastitis management input as a basis for appropriate use. PhD Thesis, Ghent University.
- Lipkens, Z. (2019). Selectively drying off cows. Impact on future performance and antimicrobial consumption. PhD Thesis, Ghent University.

## Effects of antibiotics on sessile bacteria in drinking water systems in poultry farms: A model approach

R. H. Mateus-Vargas<sup>1</sup>, F. Hahne<sup>2</sup>, S. Jensch<sup>2</sup>, N. Kemper<sup>1</sup>, G. Hamscher<sup>2</sup> and J. Schulz<sup>1</sup>

<sup>1</sup>Institute of Animal Hygiene, Animal Welfare and Farm Animal Behavior, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; <sup>2</sup>Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Germany

Email: rafael.mateus@tiho-hannover.de

**Introduction** In modern animal production systems, the administration of veterinary drugs via drinking water is an important way to treat animals, mostly when treatment of big stocks is necessary. Before reaching the animals, antibiotics get in contact with biofilms present in the water distribution system. Biofilms have been shown as favorable environment for the evolution of antibiotic resistance under copiotrophic conditions and their characteristics are in close relationship to the surrounding conditions. Therefore, the question arises if antibiotics administered via drinking water may influence biofilm growth and/or contribute to the development of antibiotic resistance in animal husbandry. The aim of this study was to evaluate these aspects with model biofilms growing under oligotrophic conditions on polyvinyl chloride (PVC)-surfaces after antibiotic exposure.

**Materials and Methods** Seven-day-old biofilms of *Pseudomonads* (ATCC 13525 or ATCC 27853) and a susceptible *Escherichia* (*E.*) *coli* strain (ATCC 23631), grown in a nutrient-poor medium at 22.0 °C ± 1.0 °C on the inner surface of polyvinyl chloride (PVC)-pipe pieces, were exposed to different antibiotics for 5-7 days. Compounds included authorized veterinary drugs containing the combination Sulfadiazine/Trimethoprim (SDZ/TMP; Trimetostat, aniMedica GmbH, Börsensell, Germany) and Tylosin A (TYL; Vetranal, Sigma-Aldrich, Germany) in concentrations recommended for therapeutic application in drinking water. Biofilm population distribution was controlled in treated and control pipes before and after antibiotic treatment through plate counts (Colony forming units; CFU). Furthermore, the minimum inhibitory concentration (MIC) of *E. coli* isolates to the following substances was determined using a broth microdilution technique (Sensititre, Trek Diagnostic Systems, West Sussex, United Kingdom): Ampicillin (AMP); Cefotaxim (CTX); Ceftazidim (CAZ); Chloramphenicol (CHL); Ciprofloxacin (CIP); Colistin (COL); Gentamicin (GEN); Nalidixic acid (NAL); Sulfisoxazole (SUL); Tetracycline (TET); Trimethoprim (TMP); Sulfadiazine/Trimethoprim (SDZ/TMP). Results obtained from the susceptibility testing were evaluated with the epidemiological cut-off value (ECOFF-values) recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST). Data arrays of bacterial counts (log<sub>10</sub> CFU cm<sup>-2</sup>) were compared between treated and controls using the *t*-test with SAS software (SAS Institute Inc., Cary, NC, USA).

**Results** Bacterial population distribution in biofilms was influenced differently depending on the antibiotic used. While the exposure of seven-day-old biofilms with SDZ/TMP led to no considerable changes in the bacterial composition after treatment (Differences < 0.5 log<sub>10</sub> CFU cm<sup>-2</sup>), TYL exposure resulted in an average decrease of 0.95 log<sub>10</sub> CFU cm<sup>-2</sup> in the number of *Pseudomonads* as well as in *E. coli* counts in biofilms of the same age (*p*>0.05). Tables 1 and 2 show the compositions of the bacterial community in biofilms. Additionally, as shown in Table 3, the MICs of *E. coli* isolates were below the concentrations applied, and the antibiotic exposure of the bi-culture biofilms did not cause any measurable changes in the MICs of sessile *E. coli* isolates.

**Table 1** Population distribution (means ± SD) before (0) and after 5 days in biofilms of controls and pipes treated with 150/30 mg L<sup>-1</sup> Sulfadiazine/Trimethoprim.

Bacteria	0	control	treated
<i>Pseudomonads</i>	7.23 ± 0.27	7.16 ± 0.22	6.98 ± 0.22
<i>E. coli</i>	4.44 ± 0.47	4.70 ± 0.68	4.61 ± 0.30

**Table 2** Population distribution (means ± SD) before (0) and after 7 days in biofilms of controls and pipes treated with 500 mg L<sup>-1</sup> Tylosin A.

Bacteria	0	control	treated
<i>Pseudomonads</i>	5.90 ± 0.04	6.15 ± 0.64	5.27 ± 0.03
<i>E. coli</i>	5.67 ± 0.21	5.71 ± 0.47	4.70 ± 0.30

**Table 3** Distribution of MICs (mg L<sup>-1</sup>) among *E. coli* isolates (*n*=22) of biofilms after antibiotic exposition

Antimi-crobial	Concentration range	Epidemiologic cut-off	MIC <sub>50</sub>	MIC <sub>90</sub>
AMP	0.25 - 32	≤8	4	4
CTX	0.06 - 8	≤0.25	≤0.06	≤0.06
CAZ	0.06 - 8	≤0.5	0.25	0.5
CHL	2 - 128	≤16	8	8
CIP	0.015 - 2	≤0.06	≤0.03	≤0.03
COL	0.12 - 16	≤2	2	2
GEN	0.25 - 16	≤2	1	2
NAL	1 - 128	≤16	4	4
SUL	2 - 256	≤*	4	4
TET	0.25 - 32	≤8	2	2
TMP	0.25 - 32	≤2	≤0.25	≤0.25
SDZ/TMP	0.59/0.03 – 76/4	≤19/1	≤0.59/0.03	≤0.59/0.03

\*No epidemiologic cut-off value was reported by EUCAST.

**Conclusions** Our model approach demonstrated that the application of antibiotics affects biofilm's bacterial composition depending on the compound used. Since non-autochthonous bacteria of aquatic environments (*E. coli*) survived noxious concentrations of antibiotics, protective characteristics of biofilms growing in drinking water systems should be considered, e.g. during bacterial disease outbreaks in poultry farms. Furthermore, results of sensitivity testing suggest that the selective pressure imposed by the use of these antibiotics during a single application may not lead to the development of resistant bacteria.

**Acknowledgement** This study was financially supported by the "Deutsche Bundesstiftung Umwelt" (DBU, grant 32732-01-31).

### Effects of a bacterial biofilm on various antibiotics

F. Hahne<sup>1</sup>, S. Jensch<sup>1</sup>, R. H. Mateus-Vargas<sup>2</sup>, J. Schulz<sup>2</sup> and G. Hamscher<sup>1</sup>

<sup>1</sup>Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Germany; <sup>2</sup>Institute of Animal Hygiene, Animal Welfare and Farm Animal Behavior, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

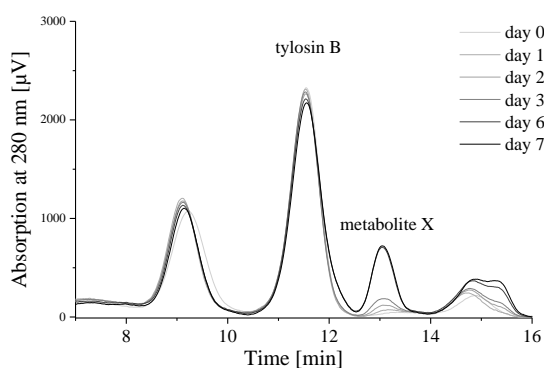
Email: Friederike.Hahne@lcb.Chemie.uni-giessen.de

**Introduction** Since the implementation of a reporting obligation in Germany regarding the application of antibiotics in livestock farming in 2011, their use decreased by 733 t (–57%) in 2017 [Wallmann *et al.*, 2018]. Despite the downward trend, the efficient use of antibiotics is an important goal in animal husbandries to avoid unnecessary dissemination of resistance. Furthermore, the persistence of various compounds and their transformation products are an emerging environmental issue. In livestock farming the drugs are administrated via feed, drinking water or injections. Biofilms, a sessile formation of microorganism with resistance to extreme conditions, develop in pipes for feed and drinking water and may interact with the administered drugs. The consequences of this exposition are poorly understood. Therefore, a static biofilm model was established to recreate the interactions of biofilms with selected antibiotics taking place in the environment of drinking water pipes of animal production premises.

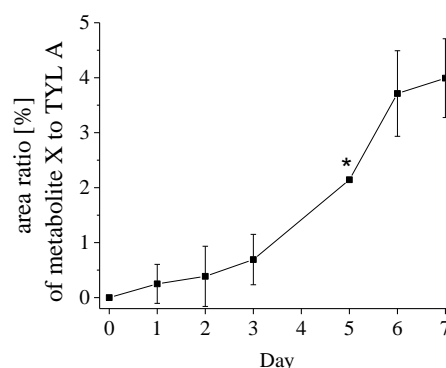
**Materials and Methods** Antibiotics were administrated to seven-day-old biofilms of *Pseudomonas fluorescence* (ATCC 13525) or *P. aeruginosa* (ATCC 27853) and a susceptible *Escherichia coli* strain (ATCC 23631). The strains were grown in nutrient-poor medium in the inner face of PVC-pipes at  $22 \pm 1^\circ\text{C}$ . The antibiotics are authorized veterinary drugs containing sulfadiazine and trimethoprim (SDZ/TMP: 150/30 mg/L, Trimetotat, aniMedica GmbH, Bösensell, Germany) or tylosin A (TYL A: 0.5 g/L, tylosin tartrate, Sigma-Aldrich vetranal<sup>TM</sup>, Steinheim, Germany). Dosages were applied as recommended by manufacturers and the duration of treatment was 5 for SDZ/TMP and 7 days for TYL A. The biotic control consisted of a pipe coated with a biofilm without antibiotics. Abiotic control consisted of a pipe with the antibiotics in nutrient-poor medium. The solutions in the pipes were sampled daily and sterile filtered prior analysis. The concentrations of SDZ and TMP were determined with HPLC-UV (Shimadzu, Japan) employing a C18 column (Hypersil Gold<sup>TM</sup>, Thermo Scientific). The detection wavelength was set 270 nm. TYL A was determined with HPLC-UV using a C18 PFP column (Hypersil Gold<sup>TM</sup> PFP, Thermo Scientific), detection was performed at 280 nm. Gradient elution with methanol was used for all analyses.

**Results** The levels of the combination preparation in the drinking water pipe with biofilm only slightly changed over the treatment period of 5 days. The concentrations averaged for SDZ (means  $\pm$  SD)  $143.2 \pm 0.2$  and for TMP  $29.4 \pm 0.1$  mg/L. In the abiotic control the concentration of SDZ averaged to  $146.6 \pm 0.3$  mg/L and TMP to  $30.4 \pm 0.2$  mg/L. Over the whole period no degradation or transformation of both active agents was visible.

In contrast, the starting concentration of TYL A in the pipe with biofilm was  $520 \pm 6$  mg/L and averaged after seven days to  $497 \pm 10$  mg/L. In abiotic control, the concentration of TYL A decreased by 0.65 % in trial period. In the corresponding UV-chromatogram a new peak (metabolite X) was detected in the treated pipe with biofilm. Metabolite X increased over the treatment period to approximately  $4.0 \pm 0.5$  % (see Figure 1 and 2). However, after isolation this substance was not stable in acidic solution and turned back to TYL A.



**Figure 1** Enlarged UV-chromatogram sections of samples from starting day to trial end (day 7) with tylosin A in the treated pipe. Retention time of metabolite X is 13 min, clearly separated from tylosin B, which already occurred in the standard.



**Figure 2** Estimated percentage contents of metabolite X of TYL A from starting day to trial end (day 7) in the treated pipe. (n=4, \* n=2).

**Conclusions** The newly established model biofilm doesn't show any effect to the combination preparation sulfadiazine / trimethoprim as demonstrated by HPLC-UV detection. Tylosin A is transformed partially to a metabolite. Our initial experiments are a first quantitative approach to investigate possible interactions between antibiotics and bacterial biofilms under standardized conditions. Furthermore, this model may demonstrate possible transformations in the drinking water system as well. Therefore, this interaction should be considered when medicating animals.

### References

Wallmann, J., Bode, C., Bender, A., & Heberer, T. (2018). Abgabemengenerfassung von Antibiotika in Deutschland 2017. Deutsches Tierärzteblatt, 66(9).

**Acknowledgement** The project is supported by the German Federal Environmental Foundation (DBU, grant 32732-01-31).

## Evaluation of antibiotic sensitivity of bacteria isolated from mastitis cases in dairy cows in the 4-year period

K. Wódz, T. Nowak and P. Kwieciński

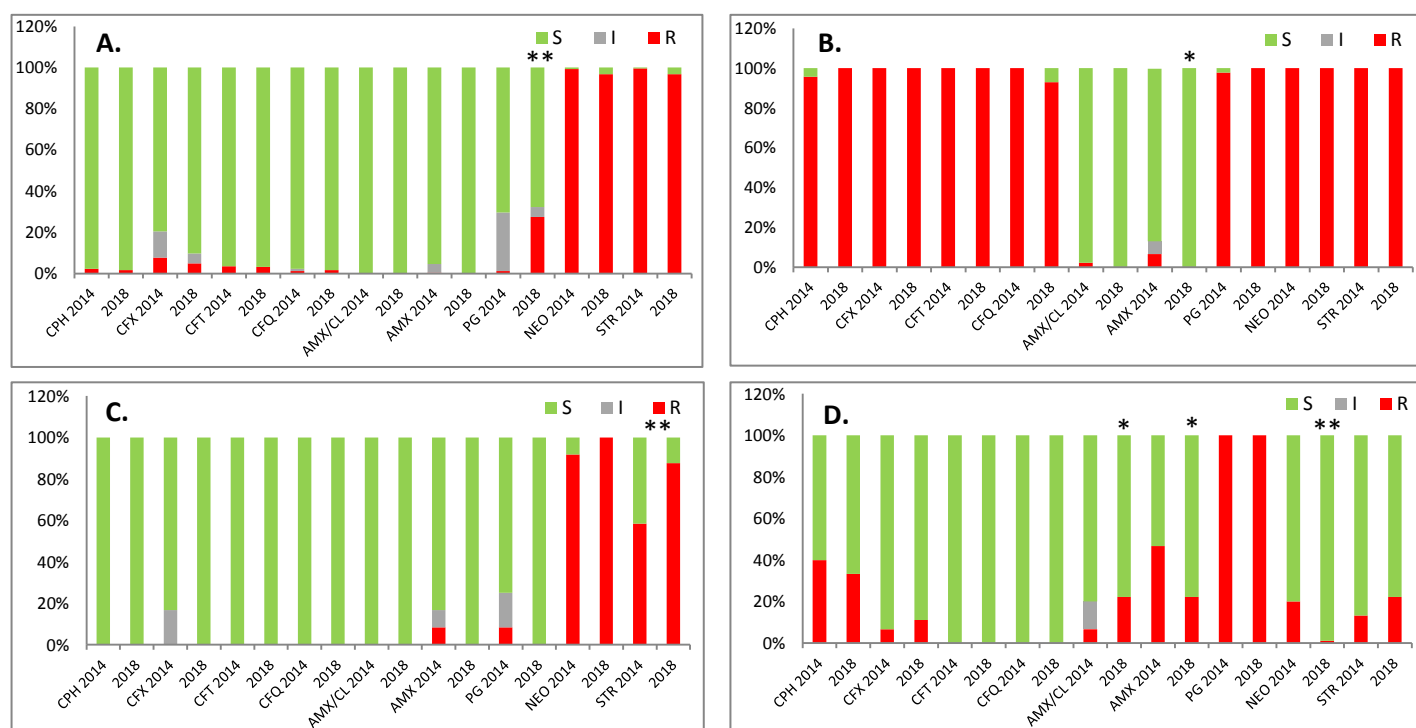
VET-LAB BRUDZEW, Department of Microbiology, Department of Molecular Biology, Poland

Email: karolina.wodz@labbrudzew.pl

**Introduction** Mastitis is the most common disease of high-yielding dairy cows, affecting the quality and quantity of milk and causing significant economic losses in production and dairy industry around the world. Antibiotics such as cephalosporin, penicillin, and aminoglycoside are widely used for the treatment and prevention of diseases affecting dairy cows caused by gram-positive and gram-negative bacteria. An increase in the occurrence of mastitis result in increased use of antibiotics, which in turn might elevate the risk of increased bacterial resistance to antimicrobial agents. The clinical consequences of antimicrobial resistance of dairy pathogens affecting humans seems to be slight. Antimicrobial resistance among dairy pathogens found in milk will probably not affect human health as long as the milk is pasteurized. However, there is an increasing number of people who consume raw milk. Therefore, transmission of a resistant mastitis pathogen to humans can occur if contaminated unpasteurized milk is consumed. The aim of the study was to compare changes in the sensitivity of bacteria that cause mastitis in the period of 4 years to antibiotics.

**Materials and Methods** In 2014 and 2018 1108 milk samples were examined. From the clinical and subclinical forms of mastitis 482 *Streptococcus* sp., 77 *Enterococcus* sp., 135 *Staphylococcus* CNS, 73 *Staphylococcus aureus*, 49 *Escherichia coli*, 25 *Trueperella pyogenes* were isolated. Bacteria were identified based on phenotypic and biochemical properties. Species identification was performed using an automated system VITEK2 (Biomerieux). For the evaluation of antimicrobial susceptibility minimum inhibitory concentration (MIC) method was used (MERLIN platform). The results were interpreted as sensitive (S), medium-sensitive (I) and resistant (R), in accordance with CLSI guidelines. Statistical analysis was performed using Statistica 12 software. The results were analyzed using the Mann-Whitney U test and  $P < 0.05$  was considered to indicate a statistically significant difference.

**Results** All the predominant mastitis streptococci and enterococci were susceptible to tested  $\beta$ -lactams. However, increase in penicillin G (PG;  $P = 0.033$ ) in case of *Streptococcus* (Fig. 1 A) and decrease in amoxicillin (AMX,  $P = 0.048$ ) resistance in case of *Enterococcus* (Fig. 1 B) was observed. Slight but not statistically significant ( $P > 0.5$ ) increase in penicillin G and amoxicillin in case of *Staphylococcus* CNS was observed. Similarly, slight but not statistically significant ( $P > 0.5$ ) decrease in penicillin G and amoxicillin in case of *S. aureus* was observed. In case of *T. pyogenes* (Fig. 1 C) increase in streptomycin resistance (STR,  $P = 0.034$ ) was observed. In case of *E. coli* (Fig. 1 D) increase in amoxicillin and clavulanic acid (AMX/CL;  $P = 0.046$ ), and decrease in amoxicillin (AMX,  $P = 0.041$ ) and neomycin (NEO,  $P = 0.038$ ) in resistance were observed.



**Figure 1** Resistance of mastitis pathogen in 2014-2018. A. *Streptococcus* sp., B. *Enterococcus* sp., C. *T. pyogenes*, D. *E. coli*

**Conclusions** In conclusion, analysis of antimicrobial sensitivity does not reveal emerging resistance among mastitis pathogens even though many of these antibiotics have been used in the dairy industry for treatment and prevention of disease for several decades. However, it is clear that use of antibiotics in dairy cows may contribute to increased antimicrobial resistance. Hence the importance of epidemiological studies monitoring the occurrence of antibiotic resistance of bacteria in case of mastitis.

### Does the feeding of discard milk to dairy calves lead to antimicrobial resistance on farm?

C. L. Firth<sup>1</sup>, A. Käsbohrer<sup>1</sup>, P. Pless<sup>2</sup>, B. Springer<sup>3</sup>, C. Egger-Danner<sup>4</sup> and W. Obritzhauser<sup>1</sup>

<sup>1</sup>Unit of Veterinary Public Health and Epidemiology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria; <sup>2</sup>Animal Health Service, Veterinary Laboratory, Styrian State Veterinary Authorities, Graz, Austria; <sup>3</sup>Centre for Foodborne Infectious Diseases, Institute for Medical Microbiology & Hygiene, Austrian Agency for Health and Food Safety, Graz, Austria; <sup>4</sup>ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria

Email: Clair.Firth@vetmeduni.ac.at

**Introduction** The use of antimicrobials in livestock production is viewed extremely critically by consumers. Nevertheless, animals are occasionally sick and need treating to regain their health and ensure their welfare is maintained. Mastitis is the most common indication for the use of antibiotics in dairy cattle. An undesirable side effect of treatment is the production of discard milk containing antimicrobial residues. This milk cannot enter the human food chain and is considered a waste product. Although not recommended, this waste milk is commonly fed to calves on farm. The aim of this analysis was to assess how frequently dairy farmers in Austria fed this discard milk to calves, and whether this feeding appeared to directly influence the prevalence of antimicrobial resistance (AMR) in commensal bacteria.

**Materials and Methods** As part of a larger multidisciplinary study into antimicrobial use in dairy cows (ADDA – Advancement of Dairying in Austria), approximately 250 farmers were sent a farm management questionnaire via email (for details, see Firth et al, 2019a). The survey included a variety of questions including a section on calf feeding and management. Farmers were enrolled by their local veterinarian. Veterinarians were enrolled through convenience sampling by the authors. The vast majority (>74%) of dairy cows included in this study were of the Austrian *Fleckvieh* (dual-purpose Simmental) breed. On a subset of 50 farms, environmental samples (feces & dust) were collected from the main cow shed, calf housing and youngstock areas (Firth et al, 2019b). These samples were then tested for the presence of extended spectrum beta-lactamase producing (ESBL) *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) using selective bacteriological methods.

**Results** A total of 211 farmers responded to the online questionnaire (response rate 84.1%). Of these, 128 (60.7%) farmers reported that they fed discard milk containing antimicrobial residues to calves. However, 91 of these farmers stated that they only fed this milk to bull calves. No farmers pasteurized discard milk before feeding it to calves.

Of the 50 dairy farms sampled for the presence of antibiotic-resistant bacteria, a total of 13 (26.0%) of farms tested positive for ESBL-*E.coli* in at least one sample (cows, calves and/or youngstock samples) (Firth et al, 2019b). Eleven of these farms were found to have ESBL-*E.coli*-positive fecal samples from calves. Only one of the 50 dairy farms tested positive for MRSA. Forty farmers from the AMR subgroup completed the farm management questionnaire. The results are shown in Table 1. Statistical analysis using the Fisher's exact test demonstrated that there was no statistically significant association between feeding discard milk to dairy calves and the presence of ESBL-*E.coli* in fecal samples collected from calves on these farms (Table 1, p=0.44).

**Table 1** 2x2 contingency table of environmental sampling results and farmers' responses to the management questionnaire

	ESBL- <i>E.coli</i> positive	ESBL- <i>E.coli</i> negative	Total
Discard milk fed – yes	5	22	27
Discard milk fed - no	4	9	13
Total	9	31	40

**Conclusions** The results of this preliminary study were inconclusive, which may be due to the small sample size, as well as the relatively low overall antimicrobial use on Austrian dairy farms (Firth et al, 2017). Furthermore, it is unclear whether the particular calves sampled on these farms had received discard milk containing antimicrobial residues or whether such feeding was just a regular practice on the farm. Going forward, the results of this study are being used to run a follow-up study which will concentrate on taking regular fecal samples from calves being fed waste milk in an attempt to assess the impact of this feeding practice on antimicrobial resistance rates in these animals over time. This follow-up study, as part of the D4Dairy (www.d4dairy.com) project, will also assess alternatives and interventions aiming to reduce the risk related to feeding this milk to young calves.

### References

- Firth, CL; Käsbohrer, A; Schleicher, C; Fuchs, K; Egger-Danner, C; Mayerhofer, M; Schobesberger, H; Köfer, J; Obritzhauser, W (2017): Antimicrobial consumption on Austrian dairy farms: an observational study of udder disease treatments based on veterinary medication records. *PeerJ*. 2017; 5:e4072
- Firth, CL; Laubichler, C; Schleicher, C; Fuchs, K; Käsbohrer, A; Egger-Danner, C; Köfer, J; Obritzhauser, W. (2019a): Relationship between the probability of veterinary-diagnosed bovine mastitis occurring and farm management risk factors on small dairy farms in Austria. *Journal of Dairy Science* (in press)
- Firth, CL; Käsbohrer, A; Springer, B; Pless, P; Köfer, J; Obritzhauser, W (2019b): Is there a link between antimicrobial use and the prevalence of MRSA and ESBL-producing *Escherichia coli* on Austrian dairy farms? *International Meeting on Emerging Diseases and Surveillance (IMED) 2018 Abstracts*, Vienna, Austria, NOV 9-12, 2018. *International Journal of Infectious Diseases* (79), S1 51-52.



### Antibiotic use and resistance in *E. coli* from poultry – a global overview

N. Roth<sup>1,2</sup>, A. Kaesbohrer<sup>3</sup>, S. Mayrhofer<sup>1</sup>, U. Zitz<sup>1</sup>, C. Hofacre<sup>4</sup>, J. Wirges<sup>5</sup> and K. J. Domig<sup>1</sup>

<sup>1</sup>University of Natural Resources and Life Sciences Vienna, Department of Food Science and Technology, Institute of Food Science, Austria; <sup>2</sup>BIOMIN Holding GmbH, Austria; <sup>3</sup>University of Veterinary Medicine Vienna, Department for Farm Animals and Veterinary Public Health, Institute of Veterinary Public Health, Austria; <sup>4</sup>Poultry Diagnostics and Research Center, University of Georgia, USA; <sup>5</sup>Biomim Deutschland GmbH, Germany

Email: jochen.wirges@biomin.net

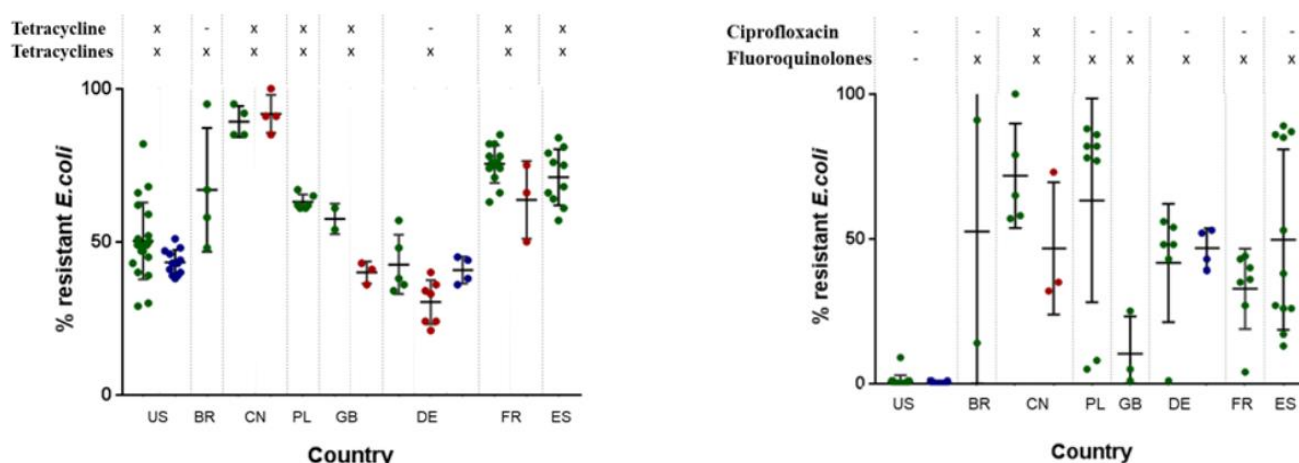
**Introduction** The increase in antibiotic resistance is a global concern for human and animal health. Resistant microorganisms can spread between food-producing animals and humans by direct contact, through the food chain and/or the environment. Poultry is one of the world's fastest growing sources of meat production. The objective of this study was to identify the legalized antibiotics, the amounts thereof administered and the level of AR monitored in *E. coli* isolated from broilers originating from the large poultry producing regions: US, China, Brazil, Poland, United Kingdom, Germany, France and Spain (Roth, et al., 2019). We hypothesize that the application of antibiotics leads to high and consistent resistance levels in *E. coli* isolates, whereas ban of some classes of antibiotics results in low resistance levels.

**Materials and Methods** Data about antibiotic use resistances in *E. coli* in broilers are shown for the largest broiler meat producers worldwide: the United States, Brazil, China and countries of European Union - Poland, United Kingdom, Germany, France and Spain. These regions count for approximately 60% of the total worldwide broiler production. Information was obtained from national monitoring and registration programs and research studies conducted between 2000 and 2018.

**Results** There is no public long-term quantitative data available on the amount of antibiotics used in poultry, with the exception of France. Data from France indicate that the decreased use of tetracyclines leads to a reduction in the detected resistance rates. Qualitative data of registered antibiotics enabled their evaluation. The fluoroquinolones, 3rd generation cephalosporins and macrolides are approved for use in large poultry-producing regions, with the exception of fluoroquinolones in the US and cephalosporins in the EU.

Data on antibiotic resistant *E. coli* is available for most regions but detection of resistance and number of isolates in each study differs among regions, therefore statistical evaluation was not possible. Tetracyclines, aminoglycosides, sulfonamides and penicillins are registered for use in poultry in all evaluated countries. The average resistance rates in *E. coli* to representatives of these antibiotic classes are higher than 40% in all countries, with the exception of ampicillin in the US. The resistance rates to fluoroquinolones in the US, where fluoroquinolones are not registered for use, are below 5%, while the average of resistant *E. coli* is above 40% in Brazil, China and EU, where use of fluoroquinolones is legalized.

**Figure 1** Resistance rates in *E. coli* to antibiotics



*E. coli* Isolates from healthy animals (green dots), chicken retail meat (blue dots) and diseased chickens (red dots) detected within scientific studies or national monitoring programmes. Each dot represents one study or data set in one year. On the top of the figure, status of approval for the specific antibiotic tested for resistance (first line), the antimicrobial class (second line)

**Conclusions** The global harmonized approach in the monitoring of antibiotic use and evaluation of resistances using the same methodology is needed. The findings from the US demonstrate the possibility to produce broilers without fluoroquinolones, which may result out in low resistance rates, but there was no elimination of the occurrence of resistant population.

#### References

Roth, N., A. Kasbohrer, S. Mayrhofer, U. Zitz, C. Hofacre, and K. J. Domig. 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. 98(4):1791-1804.

### Antimicrobial Resistance in Commensal *Escherichia coli* from fecal samples on Bavarian Dairy Farms

I. Lorenz

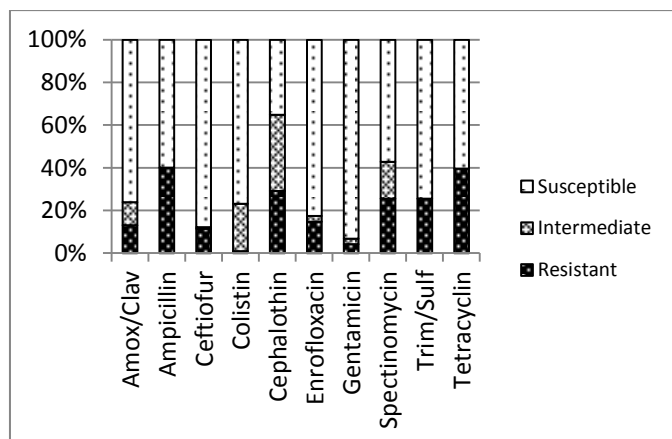
Bavarian Animal Health Service, Poing, Germany

Email: [Ingrid.Lorenz@tgd-bayern.de](mailto:Ingrid.Lorenz@tgd-bayern.de)

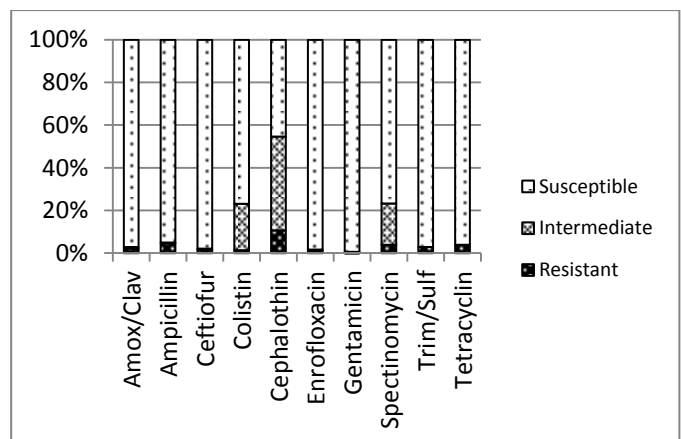
**Introduction** Monitoring of resistance against antimicrobial substances in Germany is performed using samples derived from diseased animals (Germ-Vet\*). It is not clear whether these are samples from problem farms, but the risk is at least increased in the calf area. Therefore, the aim of this study was to collect antibiotic resistance data in non-preselected Bavarian dairy farms.

**Materials and Methods** Between February 2017 and March 2018, dairy farm owners from the customer base of the Bavarian Animal health Service were invited to participate in the study. The agreement of the farmer was the only selection criterion (convenience sample). Other operations participated in the investigation as part of the PraeMAP project of the Thuringian Bovine Health Service. Geographically, the participating dairy farms cover all Bavarian government districts. On each farm, operational data were collected and fecal samples were taken from five healthy calves (if available) and five healthy cows. Feces samples were incubated on eosin methylene blue agar at 37° C for 22-48 hours. One colony was subcultured from each primary culture. The subcultures were verified by proteome analysis (MALDITOF, Bruker) as *E. coli*. Resistance was determined on the Micronaut-S plate large animals 3 (Merlin Diagnostika). Furthermore, the isolates were investigated for formation of Extended Spectrum Betalactamase (ESBL) (Vitek 2 compact, bioMerieux). Only farms from which at least three fecal samples from calves were available were included in the evaluation.

**Results** From 204 farms, 206 fecal samples were available for examination. 945 *E. coli* strains were selected from calf and cow samples, respectively. The proportion of ESBL-producing strains was 19% for calf samples and 4% for cows. The proportion of resistant *E. coli* against the tested antibiotics is shown in Fig. 1 (calves) and Fig. 2 (cows). The proportion of all resistant findings per operation varied between 0 and 68 % (median: 18 %) in calves and 0 and 40 % (median: 2 %) in cows. In the calf samples, the proportion of resistant findings increased with the stock size and the average milk yield of the stock. There was no such connection in the samples of cows. The frequency of antibiotic usage in calves perceived by farmers correlated positively to the proportion of resistances in *E. coli* from calf feces.



**Figure 1** Resistance pattern of *E. coli* from 945 fecal samples of healthy calves from 204 Bavarian dairy farms



**Figure 2** Resistance pattern of *E. coli* from 945 fecal samples of healthy cows from 204 Bavarian dairy farms

**Conclusions** The resistance rates of *E. coli* strains obtained from fecal samples of healthy calves in this study are significantly lower in all directly comparable tested antibiotics than those determined in the Germ-Vet Monitoring\*. The samples used for the Germ Vet examination are samples from untreated calves. However, the results suggest that a high proportion of samples from herds with calf health problems and therefore increased antibiotic use were included in the study. In the present study, there are large differences in antibiotic resistance numbers and patterns between farms. Owners with higher resistance rates indicated a higher usage of antibiotics in calves, however, since the antibiotic usage was not quantified in this study the subjective comments of the farm owners could not be verified.

#### References

\*) Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), 2017: BVL-Report 11.5

Berichte zu den Resistenzmonitoringstudien 2014 und 2015: Resistenzsituation bei klinisch wichtigen tierpathogenen Bakterien

[https://www.bvl.bund.de/DE/09\\_Untersuchungen/03\\_Fachmeldungen/2017/2017\\_09\\_22\\_Resistenz-Monitoring-2014-2015.html](https://www.bvl.bund.de/DE/09_Untersuchungen/03_Fachmeldungen/2017/2017_09_22_Resistenz-Monitoring-2014-2015.html)

### **Associations between antimicrobial treatment modalities and antimicrobial susceptibility in *Pasteurellaceae* and *E. coli* isolated from veal calves under field conditions**

L. Schönecker<sup>1</sup>, P. Schnyder<sup>1</sup>, G. Overesch<sup>2</sup>, G. Schüpbach-Regula<sup>3</sup> and M. Meylan<sup>1</sup>

<sup>1</sup>Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland; <sup>2</sup>Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland; <sup>3</sup>Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Switzerland

Email: lutz.schoenecker@vetsuisse.unibe.ch

**Introduction** Antimicrobial consumption, with calf pneumonia as main indication, is higher in the veal fattening industry compared to other livestock production branches. The aim of this study was to investigate possible associations between antimicrobial drug use and resistance rates in *Pasteurellaceae* and indicator *Escherichia (E.) coli* in veal calves under field conditions in a prospective trial.

**Materials and Methods** Nasopharyngeal and rectal swabs were collected from 2587 animals on 12 and 43 farms, respectively, over one year. Antimicrobial susceptibility testing (by minimal inhibitory concentration determination) was performed on 346 *Mannheimia (M.) haemolytica*, 1162 *Pasteurella (P.) multocida* and 2138 *E. coli*. Drug use was quantified as treatment incidences for each farm based on the used daily dose methodology (TI<sub>UDD</sub>), separately for group and individual treatments and for antimicrobial classes.

**Results** In mixed logistic regression analyses, a higher group TI<sub>UDD</sub> with penicillins and tetracyclines was significantly associated with increased odds for reduced susceptibility in *M. haemolytica* and *E. coli* to the corresponding antimicrobial drugs. Higher individual treatment TI<sub>UDD</sub> was associated with decreased odds for reduced susceptibility for macrolides and tetracyclines in *M. haemolytica* and for aminoglycosides in *E. coli*. No significant associations were observed for *P. multocida*.

**Conclusions** These findings suggest that targeted individual treatment of veal calves reduces antimicrobial resistance development compared to oral group treatment under field conditions. *E. coli* was confirmed as a suitable organism to detect such effects while the findings differed for the two *Pasteurellaceae* species, highlighting the importance of a differentiated assessment regarding resistance rates.

### Effects of garlic (*Allium sativum* L.) in postweaning pigs – a placebo controlled study

H. Ayrlé<sup>1,3</sup>, H. Nathues<sup>2</sup>, A. Bieber<sup>1</sup>, M. Durrer<sup>1,3</sup>, N. Quander<sup>1</sup>, M. Mevissen<sup>3</sup> and M. Walkenhorst<sup>1</sup>

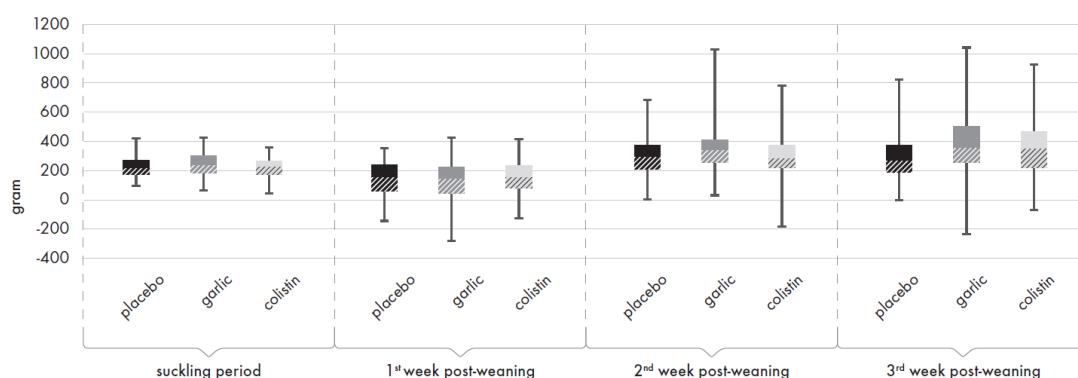
<sup>1</sup>Research Institute of Organic Agriculture (FiBL), Frick, Switzerland; <sup>2</sup>Department of Clinical Veterinary Medicine, Clinic for Swine, Vetsuisse Faculty, University of Bern, Switzerland; <sup>3</sup>Division Veterinary Pharmacology & Toxicology, Department Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Switzerland

Email: michael.walkenhorst@fibl.org

**Introduction** Postweaning diarrhoea is one of the most serious problems in pig production. It leads to impaired growth performance, decrease of animal welfare, morbidity or even death. Besides several environmental factors, *Escherichia coli* (*E. coli*) is closely associated with postweaning diarrhoea. The use of antimicrobials to prevent or treat postweaning diarrhoea is still common, but due to the rising incidence of antimicrobial resistance, it needs to be reduced. The preventive oral administration of medicinal plants like *Allium sativum* L. (AS, garlic), showing antimicrobial, anti-inflammatory, immunomodulatory and antidiarrheal properties (Ayrlé et al., 2016), might be an option to improve health and performance in postweaning pigs.

**Materials and Methods** The trial was conducted on a commercial Swiss farm (Ayrlé et al., 2019). During their first 72 hours of live, a total of 600 piglets (all half-siblings) was randomly assigned to three treatment groups within each litter. For the first two weeks post weaning, the piglets received 0.3 g dried garlic-powder/kg body weight/day, 6 mg colistin-sulphate/kg body weight /day or a placebo (PL) as top dressing divided to two daily administrations. Piglets were observed from birth until three weeks post weaning. For the first three weeks post weaning, a clinical score, body weight and the daily weight gain were measured individually and faeces dry matter as well as coliform bacteria were analysed weekly on pen level. Data were analysed using generalized mixed effect models.

**Results** The body weight of garlic-treated piglets was significantly higher (0.99 kg;  $p=0.003$ ) compared to placebo and it was also higher compared to colistin-treated piglets (0.63 kg;  $p=0.064$ ) in the end of the third week post weaning even though this effect was not statistically significant. The daily weight gains of garlic- and colistin-treated piglets were significantly higher compared to placebo-treated piglets (garlic: 61 g,  $p=0.008$ ; colistin: 61 g;  $p=0.001$ ) in the third week post weaning without differences between garlic and colistin (Figure 1). The clinical score of garlic-treated piglets was significantly lower compared to colistin-treated piglets ( $p=0.04$ ), but no differences were found when garlic was compared to placebo. No significant differences between the groups were observed for faeces dry matter. As expected, the number of colony forming units of *E. coli* in the faeces of the colistin-treated piglets was significantly lower compared to the placebo and the garlic-group after the first and the second week post weaning. No significant difference was found for the coliform bacteria count when garlic was compared to placebo. Due to severe diarrhoea, three out of nine (33%) of the garlic and the placebo pens had to be treated with antibiotics.



**Figure 1** Daily weight gain per treatment group from weaning until the third week post-weaning

**Conclusions** In conclusion, oral administration of 0.3 g dried garlic-powder/kg body weight/day did not prevent severe postweaning diarrhoea. Nevertheless, the observed improvement in growing performance in garlic-treated piglets compared to placebo suggests that garlic supplementation might be suitable to limit the use of antimicrobials to strictly therapeutic indications.

### References

Ayrlé H, Mevissen M, Kaske M, Nathues H, Grützner N, Melzig M, Walkenhorst M: Medicinal plants – prophylactic and therapeutic options for gastrointestinal and respiratory diseases in calves and piglets? A systematic review. BMC Veterinary Research, 2016; 12:89  
Ayrlé H, Nathues H, Bieber A, Durrer M, Quander N, Mevissen M, Walkenhorst M: Placebo-controlled study on the effects of oral administration of *Allium sativum* L. in postweaning piglets. Veterinary Record 2019; DOI: 10.1136/vr.10513

### The respiratory tract microflora sensitivity to antimicrobial drugs in newborn calves with different levels of vitality

A. Chernitskiy<sup>1</sup>, S. Shabunin<sup>1</sup>, O. Manzhurina<sup>1</sup> and V. Safonov<sup>2</sup>

<sup>1</sup>All-Russian Veterinary Research Institute of Pathology, Pharmacology and Therapy, Voronezh, Russia; <sup>2</sup>Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russia

Email: makhova@inbox.ru

**Introduction** Calhoo disease is an important issue of dairy farming that can considerably affect heifers' survival and productivity and has economic and welfare impacts. Neonatal calf diarrhea and bovine respiratory disease (BRD) are the most common causes of morbidity, mortality and antimicrobial use in young dairy cattle. Multidrug resistance of the respiratory tract microflora to antimicrobial medication can reduce the effectiveness of treatment and prophylaxis of BRD in calves. Presumably, low vitality and colostral immunodeficiency, associated with it, in calves may lead to microorganism strains highly resistant to antibacterial agents. The aim of this study was to review the respiratory tract microflora sensitivity to antimicrobial agents in newborn calves with different levels of vitality.

**Materials and Methods** 50 Holstein calves aged from the birth to the 28th day of life were studied, kept in the same conditions and fed on the same farm. During the study, the calves were not given any antimicrobial agents. The vitality of calves was determined shortly after birth using the VIGOR score, estimating the following data from 0 to 3: meconium staining, appearance of the tongue, time to initiation of selective calf movements such as sitting, standing attempts, suckling, head shaking, tongue withdrawal after a pinch, and eye blinking, mucous membrane color, heart and respiration rates. As a result, 5 groups were formed  $n = 10$  each: (1) excellent vitality calves with 26-27 VIGOR score (VS); (2) very good vitality calves 23-25 VS; (3) good vitality calves 21-22 VS; (4) marginal vitality calves 17-20 VS; (5) poor vitality calves 17-20 VS. Samples of nasal and tracheal swabs for bacteriological studies were obtained from calves on days 1, 3, 7, 14 and 21 after birth. For cultures isolation and microorganisms typing, meat-peptone broth and agar, milk-salt, enterococcal agar, Endo medium, blood agar, glucose-serum broth and agar produced by Research Center for Pharmacotherapy (St. Petersburg, Russia) were used. The typification of isolated *Escherichia coli* was carried out in an agglutination reaction using O-colours. The isolated cultures of microorganisms were tested for sensitivity to 16 antibacterial drugs by diffusion method - ampicillin (AM), cephalothin (CF), lincomycin (LM), erythromycin (EM), tylosin (TL), tetracycline (TC), doxycycline (DC), chloramphenicol (LV), rifampicin (RF), gentamicin (GM), streptomycin (SM), polymyxin (PM), norfloxacin (NF), enrofloxacin (EF), furazolidone (FL) and furadonin (FD), related to 11 groups, using ready-made disks produced by Research Center for Pharmacotherapy (St. Petersburg, Russia). All data were expressed as mean  $\pm$  standard deviation (SD) and median. The differences accuracy between the groups of calves was determined by comparing the median using the non-parametric Wilcoxon test in Statistica 8.0 (Stat Soft Inc., USA). The null hypothesis was rejected when  $P < 0.05$ .

**Results** Bacteria of 6 species were extracted from the nasal cavity of the calves aged 1-28 days (*Escherichia coli*, *Enterobacter cloacae*, *Citrobacter diversus*, *Enterococcus faecalis*, *Enterococcus faecium* и *Staphylococcus epidermidis*); 3 types from trachea (*E. coli*, *Ent. faecalis*, *Ent. faecium*). *E. coli* was represented by serotypes O2, O8, O9, O26 и O115. In calves of groups 3, 4 and 5, the tracheal mucosa was colonized by *E. coli* in 80-100% and *Ent. faecalis* in 30-70% of cases, in animals of groups 1 and 2, these bacteria were not detected from the trachea. The microflora contaminating the upper respiratory tract of the studied calves found to be highly resistant to the antibacterial drugs effect. Only 5 out of 16 drugs (AM, CF, DC, LV, FD) were effective against 19.7-25.8% of cultures isolated from the nasal cavity of calves. The efficiency of 10 drugs (LM, TL, TC, RF, GM, SM, PM, NF, EF, FL) was 1.5-10.6%. All studied cultures were resistant to EM, with 22.7% multiplied in the presence of EM. The tracheal microbiota in 14.3-23.8% of cases was sensitive to only 4 drugs (AM, CF, LV, RF), with 42.9% of cultures multiplied in the presence of GM, 33.3% of AM and TC, 28.6% CF, TL, EF. The highest resistance was found in bacteria isolated from the nasal cavity and trachea of calves of group 5 (100% resistance to 16 drugs, with 3 drugs (TC, SM, NF) in 50.0% and GM in 100.0% of cases stimulated crop growth). The second place in resistance was in calves of group 4 microflora of the upper respiratory tract, especially in cultures isolated from the trachea (100% resistance to 16 drugs, and 15 of them, except for TL, increased the growth of cultures in 16.7-66.7% of cases). In calves of group 3, the microbiota of the nasal cavity in varying degrees (from 7.1 to 23.5%) was sensitive to the effect of 5 drugs (AM, CF, DC, GM, FD), and trachea - in 20.0% to 2 drugs (CF, FL). In calves of group 2, the upper respiratory tract microbiota was in varying degrees (12.5-25.0%) sensitive to 8 out of 16 preparations (CF, LM, DC, LV, PM, NF, EF, FL), and in animals of group 1 - to 13 out of 16 drugs (except for LM, EM, FL) in relation to 5.0-45.0% of the isolated cultures, while there were no cases of enhanced growth in the presence of any of the 16 drugs.

**Conclusions** In conclusion, the sensitivity of respiratory tract microflora to antimicrobial medication in newborn calves with different levels of vitality is considerably different. In calves with marginal and poor vitality, the microbiota of the upper respiratory tract is most resistant to the antibacterial drugs effect. The results suggest that low vitality and colostral immunodeficiency in calves may lead to microorganism strains highly resistant to antibacterial agents.

The work is performed on Grant by the Russian Science Foundation of the state contract N 18-76-10015.

### Antimicrobial use indicators and their effect on relative ranking of grower-finisher herds

A. L. Bosman<sup>1,2</sup>, C. A. Carson<sup>2</sup>, A. Deckert<sup>1,2</sup>, R. J. Reid-Smith<sup>1,2</sup>, Z. Poljak<sup>1</sup>, S. Gow<sup>2</sup>, D. F. Léger<sup>2</sup> and S. A. McEwen<sup>1</sup>

<sup>1</sup>Department of Population Medicine, University of Guelph, Ontario, Canada; <sup>2</sup>Centre for Food-borne, Environmental Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, Ontario, Canada

Email: [bosmana@uoguelph.ca](mailto:bosmana@uoguelph.ca)

**Introduction** Antimicrobial use (AMU) information can be analyzed using various indicators. Indicators are units of measurement adjusted by a denominator that represents the population at risk. Examples include milligrams of active antimicrobial ingredient per population correction unit (PCU), defined daily doses for animals per PCU, and defined daily doses for animals per 1000 animal-days. The PCU is a measurement of the biomass of animals at risk of treatment with antimicrobials and is determined by multiplying the number of animals at risk by their standard weight (kg) at treatment (1). Defined daily doses for animals (DDDvet) is a unit of measurement adapted from human medicine by the European Medicines Agency for use in veterinary medicine (2). The DDDvet is based on the average labelled daily dose of an antimicrobial per kg of animal per species and route of administration (2). In Canada, Canadian defined daily doses for animals (DDDvetCA) are based on doses from Canadian labelled antimicrobial products (3).

We analysed AMU surveillance data collected between May 2017 and April 2018 from 23 grower-finisher sentinel pig herds in the province of Ontario by the Public Health Agency of Canada's Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (3).

**Materials and Methods** AMU data were collected by the farm veterinarians using questionnaires. Veterinarians purposively selected herds that proportionally represented the variety of farms in their practice and visited each herd once near the end of a production cycle. Questionnaire data were entered into a PostgreSQL database, and antimicrobial use indicators were applied to the data using R 3.5.2 (packages: dplyr, data.table, openxlsx, tidyr). If a herd was sampled in 2017 and in 2018, the 2017 sampling was removed.

Denominators used were the population correction unit (PCU) and 1000 pig-days at risk, using an average weight at treatment of 65 kg. Indicators used to determine the amount of antimicrobial used by each herd included milligrams of active ingredient per PCU (mg/kg pig), Canadian defined daily doses per PCU (DDDvetCA/pig) and Canadian defined daily doses per 1000 pig-days (DDDvetCA/1000 pig-days). The herds were ranked according to the amount of antimicrobials used as measured by each indicator. The relative ranking of the herds was compared among indicators.

**Results** Twenty-three farms were sampled, ranging in size from 750 to 2400 grower-finisher pigs. Twenty-two (96%) of the herds were conventional and one herd was part of a raised without antibiotics program (RWA). Eight herds (35%) did not use antimicrobials during the grower-finisher period. One herd was consistently ranked the highest user and another herd the lowest as measured in mg/kg pig, DDDvetCA/pig and DDDvetCA/1000 animal-days. However, the relative ranking of other herds changed according to the indicator used; therefore, the indicator used must be borne in mind when interpreting AMU data. In addition, if there is a specific AMU target, the choice of indicator used may influence whether the herd's AMU falls above or below the target value. Future work in this study includes examining AMU data collected from farrowing and nursery herds during the same time frame, and assessing producer and veterinarian understanding and preferences for different AMU measures and indicators.

**Conclusions** We observed that quantitative comparisons of AMU among study herds was affected by the choice of AMU indicator, which is an important consideration when interpreting comparative AMU data. While ranking of herds with potentially outlying AMU may not be affected by the choice of indicator, other herds may fall above or below a particular level or change in relative AMU depending on the indicator used.

### References

- (1) European Medicines Agency. Trends in the sales of veterinary antimicrobial agents in nine European countries (2005-2009). 2011;(EMA/238630/2011).
- (2) European Medicines Agency. Principles on assignment of defined daily dose for animals (DDDvet) and defined course dose for animals (DCDvet). 2015;(EMA/710019/2014).
- (3) Government of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2016 Annual Report. 2018.

### **Etiopathogenesis of non-infectious claw lesions in cattle and pigs – Is it all about biomechanics?**

C. K. W. Mülling

Institute of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Leipzig University, Germany

Email: [c.muelling@vetmed.uni-leipzig.de](mailto:c.muelling@vetmed.uni-leipzig.de)

**Introduction** Lameness is one of the major challenges in modern dairy husbandry and it is likewise a major concern in pigs, and in particular in breeding sows. Lameness in dairy cows is primarily caused by claw lesions. In sows, claw lesions and joint pathologies are the major causes for lameness. In cattle and pigs alike, claw lesions not only cause considerable animal welfare problems but also economic losses due to decline in performance, longevity and productivity. For successful claw lesion prevention knowledge of the etiology and pathogenesis are imperative. Despite substantial research activities in this field our understanding on the etiopathogenesis of non-infectious claw lesions is still lacking important pieces of information. This paper will present a comparative overview of our current understanding of the etiopathogenesis of non-infectious claw lesions in cattle and pigs.

The claw is the interface between the animal and the environment. Anatomically, the claw is designed for stance and locomotion on pasture type ground with variable hardness, and requires limited periods of standing. Standing and walking on hard floors and extended periods of standing inevitably lead to damage of the interior structures of the claw. Mechanical risk factors originate from the claw floor interaction, with the quality and resilience of the claw tissues, the properties of the flooring system and the animal's behaviour being the key factors. Biomechanical risk factors also originate from the shape and geometry of the claw capsule and are therefore directly linked to claw trimming.

Our evidence based understanding of the etiopathogenesis of non-infectious claw lesions in cows as a primarily metabolic or nutrition related problem has changed to understanding it as an environment associated, mechanical induced disease. And there is a growing body of evidence that this is true for pigs alike. It is nowadays well established that non-infectious claw lesions in cattle and pigs have a multifactorial etiology. In cattle, we have gained considerable insight in the impact of the cow's environment and management on claw structure and function. In comparison, the amount of information for the porcine claw is much smaller. The impact of the environment is either directly mechanical, chemical or biological or indirectly by altering the animal's behavior and secondary causing damage to the claw tissues. Environmental factors, comfort and behavior are understood as key factors in the multifactorial etiology of claw diseases and in lameness prevention. In both species, claw lesions are inevitably associated with indoor housing systems. It has been suggested more recently that mechanical influences and mechanically caused or initiated tissue alterations in the claw maybe the predominant or even exclusive cause for a majority of the non-infectious lesions. A strong biomechanical component in the etiopathogenesis of non-infectious claw lesions is well established in both species.

Pioneering studies in dairy cows have demonstrated that the strength of the suspensory apparatus of the claw was affected by housing in cubicles but not by nutrition. Parturition and lactation amplified this effect whereas feeding had no significant influence. The claw dermis is exposed to high local mechanical pressure, particularly when animals are standing for excessively long periods. Evidence has been generated that housing and claw trimming are a major hazard in dairy cows. Bone development (exostoses) on the caudal aspect of the distal phalanx has been linked with claw lesions and lameness; chronic inflammatory changes in the bone initiated or caused by pressure are discussed. Evidence for the biomechanical etiopathogenesis is growing. However, we are still lacking information on what exactly happens inside the claw during standing/excessive standing times and during locomotion. What are influences of different floor systems/types? What is the correct or best possible approach to preventive trimming? Understanding the claw-floor interaction and the precise influences of good and bad trimming on the interior structures of the claw provides the key to understand the pathogenesis of non-infectious claw lesions and to successful prevention of lesions. We therefore need an objective evaluation of floor systems and trimming to generate scientific evidence for what should be best practice.

Today, we have the methods and tools in biomechanical research to shed light on key events inside the claw and reveal mechanisms involved in the biomechanical pathogenesis of non-infectious lesions. In the context of claw lesions, the general aims of biomechanical research are data acquisition of physiological motion and strain, analysis of locomotive patterns linked to pathologies and the analysis of different influences on motion, e.g. claw trimming, ground conditions. Biplane high-speed fluoroscopy or XROMM is a relatively new method for three-dimensional analysis of bone motion with the promise of submillimeter and subdegree accuracy. It is a promising tool for gaining further insight into the claw floor interaction and for evaluation of trimming methods and flooring systems. Very limited attention has been given to using the technologies available for biomechanical research on the claw floor interaction in cattle under field conditions. Mobile pressure sensor systems available for research are too expansive or not technically developed yet to a stage where they can be used on farms under field conditions.

**Conclusions** We have strong and still increasing evidence, from field studies and biomechanical and kinematic studies in vivo and ex vivo, for cows and, to a lesser extent, for pigs, that biomechanical forces are a predominant risk factor for the development of non-infectious claw lesions. The main factors influencing biomechanics of the foot are: locomotion/standing time, behaviour, flooring, claw trimming, claw conformation, tissue properties – genetics determining claw tissue resilience and functional adaptation capacity. We have established and know for sure that preventive measures must be geared to reducing the mechanical stress level for the sensitive claw tissues. Soft elastic flooring more and more turns out to be a measure of eminent importance. Routine claw trimming, in both species, is essential and helps to balance the load born by the claw, distributed the load evenly, unload anatomical sensitive regions and avoid local pressure peaks. The interactions between the foot of an animal and its internal and external challenges are an integral component of comfort and well-being

### Inflammation, part of the foot print of sow lameness

M. Wilson<sup>1</sup>, W. Schweer<sup>1</sup>, A. Tinkle<sup>2</sup>, C. R. Dove<sup>2</sup>, A. Cornelison<sup>1</sup>, J. Torrison<sup>3</sup> and Z. Rambo<sup>1</sup>

<sup>1</sup>Zinpro Corporation, Eden Prairie, MN, USA; <sup>2</sup>University of Georgia, Athens, GA, USA; <sup>3</sup>University of Minnesota Veterinary Diagnostic Lab

Email: [MWilson@Zinpro.com](mailto:MWilson@Zinpro.com)

**Introduction** Claw lesions and lameness are often accepted as part of the business of running a sow farm accounting for approximately 15% of culls. Recording events of sow lameness are generally underestimated because sows that are culled due to reproduction or age may also be lame. A national project on sow mortality run by the Iowa Pork Industry Centre at Iowa State University shows the second greatest reason for sow death is due to lameness/injured or downer sows (29%), further demonstrating that lameness is a problem in the sow herd. This abstract will discuss five experiments with objectives to collect and determine the difference effects of trimming, environment or nutrition on locomotion scoring. A goal of these project was to establish objective measurements of locomotion.

**Experimental Design** All experimental protocols were approved by the institutional animal care use and committees. The objective of experiment one was to measure sow gait and locomotion pre-trimming verses 1 and 48-hours post trimming. Fifty-two sows were utilized in this experiment, with each sow serving as her own control. All trimming was done at 40 to 50 days gestation with the Zinpro elevated chute utilizing accepted trimming practices. The sows walked a circular tract with a straight portion containing two GigEye Ethernet Cameras, capturing 60 frames per second until 5 usable repetitions were recorded with < 10% difference in any recording for each sow. Similar to experiment one, experiment two utilized 19 sows to compare different trimming treatments on gait analysis, again sows were their own control. Sows were trimmed at three time points, day 1, 4 and 8 for dew claws, blunt trimming and functional trimming, respectively. Following each trimming sows walked a GAITFour electronic walkway (23,040 sensors) to record footfalls on the mat until 6 usable repetitions were collected. The objective for experiment three was to determine the cost of trimming and to observe production benefits from trimming, on a 5,000 sow commercial farm with 6% of sows having long rear toes. Seven hundred sixty sows were split into two groups trimmed or not trimmed. Both sow treatments were put into the chute at around 50 days of gestation for an average trim time of 8 minutes. In the fourth experiment, 70 gilts were split into two groups to determine the addition of 680 g of Availa<sup>®</sup>Sow per metric ton of feed on performance and locomotion over two parities. In a fifth and final experiment, 28 different mRNA types were analysed from the corium of the sole of dairy cows to identify inflammation responses. Data for all experiments were analysed using appropriate and accepted statistical models of SAS and SPSS.

**Results** In experiment 1 stance duration decreased ( $P < 0.01$ ) for both front and rear limbs from pre to 48 hours post trimming. Sows showed improvement in gate by functional trimming pre to post 48 hours by decreasing swing stride duration, breakover, velocity and increased swing to stance ratio ( $P < 0.05$ ). This study showed that functional trimming of sows improved forward movement with increased efficiency of gate following claw-trimming. In experiment 2, trimming dew claws to the coronary band of the claw improved velocity and stride length ( $P < 0.03$ ). Dew claw trimming also improved stride duration, stance and rear percent stance ( $P < 0.04$ ) compared to the control. Blunt trimming did not improve gait of the sow. Functional trimming showed improvement over control, dew claw trimming and blunt trimming. Functional trimming showed smaller stance, percent stance, stride duration, stride length and increased velocity over the control ( $P < 0.01$ ). For the second and third farrowing of experiment 3, trimmed sows improved pigs born alive over control, 13.1 vs 12.7 and 12.6 vs 12.0, respectively. It is intriguing that simply trimming the rear claws resulted in this boost on reproductive performance over two farrowings. In experiment 4, gilt performance and claw data were not different over two parities with the addition of Availa-Sow in the feed. However, in the second parity the gilts which received Availa-Sow had shorter stride duration, swing and stance over control ( $P < 0.01$ ). Control sows had an increase in stride duration, swing and stance when comparing their first parity to the second parity ( $P < 0.03$ ). Additionally, control sows had a shift in weight from the front limbs to the hind limbs. The lack of change from first to second parity in the Availa-Sow treated sows shows they did not experience near the amount of foot pain, suggesting Availa-Sow had a protective effect against aging compared to the control. Finally, in experiment five, keratinocytes proliferation was up regulated in damaged horn tissue for KRT5. Additionally, IL-1 $\beta$  and TNF $\alpha$  were up regulated during inflammation. Revealing that when there is inflammation, keratinocytes proliferation is signalled to increase but doesn't due to the inflammatory responses. Inflammation hinders repair of horn tissue triggering a pain response in sows.

**Conclusions** The utilization of high-speed cameras and pressure sensitive mats has provided a greater understanding of lameness and the positive effects of feet trimming on locomotion scores. Several papers have shown that inflammation decreases keratinocyte proliferation, negatively impacting the healing process and causing weakness in horn tissue. Feeding the combination mineral amino acid complex (Availa-Sow) of zinc, manganese and copper to gilts through the second parity improved locomotion pressure and gait over the inorganic mineral treatment. Suggesting that amino acid complex minerals can be used as a tool to provide mitigation of claw problems through decreasing the horn tissue damage and improving locomotion scores of sows.



### Lameness in dairy cows; where have we come from and where are we going?

L. V. Randall

University of Nottingham, School of Veterinary Science and Medicine, UK

Email: [Laura.Randall@nottingham.ac.uk](mailto:Laura.Randall@nottingham.ac.uk)

#### Introduction

The impacts of lameness in dairy cattle are well recognised, including welfare implications and the negative effects on herd performance. Claw horn disruption lesions (CHDL) represent a major cause of lameness and despite concerted efforts to understand the pathogenesis of these lesions, some major gaps in our knowledge and understanding still exist. This abstract aims to highlight some aspects of how our understanding of the pathogenesis of claw horn disruption lesions have developed, through to some key areas for future research.

#### Historical perspective and laminitis

Since the 1920s when Rusterholz disease was the term used to describe sole ulceration (Rusterholz, 1920), our understanding of the aetiopathogenesis of CHDLs has evolved considerably as research methods and capabilities have advanced with time. Research related to investigating laminitis in dairy cattle made a significant contribution to the lameness literature published from the 1960s and over the next 40 years. By definition laminitis refers to inflammation of the hoof laminae (rather than presence of CHDLs), however historically CHDLs have been thought of as a manifestation of a subclinical form of laminitis believed to be associated with a lowered rumen pH (or subacute ruminal acidosis) in dairy cows. Despite more than 40 years of research, there is a lack of evidence to demonstrate a causative relationship and more recently it has been highlighted that the use of the terminology 'laminitis' should be reserved for where inflammation of the laminae is present.

#### Environmental and animal-based risk factors

Major advances in our capability to explore the complex and multifactorial nature of lameness and CHDLs using statistical methods has provided us with a greater understanding of the role of environmental and animal-based risk factors in the development of CHDLs.

There is clear evidence that the environment contributes towards the development of CHDLs and studies have reported the effects of flooring and cubicle comfort on the development of lesions. Complexities in understanding the effect of the environment comes from unravelling the temporal relationships; previous lameness history may influence how a cow interacts with her environment which may in turn influence her future risk of lameness. Further work is needed to identify the optimum environmental conditions for housing the modern commercial dairy herd to reduce lameness.

Another area where it is increasingly apparent there are complex and potentially interacting factors, is the time around calving and to peak lactation. Previous work suggested that hormonal and metabolic changes associated with calving may contribute towards disruption to the laminar connective tissue structure and integrity, resulting in development of CHDL. Further work is needed to substantiate this causative effect as well as fully understand the mechanisms, however calving appears to be a primary factor. Energy balance has also been highlighted as playing a key role in hoof health, with milk yield increasing to peak lactation and a concurrent reduction in body condition score (BCS). A number of longitudinal studies have now demonstrated BCS as a risk factor for lameness, although underlying mechanisms are still not fully understood. Recent evidence also suggests that the role of previous lameness events is a critical factor in the development of future lesions. Changes to the pedal bone have been demonstrated to be associated with the occurrence of claw horn lesion, which may predispose to future lesions.

#### Future research directions

Ongoing research aims to address some of the knowledge gaps mentioned here. One such current study is aiming to investigate the role of inflammation in the development of CHDLs as well as further understanding the structure and function of the digital cushion. Further to this work, there are a number of key areas where we urgently need further research to be conducted including methods for early detection of lameness, where there is a role for technologies to contribute towards major advances. Use of technologies also provides us with the opportunity to further understand risk factors for lameness and CHDL. Prevention of the first case of lameness, or extending the time to first case could also have the potential to have a huge impact on lameness prevalence.

#### Acknowledgements

Work of the lameness group at the University of Nottingham, mentioned here has been funded by Biotechnology and Biological Sciences Research Council, Boehringer Ingelheim and AHDB Dairy ([www.ahdb.org.uk](http://www.ahdb.org.uk)). The author gratefully acknowledges the support of funders as well as colleagues, including Jon Huxley, Martin Green, Laura Green, Colin Mason, Mizeck Chagunda and James Wilson, who have contributed to some of the research cited here.

### **Digital Dermatitis Update – What Should Artificial Intelligence Do for You in the Near Future?**

D. Döpfer

Food Animal Production Medicine Section, School of Veterinary Medicine, UW-Madison, Wisconsin, USA

*Email: dopfer@wisc.edu*

#### **Background**

With the advent of increasing data volumes, freeware, pipelines and computing capacity, a process termed “democratization of deep learning” (Chollet, 2018) has been initiated that promotes access to state-of-the-art machine learning approaches including data mining, dimension reduction and parameter shrinkage. When working with large data sets, it is necessary to always remember that the performance of prediction models depends on the data quality, data inclusiveness and data representativeness.

#### **Application of Computer Vision**

In addition to prediction models for large data sets, computer vision for classification of images and videos, optical character recognition, and translators are examples for the application of artificial intelligence to real world problems that have gained entrance into the medical, applied and basic sciences. The systematic optimization of prediction models is necessary to result in better decisions guided by the implementation of prediction models.

#### **What should be done to optimize Computer Vision Models?**

The process of optimization should include the end users of such models rather than remote developments and implementation of decision aids by commercial entities alone. Herdsmen, producers and veterinarians should be involved in the generation and labelling of image and video data bases meant for training computer vision models in order to provide useful decision aids aimed at improving production, animal health and well-being.

#### **What should Artificial Intelligence do for end users?**

In answer to what Artificial Intelligence should do for end users in the near future, it should be emphasized that early and consistent detection of health and production outcomes should be at the focus of developments for computer vision models. Monitoring of long-term success of health management programs, treatment lists, proper drug use and follow-up on interventions to improve health and well-being of food animals should be top priorities of developments for machine learning and computer vision models.

The presentation will provide examples for computer vision models applied to the detection of claw lesions and lameness in cattle. The application of such models results in the discussion of performance and strategies necessary to generate meaningful data sets for predictions from computer vision models.

#### **References**

Chollet, F. 2018. Deep Learning with Python. Manning Publications Co, Shelter Island, NJ, USA.

### Hoof care and lameness: Learning from commercial dairy goat farms

G. Zobel<sup>1,2</sup> and J. Webster<sup>1,2</sup>

<sup>1</sup>AgResearch Ltd., Animal Welfare Team, Hamilton, New Zealand; <sup>2</sup>The Centre for Animal Welfare Science and Bioethical Analysis, the David Bayvel Consortium, Wellington, New Zealand

Email: [Gosia.Zobel@agresearch.co.nz](mailto:Gosia.Zobel@agresearch.co.nz)

Lameness in commercially managed dairy goats is a serious, yet often underestimated, welfare issue. It is recognised by an altered gait, due to it being a painful condition. Reported prevalence varies widely, undoubtedly linked to the various assessment methods used on-farm. Discussions with farmers highlight that identifying lameness, particularly low severity cases, is difficult, and that the causes and effects of lameness are not well understood. Goats become lame for a multitude of reasons, including infectious diseases (e.g., caprine arthritis encephalitis – CAE), overnutrition (e.g., laminitis) and inadequate management (e.g., hoof care, housing). It is not surprising that as goats' walking ability is impaired, regardless of the cause, their behavioural repertoire changes. This can be manifested as increased time spent lying and subsequently reduced time spent eating. Reduced milk production has also been reported.

Our research has focused on the impact of hoof care and overgrown hooves on dairy goats. Our work includes reviewing hoof management practices, a longevity assessment of goats on multiple commercial farms, and an observational study of the hooves of extensively managed dairy goats outdoors. The work outcomes, include a description of typical hoof growth, disproving a common misconception that frequent trimming increases hoof growth, and demonstrating hoof structural changes caused by poor hoof conformation and management. We have an immense photographic database tracing hoof conformation over multiple lactations. From our examination of the hooves of extensively managed milking goats, we now have a clear understanding of the factors needed to promote good hoof conformation; these include exercise, and dry, hard surfaces that promote hoof wear.

Commercial systems that promote good goat welfare, must include a program for reducing or eliminating lameness caused by hoof management issues. We have shown that there are a variety of options available for use in such a program. We suggest that a plan that relies on the goats' natural behaviour to help improve hoof management will be most successful. For example, evidence exists that goats gravitate towards hard surfaces in both outdoor and indoor environments; therefore, incorporating hard, rough surfaces into commercial housing systems will increase hoof wear in a natural pattern, decreasing hoof overgrowth and trimming needed. As well as addressing goat behavioural needs, the outcome will be less work for the farmer. Our research has therefore identified solutions that can be readily implemented and deliver benefits to commercial dairy goat systems.

### Evaluation of intra- and interrater agreement and reliability of visual locomotion scoring during the daily routine of dairy cows

A. Hund<sup>1</sup> and M. Dolezal<sup>2</sup>

<sup>1</sup>University Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria; <sup>2</sup>Platform Bioinformatics and Biostatistics, University of Veterinary Medicine Vienna, Austria

Email: Alexandra.Hund@vetmeduni.ac.at

**Introduction** Lameness is a major problem in dairy cows and affects their welfare and productivity. Therefore, locomotion scoring (LS) is an integral part of routine management as well as numerous research efforts. And while automated LS systems are being researched increasingly, visual LS is still most commonly performed using several LS systems that share a common impairment: the lack of a gold standard. Therefore, it is crucially important to assess intra- and interrater agreement and reliability (IIRAR) when performing visual LS in research. High IIRAR can be achieved through training and it seems obvious that enough time for the evaluation of individual cows would lead to better results.

Our aim was to determine agreement and reliability of locomotion scores obtained by three trained raters using video footage of cows returning from the milking parlor. Furthermore, it was our goal to compare their results to these of three untrained but experienced clinicians as well as one unexperienced rater to assess the effect of training on these parameters.

**Materials and Methods** As part of a research project visual locomotion scoring (LS) was performed in regular intervals over a prolonged period of time on a large dairy operation (about 2,500 cows in freestall barns milked twice daily). On-farm LS data was collected by three raters (Pro) using the system according to Flower and Weary (2006). For the assessment of IIRAR, a total of 7 raters were included: A person (Pro1) experienced in LS trained two more raters (Pro2 and Pro3) in LS on the project farm using at least 1000 cows. Three other clinicians experienced in LS were either familiar (Exp1) or unfamiliar (Exp2 and Exp3) with the Flower scoring system used. The unexperienced rater (New) was a veterinarian who had not performed any LS routinely before and was not familiar with any scoring system.

Video tapes of 109 different Holstein Friesian dairy cows were used for the assessment of IIRAR, allowing an average of 5s per cow for evaluation. The cows had been recorded on the project farm as they were returning from the milking parlor after morning milking walking on both slatted and concrete floors with grooves. The Exp and New raters received no visual training, only the LS system was explained in theory to all raters before they watched the video recordings. All 109 cows were scored twice by each rater two weeks apart. We assessed distributions of locomotion scores per and across raters and the intra and interrater differences in scores. Percent agreement (PA), weighted kappa ( $\kappa_w$ ), as well as Fleiss kappa were calculated using the R version 3.5.0 (R Core Team, 2018) and package *irr* (Gamer et al., 2019). Results of kappa statistics were interpreted in accordance with Landis and Koch (1977).

**Results** Wilcoxon tests showed that Pro3 rated systematically lower than Pro1 and Pro2, whereas no significance difference was observed between Pro1 and Pro2. The intrarater PA ranged from 34.6% (Exp3) to 89% (Pro3) and PA between raters from 7.4% (Pro3 and Exp3) to 51.9% (Pro1 and Pro2). Table 1 shows that intrarater reliability ( $\kappa_w$ ) was fair (Exp3) to very good (Pro3). However, interrater reliability ( $\kappa_w$ ) was fair to moderate with only one good agreement between Pro1 and Pro2. Fleiss kappa was 0.038 across all 7 raters.

**Table 1**  $\kappa_w$  of locomotion scores between all raters

Rater	Pro1	Pro2	Pro3	Exp1	Exp2	Exp3	New
Pro1	0.710						
Pro2	0.637	0.771					
Pro3	0.268	0.252	0.898				
Exp1	0.511	0.398	0.45	0.649			
Exp2	0.522	0.450	0.426	0.479	0.678		
Exp3	0.244	0.391	0.089	0.172	0.16	0.316	
New	0.286	0.505	0.148	0.214	0.303	0.262	0.470

**Discussion** We found that untrained raters were inferior to outcomes of other studies evaluating IIRAR in different LS systems as reviewed in Schlageter-Tello et al. (2014). However, the results in trained raters agreed with those other researchers found. Pro3 showed the highest intrarater reliability but would benefit from additional training to mitigate the downward bias in their LS. As expected Pro1 and Pro2 had the highest interrater reliability ( $\kappa_w$ ) which demonstrates the benefit of extensive on-site training. Training is particularly important under severe time constraint when performing LS as part of a daily routine in a milking herd. This might also explain the lower than previously described IIRAR among less experienced raters in this study.

**Conclusions** In research using visual LS evaluating IIRAR on a regular basis is an indispensable tool to identify rater bias and ensure reliable and repeatable lameness assessments.

#### References

- Flower, F.C., Weary, D.M., 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. *J Dairy Sci* 89, 139-146.
- Gamer, M., Lemon, J., Fellows, I., Singh, P. 2019. irr: various coefficients of interrater reliability and agreement, R package version 0.84.1.
- Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics* 33, 159-174.
- Schlageter-Tello, A., Bokkers, E.A.M., Koerkamp, P.W.G.G., Van Hertem, T., Viazzi, S., Romanini, C.E.B., Halachmi, I., Bahr, C., Berckmans, D., Lokhorst, K., 2014. Manual and automatic locomotion scoring systems in dairy cows: A review. *Prev Vet Med* 116, 12-25.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

### Advances in turkey lameness and gait analysis

M. Erasmus

Purdue University Department of Animal Sciences, West Lafayette, IN, USA

Email: [merasmus@purdue.edu](mailto:merasmus@purdue.edu)

**Introduction** Leg and skeletal problems are important health and welfare concerns of commercial turkeys at all stages of production, leading to reduced feed efficiency, lameness, and in some cases, culling and mortality. A recent survey of turkey industry representatives and veterinarians ranked leg problems among the top 10 turkey health issues (1). The importance of leg problems is supported by on-farm research which determined that over 40% of turkey broilers (examined between 16 and 19 weeks of age) had visible gait impediments (2). Abnormalities of the skeletal system that affect turkey gait include long bone distortions such as varus and valgus deformities, twisted leg, shaky leg, tibial dyschondroplasia and crooked toes. In male turkey breeders, additional problems such as femoral fractures lead to lameness (3). Lameness and poor gait can contribute to other welfare issues, in addition to pain and difficulty accessing feed and water. Turkeys that are behaviorally less active or immobile may be more likely to be recipients of injurious pecking. Problems that affect turkey gait, such as arthritis and footpad swelling, have also been correlated with pecking damage in slaughter-age turkeys (4).

A major objective within the turkey industry is to develop methods for reducing the incidence and severity of skeletal problems (5). However, causes of leg and skeletal problems are multifactorial and include infectious (e.g. osteomyelitis, footpad dermatitis and synovitis) as well as non-infectious causes. There are many different non-infectious causes of skeletal problems, including diet, photoperiod, rapid weight gain, environmental temperature and humidity levels, wet litter leading to footpad dermatitis, and genetic factors (reviewed in 6). The complexity of causes makes prevention of skeletal disorders difficult, but recent advances in gait analysis have provided important insights into turkey skeletal development and lameness, and have identified areas for further research.

**Gait scoring and Analysis** Methods of assessing turkey gait include i) visual scoring systems, whereby observers visually examine and score turkeys as they walk in real-time (e.g. 2, 7, 8), ii) analyzing video recordings of turkeys walking (e.g. 9), iii) using force plates and video cameras (e.g. 10) and more recently, iv) using pressure sensing walkways (e.g. 5; 11, 12). Visual scoring systems score turkeys' walking ability on a scale (e.g. 0: no detectable abnormality to 5: complete lameness, e.g. 8). Pressure sensing walkways have the advantage of being able to objectively measure, quantify and digitally record various aspects of turkeys' gait, such as the maximum force exerted on the walkway, step length and number of steps taken/min, among others (e.g. 11). However, a limitation of the pressure sensing walkway is that turkeys need to be habituated to walking on the walkway before data can be collected. Regardless of the method used to evaluate turkey gait, studies are in agreement that in healthy turkeys, gait worsens with age. Some studies have also reported that the largest change in turkey body weight is observed between 11 and 13 weeks of age (11, 13), corresponding with some of the changes that were observed in turkey gait. Gait differences have also been observed among turkeys of different genetic lines, suggesting that higher growth rates may be associated with higher incidences of leg disorders (5, 14). One study (15) has compared gait parameters between sound (no visible defects) turkeys and turkeys with leg defects using a pressure sensing walkway. Differences in gait parameters of sound turkeys and turkeys with crooked toes, shaky legs or valgus deformities depended on the age of the turkeys, but turkeys with crooked toes exhibited consistently different gait measurements compared to sound turkeys (15).

**Conclusion** Skeletal problems and lameness represent important welfare concerns for commercial turkeys at all production stages. These problems are associated with pain, inability to access feed and water, and in severe cases, mortality. Research examining turkey gait has demonstrated that turkey gait deteriorates with age, leg problems differ among genetic lines of turkeys, and changes in gait are associated with changes in body weight. Research using pressure sensing walkways to quantify turkey gait parameters has identified certain aspects of turkey gait, such the amount of time that the foot is in contact with the walkway for example, that are affected by leg disorders. With the use of technology such as pressure sensing walkways, it may be possible to refine genetic selection for improved gait, thereby leading to improved turkey welfare. Further research identifying aspects of turkey gait that can be used to genetically select for soundness will be valuable in reducing leg problems in turkeys and improving turkey welfare. The relationships between lameness, other welfare issues such as injurious pecking, and changes in body weight require further investigation.

### References

- 1) Clark, S. R. and V. Ahlmeyer. 2018. Turkey Industry Annual Report. 2) Da Costa, M. J., J. L. Grimes, E. O. Oviedo-Rondón, I. Barasch, C. Evans, M. Dalmagro and J. Nixon. 2014. *Appl. Poult. Res.* 23: 268-279. 3) Crespo R., S. M. Stover, R. Droual, R. P. Chin and H. L. Shivaprasad. 1999. *Avian Dis.* 43: 150-4. 4) Allain V., D. Huonnic, M. Rouina and V. Michel. 2013. *Br. Poult. Sci.* 54: 33-41. 5) Oviedo-Rondón, E. O., B. D. X. Lascelles, C. Arellano, P. L. Mente, P. Eusebio-Balcazar, J. L. Grimes and A. D. Mitchell. 2017. *Poult. Sci.* 96: 1989-2005. 6) Erasmus, M. A. 2018. Chapter 13. In: *Advances in poultry welfare*. Mench, J. A. (ed.). Woodhead Publishing, Cambridge, MA. 7) Quinton C. D., B. J. Wood and S. P. Miller. 2011. *Poult. Sci.* 90: 2479-86. 8) Vermette C., K. Schwan-Lardner, S. Gomis, T. G. Crowe and H. L. Classen. 2016. *Poult. Sci.* 95: 985-96. 9) Abourachid, A. 1991. *Br. Poult. Sci.* 32: 271-277. 10) Stover K. K., E. L. Brainerd and T. J. Roberts. 2018. *J. Exp. Biol.* 221. 11) Stevenson, R., H.A. Dalton and M. Erasmus. 2019. *Front. Vet. Sci.* 5: 319-331. 12) Kremer J., C. Robinson, D. Karcher. 2018. *Front Vet Sci.* 5:244-250. 13) Dalton H. A., B. J. Wood, T. M. Widowski, M. T. Guerin and S. Torrey. 2016. *Appl. Anim. Behav. Sci.* 178:40-50. 14) Eusebio-Balcazar, P., E. O. Oviedo-Rondón, J. L. Grimes and J. Scott. 2015. *Int.J. Poult. Sci.* 14:497-505. 15) Oviedo-Rondón, E. O., P. L. Mente, C. Arellano, B. D. X. Lascelles and A. Mitchell. 2018. *Poult. Sci.* 97: 2621-2637.

### The Prevalence of Carpal, Hock, and Stifle Lesions and potential Risk Factors in Swiss Dairy Cows kept in Tie Stalls

J. Bernhard<sup>1</sup>, B. Vidondo<sup>2</sup> and A. Steiner<sup>1</sup>

<sup>1</sup>Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>2</sup>Veterinary Public Health Institute, Vetsuisse-Faculty, University of Bern, Liebefeld, Switzerland

Email: [johanna.bernhard@vetsuisse.unibe.ch](mailto:johanna.bernhard@vetsuisse.unibe.ch)

**Introduction** Animal welfare in modern dairy production is affected by the occurrence of so called technopathies. Skin lesions at exposed periarticular areas of a cow's legs are frequently reported, where the presentations range from hairloss to ulceration and swelling. Most of the studies that examined potential risk factors for such skin lesions were conducted on large tie stall farms in North America or the United Kingdom that differ from the traditional tie stall housing system typical for alpine areas; thus little is known about specific risk factors for skin lesions in dairy cows that are kept in tie stalls.

**Materials and Methods** Each tie stall farm was visited  $\geq 4$  weeks after the start of the Winter housing period in the course of this cross-sectional study. In all lactating and dry cows of the herd, both left and right carpal, tarsal, and stifle joints were visually assessed for the presence of hairloss, ulceration, and swelling and scored on a 4-point-scale (score 0-3; Potterton et al. (2011)). Cows that were purchased less than six weeks before the farm visit were excluded from the statistical analysis.

Herd-associated risk factors were assessed by a questionnaire the observer filled in with the farmer; stall- and cow-associated risk factors were measured for a random sample of tie stall places and cows in each herd; missing values were imputed prior to statistical analysis. Initially, all continuous variables were divided into four groups by the three quartiles; where category 1 served as the control. The odds ratio was calculated for each significant category.

A multivariable two-level mixed-effects model with the random effect "farm" was fitted ( $\alpha \leq 0.05$ ) and odds ratios were calculated. The low prevalence of stifle lesions did not allow for valid multivariable two-level mixed effects models; therefore, the models were exclusively fitted for carpal and tarsal lesions.

**Results** Between December 2017 and April 2018, 627 cows of 27 farms were examined. Of the 607 cows included in the dataset for carpal lesions, 54.4% presented with moderate to severe hairloss, 7.7% with mild to severe ulceration, and 6.1% with moderate to severe swelling. Tarsal lesions (n = 608 cows) were recorded most frequently with a prevalence of 62.3%, 34.4%, and 24.0%, while stifle lesions (n = 608 cows) were recorded less frequently with a prevalence of 18.6%, 8.9%, and 3.5% for hairloss, ulceration and swelling, respectively. The risk to develop several types of skin lesions increased in January and decreased in December and April. A bedding depth of  $>2\text{cm}$  (according to Solano et al. (2016)) significantly decreased the risk of a cow to present with skin lesions. A free lunge space significantly decreased the risk to develop carpal ulceration and swelling. Dirtier cows more often suffered from tarsal lesions, as well as cows that were kept on a rubber mat surface. Furthermore, a higher rear step and shorter feeding intervals decreased the risk for tarsal swelling.

**Conclusions** The results of the present study highlight the importance of an adequate amount of bedding in the stall and reveal that the cows are at highest risk to develop skin lesions a few months after the Winter housing period starts. It might be hypothesized that the lesions improve with time, when the climatic conditions allow for a more frequent outdoor access in March and April.

#### References

- Potterton, S. L., M. J. Green, J. Harris, K. M. Millar, H. R. Whay, and J. N. Huxley. 2011. Risk factors associated with hair loss, ulceration, and swelling at the hock in freestall-housed UK dairy herds. *J Dairy Sci* 94(6):2952-2963.
- Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, C. G. Nash, D. B. Haley, D. Pellerin, J. Rushen, A. M. de Passille, E. Vasseur, and K. Orsel. 2016. Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in freestall barns. *J Dairy Sci* 99(3):2086-2101.

### Use of automated methods of lameness detection for evaluating the efficacy of ketoprofen in lame cows

M. Alsaad, M. Fadul, R. Deiss, E. Bucher and A. Steiner

Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland

Email: maher.alsaad@vetsuisse.unibe.ch

**Introduction** Cattle lameness is a painful disorder, with an important welfare and economic impact. As cattle are prey species with a stoic character, they rarely show overt signs of pain until the stimulus is severe. Therefore, recognition and assessment of pain in cattle remain challenging (Coetzee, 2013). Consequently, valid and reliable methods for pain assessment of the locomotor apparatus in cattle are needed. Ketoprofen was shown to moderately reducing hyperalgesia of lame cows in the recovery period after therapeutic claw trimming (Whay et al., 2005) and improving the weight distribution between sound and lame legs (Flower et al., 2008). The newly developed tools for objectively assessing the cows' gait represent a promising approach to detect cows with foot pathologies and even very slight cases of lameness (Nechanitzky et al., 2016; Alsaad et al., 2017). Therefore, the aim of this study was to evaluate the effect of the NSAID Ketoprofen (Rifen® Streuli AG, Switzerland) during the time period when ketoprofen is thought to be active (24h) on cows affected with limb pathologies using locomotion scoring and validated automated tools of weight bearing and gait analysis.

**Materials and Methods** The study protocol was approved by the animal experimentation committee of the canton of Bern, Switzerland (permission #25601). A total of forty-one lame cattle referred to the clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland, were included in this study. The inclusion criteria for the study animals were: cattle with unilateral fore- or hindlimb lameness, referred to the clinic for further assessment of this health problem. Cattle must not exhibit any relevant systemic concentration of analgesics at the beginning of the study. The study was performed as blinded, randomized, and placebo-controlled clinical trial. Cattle were randomly allocated to either the ketoprofen (group K; n = 21) or placebo group (group P; n = 20), receiving one dose of ketoprofen (3 mg/kg of BW i.v.; Rifen® Streuli Pharma AG, Switzerland, <http://www.streuli-pharma.ch/>) or an equivalent volume of sterile isotonic saline solution (NaCl 0.9% steril® Laboratorium Dr. G. Bichsel, Interlaken-Switzerland), respectively. Three data collection time points - before treatment (basis; T0), one hour (hr) after treatment (T1) and 18 hours (hrs) after treatment (T2) were defined. The parameters included the locomotion score, weight distribution parameters (weight distribution and SD of the weight derived from a 4-scale weighing platform as described by Nechanitzky et al., 2016) and gait variables of the cow pedogram (400 Hz accelerometer; kinematic outcome = stance phase duration; kinetic outcome = foot load and toe-off) as described by Alsaad et al., 2017. All variables were expressed as differences across limbs, and the measurements at 1 hr and 18 hrs were compared to the baseline. A repeated measures ANOVA was used to determine the differences between groups K and P.

**Results** The NSAID ketoprofen had a significant effect on one parameter each of the weighing platform (weight distribution) and the cow pedogram (stance phase duration) within 1 hr of intravenous administration. This effect had disappeared at 18 hrs after administration. An effect of ketoprofen on locomotion scores was not found.

**Conclusions** In conclusion, the results of this study reveal that measuring stance phase duration of the cow pedogram while cows are walking and weight bearing across limbs while standing show great potential as automated methods for evaluating the effect of NSAIDs on motion and weight bearing characteristics of the musculoskeletal apparatus of lame cattle.

### References

- Alsaad, M., M. Luternauer, J. T. Hausegger, R. Kredel, and Steiner A. (2017) The cow pedogram – analysis of the gait cycle variables allows for detection of lameness and foot pathologies. *Journal of Dairy Science* 100(2):1417-1426.
- Coetzee, J.F. (2013) Assessment and Management of Pain Associated with Castration in Cattle. *Veterinary Clinics of North America: Food Animal Practice* 29(1):75-101.
- Flower, F.C., M. Sedlbauer, E. Carter, M.A.G. von Keyserlingk, D.J. Sanderson, and Weary D.M. (2008) Analgesics improve the gait of lame dairy cattle. *Journal of Dairy Science* 91(8):3010-3014.
- Nechanitzky, K., A. Starke, B. Vidondo, H. Muller, M. Reckardt, K. Friedli, and Steiner A. (2016) Analysis of behavioral changes in dairy cows associated with claw horn lesions. *Journal of Dairy Science* 99(4):2904-2914.
- Whay, H. R., A. J. F. Webster, and Waterman-Pearson A.E. (2005) Role of ketoprofen in the modulation of hyperalgesia associated with lameness in dairy cattle. *Veterinary Record* 157(23):729-737.

### Detection of digital dermatitis treponemes and *Dichelobacter nodosus* from Mortellaro lesions in Swiss cattle

M. Alsaad<sup>1,2</sup>, I. Locher<sup>1,2</sup>, J. Jores<sup>2</sup>, P. Grimm<sup>3</sup>, I. Brodard<sup>2</sup>, A. Steiner<sup>1</sup> and P. Kuhnert<sup>2</sup>

<sup>1</sup>Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>2</sup>Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>3</sup>Clinic for Ruminants and Swiss Bovine Health Service (RGD), Switzerland

Email: maher.alsaad@vetsuisse.unibe.ch

**Introduction** Digital dermatitis (DD) in cattle is a widespread, contagious, multifactorial infectious foot disease that causes severe lameness in cattle worldwide. The three *Treponema* species *T. pedis*, *T. medium* and *T. phagedenis* are known to be the primary causative agents of DD. Besides *Treponema* species, *Dichelobacter nodosus* has been reported to contribute to the development of DD. The aim of this study was therefore to determine the prevalence of the three *Treponema* species as well as *D. nodosus* in DD lesions and the slurry environment of Swiss cattle using PCR-based techniques.

**Materials and Methods** Twenty-two farms with clinical DD and two farms without clinical DD were enrolled in this study. A total of 86 specimens from DD cases were collected (M1, n=15; M2, n=19; M3, n=9; M4, n=2, M4.1, n=16 and M5, n=25) using sterile, dry cotton swabs by rubbing the swab over the lesion (M1 to M4.1) and from the interdigital space in cases of clinically unaffected tissue (M5).. In addition, to assess the potential of environmental transmission, slurry samples from DD affected farms (n=21) were collected. Nested and real-time PCR were performed on DNA extracted from the specimens to detect *Treponema* species and *D. nodosus*, respectively. Chi-square test (with Pearson's Chi-Square) was used to investigate the associations between the presence of *Treponema* species, *D. nodosus* and the DD status of the animals.

**Results** The DD-stages (M1 to M4.1) and M5 (healthy skin) were positive for at least one or more of the DD-associated *Treponema* species (50/61 (81.97%)) and (9/25 (36%)), respectively. M1 showed lower prevalence (14.75%) compared to the other DD stages (M2, M3, M4 and M4.1) (67.21%) ( $P=0.011$ ). Most prevalent was *T. phagedenis* (65.12%). *D. nodosus* was detected in 51.81% (M1 to M4.1) and 24.10% in case of (M5) samples but not significantly associated with DD-stages. None of the *D. nodosus* strains detected contained the acid protease gene *aprV2*, that is associated with virulence in sheep foot rot but all strains contained the acid protease gene *aprB2*. Control farms were negative for all DD-associated *Treponema* species while *aprB2*-positive *D. nodosus* was present in 77.78% of specimens investigated. With respect to the slurry samples, three out of 21 specimens (14.29%) were positive for at least one or more of the DD-associated *Treponema* species and eleven out of 21 specimens (52.38%) were positive for *aprB2*-positive *D. nodosus*.

**Conclusions** DD lesions were associated with specific *Treponema* species, while *D. nodosus* did not reveal such a close link with DD lesions. DD specific *Treponema* species were present in a low number of slurry samples, that may present a potential reservoir of DD treponemes.

### References

Alsaad, M., Locher, I., Jores, J., Grimm, P., Brodard, I., Steiner, A., Kuhnert, P. (2019). Detection of specific *Treponema* species and *Dichelobacter nodosus* from digital dermatitis (Mortellaro's disease) lesions in Swiss cattle. Accepted for publication in Schweizer Archiv für Tierheilkunde.



### Supporting German dairy farmers: Development of an e-learning tool to improve hoof health in dairy cows

M. Au<sup>1</sup>, B. Behr<sup>1</sup>, S. Hachenberg<sup>1</sup>, M. Stumpf<sup>2</sup> and A. Fiedler<sup>3</sup>

<sup>1</sup>German Association for Performance and Quality Testing; <sup>2</sup>Self-employed hoof trimmer; <sup>3</sup>Practice for Hoof Health

Email: Marie.Au@dlq-web.de

**Introduction** Only one in three lame cows is detected as such in due time (Cutler et al. 2017; Leach et al. 2010). This often originates in the farmer's lack of experience in lameness detection, apart from having limited observation time. However, the chance of complications or consequential damages is lower the sooner a lame cow is detected and treated accordingly (Huxley, 2013; Maxwell et al. 2015, Miguel-Pacheco et al., 2017). These results highlight the demand for a supporting tool in hoof health management. While extensive research on hoof health is done at national and international level, the practical transfer of basic knowledge to the farmers, such as lameness detection, is often difficult.

The German Association for Performance and Quality Testing is committed to promoting knowledge transfer, benefitting from being the umbrella association of German milk recording organizations with a coverage of 31,000 dairy farms farming over 2,2 million cows. The services offered by the member organizations range from DHI to management support tools. Due to their monthly on-farm visits, the milk recording organizations are close to the farmers and understand the farmers' needs.

E-learning programs are a contemporary tool for sharing knowhow and combine several advantages: The user can access it whenever he has time to spare, the content can easily be added to or altered according to the latest research results and it reaches out to the younger or more digitalized generation of farmers, hoof trimmers, vets and students. This contribution presents the implementation of an e-learning tool regarding the improvement of hoof health management. It has been realized within the joint project KLAUENfitnet.

**KLAUENfitnet E-Learning** 6 modules cover the most important topics regarding hoof health. As a key to hoof health management, the main focus is on the improvement of lame cows being detected as early as possible. Several videos are included to highlight the characteristics in the gait pattern of healthy and lame cows and to self-test the ability to detect early stages of lameness in a quiz. Detailed background knowledge on the anatomy and the proper grooming of the hoof is the key to identifying the correct farm-individual prophylactic measures. Hence, mechanical stress in the hoof is visualized in different states of movement and/or hoof condition and, consequently, the importance of appropriate hoof trimming is illustrated.

Additionally, common hoof issues and the best practice of treatment are characterized via detailed footage of on-farm hoof trimming. The significance of digital and standardized recording of findings during a hoof trimming session is highlighted as a crucial parameter for operational analysis and sustainable improvement of lameness. This is ensured by providing the German key to Hoof Health Diagnosis (DKDS 1.0) for download, which is based on the ICAR atlas of Claw Health.

As a current hot topic dermatitis digitalis is covered in a separate module.

Special attention has been paid to the practical relevance of all modules. In order to attract students' attention and to simplify the learning process, an effort has been made by using different presentation methods, which resulted in the use of interactive charts, animated graphics and photos as well as plain text. In addition, video footage has been taken on a farm in Bavaria in order to be able to show hoof trimming in action as well as a vet and a hoof trimmer giving their detailed knowledge on hoof health management.

KLAUENfitnet e-learning has been developed in cooperation with vets, hoof trimmers and farmers and is accessible for free for anyone who is interested: <https://elearning.klauenfitnet.de>. It has been promoted in various conferences and meetings as well as newspaper and online articles.

**Results and Discussion** Since first being published in February 2018, the tool had a wide range of positive response: more than 7,500 users have accessed the homepage, with over 40% of the users being farmers. Also, more than 40% of the users visited the homepage repeatedly. The videos, which are hosted by YouTube, have partly been clicked for over 100,000 times in one year.

Since the program is in German, a translation would be advised for international use.

**Conclusion** The number of users highlights the strong interest of the dairy sector in improving hoof health in dairy cows and shows the relevance of adjusting knowledge transfer methods to the digital age.

The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

### References

1. Cutler, J. H. Rushen, H. J., de Passillé, A. M., Gibbons, J., Orsel, K., Pajor, E., Barkema, H. W., Solano, L., Pellerin, D., Haley, D., Vasseur, E., 2017. Producer estimates of prevalence and perceived importance of lameness in dairy herds with tiestalls, freestalls, and automated milking systems. *Journal of Dairy Science* 100: 9871-9880.
2. Huxley, J. N., 2013. Impact of lameness and claw lesions in cows on health and production. *Livestock Science* 156: 64-70.
3. Leach, K.A., Whay, H.R., Maggs, C.M., Barker, Z.E., Paul, E.S., Bell, A.K., Main, D.C.J., 2010. Working towards a reduction in cattle lameness: Understanding barriers to lameness control on dairy farms. *Research in Veterinary Science* 89: 311-317.
4. Maxwell, O., Miguel-Pacheco, G., Newsome, R., Randall, L., Remnant, J., Thomas, H., Huxley, J., 2015. Lameness in cattle 1. Recent research to inform clinical practice. In *Practice* 37: 127-138.
5. Miguel-Pacheco, G.G., Thomas, H.J., Huxley, J.N., Newsome, R.F., Kaler, J., 2017. Effect of claw horn lesion type and severity at the time of treatment on outcome of lameness in dairy cows. *The Veterinary Journal* 225: 16-22.

### Risk factors for pododermatitis (PD) in group housed does

M. Braconnier<sup>1</sup>, S. G. Gebhardt-Henrich<sup>1</sup>, S. Ruchti<sup>1</sup>, A. Meier<sup>2</sup>, H. Würbel<sup>3</sup>, R. Furrer<sup>4</sup>, G. Kratzer<sup>4</sup> and S. Hartnack<sup>5</sup>

<sup>1</sup>Centre for Proper Housing: Poultry and Rabbits (ZTHZ), Animal Welfare Division, VPH Institute, University of Bern, Zollikofen, Switzerland; <sup>2</sup>Department of Biostatistics, University of Zurich, Switzerland; <sup>3</sup>Animal Welfare Division, Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Switzerland; <sup>4</sup>Department of Mathematics, University of Zurich, Switzerland; <sup>5</sup>Section of epidemiology, Vetsuisse Faculty, University of Zurich, Switzerland

Email: michele.braconnier@vetsuisse.unibe.ch

**Introduction** In rabbits (*Oryctolagus cuniculus* L.), pododermatitis is a chronic multifactorial skin disease that appears mainly on the plantar surface of the hind legs. This presumably progressive disease can cause pain leading to poor welfare, yet the progression of this disease has not been thoroughly assessed on the level of individual animals. The aim of the longitudinal and cross-sectional studies was to investigate the possible risk factors and the prevalence of pododermatitis in group housed breeding does in Switzerland on litter and plastic slats.

**Materials and Methods** Cross-sectional study (CS): 30% (1090 animals) of female breeding rabbits on 17 farms were palpated on their behind feet (middle) und heels. Animal-related and environmental risk factors were recorded.








Longitudinal study (LS): 201 does on 3 farms were evaluated every four weeks throughout one year and risk factors were recorded. The risk factors were analyzed through general linear models and additive Bayesian network (ABN) modelling using a directed acyclic graph (DAG) for visualizing associations between potential risk factors.

**Results** CS: On average, 25% of the rabbits displayed ulcerative pododermatitis likely to be painful on at least one hind leg, while the prevalence varied between farms from 4 to 49%.

LS: Relative humidity inside the barns (-0.02; CI95% (-0.04, 0)), body weight (0.44; CI95% (0.22, 0.66)), number of kindlings (0.28; CI95% (0.18, 0.38)), age, and claw length were the most important risk factors, all except relative humidity being positively associated with pododermatitis.

In young does, the severity of pododermatitis quickly increased and in some rabbits proceeded to ulcerated spots. It was shown that 60.00%, 14.17% and 3.33% of ulcerated lesions recovered to a state without ulceration within 4, 8 or >12 weeks, respectively.

**Table 1** Prevalence of PD

N = 1090							
Severity	0	1	2	3	4	5	6
%	10.1	2.3	17.7	51.3	13.6	3.6	0.2

**Figure 1** Painful lesions (Severity 4 – 6) occurring on 25 % of the does. Prevalence of painful lesions/farm: 4 – 49%.

**Conclusions** Pododermatitis was present in Swiss group housing systems with litter and plastic slats. The prevalence varied between farms and the age, body weight and claw length of the rabbits were positively associated with pododermatitis as the most important risk factors, next to the relative humidity inside the barns (positive association) and the cleanliness of the plantar surface of the paws (weak negative correlation). To further investigate the causation between the moisture of the litter and the occurrence of pododermatitis, the litter moisture should be experimentally manipulated.

### Impact of farm individual action plans on lameness prevalence, productivity and welfare of dairy cattle

F. Ebert<sup>1</sup>, A. Cerna<sup>1</sup>, M. Schären<sup>2</sup>, R. Weck<sup>1</sup>, A. Wöckel<sup>1</sup>, W. Wippermann<sup>1</sup>, P. Hufe<sup>2</sup>, E. Ullrich<sup>3</sup> and A. Starke<sup>1</sup>

<sup>1</sup>Clinic for Ruminants and Swine, Veterinary Faculty, University of Leipzig, Germany; <sup>2</sup>Lehr- und Versuchsanstalt für Tierzucht und Tierhaltung (LVAT) e.V., Groß Kreutz, Germany; <sup>3</sup>Saxon State Ministry of Environment and Agriculture, Dresden, Germany

Email: [fanny.ebert@vetmed.uni-leipzig.de](mailto:fanny.ebert@vetmed.uni-leipzig.de)

**Introduction** Orthopedic disorders causing lameness belong to the most common and economically most relevant production diseases of dairy cattle worldwide. Lameness has severe economic implications while causing a serious impact on animal welfare. Studies conducted approximately 10 years ago in European countries and the United States found lameness prevalence between 25 % and 34 % (Dippel et al., 2009). Although in recent years orthopedic disorders were discussed in many studies, the unaltered lameness prevalence assessed in current studies showed, that previous concepts had no impact. Reasons for orthopedic disorders are multifactorial and can be divided in cow-, housing- and management-related risk factors. Assessing and eliminating possible risk factors should reduce orthopedic disorders and improve dairy productivity and welfare. The aim of the study was to assess the impact of farm individual action plans on lameness prevalence, productivity and welfare of dairy cattle.

**Materials and Methods** Five dairy farms in eastern Germany with high prevalence of lameness were visited between January 2017 and February 2019. The average number of milking cows per herd was 675, ranging from 257 to 1137 cows. All farms housed German Holstein cows as the predominant breed in freestall barns with cubicles and fed total mixed ration (TMR) and used the herd management system “Herde”<sup>®</sup> (dsp agrosoft). Regular hoof trimming was conducted at a minimum of twice per year and new cases of lame cows were treated at least once per week. The same veterinary hoof care professionals always visited farms. Information regarding animals, performance, housing, diet, management practices, biosecurity and claw health management were collected using direct observation of the cows and their environment, interview with the herd manager during the visit and analysing of herd data. Cows were evaluated for lameness using a 6-point locomotion scoring (LS) system (modified according to Starke et al. 2007), where 1 = regular locomotion, without lameness, 2 = imperfect locomotion, 3 = slight lame, 4 = moderately lame, 5 = severely lame and 6 = highly severely lame. Clinical case of lameness was indicated by a LS  $\geq 3$ . Furthermore body condition score (Edmonson et al., 1989), integument alterations (Lombard et al., 2010) and cleanliness (Reneau et al., 2005) of cows were assessed. Regarding the aims of the farm and the collected data, we developed an individual action plan together with the farms management, the herd manager and the farms external consultants and accompanied the implementation. Frequency, interval and topic of the following farm visits were adapted to the action plan.

**Results** Farms were visited between 2 and 24 times. At the first farm visit the farms were characterized by: average annual milk yield per cow and lactation of 9,779 kg (range from 8,387 kg to 11,542 kg), average life span production of 31,635 kg (17,631 kg to 54,908 kg), 31 % (20 % to 46 %) average culling rate and an average of 3 (2.4 to 4.6) lactations in herd until culling. The median lameness prevalence was 54 % (35 % to 80 %). The following conditions were considered when developing the farms individual action plans: Efficiency, feasibility, sustainability and profitability. Optimizing herd health documentation, raising the knowledge level about claw health and intensifying the hoof trimming and treatment were the most common objectives. Possible effects of the action plan were steadily monitored. Resulting conclusions led to adjustments to the action plan. About six month after the first farm visit, the mean lameness prevalence decreased from 54 % to 32 % (15 % to 50 %). Annual milk yield per cow and lactation increased to 9825 kg (8,424 kg to 11,747 kg) and life span production increased to 32,616 kg (21,419 kg to 53,042 kg).

**Conclusion / Outlook** Using an in-depth analysis to assess and eliminate the farm-related risk factors for orthopedic disorders helps to develop an effective farm individual action plan. With consistent implementation, decreasing of lameness prevalence is possible. Hence, productivity and welfare of dairy cattle increase.

### References

- Dippel, S., M. Dolezal, C. Brenninkmeyer, J. Brinkmann, S. March, U. Knierim, and C. Winckler. 2009. Risk factors for lameness in freestall-housed dairy cows across two breeds, farming systems, and countries. *J. Dairy Sci.* 92(11):5476–5486.
- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72(1):68–78.
- Lombard, J. E., C. B. Tucker, von Keyserlingk, M A G, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms. *J. Dairy Sci.* 93(10):4668–4676.
- Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Association between hygiene scores and somatic cell scores in dairy cattle. *J. Am. Vet. Med. Ass.* 227(8):1297–1301.
- Starke, A., Heppelmann, M., Beyerbach, M., Rehage, J. (2007): Septic arthritis of the distal interphalangeal joint in cattle. Comparison of digital amputation and joint resection by solar approach. *Vet. Surg.* 36 (4), S. 350–359.

### Validation of a visual scoring system for footpad dermatitis in broiler chickens on farm level

S. Heitmann<sup>1</sup>, J. Stracke<sup>1</sup>, H. Petersen<sup>2</sup>, N. Kemper<sup>1</sup> and B. Spindler<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Animal Hygiene, Animal Welfare and Farm Animal Behavior, Germany; <sup>2</sup>University of Veterinary Medicine Hannover, Foundation, Clinic for Poultry, Germany

Email: [sophia.heitmann@tiho-hannover.de](mailto:sophia.heitmann@tiho-hannover.de)

**Introduction** Footpad dermatitis (FPD) is an important indicator for animal welfare in the poultry industry. Therefore, measurement of the severity of FPD is used to monitor broiler welfare and giving hints to the animal's health state. Furthermore, FPD can give information on the management, as it is associated with the litter quality. However, no standardized scoring scheme exists. The aim of the presented study was, to analyze feet histopathologically after being scored by a common visual scoring system, and to find linkages between the visual and the histopathological outcome.

**Materials and Methods** Chicken feet (n=166) were collected randomly at a slaughter plant in Lower Saxony, Germany. They were collected and scored visually. This was done by an experienced observer (observer 1), using a visual scoring system for broiler chickens, approved by regulations implemented by the Lower Saxonian Ministry of Nutrition, Agriculture and Consumer Protection in §20 (4; 5) (2015) from the German Order on the Protection of Animals and the Keeping of Production Animals (2006). This scoring system is used in quality assessment for broiler health and welfare in Northern Germany.

The score has four levels:

0	no lesion, no hyperkeratosis, no ulcers
1	small lesion on the surface
2a	medium lesion, with erosions in individual cases only
2b	large and deep lesion, ulcers and scars

Subsequently, 40 feet (10 feet per score level) were examined macroscopically in more detail. For histological analysis, skin tissues (3 mm x 20 mm x 7 mm) were cut out from the center of the lesion. Tissues were kept in 10% buffered formalin and were embedded in paraffin wax. Afterwards, they were sectioned into approximately 5 µm thick slices using a standard microtome. Slices were stained with haematoxylin/eosin (HE). For cutaneous analysis, tissues were divided in five different skin segments. The thickness of each segment was measured at three different positions. For further analysis, the mean value was calculated. Additionally, the occurrence of hyperkeratosis was assessed using a binomial score. Moreover, the occurrence of ulcers was also determined. Ulcers were classified by their severity. Data were analyzed statistically by performing a cluster analysis, using the single linkage procedure in SAS (SAS Version 9.4, SAS Institute Inc., Cary, NC, USA) to classify the feet based on their histopathological findings. To test the validity of the visual scoring system, an interobserver reliability between different observers was determined (3 different observers, observers were compared pairwise). Additionally, Kendall tau correlations were calculated in order to find suitable parameters to assess the severity in a visual score more reliably.

**Results** The number of clusters was set at four (1-4), in order to allow comparability to the four-level-visual score (0, 1, 2a, 2b). Results revealed a tendency for a difference between both classification types (visual vs. cluster) ( $p=0.0884$ ). Here, most agreement was found for the score levels 0 and 1, whereas results for score levels 2a and 2b were more divergent, with only one foot being characterized as cluster 4 by cluster analysis (vs. ten feet in the visual score level 2b).

Similar results were also found in the interobserver reliability: most agreement between observers was found for score levels 0 and 1, whereas the evaluation of feet scoring 2a and 2b was inconclusive.

Furthermore, among others, a positive correlation of the size of lesion with the depth of the ulcer was detected. The size of lesion and depth of ulcer revealed a Kendall Tau correlation coefficient of 0.73.

**Conclusions** It was found that histological findings matched well with the less severe visual scores (0 and 1), whereas the distinction between the severe scores (2a and 2b) seemed to be less valid. For evaluating footpads on farm level and production systems, it is advisable to keep visual scoring systems as simple as possible. However, the scoring scheme should reflect the histopathological findings. In addition, as the correlation coefficient between both was high, the size of the lesion can serve as an indirect indicator of the depth.

### References

Implementary Rules of the Lower Saxonian Ministry of Nutrition, Agriculture and Consumer Protection for §20 (4; 5) (2015). Latest edition: July 31st 2015, from the German order on the Protection of Animals and the Keeping of Production Animals (2006). Reformed: August 22nd 2006; last amended: June 30th 2017. [http://www.nds-voris.de/jportal/portal/t/unb/page/bsvorisprod.psml;jsessionid=74A3617A5DCC73478ED097AC3A9BC276.jp24?pid=Dokumentanzeige&showdoccase=1&js\\_peid=Trefferliste&documentnumber=1&numberofresults=1&fromdoctodoc=yes&doc.id=VVND-VVND000035929#focuspoint](http://www.nds-voris.de/jportal/portal/t/unb/page/bsvorisprod.psml;jsessionid=74A3617A5DCC73478ED097AC3A9BC276.jp24?pid=Dokumentanzeige&showdoccase=1&js_peid=Trefferliste&documentnumber=1&numberofresults=1&fromdoctodoc=yes&doc.id=VVND-VVND000035929#focuspoint) (accessed: 27.03.2019)

### The causes of keel bone fractures in laying hens

M. J. Toscano

Center for Proper Housing: Poultry and Rabbits (ZTHZ), Division of Animal Welfare, VPH Institute, University of Bern, Switzerland

Email: [michael.toscano@vetsuisse.unibe.ch](mailto:michael.toscano@vetsuisse.unibe.ch)

**Introduction** Fractures to the keel bone is one of the largest problems facing the laying hen industry with more than 50% of hens being affected by end of lay (Harlander-Matauschek et al., 2015). Fractures are generally believed to result from the modern commercial hen's relatively high and sustained egg production which leave skeletal system relatively weak and brittle (Whitehead, 2004) and thus more susceptible to bone injury resulting from collisions (Wilkins et al., 2011). Although high and sustained egg production is likely a major cause of keel fractures, recent information suggests the role of additional causes that should be investigated. For instance, despite a relatively sustained egg production, susceptibility to fractures is decreased beyond 49 weeks of age using an impact testing protocol (Toscano et al., 2018). To assess these alternative possibilities, a meeting organized by KeelBoneDamage Net ([www.keelbonedamagenet.eu](http://www.keelbonedamagenet.eu)), an EU-COST Action, was held in Scotland in January 2019 to consider alternative explanations for the causes of keel bone fractures. In brief, those alternative explanations include:

- Reduced age of the first egg laid leading to delayed ossification
- Underlying pathological conditions resulting in poor bone structure including congenital malformations and metabolic diseases
- Selection for increased hen performance indirectly selecting for qualities leading to inferior bone health
- Reduced activity leading to insufficient osteogenic load and consequent poor bone health
- Fracture at the tip are not due to trauma, but poor or delayed ossification of this area

**Conclusions** The problem of fractured keels is an important issue for hen welfare and productivity. In order to develop effective intervention strategies, research must identify mechanisms which can explain the patterns of variation seen.

### References

- Harlander-Matauschek, A., T. B. B. Rodenburg, V. Sandilands, B. W. Tobalske, and M. J. Toscano. 2015. Causes of keel bone damage and their solutions in laying hens. *Worlds Poult. Sci. J.* 71:461–72.
- Toscano, M. J., F. Booth, G. Richards, S. N. Brown, D. M. Karcher, and J. F. Tarlton. 2018. Modelling collisions in laying hens as a tool to identify causative factors for keel bone fractures the means to reduce their occurrence and severity. *PLoS One* 13:e0200025 Available at <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0200025>.
- Whitehead, C. C. 2004. Skeletal disorders in laying hens: the problem of osteoporosis and bone fractures. Pages 259–270 in *Welfare of the Laying Hen*. Perry, G.C., ed. CABI Publishing, Wallingford.
- Wilkins, L. J., J. L. McKinstry, N. C. Avery, T. G. Knowles, S. N. Brown, J. Tarlton, and C. J. Nicol. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. *Vet. Rec.* 169:414 Available at <http://veterinaryrecord.bmj.com/content/169/16/414.abstract>.

### Environmental factors contributing to keel bone damage sustained by laying hens

M. Makagon

University of California, Davis, Canter for Animal Welfare & Department of Animal Science, Davis, CA, USA

Email: [mmakagon@ucdavis.edu](mailto:mmakagon@ucdavis.edu)

**Summary** Keel bone fractures have been detected in laying hens from all types of housing system. The type and design of housing system impact fracture prevalence. Paradoxically, environments that promote welfare by providing hens with the ability to engage in important behaviors, such as perching and roosting, tend to put hens at a higher risk for fractured keels. The heights of perches and other elevated structures in a hen house affect the prevalence of keel bone fractures within the flock (Wilkins et al., 2011). Installing low perches does not, however, eliminate the presence of broken keels. A recent study conducted in enriched colony cage systems, which provide hens with limited vertical access, found that the number of collisions a hen sustains at the keel affects her likelihood of developing a keel fracture. The same study found that the majority of collisions were with a perch (Baker et al., 2016). Not all physical enrichments increase keel bone fracture risk. Stratmann et al., (2015) reported less keel bone fractures among hens housed in pens with polyurethane covered metal perches as compared to hens housed with traditional metal ones. Improving the hen's ability to access and navigate between elevated spaces, for example through the provision of ramps, can also lead to a reduction in keel bone fracture prevalence (Heerkens et al., 2016). Furthermore, preparing hens for successfully navigating vertically complex environments by rearing them with access to vertical space has been shown to reduce the numbers of impacts the hens sustain at the keel as adults (Pullin et al., 2018) and reduce keel bone fracture prevalence (Casey-Trott et al., 2017).

**Conclusions** The prevalence of keel bone fractures is affected by housing design. The presence of elevated areas, such as perches, has been associated with an increased prevalence of keel bone fractures. Fracture risks can be mitigated by modifications of the environment, such as installation of soft perches or ramps, and by preparing pullets for their adult environments.

### References

- Baker SL, Robison CI, Karcher DM, Toscano MJ, Makagon MM. 2016. Behavior associated with high impacts experienced at the keel by hens housed in enriched colony systems. In Dwyer c, Haskell M, Sandilands V, editors. Proceedings of the 50<sup>th</sup> Congress of the International Society for Applied Ethology. Wageningen, The Netherlands: Wageningen Academic Publishers. p. 387
- Casey-Trott TM, Guerin MT, Sandilands V, Torrey S, Widowski TM. 2017. Rearing system affects prevalence of keel-bone damage in laying hens: a longitudinal study of four consecutive flocks. *Poultry Science*, 96(7):2029-2039
- Heerkens JLT, Delezie E, Ampe B, Rodenburg TB, Tuytens FAM. 2016. Ramps and hybrid effects on keel bone and foot pad disorders in modified aviaries for laying hens. *Poultry Science*, 95(11), 2479-2488.
- Pullin AN, Temple M, Bennett DC, Blatchford RA, Makagon MM. 2018. The effect of rearing environment on laying hen keel bone impacts sustained in enriched colony cages. Proceedings of the 52<sup>nd</sup> Congress of the International Society for Applied Ethology, p. 184
- Stratmann A, Fröhlich EK, Harlander-Matauschek A, Schrader L, Toscano MJ, Würbel H, Gebhardt-Henrich SG. 2015. Soft perches in an aviary system reduce incidence of keel bone damage in laying hens. *PloS ONE*, 10(3):e0122568.
- Wilkins LJ, McKinstry JL, Avery NC, Knowles TG, Brown SN, Tarlton J, Nicol CJ. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. *The Veterinary Record*, 169(16):414

### Quality of the laying hen skeleton; insights and solutions from genetics

I. C. Dunn<sup>1</sup>, B. Andersson<sup>2</sup>, R. H. Fleming<sup>1</sup>, D. J. De Koning<sup>3</sup>, H. M. McCormack<sup>1</sup>, A. B. Rodriguez-Navarro<sup>4</sup>, M. Schmutz<sup>2</sup> and P. W. Wilson<sup>1</sup>

<sup>1</sup>The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, Scotland, UK; <sup>2</sup>Lohmann Tierzucht, Cuxhaven, Germany; <sup>3</sup>Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>4</sup>Departamento de Mineralogía y Petrología, Universidad de Granada, Spain

Email: [ian.dunn@roslin.ed.ac.uk](mailto:ian.dunn@roslin.ed.ac.uk)

Bone fractures and other forms of skeletal damage including Keel Bone damage are a challenge for laying hens (Sandilands 2011), as the physiological adaptations for egg laying make them susceptible to osteoporosis (Whitehead 2004). There is also a welfare paradox with skeletal health in laying hens, alternative housing which allows greater movement consistently increased bone quality but at the same time they are associated with greater incidence of bone damage, often featuring the keel (e.g. Sandilands 2011). We are also starting to see the 'Long Life Layer' appear which has great benefits for sustainability but with a longer period of production this may be a greater risk for bone quality at the end of lay (Bain *et al.* 2016).

We have shown that genetic factors explain around 40% of variation for bone quality traits. So, whilst undoubtedly housing and nutrition must be optimised to ensure good bone health in laying hens, we believe genetics offers an important route to delivering improvements in bone quality in laying hens. Some years ago divergent selection from a commercial pedigree founder breed on the basis of a Bone Index, comprising several bone strength and other traits, resulted in the production of high and low bone strength lines of laying hen (Bishop *et al.* 2000). The high line showing an improvement in tibia strength of over 50%, without any adverse effects on egg production or egg quality. The bones of these hens had fewer osteoclasts resulting in a lower rate of endosteal cortical bone loss and greater medullary bone accumulation. This successful selection underlined the observations on the importance of genetics to bone quality traits and was used to investigate the genetic loci underlying the traits (Dunn *et al.* 2007).

When osteoporosis in hens does occur it is ultimately the result of the physiological changes linked to reproductive activity commencing. The hen starts to form medullary bone (Dacke *et al.* 1993), which is an adaption for laying a calcareous cleidoic egg. Medullary bone provides a reserve of calcium for mineralisation of the eggshell and it is very labile, turning over rapidly with the daily cycle of egg laying. This rapid turnover is characterised by osteoblastic and osteoclastic remodelling. Osteoblast activity in structural cortical bone at this time is minimal, as resources transfer to the medullary bone whilst osteoclast bone resorption continues. This ultimately will lead to a reduction in the integrity and mass of the structural bone which will be exacerbated by imbalances in calcium supply from the diet (Fleming *et al.* 2003). These factors are reflected in reduced breaking strength, which in turn are predictive of likelihood of fracture or deformations (Fleming *et al.* 2004). Because of this, it is frequently stated that egg production and specifically selection for increased egg production is why hens suffer from osteoporosis. However, there is little direct evidence this is the case. Indeed, there seems to be evidence that bone damage in poultry has been an issue long before the modern industry was established and the highly productive hens of today existed (Darwin 1868).

We have now calculated genetic parameters based on data from thousands of hens of two phylogenetically unrelated breeds to assess the relationship between bone quality and egg production. This has supported the genetic contribution to variance of the trait, but we can find no evidence of genetic correlation between the post peak rate of lay and bone quality. Additionally, we have evidence that the quality of the medullary bone, in terms of its mineralisation, may be important for aspects of bone quality. Therefore, we need to look for other explanations for the genetic variation in bone quality than simply egg numbers. That does not of course preclude progress in improving bone quality through genetic selection.

Unfortunately, in the past we have had to look at the end of lay to test bone quality so it has been difficult to get data to further this aim. This is because the methods required dissecting out bones to test mechanical properties. This has also been a major impediment to making genetic progress, as it is not possible to breed from dead hens and the measurements were time consuming. To remove this bottleneck we have tried to find methods to measure bone quality in living hens. Despite some limited success, it has proved difficult. Thankfully, technology advances and digital x-ray means it is possible to examine living hens in a way that was previously unthinkable. We are now able to derive measurements from living hens that are being validated for use on a scale suitable to deliver practical and reliable measurements for genetic selection. Combining this with genomic selection, which was possible for existing bone quality traits it should be possible to make progress (Raymond *et al.* 2018).

*The authors acknowledge funding from COST action on Keel bone damage, ERA-NET, BBSRC and FFAR.*

### References

- Bain MM, Nys Y & Dunn IC 2016 Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *British Poultry Science* 57, 330-8.
- Bishop SC, Fleming RH, McCormack HA, Flock DK & Whitehead CC 2000 Inheritance of bone characteristics affecting osteoporosis in laying hens. *British Poultry Science* 41, 33-40.
- Dacke CG, Arkle S, Cook DJ, Wormstone IM, Jones S, Zaidi M & Bascal ZA 1993 Medullary Bone and Avian Calcium Regulation. *Journal of Experimental Biology* 184, 63-88.
- Darwin CR 1868 *The variation of animals and plants under domestication*. John Murray, London
- Dunn IC, Fleming RH, McCormack HA, Morrice D, Burt DW, Preisinger R & Whitehead CC 2007 A QTL for osteoporosis detected in an F-2 population derived from White Leghorn chicken lines divergently selected for bone index. *Animal Genetics* 38, 45-9
- Fleming RH, McCormack HA, McTeir L & Whitehead CC 2003 Effects of dietary particulate limestone, vitamin K-3 and fluoride and photostimulation on skeletal morphology and osteoporosis in laying hens *British Poultry Science* 44, 683-9
- Raymond B, Johansson AM, McCormack HA, Fleming RH, Schmutz M, Dunn IC & De Koning DJ 2018 Genome-wide association study for bone strength in laying hens *Journal of Animal Science* 96, 2525-35
- Sandilands V 2011 The laying hen and bone fractures. *Veterinary Record* 169, 411-2
- Whitehead CC 2004 Overview of bone biology in the egg-laying hen. *Poultry Science* 83, 193-9

### Identifying genetic components contributing to bone strength in laying hens

D. J. de Koning<sup>1</sup>, F. Lopes Pinto<sup>1</sup>, H. A. McCormack<sup>2</sup>, R. H. Fleming<sup>2</sup>, I. C. Dunn<sup>2</sup>, A. Kindmark<sup>3</sup>, A. B. Rodriguez-Navarro<sup>4</sup> and H. Wall<sup>1</sup>

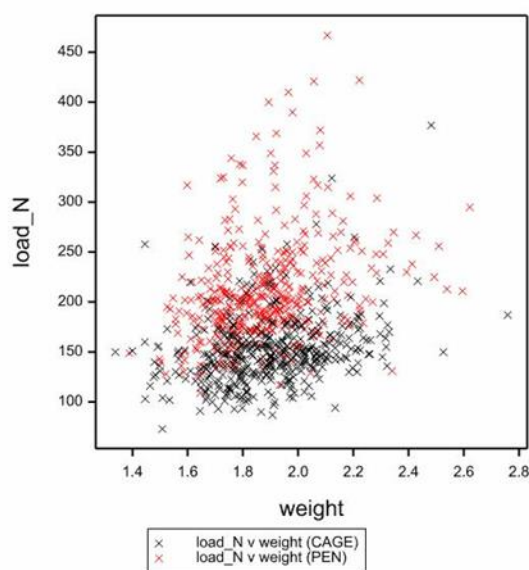
<sup>1</sup>Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>2</sup>The Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Midlothian, Scotland; <sup>3</sup>Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden; <sup>4</sup>Departamento de Mineralogía y Petrología, Universidad de Granada, Spain

Email: dj.de-koning@slu.se

**Introduction** Bone weakness and the consequent fractures represent a considerable welfare and economic problem in the layer industry. About 30% of commercial egg laying hens experience at least one bone fracture during their laying period, prior to depopulation and processing. Bishop et al. (2000) reported that about 40% of the variation in the bone strength phenotype was explained by genetic differences between the hens. A recent GWAS in a grandparent population of laying hens at Lohmann Tierzucht revealed two strong QTL regions on chromosomes 3 and 8 as well as suggestive regions on other chromosomes. However, the housing and management of the grandparent population is not representative for the practical farming conditions under which crossbred laying hens are kept. The purpose of this study is to evaluate whether significant SNPs from the GWAS, complemented by other QTL affecting bone strength from the literature, had an effect in crossbred layers under different production circumstances.

**Materials and Methods** The experiment included two commercial lines: LSL Classic (Lohmann Tierzucht GmbH, Cuxhaven, Germany) and Bovans Robust (Hendrix-Genetics, Boxmeer, The Netherlands). At our experimental farm Lövssta, 1620 birds from each breed were divided over 18 floor pens (100 birds/pen) and 180 furnished cages (8 birds/cage). Birds were also assigned one of two experimental diets. This allowed us to test any interactions between the breed, diet, and housing systems as well as their respective interactions with the putative genetic effects. At the end of lay, about 850 birds were selected for further studies. After birds were euthanized, they were necropsied to determine if they were still in lay. For birds that were still producing eggs, we stored a liver sample for DNA extraction. From around 850 birds, the right tibia was analysed using pQCT and breaking strength, while the left tibia was analysed using thermogravimetric analysis (TGA). TGA measures different bone components by weighing the bone during a constant heating ramp and determining the weight loss at characteristic temperatures. From the recent GWAS as well as other published studies we selected 111 SNPs from 11 significant and suggestive GWAS regions and 17 QTL regions with functional candidate genes. All phenotyped birds were genotyped for this candidate SNP panel (LGC Ltd Teddington, Middlesex, UK).

**Results** In the analyses we included 856 birds with phenotype and genotype data. The phenotypic analyses showed highly significant ( $P < 0.001$ ) effects of body weight and housing system on bone strength. The regression coefficient for body weight was 69.2 Newton per kg body weight (s.e. 7.49). Birds from floor pens had on average 59.7 Newton higher breaking strength (s.e. 2.89) compared to birds from furnished cages. There were no significant differences for breed or diet on breaking strength ( $P > 0.10$ ). TGA data show highly significant ( $P > 0.001$ ) differences in the composition and degree of mineralization of cortical bone. The TGA analyses show that the differences in breaking strength can be better studied focusing on the cortical bones. The relation between body weight, housing system and breaking strength is clearly illustrated in Figure 1. The strong effect of housing system was reflected in the association analysis where the first analyses across all the data did not show any significant SNP effects. Subsequent analyses within housing systems showed significant SNP affecting bone strength but almost exclusively within that housing system. The SNP associated effects vary between 10 and 45 Newton with some clear evidence of dominance and even over-dominance on a number of chromosomes.



**Figure 1** The relation between body weight (weight in kg) from birds in furnished cages (CAGE) or floor pens (PEN) and breaking strength (load\_N in Newton)

**Conclusions** In conclusion, we have validated several QTL affecting bone strength in laying hens. However, the QTL show a clear gene x environment interaction with the housing system. This causes complication in the way breeding companies should select for bone strength. Given the multidisciplinary nature of bone weakness in laying hens, the KeelBoneDamage Net EU COST Action (CA15224) has a crucial role in providing solutions to the egg producing industry.



### Comparing bone stability of laying hens differing in phylogeny and performance level in two husbandry systems

S. Jansen<sup>1</sup>, U. Baulain<sup>1</sup>, A. Weigend<sup>1</sup>, I. Halle<sup>2</sup>, A. R. Sharifi<sup>3,4</sup>, H. Simianer<sup>3,4</sup> and S. Weigend<sup>1,4</sup>

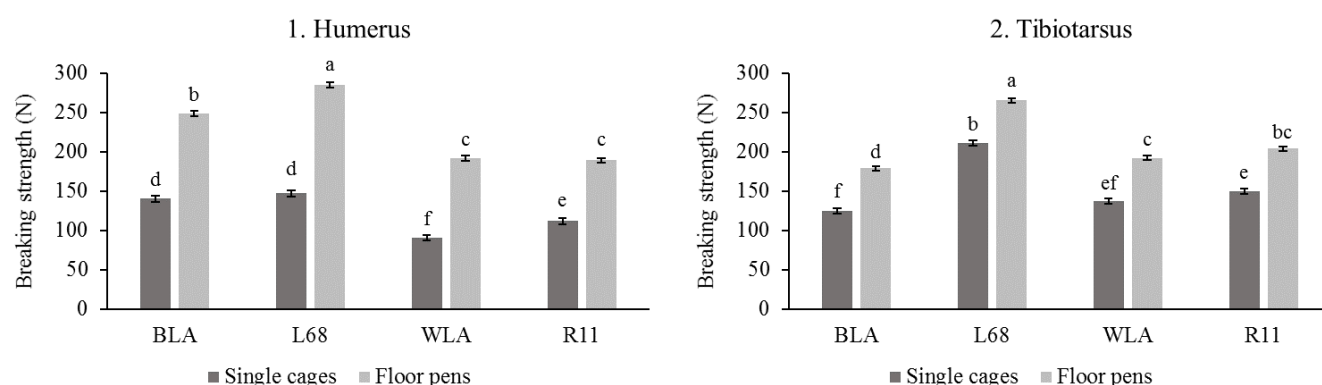
<sup>1</sup>Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt, Germany; <sup>2</sup>Institute of Animal Nutrition, Friedrich-Loeffler-Institut, Braunschweig, Germany; <sup>3</sup>Animal Breeding and Genetics Group, Department of Animal Sciences, University of Goettingen, Germany; <sup>4</sup>Center for Integrated Breeding Research, University of Goettingen, Germany

Email: Simon.Jansen@fli.de

**Introduction** Poor bone quality and fractures due to by osteoporosis cause major welfare and economic problems in laying hens (WEBSTER, 2004). In this context, reduced bone stability is often attributed to long-term selection for increased egg production (SILVERSIDES et al., 2012; JOHNSON et al., 2015). The aim of this study was to characterize the hens' bone stability in relation to their laying performance and phylogenetic origin when kept in different housing systems.

**Materials and Methods** The animal model comprised four purebred layer lines (*Gallus gallus domesticus*), differing in phylogeny (brown vs. white-egg layers) and performance level (low vs. high performing strains): BLA (brown/high), WLA (white/high), L68 (brown/low), and R11 (white/low). In this context, laying rate of low performing lines were one third lower than of high performing strains. The study was designed in two consecutive generations with 1.315 hens in total, kept in floor pens (n=771) and individual cages (n=544), respectively. In cages, individual laying rate was recorded from the beginning of the 18th week of age. At the end of the 70th week of age, all hens were sacrificed and dissected. Both, left humerus and tibiotarsus were removed from muscles and tendons and used to assess bone breaking strength via three-point bending Instron Materials Testing System. Analogous to FLEMING et al. (2006), breaking strength was defined as maximum load achieved during the test. Hens were excluded from the analysis if the total number of eggs was decreased by three standard deviations within line and/or if they had zero egg production over the last three weeks, respectively. All procedures conducted in this study were in accordance with the guidelines issued by the German animal protection law and were reviewed and approved by the relevant authorities.

**Results** Analysis of variance revealed a highly significant ( $p < 0.0001$ ) influence of both the genetic line and the housing system on humerus' and tibiotarsus' breaking strength, while the interaction was only significant ( $p < 0.0001$ ) for humerus (tibiotarsus:  $p = 0.9954$ ). No considerable differences between generations were observed, although the effect was significant. Generally, single cages led to lower bone stability but as shown in Figure 1 the two types of bones studied reacted differently. Humerus breaking strength of cage-housed animals was reduced by 46%, while for tibiotarsus, the average difference was only 26%. There was no consistent pattern of breaking strengths in relation to phylogeny or performance type. A correlation analysis within each line for hens housed in cages revealed rather low and non-significant ( $p > 0.05$ ) associations between egg production and bone stability.



**Figure 1** Bone breaking strength of humerus (1.) and tibiotarsus (2.) in four different layer lines kept in single cages (n=136) or floor pens (n=192) (LSM ± SEM, Tukey's HSD test).

**Conclusions** Our study supports earlier findings that both, genetic line and husbandry system have a major effect on bone stability (RAYMOND et al., 2018), but does not provide evidence for an effect of laying performance within the lines studied.

### References

- FLEMING, R.H., MCCORMACK, H.A., McTEIR, L. and WHITEHEAD, C.C. (2006): Relationship between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. *Br Poult Sci.* 47(6): 742-755.
- JOHNSON, M., JONSSON, K.B., ANDERSSON, L., JENSEN, P. and WRIGHT, D. (2015): Genetic Regulation of Bone Metabolism in the Chicken: Similarities and Differences to Mammalian Systems. *PLoS Genet.* 11(5): 1-19.
- SILVERSIDES, F.G., SINGH, R., CHENG, K.M. and KORVER, D.R. (2012): Comparison of bones of 4 strains of laying hens kept in conventional cages and floor pens. *Poult Sci.* 91(1): 1-7.
- RAYMOND, B., JOHANSSON, A.M., MCCORMACK, H.A., FLEMING, R.H., SCHMUTZ, M., DUNN, I.C. and DE KONING, D.J. (2018): Genome-wide association study for bone strength in laying hens. *J Anim Sci.* 96(7): 2525-2535.
- WEBSTER, A.B. (2004): Welfare implications of avian osteoporosis. *Poult Sci.* 83(2): 184-192.

### Effect of egg production on bone quality in laying hens

S. Petow<sup>1</sup>, B. K. Eusemann<sup>1</sup>, A. B. Rodriguez-Navarro<sup>2</sup>, E. Sanchez-Rodriguez<sup>2</sup>, A. Patt<sup>1</sup>, C. Benavides-Reyes<sup>2</sup>, N. Dominguez-Gasca<sup>2</sup>, L. Schrader<sup>1</sup> and R. Ulrich<sup>3</sup>

<sup>1</sup>Institute of Animal Welfare and Animal Husbandry, Friedrich-Loeffler-Institut, Celle, Germany; <sup>2</sup>Departamento de Mineralogia y Petrologia, Universidad de Granada, Spain; <sup>3</sup>Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

Email: [Stefanie.Petow@fli.de](mailto:Stefanie.Petow@fli.de)

**Introduction** Laying hens show a very high prevalence of keel bone fractures in all housing systems, reaching up to 97 % of hens near the end of a production cycle. It is likely that affected hens suffer pain. Hence, bone fractures are one of the most serious animal welfare problems in the egg production industry. Since a lot of energy, protein and calcium is required for egg formation, high egg production might be one factor leading to metabolic stress contributing to this multifactorial problem.

**Material and Methods** The aim of this study was to investigate how egg production affects keel bone health and characteristics of long bones. A total of 76 laying hens of a pure-bred layer line (WLA) with an average laying performance of 320 eggs per year were kept in a group floor housing system from hatch until 62 weeks of age. 38 hens were given a Suprelorin® implant with the active component deslorelin acetate that suppressed follicle maturation and, thus, prevent egg production for three months. Subcutaneous implants were renewed every three months to cover the whole production cycle (group S). The other 38 hens were kept as control hens (group C). To detect keel bone fractures, all hens were radiographed monthly. In the 62<sup>nd</sup> week of age, hens were euthanized and dissected. Formaldehyde-fixed, paraffin embedded specimen from keel bones and tibiotarsi of selected hens were used for histopathology and morphometric analysis. Strength and chemical composition of tibiotarsi were analyzed using the following methods: three-point bending test, thermogravimetric analysis, infrared spectroscopy, X-ray diffraction and scanning electron microscopy.

**Results** Hens of group S showed significantly fewer keel bone fractures from the 32<sup>nd</sup> week of age onwards (group S: 1.64 %; group C: 67.46 %;  $p < 0.001$ ). Tibiotarsi of group S had a significantly larger diameter ( $p < 0.05$ ) and were mechanically stronger ( $p < 0.001$ ) compared to group C. Histopathology of selected keel bones from hens with and without fractures revealed that all hens displayed a variable degree of osteopenia of cortical and trabecular bone despite obvious medullary bone formation. In contrast, a much thicker cortical and trabecular bone consisting mainly of mature lamellar bone and no medullary bone was present in group S. Accordingly, morphometric assessment of tibiotarsal cross sections revealed a decreased relative cortex area in group C as compared to group S. The degree of mineralization of cortical bone was significantly lower in group S hens compared to group C hens ( $p < 0.05$ ). Medullary bone was present in tibiotarsi of group C but not of group S hens.

**Conclusions** These results give strong evidence that egg production plays an important role in the etiology of keel bone fractures. This is consistent with the differences in long bone characteristics that have been found: bone diameter and bone strength seem to be negatively influenced by egg production. The negative contribution to bone breaking strength of a smaller bone diameter cannot be compensated by the higher degree of mineralization of cortical bone and the presence of medullary bone in productive laying hens.

### Improving intra- and inter-observer repeatability and accuracy of keel bone assessment in laying hens by training with radiographs

S. G. Gebhardt-Henrich, C. Rufener and A. Stratmann

Center for Proper Housing of Poultry and Rabbits (ZTHZ), Division of Animal Welfare, VPH Institute, University of Bern, Zollikofen, Switzerland

Email: [sabine.gebhardt@vetsuisse.unibe.ch](mailto:sabine.gebhardt@vetsuisse.unibe.ch)

**Introduction** A majority of laying hens damage their keel bone during their lifetime which includes fractures and deviations of various severity at different locations of the bone constituting a severe welfare problem (see Riber et al., 2018). Assessing keel bone damage reliably and accurately is a requirement for all research on this topic. Most commonly, assessment is done on live birds by palpation and is therefore prone to bias. A two-day Training School of the COST Action “Identifying causes and solutions of keel bone damage in laying hens” with 16 participants of various experience was held where palpation of live hens was followed by consulting corresponding radiographic images of keel bones. We hypothesized that the inter-observer and intra-observer repeatabilities as well as the agreement between palpation and assessment from the radiograph (considered as the accuracy) would increase with training.

**Materials and Methods** On the first day of the Training School 20 Lohmann Selected Leghorn (LSL) and 20 Lohmann Brown (LB) laying hens representing a wide variety of keel bone damage, i.e., including minor and major damage at different locations of the keel bone, were collected from a pen equipped with an aviary system and radiographed following Rufener et al. (2018). Sixteen participants with varying levels of experience with palpations conducted palpations of keel bones three times: 1) w/o seeing the radiograph, 2) again w/o seeing the radiograph to assess intra-observer reliability, 3) while consulting the radiograph. This protocol was repeated with another 40 hens the following day. Repeatability estimates were calculated using the R-package rptR (Nakagawa and Schielzeth, 2010) and the change in level of accuracy on day 1 and day 2 was analyzed with generalized linear models.

**Results** As predicted, the inter-observer repeatabilities of the fractures and deviations were improved by training and increased from  $R = 0.11$  (Confidence Interval CI 0.1 – 0.24) for fractures) and  $R = 0.18$  (CI 0.16 – 0.33) for deviations to  $R = 0.43$  (CI 0.28 – 0.58) for both measures on the second day of the Training School. Training ( $F_{1, 894} = 8.74$ ,  $P = 0.003$ ), location at the keel bone ( $F_{2, 894} = 6.57$ ,  $P = 0.002$ ) and the interaction between these two factors ( $F_{2, 894} = 4.06$ ,  $P = 0.018$ ) but not the assessor ( $F_{14, 894} = 1.31$ ,  $P = 0.19$ ) predicted the accuracy in the assessment of fractures. In contrast to fractures the assessors differed in the percentage of accurate assessments of deviations but again a training effect was present (training:  $F_{1, 676} = 13.75$ ,  $P = 0.0002$ , assessor:  $F_{14, 676} = 1.84$ ,  $P = 0.03$ ). There was a trend that the training effect depended on the location of the deviation (interaction training x location:  $F_{2, 676} = 2.48$ ,  $P = 0.09$ ). The estimation of intra-observer repeatabilities of fractures and deviations was impaired because data directly after training were only available from one person. Concentrating on day 1 before training, intra-observer repeatabilities of fractures ( $R = 0.3$ , CI 0.20 – 0.40) were similar to the intra-observer repeatabilities of deviations ( $R = 0.32$ , CI 0.24 – 0.42) before training.

**Conclusions** In conclusion, the training school involving radiographs improved inter-observer repeatabilities in the diagnosis of fractures and deviations of keel bones and thus had the potential to lead to more comparable results among research groups.

#### References

- Nakagawa, S., and H. Schielzeth. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev. Camb. Philos. Soc.* 85:935–956. doi: 10.1111/j.1469-185X.2010.00141.x.
- Riber, A. B., T. M. Casey-Trott, and M. S. Herskin. 2018. The Influence of Keel Bone Damage on Welfare of Laying Hens. *Front. Vet. Sci.* 5:395–407. doi: 10.3389/fvets.2018.00006.
- Rufener, C., S. Baur, A. Stratmann, and M. J. Toscano. 2018. A Reliable Method to Assess Keel Bone Fractures in Laying Hens From Radiographs Using a Tagged Visual Analogue Scale. *Front. Vet. Sci.* 5:6–13. doi: 10.3389/fvets.2018.00124.

## Black food for fowl – Effects of enriched charcoal in a temporary crude protein reduced diet on male Turkeys fattened under on-farm conditions

K. Hinz, J. Stracke, N. Kemper and B. Spindler

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behavior; University of Veterinary Medicine Hannover, Foundation; Hannover, Germany

Email: [katharina.hinz@tiho-hannover.de](mailto:katharina.hinz@tiho-hannover.de)

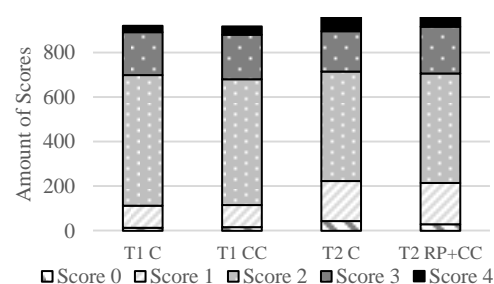
**Introduction** Foot pad dermatitis (FPD) is a very important animal-related welfare indicator in turkey farming. Major cause is wet litter, which among other things, can result from moist feces. Dietary charcoal is a potential way to absorb liquid in feces due to its characteristics as unspecific absorber. Therefore, it is used as household remedy for treatment of diarrhea and intoxications. Studies show advantages in improving growth performance, feed efficiency and intestinal morphology in different species including poultry, but the results are inconsistent. Another approach to improve FPD is the reduction of dietary protein level. In broiler, there is evidence that this can affect FPD positively. Furthermore, a protein-reduced diet can decrease nitrogen emission via feces being important for environmental issues. The problem of economic losses due to reduced weight gain can be compensated by only feeding the protein-reduced diet in the first half of the fattening period. In this study, effects of both, dietary supplementation of 0.2% enriched charcoal to a standard diet and a diet with temporary reduced crude protein by 1% were investigated in male turkeys during the fattening period.

**Materials and Methods** Data were collected on three commercial farms fattening male turkeys (BUT6, TP7). On every farm, turkeys were kept in two identical stables (flock size 3,500 to 4,950 animals). Barns were littered with straw and new straw was applied every second day. Stocking density was limited to 53 kg/m<sup>2</sup> on two farms participating on “Initiative Tierwohl” program, while on the third farm it was limited to 58 kg/m<sup>2</sup>. All turkeys were slaughtered at 147±3 days of life. Two different feeding trials were conducted successively, investigating two batches per farm. During both trials, turkeys were fed with a standard six-phased diet (control group). Treatment groups got experimental diets, starting in week six with the first grower feed. In feeding trial 1, diet of the treatment group was supplemented by 0.2% powdered charcoal, which was sprayed with an extract from fermented herbs for complete fattening period. In feeding trial 2, diet of the treatment group was crude protein reduced by 1% for the first and second grower phase; additionally they received 0.2% charcoal for complete fattening period. All farms were visited when birds were 6, 10, 14 and 18 weeks of age. Forty birds per flock were weighted and both footpads were scored using the system by Hocking et al. (2008); for further analyzes the most severe score was used per bird. Farmers registered mortality continuously. Statistical analysis was conducted using SAS Version 9.4. Body weight data were analyzed with a mixed linear model (MIXED procedure) and footpad data with the GENMOD procedure. Treatment, farm and their interaction were included as fixed factors. Random effects consisted of the individual animal (1-40), nested in age (6; 10; 14; 18 weeks) and nested in batch (1, 2). Mortality and feed consumption were analyzed using descriptive methods.

**Results** In feeding trial 1, the body weight was not influenced by the charcoal diet ( $F=0.26$ ;  $p>0.05$ ). The farm revealed a significant effect ( $F=241.52$ ;  $p<0.001$ ) with significant differences between each farm (all  $t>6.6$ ; all  $p<0.001$ ). Mortality and feed consumption did not differ. The degree of FPD was not affected by the diet ( $p>0.05$ ), but here again, the farm revealed a significant effect ( $p<0.001$ ). In trial 2, body weight of turkeys fed protein reduced and charcoal supplemented diet was significantly lower ( $F=5.57$ ;  $p<0.05$ ) compared to the control group. Weight was significantly affected by farm ( $F=537.53$ ;  $p<0.001$ ), with pairwise comparisons showing significant differences between each of the farms (all  $t>6.67$ ; all  $p<0.001$ ). Diet showed no influence on FPD ( $p>0.05$ ). Mortality was lower by 0.5 percentage points in the group receiving the experimental diet and feed consumption did not vary between the groups.

**Table 1** Performance data of feeding trial 1 (T1) and feeding trial 2 (T2), means ± SD, (n = 6 batches); <sup>1</sup> provided by slaughter house; C (Control); CC (Charcoal Group); RP (reduced protein)

	T1 C	T1 CC	T2 C	T2 RP+CC
Body Weight kg				
Week 6	1.82 ± 0.38	1.79 ± 0.41	2.15 ± 0.62	2.09 ± 0.56
Week 18	16.39 ± 1.70	16.59 ± 1.79	17.67 ± 2.11	17.54 ± 1.94
Slaughter weight <sup>1</sup>	21.52 ± 1.27	21.77 ± 1.01	21.67 ± 0.63	21.66 ± 0.59
FCR kg/kg	2.67 ± 0.1	2.64 ± 0.15	2.74 ± 0.03	2.70 ± 0.08
Mortality %	5.84 ± 1.9	5.84 ± 1.67	5.33 ± 1.7	4.78 ± 1.4



**Figure 1** Results of FPD Scoring of T1 and T2

**Conclusions** In conclusion, this study revealed no effects of the enriched charcoal on the birds’ performance or the degree of FPD under on-farm conditions. However, in combination with a temporary protein reduced diet it resulted in significantly reduced weights during the fattening period, but showed no negative effect on the slaughter weight of the turkeys. This could be a promising base for future research aiming for an enhanced nitrogen efficiency. Even if FPD was not improved in the second trial, the treatment indicated benefits for mortality during the fattening period. Further research is required for clarifying if charcoal is relevant for the positive results of the performance presented in this study and for examining the limits of temporary protein reduction.

This research was conducted within EIP Agri Project “CarboFeet” which is kindly funded by European Union and European Agricultural Fund for Rural Development.

## References

Hocking, P.M.; Mayne, R.K.; Else, R.W.; French, N.A.; Gatcliffe, J. Standard European footpad dermatitis scoring system for use in turkey processing plants. *Worlds Poult. Sci. J.* **2008**, *64*, 323-328, doi:10.1017/s0043933908000068.

### Feather pecking in laying hens in relation to motor impulsivity

J. Heinsius<sup>1</sup>, N. van Staaveren<sup>1</sup>, I. Y. Kwon<sup>1</sup>, A. Li<sup>1</sup>, J. B. Kjaer<sup>2</sup> and A. Harlander<sup>1</sup>

<sup>1</sup>University of Guelph, Department of Animal Biosciences, Guelph, ON, Canada; <sup>2</sup>Friedrich-Loeffler-Institut Federal Research Institute for Animal Health, Institute of Animal Welfare and Animal Husbandry, Celle, Germany

Email: aharland@uoguelph.ca

**Introduction** Abnormal-repetitive behavioural disorders such as attention-deficit hyperactivity and hair pulling in humans show similarities with repetitive feather pecking (FP) in laying hens. Repetitive feather pecking (FP) is one of the main production diseases in laying hens and can be underlined by neurological changes. A main component of these sort of behaviors is motor impulsivity - a lack of behavioral inhibition where subjects show a lack of suppression of pre-potent motor responses. The aim of the present study was to investigate whether FP in laying hens is associated with a lack of suppression of motor responses (i.e. an inability to suppress pecking behaviour).

**Materials and Methods** White Leghorn laying hens originated from a high FP line (HFP) or an unselected control line (CON) and further selection of extreme phenotypes took place by selecting peckers (P) and non-peckers (NP) based on behavioural observations (3 days, 20 min/day). All occurrence of pecks at the feather cover of other hens were recorded for individual hens and those that performed an average of >5 pecks were classified as P, while birds that performed <2.5 pecks were classified as NP. Twenty hens were selected and trained on a Go/No-Go task in an operant chamber. During visual Go cues birds had to peck at a lightened key, while during visual No-Go cues paired with an aversive sound birds had to suppress pecking at the key to receive a food reward. Following a training period to ensure all hens rapidly and accurately responded in the task (>75% success rate), birds' ability to suppress peck was assessed by measuring the number of pre-cue pecks and number of false alarms (pecks during No-Go cues). The effect of phenotype, genotype and their interaction on the outcome variables was assessed using an ANOVA model in SAS v9.3.

**Results** There was no interaction of genotype and phenotype on the outcome variables (Table 1,  $P > 0.05$ ). Birds that came from a HFP or CON line did not differ in the number of pre-cue pecks or false alarms ( $P > 0.05$ ). Similarly, birds that showed more than 5 pecks during the behavioural observations and were classified as P did not show a higher number of pre-cue pecks or false alarms ( $P > 0.05$ ).

**Table 1** The average number of pre-cue responses (A) and false alarms (B) of laying hens (n = 20) with different feather pecking genotypes (HFP: high feather pecking line, CON: unselected control line) and phenotypes (P: pecker, NP: non-pecker) during a Go/No-Go task.

Genotype	HFP		CON	
Phenotype	P	NP	P	NP
No. of pre-cue pecks	2.0 ± 0.57	1.9 ± 0.62	2.9 ± 0.85	2.6 ± 0.73
No. of false alarms	0.5 ± 0.29	1.3 ± 0.52	0.9 ± 0.47	0.9 ± 0.43

**Conclusions** In conclusion, the propensity of laying hens to perform more or less FP behavior did not influence their ability to suppress pecks. This suggests that laying hens that perform FP are still capable of suppression this behavior and that other neurological changes apart from lack of behavioral inhibition play a role in the development of FP.

### Identifying welfare issues in turkey hen and tom flocks applying the transect walk method

J. Marchewka<sup>1</sup>, G. Vasdal<sup>2</sup> and R. Oppermann Moe<sup>3</sup>

<sup>1</sup>Institute of Genetics and Animal Breeding Polish Academy of Sciences, Jastrzebiec, Magdalenka, Poland; <sup>2</sup>Norwegian Meat and Poultry Research Centre, Oslo, Norway; <sup>3</sup>Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Oslo, Norway

Email: j.marchewka85@gmail.com

**Introduction** The varying lengths of the production cycle of turkeys requires producers to separate the two sexes, but still they remain housed in the open space of the same building, under equal management and production conditions. The two sexes differ with regard to behavioral and physiological characteristics, and are slaughtered at different ages, resulting in different growth patterns and final slaughter age. Most of the work on turkey welfare has focused on toms at the end of their production cycle, since this period is considered challenging, when the barn is at its maximum capacity with regards to number of birds, ventilation capacity, litter quality and animal care. Research on the welfare of toms and hens at the time before hens are slaughtered is currently lacking. The main aim of the current study was to measure on-farm health and welfare issues of commercially reared hen and tom turkeys at 11 weeks of age using the transect walk method.

**Materials and Methods** The current study was conducted between November 2017 and March 2018 in 20 commercial turkey flocks on 16 different farms in Norway. Norwegian commercial turkey flocks are uniform with regard to stocking density and the absence of beak and toe trimming practices. On each farm, one barn, divided into tom and hen area, was evaluated using the transect walk method (Marchewka et al., 2015). An observer walked the transects in random order and recorded the total number of birds per transect that were: immobile, lame, with visible head-, tail- or wing- wounds, small, featherless, dirty, sick, terminal, or dead. In eight flocks, producers separated a small part of the rearing area in the barn in order to place any unfit birds which required treatment or separation from the whole flock, called the sick pen where birds were scored according to the indicators used in the transect walks method. To analyse the data we applied ANOVA and Spearman correlations in SAS software (v 9.3).

**Results** The most commonly observed welfare challenge in both hens and toms were dirty birds, birds with featherless areas, tail wounds and wing wounds. Across sexes, poor litter quality resulted in more head wounds ( $P<0.05$ ). Toms had significantly more tail wounds ( $P<0.001$ ), there were more sick birds ( $P<0.01$ ) and more terminal birds ( $P<0.01$ ) compared to hens at the same age. No differences were found between prevalence of birds with reduced welfare in the production area and in the sick pens, neither in the production area of the farms with sick pens and without sick pens. Several of the welfare indicators were positively correlated between the sexes, including lameness, head wounds, wing wounds, tail wounds, dirty birds and dead birds, suggesting underlying environmental or management causes. Dirtiness was positively correlated with lameness and immobility both in hens and toms, which is likely due to lame and immobile birds having more difficulties getting up from the ground and therefore spend more time in contact with the litter. Dirty feathers are known to be associated with contact dermatitis (de Jong et al., 2014) and a dirty environment could increase risk of infection in the birds. Dirty feathers reduce the feathers thermoregulatory ability, leaving the birds less able to keep warm during transportation which may lead to increased mortality during this phase.

**Conclusions** Dirtiness was suggested as a potential iceberg welfare indicator in turkey production.

### References

De Jong, I. C., H. Gunnink and J. Van Harn. 2014. Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance, and carcass yield in broiler chickens. *J. Appl. Poultry Res.* 23:51–58.

Marchewka J., I. Estevez, G. Vezzoli, V. Ferrante, and M.M. Makagon. 2015. The transect method: a novel approach to on-farm welfare assessment of commercial turkeys. *Poult. Sci.* 94:7-16.

### Decision making of high and low feather pecking line of laying hens under ambiguity

K. Pichová<sup>1</sup>, J. van der Eijk<sup>2</sup>, L. Kostal<sup>1</sup>, B. Bilcik<sup>1</sup> and T. B. Rodenburg<sup>2,3</sup>

<sup>1</sup>Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia; <sup>2</sup>Wageningen University & Research, Wageningen, The Netherlands; <sup>3</sup>Utrecht University, Utrecht, The Netherlands

Email: Katarina.Pichova@savba.sk

**Introduction** Feather pecking (FP) is a major welfare problem in egg production. Consequence of this behavior is increased heat loss and feed consumption as well as decreased egg production. Beside the economic perspective, FP is a source of stress, pain and influences physiological processes as well. The lines of laying hens selected divergently for high and low incidence of feather pecking provide a source of valuable information about the effect of FP on behavior, physiology and neurobiology. There is, however, very little information about the effect of FP on cognition and affective states of hens. The main aim of this study was to investigate whether the higher incidence of FP influences emotional state of hens using cognitive bias test.

**Material and Methods** Forty hens from lines selected for a different propensity to develop FP (high FP line – HFP n = 20 and low FP line – LFP n = 20) were trained to discriminate feeders of two colors. Half of the hens was trained to approach the white feeder (positive stimulus – PS) to obtain a reward (mealworm) and not to approach the black feeder (negative stimulus – NS) to avoid punishment (water spray). The other half of birds was trained to stimuli with swapped colors. After successful discrimination training hens were exposed to three judgement bias tests in three consecutive days, in which besides the PS and NS also the ambivalent stimulus – a grey colored feeder (AS) was presented. Latency to approach each feeder was measured and analyzed using a three-way ANOVA (Glimmix procedure in SAS). After the cognitive bias test, the quality of feather cover was assessed using infrared thermography (IRT) based on protocol by Pichová et al. (2017). Percentage of featherless areas of selected body parts (HN – head & neck, BR - back & rump, B - belly, UB - underneck & breast) were estimated by Flir Reporter software. Data were analyzed using the two-way ANOVA (Glimmix procedure in SAS).

**Results** There was a significant effect of stimulus ( $F_{2,272} = 612.56, < 0.001$ ) on latency of approach to the feeder. Tukey-Kramer analysis did not reveal differences between lines in the latency of response to PS and NS, but the latency of response to AS differed significantly. The HFP hens approached the AS faster than the LFP hens (Table 1). Repeated testing had no effect on reaction to any stimulus in either line ( $F_{4,272} = 0.08, P = 0.989$ ).

**Table 1** Latency to approach stimuli by hens from the HFP and LFP lines in the judgement bias tests (mean latency  $\pm$  SEM).

	HFP	LFP	
	mean $\pm$ SEM	mean $\pm$ SEM	P
PS	3.18 $\pm$ 0.10 s	3.07 $\pm$ 0.13 s	ns
AS	13.59 $\pm$ 0.59 s	16.68 $\pm$ 0.79 s	*
NS	28.06 $\pm$ 0.13 s	29.35 $\pm$ 0.33 s	ns

**Table 2** Feather damage in hens from the HFP and LFP line as measured by the IRT (mean percentage  $\pm$  SEM of featherless areas of selected body regions).

	HFP	LFP	
	mean $\pm$ SEM	mean $\pm$ SEM	P
HN	0.00 $\pm$ 0.00 %	0.00 $\pm$ 0.00 %	ns
BR	3.23 $\pm$ 0.85 %	0.00 $\pm$ 0.00 %	**
B	7.34 $\pm$ 1.53 %	2.44 $\pm$ 0.43 %	***
UB	2.45 $\pm$ 0.90 %	0.04 $\pm$ 0.03 %	*

There was a significant effect of line on quality of feather cover ( $F_{1,222} = 15.88, P < 0.001$ ). HFP hens had a significantly higher percentage of featherless areas than LFP hens in BR, B, and UB region. HN region was not affected by feather pecking (Table 2).

**Conclusions** We did not prove our hypothesis that the LFP are more “optimistic” than HFP hens due to less stress connected with FP. Contrary to that, HFP hens approached ambiguous stimulus faster than LFP hens. On the other side, the shorter latency to approach ambiguous stimulus does not necessarily reflect “optimistic” bias, but can also result from the higher motor activity of the HFP line or from different coping strategies of the lines, as described by other authors. The IRT revealed higher feather damage in HFP line hens in body areas typical for feather pecking: belly, back, and rump.

### References

Pichová, K., Bilčík, B., Košťál, E., 2017. Assessment of the effect of housing on feather damage in laying hens using IR thermography. *Animal* 11, 661-669.

### The influence of tactile stimulation on affective states in laying hens

Z. Skalná, K. Pichová and Ľ. Košťál

Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

Email: zu.skalna@gmail.com

**Introduction** To improve animal welfare we need to know more about influence of various aspects of animal environment on their emotional states and cognitive functions. Domestic chicken (*Gallus gallus domesticus*) is one of the most common and widespread of all domesticated animals. Even though chickens are often perceived by people only as commodity, they are intelligent emotional animals with individual personalities. Inspired by the Panksepp's "laughing rats" (2003) and work of Rygula et al (2012), we wanted to examine whether tickling evokes positive emotional states also in laying hens. To test it we used their judgement under ambiguity.

**Materials and Methods** Sixteen laying hens of the Dominant Striped breed (19 weeks old) were divided into 2 groups – 8 hens were handled (H) and 8 hens tickled on belly/chest area (T). All training and testing took part in the custom-built Skinner box with touchscreen. In the first phase of experiment hens were trained in the fixed interval schedule to peck at positive stimulus (PS, rewarded by mealworm) represented by circle of white or dark gray color (30 PS). In the next phase, hens were trained in the operant discrimination task to peck at PS and refrain from pecking at negative stimulus (NS, punished by white noise), represented by circle with color opposite to PS (15 PS, 15 NS). Training continued until all animals were able to discriminate successfully and peck at more than 75 % of PS and less than 25 % of NS during 3 consecutive daily sessions. Hens were subjected to the tactile stimulation treatments in the afternoon while the discrimination training took time in the morning. T hens were tickled on the belly/chest area (30 s) while H hens were handled by two hands (30 s). In the last phase of experiment, hens were subjected to judgement bias test following immediately after the tactile stimulation treatment, and their responses to PS, NS and ambiguous stimulus (AS, circle with shade of gray between the PS and NS) were recorded on three consecutive days (15 PS, 15 NS, 15 AS). Data were analyzed by analysis of variance (GLIMMIX procedure in SAS, SAS® University Edition, USA).

**Results** All hens discriminated significantly between the PS and NS from the 1<sup>st</sup> session ( $P < 0.001$ ), but the second criterion (respond to more than 75 % of PS and less than 25 % of NS) was met in all animals starting from the 7<sup>th</sup> training session. There was a trend towards the lower proportion of pecks at AS in T hens as compared to H hens, but it did not reach the level of statistical significance (H  $51.94 \pm 8.13$  %, T  $33.01 \pm 5.60$  %;  $P = 0.12$ ) (Table 1). However, the latency of response to AS was longer in T hens as compared to H hens (H  $2.97 \pm 0.32$  s, T  $3.70 \pm 0.18$  s;  $P < 0.05$ ) (Table 2).

**Table 1** Proportion of responses (mean  $\pm$  SEM, %) to positive (15 PS), negative (15 NS) and ambiguous stimuli (15 AS) in judgement bias test between handled (H) and tickled hens (T).

stimulus	H hens (n = 8)	T hens (n = 8)	P-value
PS	$91.11 \pm 2.71$ %	$89.72 \pm 0.47$ %	0.440
AS	$51.94 \pm 8.13$ %	$33.05 \pm 5.60$ %	0.119
NS	$3.61 \pm 0.16$ %	$3.61 \pm 1.70$ %	0.978

**Table 2** Latency of responses (mean  $\pm$  SEM, s) to positive (15 PS), negative (15 NS) and ambiguous stimuli (15 AS) in judgement bias test between handled (H) and tickled hens (T).

stimulus	H hens (n = 8)	T hens (n = 8)	P-value
PS	$1.65 \pm 0.15$ s	$1.88 \pm 0.13$ s	0.443
AS	$2.97 \pm 0.32$ s	$3.70 \pm 0.18$ s	<b>0.018</b>
NS	$4.88 \pm 0.06$ s	$4.87 \pm 0.07$ s	0.974

**Conclusions** Laying hens have successfully learned to discriminate between the PS and NS in the discrimination task using touchscreen operant chamber. Mealworms and white noise proved to be the suitable reward and punishment. The results did not confirm our hypothesis, that laying hens perceive tickling as positive stimulation. However, this applies only for this type of tickling, in this body part, at this age and with this treatment duration.

### References

- Panksepp J, Burgdorf J (2003) "Laughing" rats and the evolutionary antecedents of human joy? *Physiology & Behavior* 79  
Rygula R, Pluta H, Popik P (2012) Laughing Rats Are Optimistic. *PLOS ONE* 7(12)



### Efficacy of Ammonisation to Eliminate Aflatoxin, Ochratoxin, and Zearalenone

V. Dogan and A. Hayirli

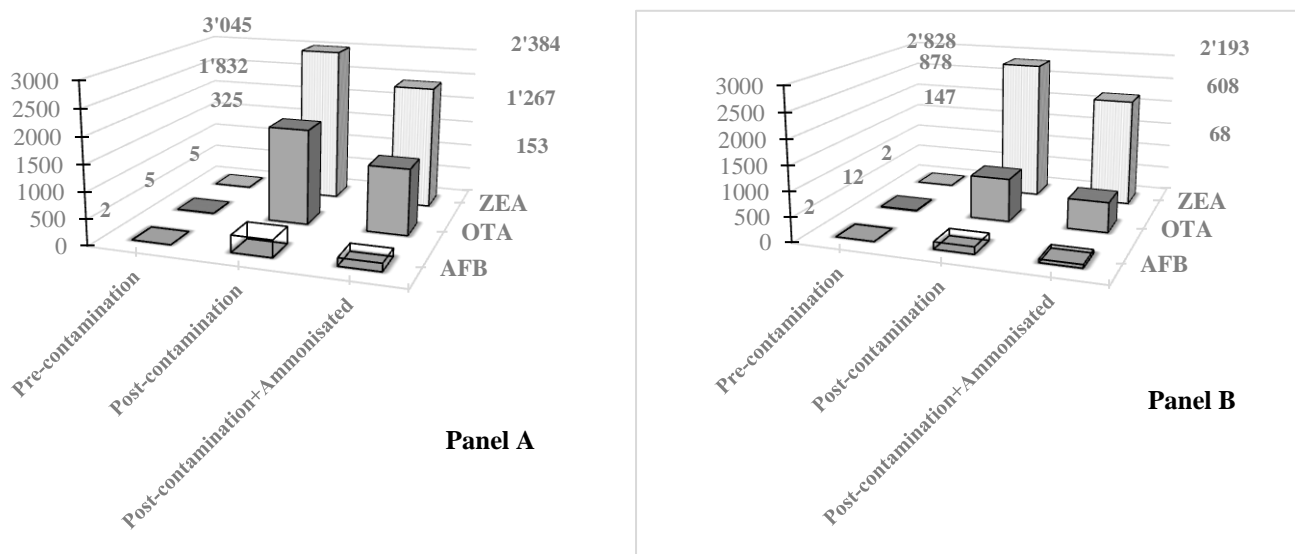
Department of Animal Nutrition & Nutritional Disorders, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

Email: d\_veysel@yahoo.com

**Introduction** Mycotoxicosis is a worldwide problem threatening economics of animal production as well as health of animals and humans. Aflatoxin (AFB), ochratoxin (OTA) and zearalenone (ZEA) produced mainly by *Aspergillus*, *Penicillium* and *Fusarium*, respectively, are common in animal nutrition (D'Mello *et al.*, 1997; Iheshiulor *et al.*, 2011). These mycotoxins are structurally similar with respect to possessing lactone ring with epoxy bind. Ammonisation destroys lactone ring and breaks down epoxy bind, leading mycotoxins to be harmless residues (Park *et al.*, 1988; Piva *et al.*, 1995). This experiment was conducted to determine alterations in mycotoxin concentration upon ammonisation.

**Materials and Methods** Commercially available compound feed for laying hens (11.09 MJ/kg, 17% crude protein, 4% crude fibre, 3.5% crude fat, and 12.5% crude ash) and total mixed ration (TMR) for dairy cows (19.4% grass hay, 19.2% barley silage, 24.0% corn silage, 9.7% rolled barley grain, and 27.8% protein-energy concentrate on a dry matter basis) were dried at 60°C for 3 days. After ground to pass 1 mm screen, feed samples were subjected to mycotoxin contamination (A6636, Aflatoxin B<sub>1</sub>; Z2125, Zearalenone; OTA32937, Ochratoxin A, SIGMA, Germany) at the 25-fold of legal limits (20 µg/kg for AFB, 200 µg/kg for OTA, and 500 µg/kg for ZEA). After that samples were subjected to 1-day ammonisation using NH<sub>4</sub>CO<sub>3</sub> (10 g/kg) (TOXIFARM DRY, Farmavet International, Turkey). Samples (pre-contamination, post-contamination, and post-contamination plus ammonised) were analysed for mycotoxins using LC-M/MS. Data were analysed using one-way ANOVA. Group mean differences were attained by the LSD option.

**Results** Contamination rate for the target of achieving 500 µg for AFB, 5000 µg for OTA, and 12500 µg for ZEA was 65, 37, and 24%, respectively in compound feed for laying hens and 29, 18, and 23%, respectively in TMR for dairy cows. After ammonisation, concentrations of AFB, OTA, and ZEA decreased, relative to post-contamination, by 53, 21, and 22%, respectively in compound feed for laying hens and 54, 31, and 22%, respectively in TMR for dairy cows. These decreases were not sufficient enough to bring levels down to legal limits.



**Figure 1** Alterations in mycotoxin concentrations (µg/kg on a dry matter basis) in compound feed for laying hen (A) and TMR for dairy cows (B) contaminated with 25-fold of legal limits for AFB, OTA and ZEA in response to ammonisation.

**Conclusions** Ammonisation is a partially effective method to destroy commonly found mycotoxins. Ammonisation technique can be used in bins while drying off concentrate items. If TMR machines are modified to block air-inlets, ammonisation can also be applied prior to feeding TMR to ruminants. Further studies are needed to elaborate alterations in starch and amino acid profiles after ammonisation.

#### References

- D'Mello JPF, Macdonald AMC. Mycotoxins. *Anim Feed Sci Tech*, 1997, 69:155-166.
- Iheshiulor OOM, Esonu BO, Chuwaka OK, Omede AA, Okoli IC, Ogbuewu IP. Effects of mycotoxins in animal nutrition: a review. *Asian J Anim Sci*, 2011, 5:19-33.
- Park DL, Lee LS, Price RL, Pohland AE. Review of the decontamination of aflatoxins by ammoniation: current status and regulation. *J Assoc Anal Chem*, 1988, 71:685-703.
- Piva GFP, Galvano FRD, Pietri AAP, Piva ARD. Detoxification methods of aflatoxins. A review. *Nutr Res*, 1995, 15:767-776.

### **Microbial colonisation is essential for the development of the gut immune system in chickens**

B. Kaspers<sup>1</sup>, S. Lettmann<sup>1</sup>, S. Härtle<sup>1</sup>, S. Röhl<sup>1</sup>, B. Schusser<sup>2</sup>, P. Velge<sup>3</sup>, and C. Schouler<sup>3</sup>

<sup>1</sup>Institute for Animal Physiology, LMU Munich, Germany; <sup>2</sup>Reproductive Biotechnology, School of Life Science Weihenstephan, TU Munich, Germany; <sup>3</sup>INRA, Infectiology and Public Health, Nouzilly, France

*Email: kaspers@tiph.vetmed.uni-muenchen.de*

Development of the immune system is controlled by genetic programs and environmental cues. While mammals acquire the initial gut flora from their mother this is not the case in modern poultry production. Whether this impacts on the development and functional maturation of the immune system is largely unclear. We therefore compare immune system development in sterile (germ free), mono- and tetra-reconstituted and SPF birds as well as birds raised in the presence of a maternal flora.

While no significant differences were observed in the development of selected parameters of the innate immune system between the groups, striking differences were observed in the adaptive immune system. Germ free birds had highly reduced numbers of B-lymphocytes in the gut which was paralleled by a complete absence of IgA production. IgA production was partially restored by mono-reconstitution and further enhanced in tetra-reconstituted and SPF birds. However, development of the B-cell system was greatly retarded in all groups in comparison with birds that acquired a maternal flora on the day of hatch. In contrast, neither B-cell maturation in the bursa of Fabricius nor circulating B-cell numbers were affected. These data predict that molecular cues induced by microbial colonization attract circulating B-cells into the mucosal tissue and regulate maturation towards IgA producing cells. Interestingly, these signals do not only activate homing and maturation of the B-cell compartment but also maturation of the epithelial IgA transporter system as poly-Ig receptor (PIGR) expression was very low in germ free birds but induced in response to microbial colonization.

### Infectious bursal disease virus infection leads to modifications of gut-associated immune parameters and microbiota composition

S. Rautenschlein<sup>1</sup>, L. Li<sup>1</sup>, T. Kubasová<sup>2</sup>, I. Rychlik<sup>2</sup> and F. J. Hoerr<sup>3</sup>

<sup>1</sup>Clinic for Poultry, University of Veterinary Medicine Hannover, Germany; <sup>2</sup>Veterinary Research Institute, Brno, Czech Rep.;

<sup>3</sup>Veterinary Diagnostic Pathology, Fort Valley, USA

Email: [Silke.Rautenschlein@tiho-hannover.de](mailto:Silke.Rautenschlein@tiho-hannover.de)

**Introduction** Infectious bursal disease (IBD) is an immunosuppressive disease of chickens with a worldwide distribution. The B lymphocytes of the bursa of Fabricius (BF) are the primary target cells of the virus (IBDV). But also macrophages may be infected. Some studies suggest effects of IBDV on the intestinal tract, and diarrhoea of IBDV-infected birds may sometimes be observed in the field. As there is a close interaction between the development of the gut-associated immune system and the colonizing gut microflora, it was of our interest to understand more about the effects of IBDV on the gut associated immune tissue. In addition we investigated the possible impact of IBDV-infection on *Campylobacter jejuni* (*C. jejuni*) colonisation of the intestinal tract.

**Materials and Methods** A total of four experiments with commercial Ross 308 broilers was conducted. Birds were raised under isolated conditions. They were inoculated at 14 or 15 days post hatch either with a very virulent (vv) strain of IBDV (Experiments 1 and 2) or with vvIBDV (eye-drop route) and subsequently with 10<sup>6</sup> colony forming units (CFU, oral route) of *C. jejuni*, when the number of circulating B cells was depressed by viral infection as determined by flow cytometric analysis (Experiments 3 and 4). Control birds received only diluent. At various days post inoculation, birds were randomly selected and n=6/group necropsied. The following parameters were determined: macroscopical and microscopical lesion development in gut-associated lymphoid tissues including the BF, caecal tonsils (CT) and caecum, local accumulation of different immune cell populations and gut microbiota composition as determined by immunohistochemical methods and by Illumina Sequencing, respectively (Li et al., 2018a, b). In addition, the IBDV-antigen load and *C. jejuni* colonisation were investigated in different immune organs and the gut by immunohistochemical antigen detection or bacterial culture on CCDA-plates. Serum antibody levels against either pathogen were evaluated by ELISA systems.

**Results** vvIBDV-replication was detected in the BF, CT and caecum. The highest IBDV-antigen loads were found in the BF, and the fastest clearance was observed in the caecum. During the acute phase of the infection, IBDV-replication was accompanied by histological lesions with depletion of lymphoid cells and infiltration of heterophils as well as goblet cells. vvIBDV infection also led to an increase in T lymphocyte numbers and macrophages in the lamina propria of the caecum and CT. Illumina sequencing analysis indicated that vvIBDV infection led to a detectable modification of the gut microflora composition. When vvIBDV-infection was combined with *C. jejuni*-inoculation, not only vvIBDV modified the gut microflora, also *C. jejuni*- mono-inoculated control birds showed changes compared to non-inoculated groups. Possible additive or synergistic effects between the pathogens were observed and the interval between inoculation time points influenced the results. CFU of *C. jejuni* in the caecal content varied between co-inoculated groups of Experiment 3 and 4. The longer interval with bacterial inoculation at 9 days post viral infection (pvi) in Experiment 4 led to a delayed clearance of *C. jejuni* associated with lower *C. jejuni*- antibody levels. Early *C. jejuni*- inoculation at 7 days pvi resulted in significantly higher bacterial loads at 7 and 14 days post bacterial inoculation in co-inoculated birds compared to mono-inoculated controls ( $P < 0.05$ ).

**Conclusions** This study indicates that vvIBDV not only affected the BF as the main target organ of the virus, it also impacted local immune cell populations in the gut, which may have subsequently contributed to changes the intestinal microflora composition of the host. These effects not only on cells of the specific immune system but also on local barrier mechanisms led to a modified colonization pattern of *C. jejuni*. What kind of mechanisms contribute to these changes is not clear. It may be the effect of vvIBDV on the humoral immunity, as this was shown to play a role in bacterial clearance (Lachame-Lora et al., 2017). Also vvIBDV-induced changes in the microflora composition may have subsequently affected the colonizing conditions for *C. jejuni*. More studies are needed to further understand the immunosuppressive mechanisms of vvIBDV on the gut immune homeostasis and the control of the microflora composition.

### References

- Lacharme-Lora L, Chaloner G, Gilroy R, Humphrey S, Gibbs K, Jopson S, Wright E, Reid W, Ketley J, Humphrey T, Williams N, Rushton S, Wigley P. (2017). B lymphocytes play a limited role in clearance of *Campylobacter jejuni* from the chicken intestinal tract. *Sci Rep.* 2017 Mar 23;7:45090. doi: 10.1038/srep45090.
- Li L, Kubasová T, Rychlik I, Hoerr FJ, Rautenschlein S. (2018). Infectious bursal disease virus infection leads to changes in the gut associated-lymphoid tissue and the microbiota composition. *PLoS One.* 2018 Feb 1;13(2):e0192066. doi: 10.1371/journal.pone.0192066. eCollection 2018
- Li L, Pielsticker C, Han Z, Kubasová T, Rychlik I, Kaspers B, Rautenschlein S. (2018). Infectious bursal disease virus inoculation infection modifies *Campylobacter jejuni*-host interaction in broilers. *Gut Pathog.* 2018 Mar 30;10:13. doi: 10.1186/s13099-018-0241-1. eCollection 2018.

### Supporting Gut Health: Principles Underlying Alternatives to Antibiotics in Food Animal Management

T. H. Elsasser<sup>1</sup> and A. Cross<sup>2</sup>

<sup>1</sup>Independent Animal Health Consultant, College Park, MD, USA; <sup>2</sup>Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, USA

Email: [teddodge@gmail.com](mailto:teddodge@gmail.com)

**Background** Presently, with the integration of phenotypic and genotypic information, animal agriculture is poised to base the optimization of its efforts on objective criteria encompassing what is termed “gene-by-environment-by-management” interactions. With that being the case, different aspects of how provided nutrients are assimilated into consumable products is intimately tied to the efficiency of those metabolic processes, the efficiency governing how much energy and protein are metabolically directed in the direction the producer wants it to go. In its fundamental essence, that “desired efficiency path” is shaped by physiologically interacting regulatory pathways now recognized to span what we term the “endocrine-immune-microbiome-metabolome” (EIMM) gradient. Here is where it gets complicated. Organisms have evolved mechanisms to detect when their environment is suboptimal, hostile in fact, and they have amassed response mechanisms to deal with the perceived insult. On the evolutionary scale, the earliest responses morphed into what we call in animals and man the innate immune response today. Over time animals further developed more sophisticated responses that were termed the adaptive immune response. Sometimes these innate and adaptive responses run in conflict, “overcompensate” towards the elimination of the factor(s) causing the immune stimulation and generate responses harmful to the host. This harm comes in the form of their capacities to evoke inflammatory responses in cells – directly and indirectly. Systemically in animals, there is no more significant factor that decreases the EIMM for anabolic purposes than inflammation directed through catabolic cytokines, arachidonic acid cascade products, neurochemicals, and reactive oxygen and nitrogen intermediates. In addition, where the epithelium is often the discriminating barrier between the “outside” and the “inside” environments, response inflammation in these cells, and in particular reference to the topic at hand the intestinal epithelial cells, promotes breakdown of the physical barrier (tight junctions, mucin layers, substructure morphology) to both infections and toxic mediators as well as compromised efficiency of nutrient uptake and transport.

**Challenge to agriculture, and animal and human health** The discovery (late 1940’s) that the addition of low concentrations certain antibiotics to the diets of animals increased their growth and production efficiency was a “game changer”. The working theory was that the drugs suppressed the pathogen load and prevented the emergence of disease-causing bacteria. Added at sub-minimal inhibitory concentrations (MIC), the drugs were present at levels too low to kill bacteria but maintained the capacity to enhance selection for bacteria that evolved the mechanisms to counteract the pharmacological action of the drug. Seventy years later these negative consequences led to their being banned. However, over time one positive outcome of research further into the actions of antibiotics emerged, namely, we now know that a part of the mechanisms of action of these drugs, the tetracycline derivatives in particular, can be attributed to their nonantimicrobial anti-inflammatory actions on the gut even when used at sub-MIC levels. Nonantimicrobial effects impact cells and tissue integrity through effects on matrix metalloproteases, proinflammatory cytokines, reactive chemical intermediates, and immune cell activation. As animal breeding has evolved to capitalize on the overall actions of these powerful drugs to enhanced productivity especially under intensive management scenarios, are there opportunities to support the desired production responses exploiting a non-drug protocol?

**Developing nonantimicrobial strategies to maintain animal efficiency** Our lab has focused on mitigating gut inflammation using naturally-derived products as test agents to probe the validity of strategies (in essence metabolite interactions) intended to provide stability to the gut environment. We investigated such factors as inappropriate inflammatory responses in target epithelial cells. We contend that there is no single factor that can satisfy all criteria but rather interacting metabolites that (1) shape/augment the integrity of cellular protective components, (2) change in respect to gut location, (3) modulate bacteria critical to gut stability, and (4) facilitate better recovery of the absorption matrix of the gut following stress. In this presentation we will explore some novel mechanisms of action of dietary colostrum and tocopherols that could be exploited towards the development of alternatives to antibiotics with an emphasis on disease prevention that could stem from overt gut inflammation.

**Summarizing our approach** We view the epithelial barrier between the “external environment” and the host’s and internal environment as the first target towards which relevant interventions should be targeted. As the primary site of nutrient substrate use regulation, the intestinal epithelial layer is of particular relevance. We have described what we call a multiple component “anti-inflammatory index” based on gut segment-specific metabolite profiling that has strong associations with decreased low to moderate level inflammatory insults associated with reactive nitrogen compound modification of intracellular villus epithelial cells. We focus on these low-level insults because they are the ones that metabolically rob the resources of the affected animal with little to no obvious outward signs other than the less than optimal performance yet set the animal up for opportunistic infection when cellular damage occurs. Recognizing tremendous animal to animal and replication to replication variation in the metabolome and microbiome as affected by dietary treatments, we tease out the commonalities between replications and use this as a structured basis for estimating the relative contributions of various metabolites to the anti-inflammatory status of the digesta for a given segment of the gut. The data suggest that different segments of the gut may benefit from different intervention metabolite patterns as the nature of the digesta contents change as a result of host and microbial processing.

### How to select gut microbiota members with increasing chicken resistance to enteric diseases

I. Rychlik

Veterinary Research Institute, Brno, Czech Republic

Email: [rychlik@vri.cz](mailto:rychlik@vri.cz)

**Introduction** Chickens evolved for millions of years to be hatched in a nest in contact with an adult hen. On the other hand, current commercial production of chickens is based on hatching chicks in a clean hatchery environment in the absence of adult hens. Colonisation of commercially hatched chickens is therefore exclusively dependent on environmental sources during which the caecum of chickens is first colonised by *Enterobacteriaceae* (phylum *Proteobacteria*), which are replaced by *Lachnospiraceae* and *Ruminococcaceae* (phylum *Firmicutes*) during the second week of life. At around one month of age, *Firmicutes* become complemented by bacterial isolates belonging to phylum *Bacteroidetes* [1]. This gradual microbiota development during the first weeks of life leaves chicks highly susceptible to different infections, e.g. with *Salmonella* [2, 3] although it is well established that inoculation of chicks with microbiota of adult hens can increase their resistance to *Salmonella* [2, 4, 5]. Unfortunately, administration of complex microbiota from adult hens to newly hatched chickens, though effective in preventing infections, is not widely accepted. The reason is that such products may contribute to the spread of yet unknown pathogens. Repeatedly confirmed efficacy of competitive exclusion products together with accumulating knowledge on chicken microbiota composition led us to systematically culture and whole genome sequence individual chicken gut microbiota members and test them as new generation of probiotics in newly hatched chicks.

**Materials and Methods** Using anaerobic culture, we obtained over 350 isolates originating from the chicken caecum. Following whole genome sequencing, 218 different species belonging to 7 different phyla - *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Elusimicrobia* and *Synergistetes* were identified among these isolates. Out of these, 79 different isolates were already tested in newly hatched chickens, *i.e.* chicks on day 1 of life were orally inoculated with the pure culture. Half of the chicks were sacrificed 7 days later to determine whether the tested anaerobe effectively colonized chicken caecum. Second half was challenged with *Salmonella* Enteritidis and additional 4 days later, *Salmonella* counts in the caecum and liver were determined.

**Results** We observed that only 18 isolates belonging to phylum *Bacteroidetes*, 3 isolates belonging to phylum *Firmicutes* class *Negativicutes*, two isolates of *Desulfovibrio* and single isolates of *Akkermansia* and *Cloacibacillus* belonging to phyla *Verrucomicrobia* and *Synergistetes* were capable of colonisation of chicken caecum after oral administration during the first week of life. We never succeeded with the colonisation of newly hatched chicks with any isolate belonging to families *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcaceae* or *Erysipelotrichaceae* (all phylum *Firmicutes*) or 4 tested isolates belonging to phylum *Actinobacteria*. Whenever only a single isolate of gut anaerobe was used for chicken inoculation, no protection against challenge with *S. Enteritidis* was observed. However, when different mixtures of gut anaerobes were prepared and tested, we were able to increase chicken resistance to *S. Enteritidis* infection by a factor of 1000.

**Conclusions** Gram negative gut anaerobes are suitable for a single dose oral administration to chickens during their first days of life. On the other hand, Gram positive gut anaerobes do not efficiently colonize after a single dose and have to be administered repeatedly. Individual strains are unlikely to be of any effect in increasing resistance against *Salmonella* infection. However, selection of defined mixtures of pure isolates is possible and these increase chicken resistance to *Salmonella* significantly. These findings should be considered when designing new generation of probiotics, competitive exclusion products or even human faecal transplants.

### References

1. Videnska P, Sedlar K, Lukac M, Faldynova M, Gerzova L, Cejkova D, et al. Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. *PLoS One* 2014;9:e115142.
2. Varmuzova K, Kubasova T, Davidova-Gerzova L, Sisak F, Havlickova H, Sebkova A, et al. Composition of gut microbiota influences resistance of newly hatched chickens to *Salmonella* Enteritidis infection. *Front Microbiol* 2016;7:957.
3. Beal RK, Wigley P, Powers C, Hulme SD, Barrow PA, Smith AL. Age at primary infection with *Salmonella enterica* serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. *Vet Immunol Immunopathol* 2004;100:151-64.
4. Rantala M, Nurmi E. Prevention of the growth of *Salmonella infantis* in chicks by the flora of the alimentary tract of chickens. *Br Poult Sci* 1973;14:627-30.
5. Milbradt EL, Zamae JR, Araujo Junior JP, Mazza P, Padovani CR, Carvalho VR, et al. Control of *Salmonella* Enteritidis in turkeys using organic acids and competitive exclusion product. *J Appl Microbiol* 2014;117:554-63.

### Competitive exclusion and compartmentalization to intervene in colonization and transmission of ESBL-*E. coli* in broiler flocks

M. A. Dame-Korevaar<sup>1</sup>, E. A. J. Fischer<sup>1</sup>, J. A. van der Goot<sup>2</sup>, F. C. Velkers<sup>1</sup>, D. Ceccarelli<sup>2</sup>, D. J. Mevius<sup>2,3</sup> and J. A. Stegeman<sup>1</sup>

<sup>1</sup>Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, the Netherlands; <sup>2</sup>Department of Bacteriology and Epidemiology, WBVR, Lelystad, the Netherlands; <sup>3</sup>Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands

Email: M.A.Dame-Korevaar@uu.nl

**Introduction** Plasmid-mediated Extended Spectrum Beta-Lactamase and AmpC Beta-Lactamase (ESBL/pAmpC) producing bacteria are resistant to extended spectrum beta-lactam antimicrobials. An important reservoir of ESBL/pAmpC-producing bacteria are broilers and transmission occurs via several routes through the broiler production pyramid<sup>1</sup>. Previous studies showed that a single oral supply of competitive exclusion (CE) product before challenge with a high dose ( $10^5$ - $10^8$  CFU/mL) of ESBL-producing *E. coli* led to reduced colonization, excretion and transmission, but not to complete prevention<sup>2,3</sup>. In practice, broilers may be exposed to much lower levels of ESBL-producing *E. coli*, and because the hypothesized mechanism of CE products is competition between bacteria, a longer supply of CE product might be more effective. Next, spatial distance between animals housed in different pens delayed the contamination of broilers with *Campylobacter* and *E. coli*<sup>4</sup>, suggesting that compartmentalization might prevent the spread of ESBL/AmpC-producing bacteria for some time, giving CE products time to colonize the broiler gut. Therefore, the effect of prolonged supply of CE product and compartmentalization of a broiler flock, kept under semi-field conditions, on colonization and transmission of ESBL-producing *E. coli* after challenge with a low dose was investigated in an experiment.

**Material and Methods** One day old broilers (Ross 308) ( $n=400$ ) were housed in 4 houses. Each house was subdivided in 9 pens, with 1 infectious (I-) pen in the middle ( $n=20$  broilers), surrounded by 8 susceptible (S-) pens ( $n=10$  broilers per pen). The I-pen was separated from S-pens by a wired fence. The S-pens were separated from each other with wooden panels. In 2 of 4 houses CE product was supplied via drinking water from day of hatch until day 7. At day 5 in each I-pen 10 randomly selected broilers were challenged with *E. coli* strain E38.27 carrying *bla*<sub>CTX-M-1</sub> on plasmid IncII by individual oral inoculation (0.5 mL,  $10^2$  CFU/mL). Presence of ESBL-producing *E. coli* was determined using individual cloacal swabs. Swabs were taken just before inoculation and at day 5-21 daily, and environmental samples were collected using boot socks, at day -1, 2, 5, 7, 14, 22. Cox proportional hazard model was used to compare the risk of colonization between groups. Transmission rate within ( $\beta_w$ ) and between ( $\beta_b$ ) pens was quantified based on a SI model, with the force of infection determined by the cumulative sum of the excretion time ( $\sum \text{excrtime}$ ). Expected new cases ( $C$ ) was:  $E(C) = S * (1 - e^{-\beta * \sum \text{excrtime} * \Delta t})$  and parameters were estimated using maximum likelihood estimation.

**Results** In both houses treated with CE product, none of the broilers or environmental samples were found to be CTX-M-1-*E. coli* positive. In contrast, in the untreated houses 93.5% of the broilers were CTX-M-1-*E. coli* positive at day 21, however, time until colonization differed between the two untreated houses (HR 0.34, 95% CI 0.24 – 0.48). Compartmentalization resulted in a decreased transmission in the two untreated houses. Transmission rate (hour<sup>-2</sup>) between pens ( $\beta_b$  0.0016, 95% CI 0.0012-0.0019) was lower compared to transmission within pens ( $\beta_w$  0.0072, 95% CI 0.0057 – 0.0084).

**Conclusions** The concept of competitive exclusion can be a useful intervention as supply of a CE product during the first week after hatch prevented colonization of CTX-M-1-*E. coli* after challenge at day 5. Furthermore, compartmentalization resulted in a delay of transmission between broilers. Therefore, a combination of compartmentalization and the supply of CE product may help to reduce transmission and prevent colonization of ESBL-*E. coli* in poultry houses.

#### References

- 1 Dame-Korevaar A, Fischer EAJ, van der Goot J, Stegeman A, Mevius D. 2019. Transmission routes of ESBL/pAmpC producing bacteria in the broiler production pyramid, a literature review. *Prev Vet Med*. 162: 136-150.
- 2 Nuotio L, Schneitz C, Nilsson O. 2013. Effect of competitive exclusion in reducing the occurrence of *Escherichia coli* producing extended-spectrum beta-lactamases in the ceca of broiler chicks. *Poult Sci* 92:250-254.
- 3 Ceccarelli D, van Essen-Zandbergen A, Smid B, Veldman KT, Boender GJ, Fischer EAJ, Mevius DJ, van der Goot JA. 2017. Competitive Exclusion Reduces Transmission and Excretion of Extended-Spectrum-beta-Lactamase-Producing *Escherichia coli* in Broilers. *Appl Environ Microbiol* 83:e03439-16.
- 4 Van Bunnik BAD, Ssematimba A, Hagenaars TJ, Nodelijk G, Haverkate MR, Bonten MJM, Hayden MK, Weinstein RA, Bootsma MCJ, De Jong MCM. 2014. Small distances can keep bacteria at bay for days. *Proc Natl Acad Sci USA*. 9:3556-60.

### Impact of a mixture of mono-, di- and triglycerides of butyric, capric, and caprylic acid, formic acid and a probiotic *Clostridium butyricum* (CBM588) on performance and gut health in broiler chickens

J. Zentek<sup>1</sup>, W. Vahjen<sup>1</sup>, K. Manner<sup>1</sup> and P. Ader<sup>2</sup>

<sup>1</sup>Institute of Animal Nutrition, Freie Universität Berlin, Germany; <sup>2</sup>BASF SE, Ludwigshafen, Germany

Email: Juergen.Zentek@fu-berlin.de

**Introduction** Necrotic enteritis (NE) is one of the major causes of intestinal diseases, loss of performance and increased use of antibiotics in broilers. The cause is considered an infection with toxin-forming clostridia and coccidian as predisposing factor. Feed additives are an option to influence the occurrence of the disease and to reduce the negative effects on animal production. As such, short and medium chain fatty acids and probiotics are of particular interest. In this trial, the efficacy of the additives was characterized by performance (body weight, body weight gain, feed intake, feed-to-gain-ratio), and parameters of gut health after anticoccidial vaccination and a challenge with *Clostridium perfringens* (CP).

**Materials and Methods** The objective of the trial was to evaluate the efficacy of a mixture of mono-, di and triglycerides of butyric, caprylic, and capric acid (glycerides) (1 and 3 g/kg), formic acid (10 g/kg) and a probiotic *Clostridium butyricum* (CB) strain ( $5 \times 10^8$  cfu/kg) in broiler chickens compared to a control diet without supplementation. The diets (starter, grower, finisher) were based on wheat, soybean meal, maize and met the recommendations for broiler chickens (GfE 1999). The efficacy of the additives was compared to non-challenged and challenged control birds. All broiler chickens were vaccinated orally on d 4 and 9 with Poulvac Bursa Plus (Zoetis, Zaventem, Belgium) and on d 14 and 16 with Hipracox (Hipra, Melle, Belgium) and Paracox-8 (MSD Animal Health, Brussels, Belgium). At d 18, 19 and 20, birds were challenged with  $5 \times 10^8$  cfu of a pathogenic CP strain (Geeraerts et al. 2016), except the negative control group. Coccidial and NE lesions were scored before the challenge at d 17 and on days 22 and 36. The response of the microbiota was monitored to study the impact of the different treatments. Bacterial composition was analyzed via 16S rDNA sequencing of the intestinal microbiota in the ileum and caecum. Data pre-processing included demultiplexing (bcl2fastq 1.8.4), inline barcode sorting, clipping and combination of forward and reverse reads (BBMerge 34.48). Bacterial community analysis was done with Mothur 1.35.1 using the Silva reference classification after removal of sequences with ambiguous bases (Phred score below 33), filtering of short alignments elimination of chimera (uchime) and subsampling to 30,000 sequences per samples (inspection of rarefaction curves). OTU were picked at a 97% identity level (cluster.split method). Additionally, putative species level annotations of OTU were done with the NCBI software BLAST+ 2.2.29 ( $E < 0.1$ , % identity 90%). CP in the excreta also culturally. The statistical analysis was performed with SPSS (Version 25), ( $p < 0.05$ ).

**Results** After challenge, the number of dead birds was increased in all infected groups, lowest number of NE fatalities was observed in the groups fed with the high dosage of glycerides and CB, respectively. Except low dose rate of glycerides all treatments favourably increased the zootechnical performance compared to the challenged control group. Two days after the challenge, significantly increased excretion of CP was observed in all challenged groups, 17 days after the challenge the concentrations decreased numerically, in the group fed CB even significantly ( $p < 0.05$ ). Coccidiosis lesions did not differ between the groups. Two days after the challenge there was a significant decrease in the dry matter of the excreta in the challenged compared to the non-challenged control birds, the challenged groups fed the different feed additives did not show significant changes compared to the control birds. On day 17 after the challenge excreta dry matter was not different between all challenged groups. The NE scores increased in all challenged groups on day 2 after the challenge ( $p < 0.001$ ) compared to the unchallenged control. On day 17 after the challenge NE scores were reduced in all challenged groups compared to day 2 but no significant difference in NE scores between treated groups was observed. Never the less the numerically lowest NE score at day 2 and 17 post challenge was found in birds that have been treated with higher dose of glycerides, formic acid or CB, respectively. The composition of the microbiota in ileum and caecum was only moderately affected by the additives and treatments. Lasting effects of the CP challenge on the bacterial diversity compared to the unchallenged control was found, as all treatment groups showed a numerically or significantly reduced diversity in bird's ileum.

**Conclusions** The investigations show that the infection with CP leads to a significant impairment of the performance and health of broilers. The selected infection model was able to clearly demonstrate these effects. Overall, the available data show that the performance and the effects of the various additives were significantly improved compared to the untreated control. This is also expressed in a slight but interesting change in the excretion of CP in the excreta. Overall, the results show that the administration of the tested additives appears to have a positive effect on NE prevention. Nevertheless, the study is an orientating work, the results would have to be confirmed by further investigations. With reference to the intestinal microbiota, it can be stated that the additives also had an effect on the microbial colonisation of the digestive tract, and on the intestinal concentrations of CP. In practice, where significantly lower infection pressures can be expected, it might be interesting to further objectify the effects of the glycerides and the CB strain.

#### References

Geeraerts et al. (2016). Br Poultry Sci 57(3): 324-329; GfE (1999). Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Broiler. Frankfurt (Main), DLG Verlag.

## Changes in rumen pH, volatile fatty acids, lipopolysaccharide, and the bacterial community of Japanese Black beef cattle at different fattening stages

S. Sato<sup>1</sup>, H. Makino<sup>1</sup>, N. Ishizuka<sup>1</sup>, Y.-H. Kim<sup>1</sup>, T. Masaki<sup>2</sup>, E. Iwamoto<sup>2</sup>, S. Kushibiki<sup>3</sup> and H. Mizuguchi<sup>4</sup>

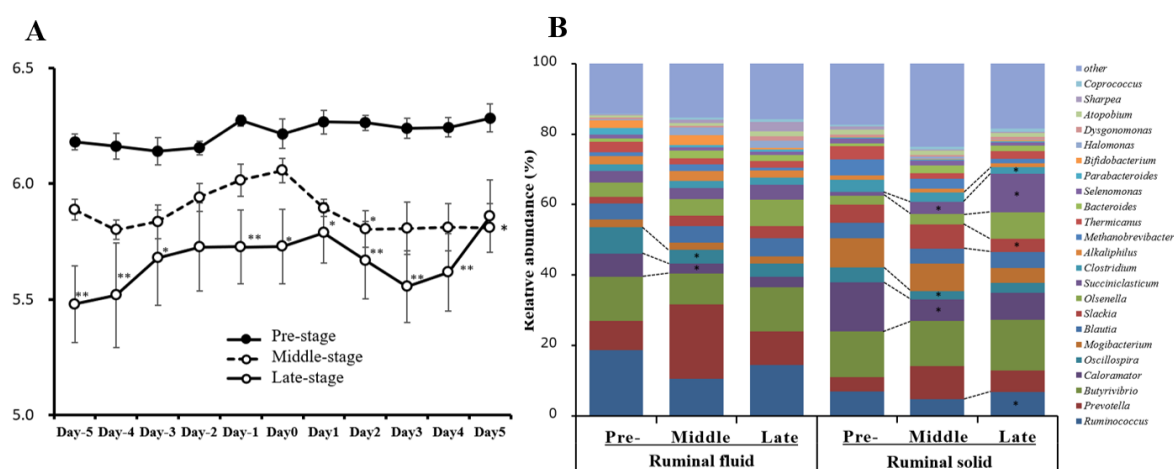
<sup>1</sup>Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan; <sup>2</sup>Hyogo Agricultural Technology Center, Kasai, Hyogo, Japan; <sup>3</sup>National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan; <sup>4</sup>Organization for Research Promotion, Iwate University, Morioka, Iwate, Japan

Email: sshigeru@iwate-u.ac.jp

**Introduction** Japanese Black beef cattle produce high-quality meat. However, when they are fed a high-grain diet with a small amount of forage, clinical or subclinical ruminal acidosis frequently occurs. When dairy cows are fed a concentrate diet, they develop a low ruminal pH and high volatile fatty acid (VFA) levels, and the composition of the ruminal bacterial community changes. However, there is little knowledge of this in Japanese Black beef cattle. Therefore, this study clarified the ruminal fluid characteristics, especially pH and VFAs concentrations and lipopolysaccharide (LPS) activity, and the bacterial community composition in Japanese Black beef cattle at different fattening stages.

**Materials and Methods** Nine Japanese Black cattle equipped with fistulas at 10 months of age were evaluated. The cattle were fed a concentrate diet with little roughage twice daily. At the pre-, middle, and late fattening stages (15, 20, and 28 months of age, respectively), the ruminal pH was measured continuously every 10 min throughout the experiment using a radio transmission system (YCOW-S; DKK-TOA Yamagata, Japan). Ruminal fluid and content samples were collected at 4 h after the morning feeding to analyze the bacterial community and levels of total VFAs, individual VFAs, ammonia nitrogen, lactic acid, and LPS. Total bacterial DNA was extracted from the ruminal fluid and content samples for 454 pyrosequencing analysis. The statistical analyses were performed using two-way repeated-measures analysis of variance with Bonferroni's multiple-comparison post-test.

**Results** The 24-h mean ruminal pH was significantly lower during the late stage than the pre- and middle stages. During the late stage, the total VFA and acetic acid concentrations were significantly lower and the LPS activity significantly higher compared with the pre- and middle stages. In the pyrosequencing analysis, the genera *Ruminococcus* (phylum Firmicutes) and *Prevotella* (phylum Bacteroidetes) were predominant in the ruminal liquid and solid fractions regardless of stage, and *Ruminococcus* was significantly more common in the fluid than solid fraction at each stage. Further, compared with the pre-fattening stage, the proportion of *Ruminococcus* was decreased and that of *Prevotella* increased during the late stage in both the fluid and solid fractions.



**Figure 1** Changes in the 24-h mean ruminal pH (A) and bacterial composition of the ruminal fluid and solid (B) in Japanese Black beef cattle at pre-, middle and late fattening stages.

Values represent mean  $\pm$  SE (n = 9). \* denotes significant difference ( $P < 0.05$ ,  $P < 0.01$ , respectively) compared with the pre-stage at the same time point (A) and with each stage of the same genus (B).

**Conclusions** The lower ruminal total VFA level and pH observed in Japanese Black cattle during the late fattening stage may have been caused by the decreased intake of dry matter and rumination, likely due to depressed salivation. Further, the bacterial community in the fluid fraction was distinguished from that in the solid fraction, and the proportions of *Ruminococcus* and *Prevotella* depended on the fattening stage. Therefore, the ruminal bacterial community in the fluid and solid fractions of Japanese Black beef cattle might adapt to long-term feeding of a high-grain diet by changing fermentative ability at different fattening stages.



### Effect of dietary phytase and lactic acid treatment of cereals on metabolic active bacteria in ileal digesta, mucosa and ileo-cecal lymph nodes and expression of innate immune genes in pigs

J. Klinsoda, J. C. Vötterl, Q. Zebeli and B. U. Metzler-Zebeli

University of Veterinary Medicine, Institute of Animal Nutrition and Functional Plant Compounds, Vienna, Austria

Email: Jutammat.Klinsoda@vetmeduni.ac.at

**Introduction** Due to the low intestinal availability of phytate-phosphorus (P) in plant feedstuffs, different strategies are used to enhance the P availability from cereals in pig diets, such as phytase supplementation and soaking of cereals (Metzler-Zebeli et al., 2014). While dietary phytase mainly increases the mineral availability in the upper digestive tract, soaking of cereals additionally modifies the complex carbohydrate fractions (Metzler-Zebeli et al., 2014). Changes in dietary minerals and carbohydrates were shown to modulate the bacterial community and alter the mucosal host inflammatory response (Metzler-Zebeli et al., 2015), whereas little is known about their potential to alter intestinal bacterial translocation. Therefore, this study examined the effect of phytase supplementation and lactic acid treatment of dietary cereals on changes in the metabolically active bacterial microbiome in ileal digesta, mucosa and ileo-cecal lymph nodes as well as on the expression of innate immune and barrier function genes in the ileal mucosa of pigs.

**Materials and Methods** Thirty-two castrated male pigs (Large White, 13.1 kg, 6-8 weeks of age) were fed 1 of 4 wheat (36.2%)-corn (36.0%)-soybean meal based diets according to a 2×2 factorial arrangement of dietary treatments with 4 replicate batches: control diet (Con), diet with phytase (500 FTU/kg; Con-Phy), diet containing lactic acid-treated cereals (LA), and diet with phytase and lactic acid-treated cereals (LA-Phy). For LA and LA-Phy diets, cereals were soaked in 2.5% LA solution for 48 hours at 21°C, dried at 70°C for 1 hour and afterwards at 60°C for 23 hours. Pigs were individually housed in metabolism pens and fed 3-times daily. Each replicate batch lasted 18 days. On day 18, pigs were euthanized 2 hours after their last feeding. Ileal digesta, ileal mucosa scrapings and ileo-cecal lymph nodes were collected to isolate total RNA, which was transcribed into cDNA. The cDNA was used for sequencing of the V3-V4 region of the 16S rRNA gene on an Illumina MiSeq platform. Expression of innate immune genes and barrier function genes at the ileal mucosa was determined using quantitative PCR. Bioinformatics were performed in QIIME. Bacterial and host gene expression data were analyzed by ANOVA using the PROC MIXED procedure in SAS with the model of the fixed effects of replicate and treatment effect. Significance was declared at  $P < 0.05$  and trends at  $0.05 < P \leq 0.10$ .

**Results** Present results showed that *Clostridiaceae* and *Lactobacillaceae* were the predominant families across dietary treatments in ileal digesta, at the mucosa and in ileo-cecal lymph nodes. The diets containing LA-treated cereals decreased several dominant families, including *Helicobacteraceae*, *Streptococcaceae* and *Fusobacteriaceae* in ileal digesta and *Campylobacteraceae* at the ileal mucosa, whereas only unclassified *Clostridiales* 2 was significantly increased in ileal digesta ( $P < 0.05$ ). The bacterial composition in ileo-cecal lymph nodes was unaffected by both phytase supplementation and LA treatment of cereal grains. Results for the expression of barrier function and innate immune genes showed that cytokines, suppressors of cytokine signalling, and tight-junction proteins were similarly expressed among diets at the ileal mucosa. The interaction between phytase supplementation and LA-treatment of grains for the relative expression of *MUC2*, however, indicated that the LA-treatment of cereals increased the expression level of *MUC2* at the ileal mucosa, whereby this effect was abolished when phytase was added to the diet ( $P < 0.05$ ). Identification of the most influential features using sparse partial least squares-discriminant analysis demonstrated negative relationships between the abundance of *Lactobacillus mucosae* at the ileal mucosa and ileo-cecal lymph nodes and *MUC2* expression levels, whereas mucosal *Actinobacillus* and several other *Lactobacillus* species in ileo-cecal lymph nodes were positively associated with *MUC2* expression ( $|r| > 0.8$ ).

**Conclusions** The present findings demonstrate that mainly the LA-treatment of cereals altered the composition of the metabolically active bacteria, whereby the effect was greatest in ileal digesta. Specifically, the LA-treatment of cereals decreased the abundance of several families comprising important porcine opportunistic pathogens in ileal digesta and at the mucosa, whereas both dietary treatments appeared not to alter the bacterial translocation into ileo-cecal lymph nodes. Despite the shift in the luminal and mucosal taxa, expression of most innate immune genes was similar among diets, whereby the upregulation of *MUC2* expression with the LA diet indicated changes in microbe-host-signalling.

#### References

- Metzler-Zebeli BU, Deckardt K, Schollenberger M, Rodehutscord M, Zebeli Q. Lactic acid and thermal treatments trigger the hydrolysis of myo-inositol hexakisphosphate and modify the abundance of lower myo-inositol phosphates in barley (*Hordeum vulgare* L.). *PLoS One*. 2014;9(6):e101166.
- Metzler-Zebeli BU, Mann E, Ertl R, Schmitz-Esser S, Wagner M, Klein D, Ritzmann M, Zebeli Q. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. *Br J Nutr*. 2015;113(7):1019-1031.

## Comparison of pH, volatile fatty acids, lipopolysaccharide, and the bacterial community in the rumen and reticulum of Japanese Black beef cattle

S. Sato<sup>1</sup>, H. Makino<sup>1</sup>, Y.-H. Kim<sup>1</sup>, T. Masaki<sup>2</sup>, E. Iwamoto<sup>2</sup>, S. Kushibiki<sup>3</sup>, T. Ichijo<sup>1</sup> and H. Mizuguchi<sup>4</sup>

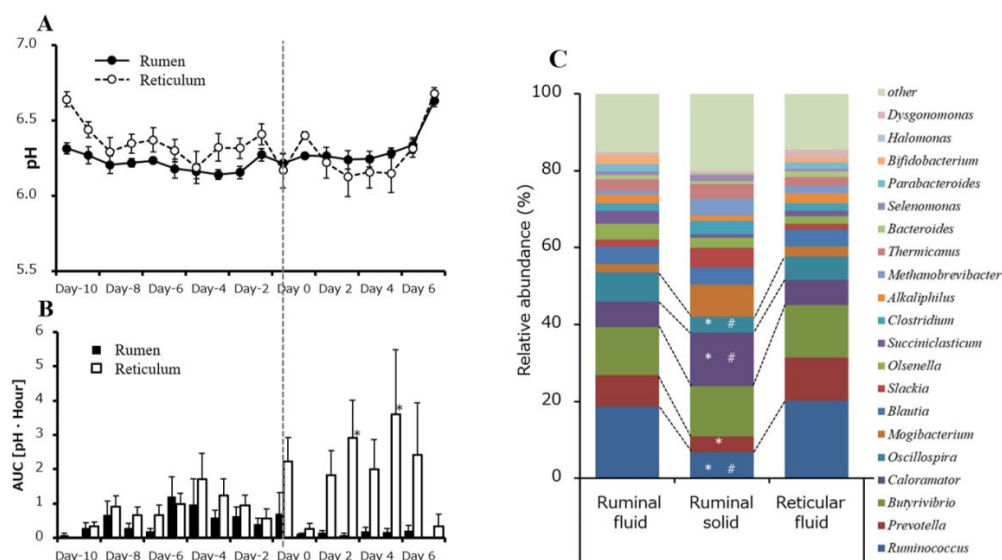
<sup>1</sup>Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan; <sup>2</sup>Hyogo Agricultural Technology Center, Kasai, Hyogo, Japan; <sup>3</sup>National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan; <sup>4</sup>Organization for Research Promotion, Iwate University, Morioka, Iwate, Japan

Email: sshigeru@iwate-u.ac.jp

**Introduction** Clinical or subclinical ruminal acidosis (RA or SARA) occurs frequently in Japanese Black beef cattle as soon as the early fattening stage because they are fed a high-grain diet and small amounts of forage. Recently, we developed a novel radio-transmission pH measurement system that can identify cattle with RA or SARA without sampling the ruminal fluid. The pH sensor may accidentally enter the reticulum when it is administered orally. It has been suggested that the reticular pH is slightly higher than the ruminal pH in cows due to the mixing and dilution of fluids with saliva, and the reticular and ruminal pH show a significant positive correlation. However, the reticulorumen fluids of Japanese Black beef cattle have not been characterized. Therefore, this study examined the ruminal and reticular fluid characteristics, especially the pH and volatile fatty acids (VFA) concentrations and lipopolysaccharide (LPS) activity, as well as the bacterial community in Japanese Black beef cattle.

**Materials and Methods** Nine Japanese Black beef cattle equipped with fistulas at 10 months of age were used. The cattle were fed a concentrate diet with little roughage twice daily. At 15 months of age, the ruminal and reticular pH were measured simultaneously every 10 min using a radio-transmission system (YCOW-S; DKK-TOA Yamagata, Japan). Ruminal and reticular fluids were collected at 4 h after the morning feeding to analyze VFA, ammonia nitrogen (NH<sub>3</sub>-N), lactic acid (LA), and LPS levels. Total bacterial DNA was extracted from the ruminal and reticular fluids and ruminal contents for 454 pyrosequencing analysis. Statistical analysis was performed using two-way repeated-measures analysis of variance followed by Bonferroni's multiple-comparison post-test. Pearson's correlation coefficients were calculated to compare the ruminal and reticular pH.

**Results** The 24-h and 1-h mean ruminal pH values were slightly lower than the reticular pH values. The ruminal total VFA and butyric acid concentrations tended to be lower than those in the reticulum, whereas no differences were observed in the total VFA, NH<sub>3</sub>-N, or LA concentrations or LPS activity between the rumen and reticulum. Although marked variation was observed among the cattle, there was a positive correlation between the ruminal and reticular pH. In the fluid fraction, the genera *Ruminococcus*, *Prevotella*, and *Butyrivibrio* were predominant in both the rumen and reticulum. The relative abundances of *Ruminococcus*, *Prevotella*, and *Caloramator* were significantly lower in the solid than fluid fraction. In addition, in principal coordinate analysis plots, the ruminal solids were separate from the ruminal and reticular fluids.



**Figure 1** Changes in the 24-h mean ruminal and reticular pH (A) and AUC (pH<5.6 · h, B) , and the relative abundance of the major bacterial genus (C) in Japanese Black beef cattle. Mean ± SE, n = 9, \*, #denotes significant difference ( $P < 0.05$ ) compared with the ruminal and reticular fluids.

**Conclusions** The ruminal pH was slightly lower than the reticular pH. The bacterial communities were similar between the ruminal and reticular fluids but different between the solid and fluid fractions. Further pathophysiological study of RA or SARA in Japanese Black beef cattle is required.

### Permanent long-term surveillance of ammonia in livestock – possibilities of a novel sensor technology

S. Dennier

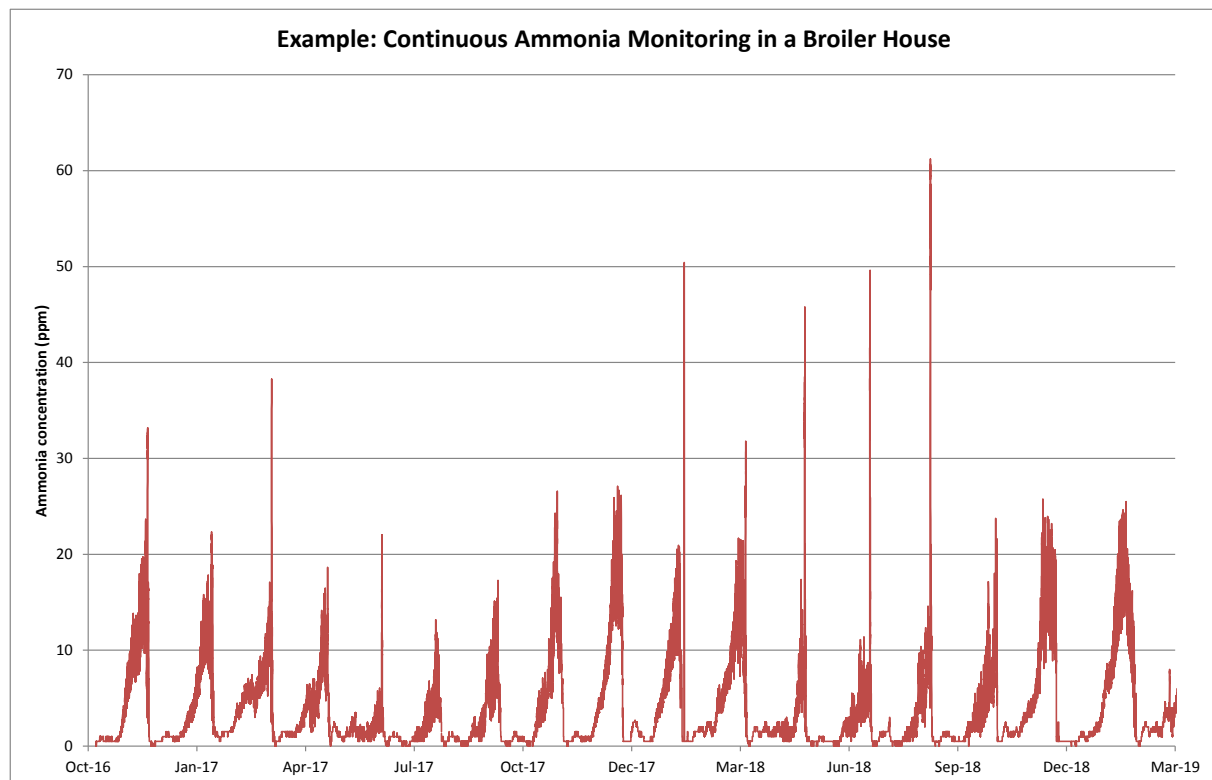
Drägerwerk AG & Co. KGaA, Lübeck, Germany

Email: [silja.dennier@draeger.com](mailto:silja.dennier@draeger.com)

**Introduction** The potential impact of ammonia on farm animal health has been described in various studies and research papers. In every-day practice, monitoring the ammonia levels in a barn is a challenge for the veterinary. Technologies for long-term measurement have been expensive and complex. Veterinaries thus often rely on spot or short term measurement that does not reflect the climate situation in a barn. In 2016, Dräger launched an easy-to-install, electrochemical sensor that is capable of monitoring the ammonia concentration in a barn over long periods without requiring recalibration. The presentation provides an insight into the possibilities of the sensor technology – as a basis for future research on farm animal health.

**Approach and Findings** In field testing as well as in cooperation with livestock climate experts and research partners, Dräger have seamlessly tracked ammonia levels in different livestock environments over long periods up to four years. While mostly not triggered by a specific research hypothesis, the compilation of the collected data allows for extracting findings on fluctuation and expansion of ammonia in farm animal barns, helping the veterinary to understand some of the interrelations of climate and animal health. One of the obvious findings is that spot monitoring of ammonia has only limited validity for stating air quality in a barn or the animals' gas exposure.

The presentation's target is to make the audience familiar with the possibilities of the technology and encourage further research on this basis.



### The impact of heat stress on reproduction and fertility in dairy cows

Z. Roth

Department of Animal Science, Faculty of Agricultural, Food and Environmental Quality Sciences, the Hebrew University of Jerusalem, Israel

Email: z.roth@mail.huji.ac.il

Reduced reproductive performance of lactating cows during the summer is mainly associated with intensive genetic selection for high milk production. It is expected to worsen in the coming years due to the concomitant global climate warming. Heat stress is not confined only to hot climates, since adverse effect of elevated environmental temperature is also reported in dairy farms located in cooler regions, such as Europe. Moreover, the effects of heat stress are not limited to the hot season as they carry over to the cooler months. The most common strategy to alleviate the effect of heat stress is providing shade and evaporative cooling, based on combining sprinkling and ventilation. Intensive cooling (i.e., 7-cumulative h/day) can prevent the decline in milk production, expressed by summer-to-winter milk-production ratio of 0.985. On the other hand, conception rate (CR) during the summer is lower by 22 percentage units relative to that of the winter. Additional strategies to improve reproductive responses are needed. However, given that multiple reproductive processes are impaired, it is difficult to restore normal functioning.

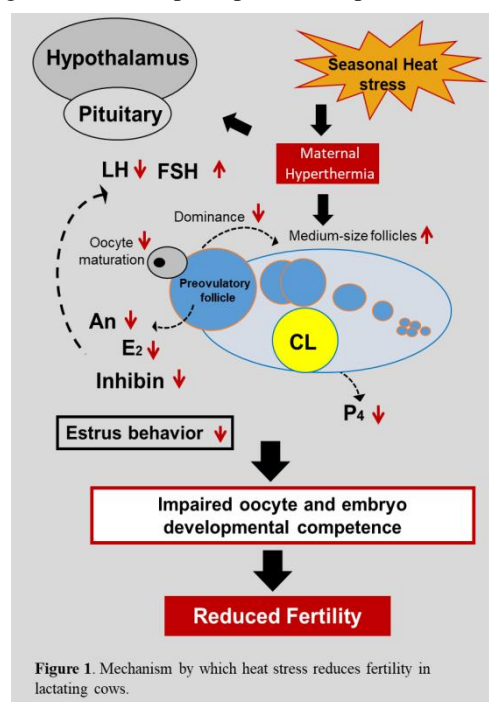


Figure 1. Mechanism by which heat stress reduces fertility in lactating cows.

The ovarian pool of oocyte is highly sensitive to thermal stress; it includes the early-antral follicles of about 0.5 to 1.0 mm in diameter. Heat stress also impairs the growth of medium sized follicles (6-9 mm), reduces the size and dominance of the dominant follicle and impairs the function of the preovulatory follicle. The endocrine background governs alterations in the hypothalamus-pituitary-ovarian axis (**Figure 1**), expressed by impaired steroidogenesis, reduced concentration of inhibin and increased that of the follicular stimulating hormone (FSH). In light of these, hormonal-treatment strategies to enhance removal of the cohort of damaged follicles has been suggested. Treatment with gonadotropin releasing hormone (GnRH) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), seems to be the most promising approach. In particular, induction of successive 9-day follicular waves with GnRH and PGF<sub>2α</sub> has a beneficial effect, as it increased estradiol concentration in the follicular fluid and improved pregnancy rate at 120 days in milking by 14%.

Heat-induced alteration in luteal function, expressed by reduced progesterone (P<sub>4</sub>) concentration in the circulation, is another mechanism suggested to reduce embryo survival which might lead to an increase in early embryonic loss. Induction of accessory corpus luteum and excess luteal tissue have been attempted to moderate the deleterious effects of heat stress on luteal function. For instance, administration of GnRH between days 5 and 15 post-AI increased conception rate by approximately 15 percentage units. Administration of controlled intra-vaginal drug-releasing (CIDR) device on day 5 ± 1 post-AI for 13 days had a substantial effect on CR in subgroup of cows; those with low body condition score, and those diagnosed with postpartum reproductive disorders. It should be pointed out, that cows were maintained under normothermic conditions by using evaporative cooling system.

The ovarian pool of oocytes is also sensitive to elevated temperature. Oocytes collected from Holstein cows during the summer exhibited a reduced developmental competence, expressed by low proportion of oocytes that were fertilized and further developed to the blastocyst stage. A period of two to three estrous cycles is required for recovery from summer heat damage and appearance of competent oocytes. This might explain the reduced fertility during the autumn, when cows are no longer exposed to environmental thermal stress. While findings indicate a carryover effect, it should be noted that only subpopulations out of the entire follicular reservoir are damaged, reflected by spontaneous recovery of conception rate through autumn to the subsequent winter. In light of this, enhanced removal of impaired follicles has been suggested to improve fertility. In particular, three consecutive follicular waves induced by GnRH and PGF<sub>2α</sub> during the summer and autumn improved conception rate, mainly in first-calving cows and cows with high body condition score postpartum.

While much of the effect of heat stress involves alterations in the follicle and its enclosed oocyte, preimplantation embryos are also sensitive to elevated temperature, in a stage-dependent manner. Two-cell-stage embryos are more sensitive to heat stress than those at 4- and 8-cell stages. On the other hand, embryos at later developmental stages (i.e., morula, blastocyst) are more resistant to heat stress most likely due to acquisition of thermotolerance i.e. a better balance between free-radical generation and antioxidant protection. One approach to improve bovine embryos' tolerance is supplementation of antioxidants such as vitamin E or selenium. But, the most promising approach is transferring of 8-day embryos during the summer; this approach has been found to increase pregnancy rate to that achieved in the winter. It should be pointed out that the body temperature of the recipient cows is critical for embryonic survival.

**In summary**, the author believes that using an efficient cooling system to maintain normothermia is a prerequisite to any additional remedial approach, whether it is hormonal treatments to remove damaged follicles, treatment to support CL function or embryo transfer. The effect of heat stress is multifactorial in nature. Therefore, effective reproductive management during the summer is suggested to combine a few treatment approaches.

### Effects of vet.smart.tubes ventilation systems on calf respiratory health

A.-C. Endres<sup>1</sup>, J. John-Wiedmer<sup>2</sup>, J. Neumayer<sup>3</sup> and W. Hasseler<sup>4</sup>

<sup>1</sup>Weihenstephan-Triesdorf University, Germany; <sup>2</sup>Tierklinik Teisendorf, Germany; <sup>3</sup>VET.SMART GmbH, Austria;

<sup>4</sup>Veterinärsgemeinschaft Papenburg, Germany

Email: j.neumayer@vetSMARTtubes.com

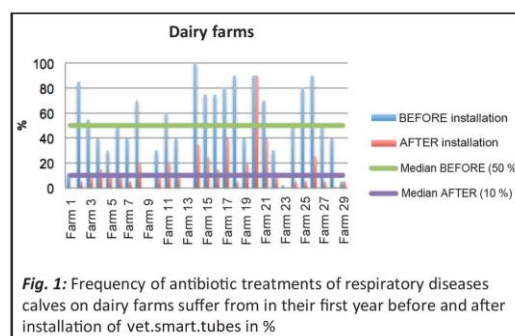
**Introduction** Draft-free ventilation in the calf barn is a key factor for optimized husbandry with reduced germ load. vet.smart.tubes ventilation systems provide calves with sufficient draft-free fresh air in a precisely controlled manner. The aim of the present study was to analyze the effect of vet.smart.tubes on calf respiratory health. In addition, the influence of husbandry conditions and management factors on calf health was examined.

**Materials and Methods** Using a questionnaire, 29 dairy farms and 8 fattening farms from Germany, Austria and Switzerland, who have installed vet.smart.tubes, were asked about the effect of the system on the respiratory health of their calves. All influencing factors were taken into account. The data analysis was carried out with Microsoft Excel 2011. The frequencies of the answers to the individual questions were recorded and evaluated as a percentage.

**Results** In addition to the optimized ventilation, management factors such as birth hygiene, colostrum care and trough have a strong influence on the success of a ventilation system in the following weeks. The positive impact of vet.smart.tubes on calf respiratory health was confirmed by the results. This was reflected in the decline in antibiotic treatments by 72% at the dairy farms and 87% at the fattening farms. Also the veterinary and drug costs declined by 57% at dairy farms and by 49% at fattening farms. The occurrence of mild bovine influenza symptoms was reduced by 80% at dairy farms, and by 78% at fattening farms, and the occurrence of symptoms such as fever and the refusal to eat decreased by 83% at the dairy farms and 53% at the fattening farms.

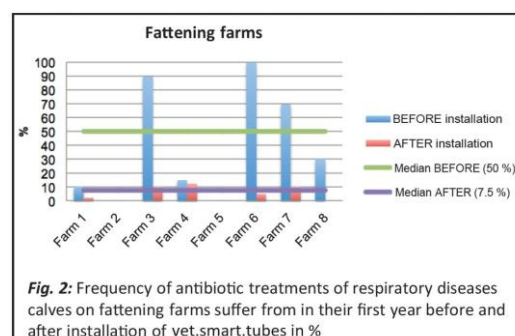
**Tab. 1:** Averages and medians of respiratory diseases on dairy farms depending on group size in %

	Dairy farms					
	Mdn ± STD			Median		
	BEFORE	AFTER	Difference	BEFORE	AFTER	Difference
≤ 10 animals/ group (n=17)	46.8 ± 27.26	13.73 ± 12.11	71	40	5	88
11-15 animals/ group (n=5)	67 ± 18.57	23 ± 10.37	66	70	20	71
> 15 animals/ group (n=7)	63 ± 30.24	17.14 ± 15.24	73	70	10	86



**Tab. 2:** Averages and medians of respiratory diseases on fattening farms depending on group size in %

	Fattening farms					
	Mdn ± STD			Median		
	BEFORE	AFTER	Difference	BEFORE	AFTER	Difference
≤ 10 animals/ group (n=4)	38.33 ± 28.43	7.33 ± 6.43	81	30	10	67
20-35 animals/ group (n=3)	52 ± 45.37	4 ± 1.73	92	45	5	89
64 animals/ group (n=1)	90	10	89	-	-	-



**Conclusions** The study proved the positive effect of a draft-free ventilation through vet.smart.tubes in the calf area. A new approach on this basis is the vet.smart.booster a precision air conditioning unit that combines draft-free ventilation in winter with high-efficiency cooling in summer. By means of two mutually displaceable half shells with precisely calculated hole patterns, defined air velocities are guaranteed according to the needs and ambient temperature.

Since in old buildings appropriate husbandry conditions for calves often cannot be realized, the vet.smart.mobarn has been developed. This mobile barn offers optimal conditions for healthy calf keeping and has a floor area of 25 m<sup>2</sup> for up to 10 calves. The roof is diffusely translucent and has a ridge opening. To clear out, the barn can be moved by means of wheel or front loader. The vet.smart.booster is part of the barn.



**Pict. 1:** vet.smart.mobarn



**Pict. 2:** vet.smart.booster



### Physiological indicators of beginning heat stress in grazing Holstein dairy cows

A. Pontiggia<sup>1,2,3</sup>, A. Munger<sup>1</sup>, F. Dohme-Meier<sup>1</sup>, L. Eggerschwiler<sup>1</sup>, W. Heuwieser<sup>4</sup>, L. Schuller<sup>4</sup>, S. Ammer<sup>5</sup>, R. Bruckmaier<sup>2</sup> and N. Keil<sup>3</sup>

<sup>1</sup>Agroscope, Ruminant Research Unit, Posieux, Switzerland; <sup>2</sup>University of Bern, Vetsuisse Bern, Switzerland; <sup>3</sup>Federal Food Safety and Veterinary Office, Proper Housing of Ruminants and Pigs, Ettenhausen, Switzerland; <sup>4</sup>Freie Universitat Berlin, Department of Veterinary Medicine, Clinic of Animal Reproduction, Berlin, Germany; <sup>5</sup>University of Gottingen, Department of Animal Sciences, Gottingen, Germany

Email: [alice.pontiggia@agroscope.admin.ch](mailto:alice.pontiggia@agroscope.admin.ch)

**Introduction** The continuous direct exposition to solar radiation coupled with their own metabolic heat production, due to ruminal fermentation and milk synthesis, make grazing dairy cows particularly susceptible to heat stress. Changes in the biological functioning of cows that suffer from heat stress may have negative effects on reproductive performance, animal welfare and health. Vaginal temperature (VT) is very sensitive to environmental conditions and known to be a good indicator to reflect physiological and pathological processes (Ammer et al., 2016). The temperature humidity index (THI) and the comprehensive climate index (CCI) are used to assess the effect of climatic conditions on dairy cattle. The THI is calculated out of the ambient temperature (T) and the relative humidity (RH) and the CCI additionally includes the solar radiations (Rad) and the wind speed (WS) (NRC, 1971, Van Iaer et al., 2015). The objective of the present study was to identify physiological indicators of beginning of heat stress in dairy cows in a pasture-based production system in Switzerland.

**Materials and Methods** The study was performed on 24 primiparous and multiparous Holstein dairy cows between the 6<sup>th</sup> of June and the 6<sup>th</sup> of September 2018 at Agroscope (Posieux, Switzerland). At the beginning of the study cows produced  $36.7 \pm 6.7$  kg of milk and were  $95 \pm 34$  days in milk. The cows were divided into 2 homogeneous treatment groups based on milk production, body weight, coat color and days in milk. Cows were paired randomly and each pair was pastured with another pair, resulting in six paddock groups. Data were collected during six periods with increasing THI. A period consisted of 2-3 days with “beginning heat stress” (daily mean THI < 65) and the following consecutive 2-3 days with “mild heat stress” (daily mean  $65 \leq \text{THI} < 72$ ). During “beginning heat stress”, all animals grazed full-time. During “mild heat stress”, three paddock groups were kept inside the barn from 1130 until 1500, while the other three paddock groups remained on pasture. For the following period the treatments for the paddock groups were switched over. Subsequently, each pair was combined with another pair to get different paddock groups for the 3<sup>th</sup>/4<sup>th</sup> and 5<sup>th</sup>/6<sup>th</sup> period. To continuously measure body temperature every 10 min, a microprocessor-controlled temperature logger (STAR-ODDI, Garabaer, Iceland) attached to a modified vaginal controlled internal drug release device (Eazy-Breed, Parsippany, NJ, USA) was inserted into the vaginal cavity of each dairy cow. Blood samples were collected once a day between 1500 and 1550 and the concentrations of plasma metabolites (glucose, BHB, NEFA) and hormones (T3, T4) were analyzed. Milk for the analysis of cortisol concentration was sampled twice a day (0430-0530 and 1600-1700). On pasture, a weather station (Onset, Bourne, MA, USA) recorded T, RH, WS and Rad every minute. In the barn, T and RH were recorded every 15 min with a logger (Testo, Monchaltorf, Switzerland). Data was analyzed in two time windows: 0900-1130 (AM) and 1140-1300 (PM). Data are represented as mean  $\pm$  SD. In the following preliminary results from univariate linear mixed regression models irrespective of treatment are shown for the 1<sup>st</sup>/2<sup>nd</sup> and the 5<sup>th</sup>/6<sup>th</sup> periods (except for cortisol that was analyzed for the 5<sup>th</sup>/6<sup>th</sup> period only).

**Results and Discussion** During the four periods, the  $\text{THI}_{\text{AM}}$  was  $64.5 \pm 4.6$ , the  $\text{CCI}_{\text{AM}}$  was  $24.0 \pm 5.3$  °C, the  $\text{THI}_{\text{PM}}$  was  $66.9 \pm 4.3$  and the  $\text{CCI}_{\text{PM}}$  was  $26.4 \pm 5.3$  °C. CCI and THI were closely correlated ( $r = 0.97$ ,  $p < 0.001$ ) so that they seem to be equally suitable to estimate the effect of climate conditions on dairy cows. The  $\text{VT}_{\text{AM}}$  was  $38.4 \pm 0.2$  °C and the  $\text{VT}_{\text{PM}}$  was  $38.5 \pm 0.3$  °C.  $\text{VT}_{\text{AM}}$  increased by 0.02 °C per increase in  $\text{THI}_{\text{AM}}$  unit ( $p < 0.001$ ) and  $\text{VT}_{\text{PM}}$  increased by 0.03 °C per increase in  $\text{THI}_{\text{PM}}$  unit ( $p < 0.001$ ). The T4 concentration was  $37.8 \pm 11.1$  nmol/l. T4 decreased by 0.1 nmol/l per increase in  $\text{THI}_{\text{AM}}$  unit ( $p < 0.05$ ) and it decreased by 0.2 nmol/l per increase in  $\text{THI}_{\text{PM}}$  unit ( $p < 0.001$ ). The T3 concentration was  $2.0 \pm 0.3$  nmol/l. T3 decreased by 0.006 nmol/l per increase in  $\text{THI}_{\text{AM}}$  unit ( $p < 0.01$ ) and it decreased by 0.008 nmol/l per increase in  $\text{THI}_{\text{PM}}$  unit ( $p < 0.001$ ) suggesting that thyroid hormones could be important for acclimation during heat stress (Weitzel et al., 2017). Associations of THI with glucose ( $3.1 \pm 0.2$  mmol/l), BHB ( $0.7 \pm 0.1$  mmol/l) and NEFA ( $0.1 \pm 0.1$  mmol/l) could not be detected but might be attributed to the cow’s variation in stage of lactation and changes in grass quality. The cortisol concentration in the milk obtained in the morning was  $2.6 \pm 1.4$  nmol/l and  $1.8 \pm 1.4$  nmol/l in the afternoon. Cortisol concentration in the milk harvested in the afternoon increased by 0.02 nmol/l per increase in  $\text{THI}_{\text{PM}}$  unit ( $p < 0.05$ ) indicating that animals perceived the increase of the THI as a stressor. An association between cortisol concentration in the milk harvested in the morning and THI could not be detected.

**Conclusions** In the range of the measured climate conditions, we could see an increase in VT and cortisol concentration and a decrease in T4 and T3 concentration with increasing THI indicating that heat stress has an impact on metabolites and hormones. They could be potentially used as physiological indicators of beginning heat stress in grazing Holstein dairy cows.

### References

- Ammer, S., Lambert, C. and Gauly, M. (2016): Comparison of different measuring methods for body temperature in lactating cows under different climatic conditions. *J. Dairy Res.* 83: 165-172.
- NRC (1971): A guide to environmental research on animals. Washington, DC: National Academy of Sciences.
- Van Iaer, E., Moons, C. P., Ampe, B., Sonck, B., Vandaele, L., De Campeneere, S. and Tuytens, F. A. (2015): Effect of summer conditions and shade on behavioural indicators of thermal discomfort in Holstein dairy and Belgian Blue beef cattle on pasture. *Animal* 9: 1536-1546.
- Weitzel, J. M., Viergutz, T., Albrecht, D., Bruckmaier, R., Schmicke, M., Tuchscherer, A., Koch, F. and Kuhla B. (2017): Hepatic thyroid signaling of heat-stressed late pregnant and early lactating cows. *Journal of Endocrinology*. 234: 129-141.

### Smart data in health management from Precision Livestock Farming

D. Berckmans<sup>1,2</sup>

<sup>1</sup>Katholieke Universiteit Leuven, KU Leuven, Belgium; <sup>2</sup>BioRICS NV, Belgium

Email: [daniel.berckmans@kuleuven.be](mailto:daniel.berckmans@kuleuven.be)

**Introduction** This year 65 Billion animals are slaughtered for food production. The worldwide demand for animals products is expected to increase with up to 75 % by 2050. At the same time the number of farmers is decreasing which means that each farm will count more animals. It is a serious challenge to guarantee good animal welfare and health for all those animals.

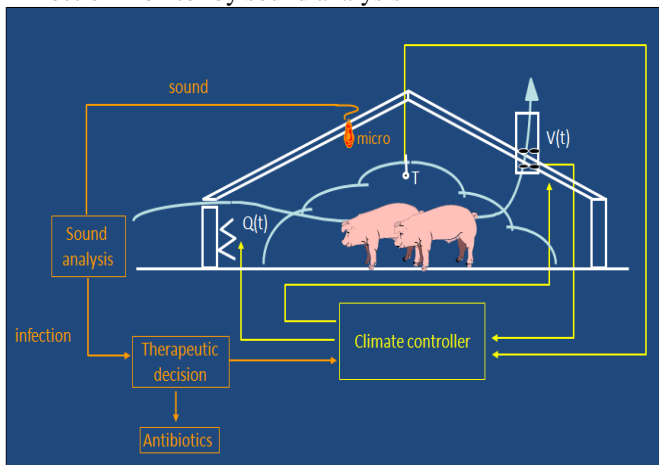
Precision Livestock Farming (PLF) aims at monitoring livestock continuously in real-time to further act as a support tool in managing livestock for better animal health, welfare, production and reduced environmental impact. This is done by using cameras, microphones and sensor in combination with intelligent algorithms to monitor animals and their behaviour 24/7 in a fully automated way.

**Materials and Methods** Since 1991 we are developing real-time monitoring techniques for living organisms starting on insects, mussels, fish, rats and mice, broilers, pigs, cow, rabbits, horses etc. The last 20 years we apply the approach also on humans.

Living organisms are Complex, Individually different, Time-varying in their responses and Dynamic (CITD). Due to their time varying behaviour we need to monitor them continuously 24/7. Continuous means 25 images per second or 20.000 sound samples or up to 250 accelerometer samples per second. These new technologies are combined with smart algorithms to turn the raw data into relevant information and only send meaning full data higher up to the early warning and management system.

**Results** We show examples of animal variables and behaviours that can be monitored with today's PLF technology. Many of them relate to animal health such as real-time pig weight monitoring by camera, pigs' water use monitoring by image analysis. Pig infection monitoring and broiler feed intake both realised by sound analysis. Cow lameness monitoring by image analysis. Looking to the future, we show animal welfare monitoring based upon real-time physiological data. We discuss the importance of animal welfare not only from ethical viewpoint but also from the aspect of efficient use of feed energy for production and for the immune system. We give an opinion of the role of the veterinarian in the digital world of Precision Livestock Farming and finally we discuss a potential business model to make this technology support veterinarians.

Infection monitor by sound analysis



Lameness monitor by image analysis



**Conclusions** In conclusion, Precision Livestock Farming will become true and has the potential to change the whole livestock sector and the work of many stakeholders. It remains a support tool for the farmer in managing his animals and it will give totally new opportunities to the veterinarians.

### Animal Health Surveillance and Smart Farming: Cyber Security in times with Robots, BigData, AI and Social Media

A. J. Dörig

Chairman Advisory Board “Advanced Cyber Security” of the Swiss Academy of Engineering Sciences SATW, Switzerland

Email: [doerig@doerigpartner.ch](mailto:doerig@doerigpartner.ch)

**Introduction:** Smart Farming is a farming management concept using modern technology to increase the quantity and quality of agricultural products. Farmers in the 21st century have access to GPS, soil scanning, data management, and Internet of Things technologies. By precisely measuring variations within a field and adapting the strategy accordingly, farmers can greatly increase the effectiveness of pesticides and fertilizers and use them more selectively. Similarly, using Smart Farming techniques, farmers can better monitor the needs of individual animals and adjust their nutrition correspondingly, thereby preventing disease and enhancing herd health.

**Challenges:** ‘The United Nations Food and Agriculture Organisation predicts that in order to keep pace with population growth, food production must increase by 70 percent by 2050; it also estimates that agriculture worldwide is currently responsible for a fifth of greenhouse gas emissions and for using some 70 percent of the world’s fresh water. ‘The development of smart farming and precision agriculture must accelerate rapidly and learn lessons from smart city projects if it is to meet the challenge set by the UN’s Food and Agriculture Organisation.

The way farmers produce their food must radically change in order to feed the growing world population of the future: Precision agriculture or smart farming makes use of GPS services, machine to machine (M2M) and Internet of Things (IoT) technologies, sensors and big data to optimise crop yields and reduce waste. Decision based support systems, backed up by publicly available data - including weather conditions and forecasts, machine status, crop information and animal health - can provide real time information at a level of granularity not previously possible. This enables better, more accurate decisions to be made and results in less waste and maximum efficiency in operations. This matters in an industry where margins can be tight, and a saving of a few percent can amount to a great deal of money and precious resources.

**Solutions:** The European Commission project Internet of Food and Farm 2020. The internet of things (IoT) has revolutionary potential. A smart web of sensors, actuators, cameras, robots, drones and other connected devices allows for an unprecedented level of control and automated decision-making. The project Internet of Food & Farm 2020 (IoF2020) explores the potential of IoT-technologies for the European food and farming industry.

A secure, robust, trustworthy, reliable and agile digital foundation would be an important advantage for ongoing innovation in smart farming. The most common concern about automation and the Industrial Internet of Things (IIoT) is about cybersecurity. Adolf J. Doerig looks at the strategic and tactical challenges for future oriented and trustworthy architectures. Something like a trustworthy Internet of Food & Farm.



### Precision Dairy Opportunities and Challenges

J. M. Bewley

Alltech, Inc. Nicholasville, KY, USA

Email: [jeffrey.bewley@uky.edu](mailto:jeffrey.bewley@uky.edu)

**Introduction.** Across the globe, the trend toward fewer, larger dairy operations continues. Dairy operations today are characterized by narrower profit margins than in the past, largely because of reduced governmental involvement in regulating agricultural commodity prices. Consequently, small changes in production or efficiency can have a major impact on profitability. The resulting competition growth has intensified the drive for efficiency resulting in increased emphasis on business and financial management. Furthermore, the decision-making landscape for a dairy manager has changed dramatically with increased emphasis on consumer protection, continuous quality assurance, natural foods, pathogen-free food, zoonotic disease transmission, reduction of the use of medical treatments, and increased concern for the care of animals. These changing demographics reflect a continuing change in the way in which dairy operations are managed. In large part, many of these changes can be attributed to tremendous technological progress in all facets of dairy farming, including genetics, nutrition, reproduction, disease control, and management.

**Precision Dairy Farming.** Precision Dairy Farming is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance. Many Precision Dairy Farming technologies, including daily milk yield recording, milk component monitoring, pedometers, automatic temperature recording devices, milk conductivity indicators, automatic estrus detection monitors, and daily body weight measurements, are already being utilized by dairy producers. Other theoretical Precision Dairy Farming technologies have been proposed to measure jaw movements, ruminal pH, reticular contractions, heart rate, animal positioning and activity, vaginal mucus electrical resistance, feeding behavior, lying behavior, odor, glucose, acoustics, progesterone, individual milk components, color (as an indicator of cleanliness), infrared udder surface temperatures, and respiration rates. The main objectives of Precision Dairy Farming are maximizing individual animal potential, early detection of disease, and minimizing the use of medication through preventive health measures. Perceived benefits of Precision Dairy Farming technologies include increased efficiency, reduced costs, improved product quality, minimized adverse environmental impacts, and improved animal health and well-being. Real time data used for monitoring animals may be incorporated into decision support systems designed to facilitate decision making for issues that require compilation of multiple sources of data. Technologies for physiological monitoring of dairy cows have great potential to supplement the observational activities of skilled herdspersons, which is especially critical as more cows are managed by fewer skilled workers. The economic implications of technology adoption must be explored further to increase adoption rates of Precision Dairy Farming technologies.

**Current State.** The list of Precision Dairy Farming technologies used for animal status monitoring and management continues to grow. Despite widespread availability, adoption of these technologies in the dairy industry has been relatively sparse thus far. Perceived economic returns from investing in a new technology are always a factor influencing technology adoption. Additional factors impacting technology adoption include degree of impact on resources used in the production process, level of management needed to implement the technology, risk associated with the technology, institutional constraints, producer goals and motivations, and having an interest in a specific technology. Characteristics of the primary decision maker that influence technology adoption include age, level of formal education, learning style, goals, farm size, business complexity, increased tenancy, perceptions of risk, type of production, ownership of a non-farm business, innovativeness in production, average expenditure on information, and use of the technology by peers and other family members.

**Investment.** Though Precision Dairy Farming is in its infancy, new Precision Dairy Farming technologies are introduced to the market each year. As new technologies are developed in other industries, engineers and animal scientists find applications within the dairy industry. More importantly, as these technologies are widely adopted in larger industries, such as the automobile or personal computing industries, the costs of the base technologies decrease making them more economically feasible for dairy farms. Because the bulk of research focused on Precision Dairy Farming technologies is conducted in research environments, care must be taken in trying to transfer these results directly to commercial settings. Field experiments or simulations may need to be conducted to alleviate this issue. Because of the gap between the impact of Precision Dairy Farming technologies in research versus commercial settings, additional effort needs to be directed toward implementation of management practices needed to fully utilize information provided by these technologies. To gain a better understanding of technology adoption shortcomings, additional research needs to be undertaken to examine the adoption process for not only successful adoption of technology but also technology adoption failures. Before investing in a new technology, a formal investment analysis should be conducted to make sure that the technology is right for your farm's needs. Precision dairy farming technologies provide tremendous opportunities for improvements in individual animal management on dairy farms. In the future, Precision Dairy Farming technologies may change the way dairy herds are managed.

### Intensive pig production systems: good or bad for animal health and welfare?

D. Maes

Unit of Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University Belgium

Email: [Dominiek.Maes@UGent.be](mailto:Dominiek.Maes@UGent.be)

**Introduction** Many countries in the world keep pigs in small groups that scavenge or are fed household waste. However, in high-income countries most of the pigs are raised in intensive pig production systems. In the European Union (EU), piglets in some farms may be reared in extensive outdoor systems along with the sow, but post-weaned pigs are almost always kept in indoor systems (EFSA 2007). Intensive production systems are characterized by higher animal density, large farms, use of concentrated feed and control of the production environment and stable climate. The aim is to maximize the efficiency of production, with emphasis on productive healthy animals with efficient use of inputs. During the last decades, major improvements have been achieved in pigs in terms of performance. The number of total born piglets per sow per year has increased dramatically, mainly because of genetic selection. The number of pigs weaned per sow per year has also increased, although the effects are somewhat flattened due to higher pre-weaning mortality. The performance of pigs post-weaning and fattening pigs (growth, feed conversion, carcass quality) has improved, but changes are less pronounced than in sows. The present abstract provides a critical reflection of intensive pig production with emphasis on health and welfare.

**Animal health** Optimal health of farm animals is a prerequisite for a profitable production, for producing safe and wholesome animal products and for the welfare of the animals. Although it is well known that health is much more than absence of disease, the health status of pig herds is often measured in terms of absence of specific diseases. Animal health in pigs has not improved to the same degree as performance. Many pig herds remain endemically infected with the major pathogens, and new pathogens or disease complexes have emerged. Prevalence figures of lung lesions in slaughter pigs are comparable to those of three decades ago. Mortality rates have generally increased. The fact that pathogenic infections remain in pig herds, even in professionally managed herds, is not that surprising given the numerous infection sources and transmission routes, international trade, and the fact that a large number of pigs are raised on one site or even share the same airspace. Proper biosecurity measures may interrupt or limit pathogen spread although they do not guarantee absence of disease problems, also because some disease conditions (“production diseases”) are not primarily caused by infections. External biosecurity refers to measures preventing pathogens from entering the herd, while internal biosecurity relates to preventing the within-herd spread of pathogens. Optimizing biosecurity and management is an everyday challenge, and scoring of biosecurity is helpful for sensitizing and benchmarking. Vaccination can be used if optimizing management and biosecurity is not sufficient. However, vaccines are available only for specific diseases, most of them provide only partial protection, do not prevent infection, and need to be administered individually mostly by injection. Antimicrobial use is still much too high in many pig herds worldwide, although major efforts are made in some countries to reduce the total use, the prophylactic use and to minimize the use of critically important molecules. Nutrition and genetics are not only important for performance but also for health. Feeding pigs according to minimal nutritional requirements can prevent deficiencies or disease, but may not be sufficient for optimal (intestinal) health and production. Further research on the role of microbiota is needed.

**Animal welfare** The strong genetic selection for more prolific sows in intensive systems has also a downside. The number of stillborn piglets has increased, the average birth weight decreased, there is less colostrum per pig, higher piglet mortality and the longevity of the sows has decreased. Genetic selection for robustness and higher piglet vitality should receive more attention. In addition, piglets are often weaned at a young age (21 days or even earlier outside the EU). All these factors might affect animal welfare and contribute to more disease problems after weaning. Although intensive systems have several advantages compared to extensive systems in terms of hygiene, biosecurity and disease prevention, there are many critical factors that may affect animal welfare. It is difficult for the animals to express natural behavior as they are limited in space. This is the case for all age groups, but is particularly applicable to sows housed in crates. Proper enrichment of the environment is helpful and may prevent or limit aggressive or stereotypic behavior. Discussion remains whether enrichment is sufficient *e.g.* when sows are housed in crates, or whether more drastic changes in the housing should be implemented. Floor characteristics are of paramount importance for animals that are permanently housed indoors. Inappropriate flooring, especially in group housing systems and in case of slatted floors, might predispose to severe locomotion problems and skin lesions. The way of feeding the animals is also important for welfare. In intensive systems, animals typically receive concentrated feed and might be fed restrictively (*e.g.* pregnant sows). There are limited occasions for foraging and animals spend much less time with eating than in natural conditions. Severe welfare problems, including aggression, may arise if feeding systems do not work properly. Welfare legislation in the EU has evolved significantly towards more animal friendly conditions, and further adaptations can be expected in the future. However, legislation varies among continents and countries. It is positive to see that many pig farmers implement extra animal welfare measures on top of what is minimally required by legislation.

**Conclusions** Intensive pig production systems are often perceived in a negative way by society, although they allow animals to grow according to genetic potential and offer many advantages in terms of animal health, hygiene and even welfare. However, stockmanship skills of the farmer are essential in intensive systems. Many critical issues related to pig health and welfare remain and consequently, there is still much room for improvement. More research on the use of technology and automated data collection in pig farms will very likely contribute to further optimize the health and welfare of pigs.

### Acoustic of dairy cows' gait: Detecting claw lesions by analyzing the footfall sound

N. Volkmann<sup>1</sup>, B. Kulig<sup>2</sup>, S. Hoppe<sup>3</sup>, O. Hensel<sup>2</sup> and N. Kemper<sup>1</sup>

<sup>1</sup>Institute for Animal Hygiene, Animal Welfare and Animal Behavior, University of Veterinary Medicine Hannover, Foundation, Germany; <sup>2</sup>Section of Agricultural and Biosystems Engineering, University of Kassel, Germany; <sup>3</sup>Landwirtschaftskammer Nordrhein-Westfalen, VBZL Haus Riswick, Kleve, Germany

Email: Nina.Volkmann@tiho-hannover.de

**Introduction** Claw lesions are a widespread health problem in dairy production and the third most frequent reason for culling. The assessment of locomotion is the most common method to identify cows affected by claw lesions. But, apart from a trained observer, this requires a lot of time which is limited especially with increasing herd sizes. Furthermore, lameness detection by visual observation is complicated because cows tend to mask the signs of pain caused by claw lesions until the stimulus becomes severe. Hence, there is a growing interest in supporting automated methods for lameness detection. To our knowledge, currently no system for an automated diagnosis of claw lesions could be implemented on farm. Claw lesions affect the well-being of dairy cows as they cause pain to the animals and that in turn leads to changing their natural behavior and their normal gait to reduce discomfort. Each step from a walking cow on solid surface (as it can be found in most of the housings) produces a signal of footfall sound. We hypothesized that this sound varies between lame and non-lame animals. Therefore, the aim of this project was to develop a system which is capable of an automated and early diagnosis of lameness in dairy cows by analyzing the footfall sounds on farm.

**Materials and Methods** The measurements were performed at the experimental farm of the North-Rhine-Westphalia Chamber of Agriculture. The experimental set-up was placed on the way back from the milking parlor. The test track was a constructed panel equipped with a knock sensor recording the footfall sound and an antenna for animal identification. Initially, seventeen cows were randomly selected to analyze their data precisely and 123 sound files of them were used for the evaluation. These animals additionally were filmed laterally by video and they were locomotion scored while they were walking along the test track. Furthermore, the cows were weighted and diagnostic findings concerning their claw lesions were recorded.

For analyses, the sound files were edited with the program R 3.5.1 and the libraries of seewave, tuneR and soundgen. The target value was the two-stage scale of locomotion scoring (lame vs. non-lame), and about 60 properties of the sound files (e.g. entropy, measures of location and variations of *fast Fourier* and the *Hilbert envelope transformation*), were used as influencing factors. The classification of the animals (lame vs. non-lame) was performed using a machine learning process (JMP Pro 14.0.4).

**Results** The footfall sound was analyzed from cows at a mean lactation day of 80 (min = 10; max = 234) and an average parity of four (min = 1; max = 11). The mean bodyweight of the animals was 680 kg (min = 574 kg; max = 753 kg). Hoof-trimming data revealed five healthy cows to show no claw lesion, nine with one diagnosis and three animals with two legs affected by a claw lesion. Sole ulcers were the most common disease, affecting five cows. Furthermore, three animals showed white line diseases, three an interdigital hyperplasia and double sole as well as digital dermatitis were detected twice, respectively. During the process of locomotion scoring 70.6% of the cows were assessed correctly, i.e. without any claw lesion as non-lame and affected by at least one claw lesion as lame. The process of machine learning generated a model with a misclassification rate, also known as error rate, of 20%.

**Conclusions** In conclusion, the developed system could be integrated in the stable and in the normal practice, i.e. on the way back from milking, on this dairy farm without disturbing the normal processes in the stable. Furthermore, the footfall sound of these animals, analyzed so far, offered the opportunity (with a misclassification rate of 20%) to classify 80% of the cows correctly regarding their claw lesions. However, further research is required on this system to improve the measurements on farms and especially a more detailed interpretation of the recorded footfall sounds. The performance of the system has to be validated with more cows.

**Acknowledgements** The study was conducted within the project "SoundHooves - Acoustic detection of lameness in cows". The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

## Monitoring calves at risk for diarrhea by use of an ear-attached accelerometer system

M. Goharshahi<sup>1</sup>, L. Lidauer<sup>2</sup>, A. Berger<sup>2</sup>, F. Kickinger<sup>2</sup>, M. Öhlschuster<sup>2</sup>, W. Auer<sup>2</sup>, D. Klein-Jöbstl<sup>1</sup>, M. Drillich<sup>1</sup> and M. Iwersen<sup>1</sup>

<sup>1</sup>Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, University of Veterinary Medicine Vienna, Austria; <sup>2</sup>Smartbow GmbH, Weibern, Austria

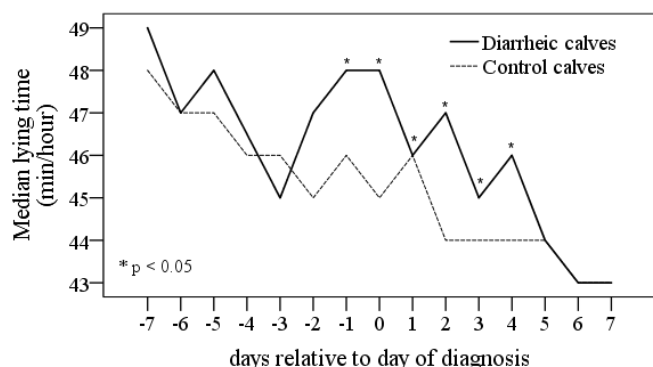
Email: Michael.Iwersen@vetmeduni.ac.at

**Introduction** One of the most important diseases in cattle worldwide is diarrhea in new born calves. Besides animal welfare issues, economic losses caused by costs for diagnosis, treatment, increased labor, increased risk for sequelae, impaired development of the calf, and, potentially, a total loss of the animal are a burden for the farmers. One important measure in minimizing the costs and the suffering of animals is an early detection of disease. Diseases are often associated with behavioral changes. Hence, a continuously monitoring of animal behaviors by use of a sensor system has the potential of early detection of disease. The aim of this study was to describe movement and activity patterns of calves suffering from diarrhea in comparison with healthy controls. For this, calves were equipped with a 3D-accelerometer integrated into an ear tag SMARTBOW (SB, Smartbow GmbH, Weibern, Austria). The SB system is commercially available for animal localization, estrus detection, and health monitoring in dairy cows. So far, it is not used in calves.

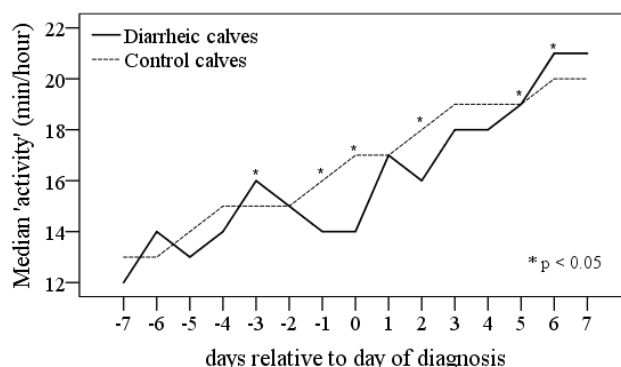
**Materials and Methods** The study was conducted in a commercial dairy farm in Slovakia, housing more than 2,500 calves and young stock. A total of 310 healthy female Holstein calves were enrolled at birth and followed up to 28 days of life, i.e. during the period of greatest risk for diarrhea. All animals were equipped with a SB tag in the middle of the right ear at the first day of life. Acceleration data were recorded with a frequency of 10 Hz and sent in real-time to the receivers (SB WallPoints). The WallPoints were connected with a local farm server on which data were processed and analyzed. Based on algorithms for cows, standing and lying times as well as ‘activity’ measures provided by SB were used as output parameters in our study.

Additionally, all calves were clinically examined once per day. Based on the scoring system by Larson (1977), calves with a fecal score of 3 (spreads readily to about 6 mm depth) or 4 (i.e., orange juice, liquid consistency, splatters) on at least two consecutive days, were categorized as ‘diarrheic’. At the time of diarrhea diagnosis, a healthy control calf matching best in age was also selected and examined. Fecal samples of diarrheic and control calves were tested for the most important pathogens with regard to newborn calf diarrhea (i.e. rotavirus, bovine coronavirus, *Cryptosporidium parvum*, and *Escherichia coli* F5) by an on-farm test (FASTest D4T bovine, Megacor Diagnostik GmbH, Hoerbranz, Austria). For statistical analysis, the SPSS software package (version 24, IBM Corporation, Armonk, NY) was used. Wilcoxon nonparametric test for two-related-sample groups were used and a  $p$ -value  $< 0.05$  was considered as significant.

**Results** In total, 13 calves left the herd before 28 days of life, because they died or were sold. In the remaining 297 calves, diarrhea occurred in 152 animals, resulting in an incidence of diarrhea of 51.2% (95% CI: 45.3–56.7%). *Cryptosporidium parvum*, rotavirus, coronavirus and *E. coli* F5 have been detected in 88.3%, 32.1%, 5.1%, and 1.3% of diarrheic calves, and in 56.4%, 29.5%, 7.7%, and 3.8% of control calves. For *Cryptosporidium parvum* the differences between diarrheic and control calves were significant ( $p < 0.05$ ). In Figure 1, SB-recorded median lying times (minutes per hour) for diarrheic and control calves are presented for the week before and after the visual diagnosis of diarrhea (day 0). SB-data showed that lying time increased in diarrheic calves already 2 days before visual observation of diarrhea. These differences were significant from day -1 to day 4. On day 0, average lying times per day were 72 minutes longer in diarrheic calves compared to their controls. ‘Activity’ estimated by SB (Figure 2) started to decrease from day -3 and showed maximum differences compared with controls on day 0 ( $p < 0.05$ ).



**Figure 1** Median lying times from day-7 to day 7 in relation to visual diagnosis of diarrhea (day 0).



**Figure 2** Median ‘activity’ measures day-7 to day 7 in relation to visual diagnosis of diarrhea (day 0).

**Conclusions** Based on algorithms developed for dairy cows, significant differences in lying times and ‘activity’ between diarrheic and healthy calves were observed by the SB system. These differences were detected already 1 day before visual diagnosis of diarrhea. Future research should focus on developing an algorithm for detecting calves at risk for diarrhea and on testing its accuracy.

### ZellDiX – A new approach to predict udder health by using DHI results and cell differentiation

A. Bartel<sup>1</sup>, F. Querengässer<sup>1</sup>, E. Gass<sup>2</sup>, F. Onken<sup>2</sup>, C. Baumgartner<sup>3</sup> and M. Doherr<sup>1</sup>

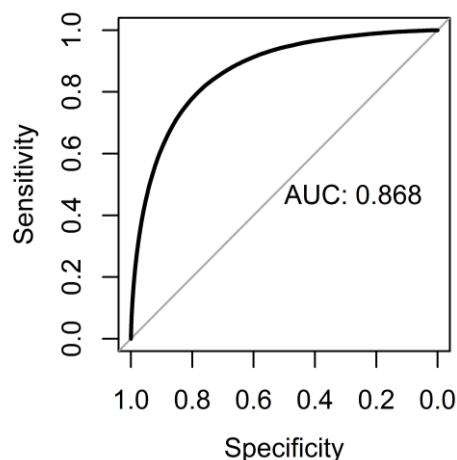
<sup>1</sup>Institute for Veterinary Epidemiology and Biostatistics, Free University Berlin, Germany; <sup>2</sup>German Association for Performance and Quality Testing, Bonn, Germany; <sup>3</sup>Bavarian Association for Raw Milk Testing, Wolnzach, Germany

Email: Alexander.Bartel@fu-berlin.de

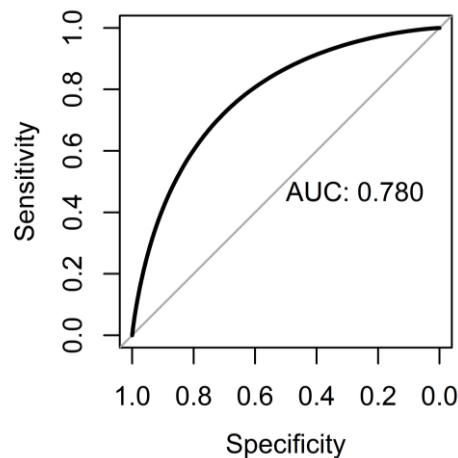
**Introduction** Management of udder health is a challenging aspect on every dairy farm. Somatic cell counts (SCC), obtained from monthly DHI testing, are helpful to monitor udder health at individual animal and herd level. In Germany, six farm-level parameters using DHI based SCC results are currently provided by a monthly report. They are a useful tool to reflect the present udder health status. However, to our knowledge, there are no standardized indicators available based on DHI results to predict the cow-level udder health status in the future. The German ZellDiX project aims to enhance the informative value of DHI results by evaluating the additional value of differential somatic cell count (DSCC) (Pilla et al. 2013) and by establishing statistical models that reliably predict future udder health.

**Materials and Methods** Using a new generation of high throughput devices (Damm et al. 2017), SCC as well as DSCC was routinely analyzed from DHI samples of over 900,000 cows from 19,160 farms in two German federal states. Over the course of two years, more than 10 million measurements were available. Our data set consisted of highly diverse farms in respect to size and management type. We fitted two statistical models, one for chronic SCC elevations and one for stable good udder health. Chronic elevations were defined as cell counts above a defined threshold in the next 2 DHI measurements. In order to meet different farmers' needs, results were derived for SCC thresholds between 200,000 and 700,000 cells/ml. Cows with stable good udder health had SCC values below 100,000 cells/ml in the next two DHI measurements. For mathematical modeling, we used generalized additive models (GAM) (Wood 2008) with cubic regression splines. Both models were 10-fold cross-validated and tested using internal and external validation data. In addition to these predictions, GAMs allowed to identify biases in the underlying data set and the impacts of individual parameters.

**Results** The predictions of the models accurately reflected the real probability, independent of region, size and breed composition of a farm as tested by multiple validation approaches. The AUC of the chronic udder impairment model (Fig. 1) at a SCC threshold of 400,000 cells/ml was 0.868 [95% CI 0.866 – 0.870] with a calibration slope of 0.995 [95% CI 0.983 – 1.006]. For the stable udder health model (Fig. 2) the AUC was 0.780 [95% CI 0.779 – 0.781] and the calibration slope was 0.993 [95% CI 0.990 – 0.996].



**Figure 1** ROC-Curve of the chronic udder impairment model



**Figure 2** ROC-Curve of the stable udder health model

**Conclusions** Using individual predictions for the risk of chronic cell count elevations can support treatment or culling decisions, while ranking animals could help farmers to prioritize resources. Identification of animals with a high probability of stable low cell counts can serve as a comparable indicator of udder health. On farm level, this can be a parameter for effective management. From April 2019 on, we will use the models in practice on cooperating pilot farms.

### References

- Damm, Malin, Claus Holm, Mette Blaabjerg, Morten Novak Bro, and Daniel Schwarz. 2017. "Differential Somatic Cell Count—A Novel Method for Routine Mastitis Screening in the Frame of Dairy Herd Improvement Testing Programs." *Journal of Dairy Science* 100 (6): 4926–40. doi:10.3168/jds.2016-12409.
- Pilla, R, M Malvisi, GGM Snel, D Schwarz, S König, C-p Czerny, and R Piccinini. 2013. "Differential Cell Count as an Alternative Method to Diagnose Dairy Cow Mastitis." *Journal of Dairy Science* 96 (3): 1653–60. doi:10.3168/jds.2012-6298.
- Wood, Simon N. 2008. "Fast Stable Direct Fitting and Smoothness Selection for Generalized Additive Models." *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 70 (3): 495–518. doi:10.1111/j.1467-9868.2007.00646.x.

### Using cognitive bias as a welfare tool in poultry

L. Košťál, Z. Skalná and K. Pichová

Center of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

Email: [Lubor.Kostal@savba.sk](mailto:Lubor.Kostal@savba.sk)

Harding et al. [1] in their pivotal study introduced the idea of using the link between cognition and emotions for the assessment of affective states in animals. Soon after that, the first studies of affect-induced judgment bias in birds were published, using European starling as a model. Although several other inspiring papers on this subject used songbird species (starlings and in one case canaries), nevertheless, substantial numbers of published avian cognitive bias studies used poultry species, such as domestic chickens (9 papers) and Japanese quail (2 papers).

Variation in design of cognitive bias tests in these poultry studies is quite large. Most of the studies use more or less modified spatial judgement task of Burman et al. [2]. In this task, birds are trained to expect a reward (corn, mealworm) in one location and no reward (empty bowl) or punishment (puffed rice soaked in a quinine sulphate, air puff) in another. After successful discrimination training follows the judgement bias testing, assessing whether birds in positive or negative emotional states respond differently to ambiguous stimuli of intermediate spatial location. The second approach used by two chicken studies adopted a Go-Go two choice visual discrimination task of Brilot et al. [3]. In this task hens have to learn to associate combination of cues (two background colours and two symbols) with a different food reward size. Hens are trained in a two-choice (left–right) test in an arena to associate a high-value reward (four mealworms) with a black cue and a low-value reward (one mealworm) with a white cue. Birds that reach training criteria are exposed to three unrewarded ambiguous cues (25, 50, and 75% black) to assess judgement bias. The third approach is based on operant Go-NoGo discrimination task, derived from the design of pivotal study of Harding et al. [1]. This is the approach used in our laboratory. We use the custom-built Skinner boxes with touchscreen (operated by Biopsychology toolbox software) designed for quail and laying hens for training the operant visual discrimination task and subsequent judgement bias testing. A custom mealworm dispenser controlled by the software delivers the reward and white noise serves as a punishment. Circles in different shades of grey are used as cues. The last, fourth design of judgement bias tests used in poultry is trying to eliminate one of the drawbacks of all previous approaches, i.e. extensive discrimination training needed before the judgment bias tests itself. (To illustrate this difficulty, in one of the papers using the two choice visual discrimination task, one third of hens did not achieve the discrimination criterion.) To avoid these problems, Salmeto et al. [4] came up with the original idea: in their experiments with young chicks, they used naturally appetitive (mirror image of chick) and aversive (horned owl silhouette) stimuli in a straight alley maze. As ambiguous cues in the judgement bias test, they used morphed images of a chick and an owl.

There is also large variation in the ways affective states (mood) are induced in the above-mentioned poultry studies: housing environment, enrichment, social isolation, different fearfulness, series of aversive events over several days, different temperatures, treatment with corticosterone, or suppression of depression induced by social isolation by the antidepressants differ from study to study. However, not all of the treatments caused the expected judgement biases. For example there were no significant differences in responses in judgement bias tests between laying hens housed in the basic and enriched pens, or Japanese quail housed in cages and in deep litter pens, suggesting that these environments did not induce large enough differences in the birds' emotional state to have a significant impact on their behaviour in the tests, or that the tests used are not sensitive enough. On the other hand, corticosterone-treated broilers showed an increased expectation of punishment in the face of ambiguous information [5], that lead authors to conclude that pessimism could be a useful welfare indicator in chickens. With respect to underlying brain mechanisms, it is of interest that the judgement bias in young hens was shown to be related to dopamine turnover rate in mesencephalon, with higher activity in individuals that had a more optimistic response.

In conclusion, the cognitive bias paradigm is a valuable tool for the assessment of poultry welfare. Nevertheless, existing judgement bias tests need further optimization and validation, improvement of the test design, and avoiding problems such as loss of ambiguity with repeated testing etc. Other types of cognitive bias tests, such as the newly proposed tests of attention and memory bias introduced recently in some species, represent another promising perspective.

### References

1. Harding, E., Paul, E., and Mendl, M., *Nature*, 2004, **427**(6972): p. 312.
2. Burman, O.H., et al., *Animal Behaviour*, 2008, **76**(3): p. 801-809.
3. Brilot, B.O., Asher, L., and Bateson, M., *Animal Cognition*, 2010, **13**(5): p. 721-731.
4. Salmeto, A.L., et al., *Brain Res*, 2011, **1373**: p. 124-130.
5. Iyasere, O.S., et al., *Scientific Reports*, 2017, **7**(1): p. 1-12.

### Automated assessment of welfare in chickens

M. S. Dawkins

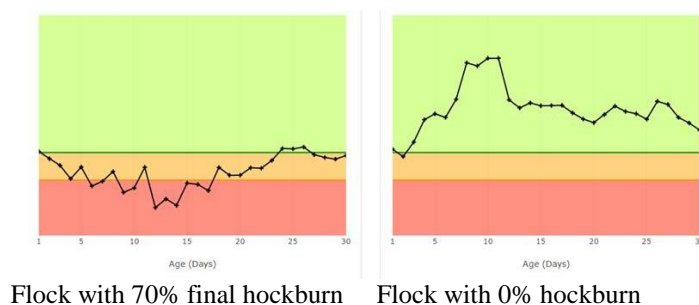
Department of Zoology, University of Oxford, UK

Email: [marian.dawkins@zoo.ox.ac.uk](mailto:marian.dawkins@zoo.ox.ac.uk)

**Introduction** OPTICFLOCK is a smart system that processes data from cameras on-farm and delivers a ‘verdict’ on the welfare of each flock. The aim of the OPTICFLOCK project is to provide farmers with a management tool that enables them achieve higher welfare standards, improved efficiency and reduced need for medication. By automatically monitoring the welfare of broiler chickens throughout their lives, it gives farmers a continuous readout of the health and welfare of their flocks in real time and provides early warning signs of problems before these become serious

**Materials and Methods** OPTICFLOCK works by analysing optical flow patterns produced by the movement of chicken flocks. It streams images directly from IP cameras inside broiler chicken houses into a small computer that immediately processes the images. Only numbers, not images are stored so that security is ensured. Previous research has shown that optical flow measures are correlated with key welfare outcomes for broiler flocks such as % hockburn and pododermatitis. In addition, flocks that subsequently tested positive for *Campylobacter* showed altered optical flow patterns when birds were less than a week old, at least two weeks before *Campylobacter* is normally detectable in faecal samples.

To be useful to farmers, OPTICFLOCK needs to be able to indicate in real time which flocks are at risk of developing welfare problems. By using the optical flow patterns of flocks with known welfare outcomes as a reference, a traffic light system for use on an app or PC has been developed. Figure 1 shows for one outcome measure - % hockburn – how two particular flocks (black lines) compare to the reference flocks. Here, the key reference is the daily median of kurtosis optical flow values of all reference flocks that had low (<10%) hockburn. The green area shows the Inter Quartile Range of the deviations from that median shown by reference flocks with low (10%) hockburn. The red area shows the IQR of the deviations from that same median shown by reference flocks with high hockburn (>40%).



The daily optical flow patterns of the two flocks were different from the start. The flock on the left had a final hockburn measure of 70% and the deviation of its kurtosis optical flow from the reference median fell into the IQR of high hockburn reference flocks (red). The flock on the right, which had 0% final hockburn, spent all of its life within the IQR of the low hockburn reference flocks.

**Conclusions** OPTICFLOCK can indicate which flocks are at risk from high levels of hockburn in very young birds, before any marks appear. It still needs to be shown, however, that camera monitoring is any better at managing hockburn or other welfare issues than existing methods, such as careful control of temperature and humidity within the house. This and other limitations of automated assessment of flock welfare will be discussed.

### References

- Dawkins, M.S., Roberts, S.J., Cain, R.J., Nickson, T., Donnelly, C.A. (2017) Early warning of footpad dermatitis and hock burn in broiler chicken flocks using optical flow, body weight and water consumption. *Veterinary Record* 180: 448 (May 20 2017). Cite as doi 10.1136/vr.104066
- Colles, F.M., Cain, R., Nickson, T., Smith, A., Roberts, S.J., Maiden, M.C.J., Lunn, D., Dawkins, M.S. (2016) Monitoring chicken flock behaviour provides early warning of infection by human pathogen *Campylobacter*. *Proceedings of the Royal Society B* 283. 20152323.

## Association of rumination time with some biomarkers from automatic milking system

D. Malašauskienė, M. Televičius, V. Juozaitienė, Ž. Prokopavičiūtė, I. Navalinskaitė, E. Pocevičienė and R. Antanaitis  
Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania  
Email: Ramunas.Antanaitis@ismuni.lt

**Introduction** The rumination process is essential for the fermentation and digestion of ingested feed and is influenced by different external conditions (Soriani et al., 2012); therefore, its monitoring may be a useful element when assessing the health status of ruminants. Rumination time, subsequent yield, and milk trait change depends on the period of lactation and reproductive status of a dairy cow (Antanaitis et al., 2018). Devices effective at measuring rumination by differentiating specific movements and sounds have been recently developed (Braun et al., 2013). The objective of this study was to determine the associations of rumination time (RT) with milk yield, bodyweight, milk temperature, milk fat – protein ratio, and electrical conductivity of milk at the udder quarters level.

**Materials and Methods** The study was performed on 775 dairy cows from a herd of 1250 cows. The cows were kept in a loose housing system, and were fed total mixed ration (TMR) throughout the year at the same time, balanced according to their physiological needs. Cow feeding took place every day at 06:00 and 18:00. 775 cows were selected for 1 – 200 days of milk (DIM). The cows were milked with Lely Astronaut® A3 milking robots with free traffic. The rations were calculated according to physiological standards. Daily milk yield, rumination time, body weight, milk fat and protein ratio, milk temperature, electric milk conductivity (from all udder quarters) were collected from the Lely T4C management program for analysis. Data of the tested cows were analysed in IBM SPSS statistics Version 20.0 for Windows. Distributions of the productivity of cows (MY), milk fat a protein ratio (F/P), body weight (BW) and milk temperature (MT), electrical conductivity of milk (EC) at the udder quarters level: left front (LF), left right (LR), rear left (RL) and rear right (RR) have been to assess according to the Kolmogorov-Smirnov test.

**Results** We found that the average rumination time of evaluated cows was  $416.35 \pm 1.68$  min/d. We estimated that the average milk electrical conductivity of all quarters was  $70.31 \pm 0.212$  S·m<sup>-1</sup>. There were similar levels of the average electrical conductivity in front quarters ( $70.30 \pm 0.23$  S·m<sup>-1</sup>) and rear quarters ( $70.36 \pm 0.21$  S·m<sup>-1</sup>) of udder. Furthermore, the average difference between cow's udder quarters maximum and minimum electrical conductivity of milk was  $4.70 \pm 0.296$  S·m<sup>-1</sup> or  $6.12 \pm 3.27\%$  S·m<sup>-1</sup> (Tab1.) The rumination time was positively correlated with productivity of cows ( $P=0.000$ ) and negatively with F/P ( $P=0.001$ ) and body weight of cows ( $P=0.001$ ). The duration of rumination tended to increase with an increase in milk temperature ( $r=0.087$ ,  $P=0.020$ ). It was observed that rumination time negatively correlated with electrical conductivity of all quarters of udder ( $r=-0.114$ ,  $P=0.002$ ), front quarters ( $r=-0.110$ ,  $P=0.003$ ) and rear quarters ( $r=-0.118$ ,  $P=0.002$ ). Additionally, a negative RT correlation with EC difference between udder quarters of cows ( $r=-0.151$ ,  $P=0.000$ ) was calculated. We detected that milk temperature positively correlated with milk yield of cows ( $r=0.467$ ,  $P=0.000$ ) and slightly negatively related with F/P ( $r=-0.124$ ,  $P=0.002$ ), body weight of cows ( $r=-0.086$ ,  $P=0.002$ ) and electrical conductivity of individual quarters of udder - from  $r=-0.085$ ;  $P=0.002$  (left front quarter) to  $r=-0.255$ ,  $P=0.000$  (front left quarter) (Fig 1). There was significant relationship between RT of cows and productivity body weight, milk temperature, fat and protein ratio and electrical conductivity at the udder quarter level.

**Conclusions** RT was positively correlated with productivity, negatively with F/P, body weight of cows and electrical conductivity of all quarters of udder.

**Table 1** Descriptive statistic

Traits	M	SE
Milk temperature, T°C	37.90	0.04
Milk yield, kg	35.68	0.41
F/P	1.14	0.01
Body weight, kg	668.50	3.32
Milk EC (S·m <sup>-1</sup> ) of udder quarters:		
Front left	70.25	0.27
Front right	70.13	0.25
Rear left	70.29	0.25
Rear right	70.42	0.27

**Figure 1** Coefficients of correlation of rumination time with investigated traits of cows.

	Group	Average	SE	Min.	Max.
Time (hour)	T	2.727	0.910593	0.44	10.5
	C	1.6	0.271277	0.21	3.24
24	T	1.306	0.262167	0.29	2.95
	C	1.626	0.300988	0.48	3.81
48	T	0.754	0.258028	0.24	2.54
	C	0.961	0.198346	0.27	1.96

## References

- Negrão, J. A., & Marnet, P. G. (2003). Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. *Small Ruminant Research*, 47(1), 69-75.
- Berci I DVM. PhD. Neuroimmune Biology. Vol 1. 2002. pp. 3-45. Elsevier Science. 2001.



## Association between social preference and development of tail biting in growing-finishing pigs

Y. Z. Li<sup>1,2</sup>, K. St Charles<sup>2</sup> and L. J. Johnston<sup>1,2</sup>

<sup>1</sup>West Central Research and Outreach Center, University of Minnesota, Morris, MN, USA; <sup>2</sup>Dept. of Animal Science, University of Minnesota, St Paul, MN, USA

Email: yuzhili@umn.edu

**Introduction** Pigs are social animals that form social structures to maintain group stability. Social structures and social preference can affect welfare of individual pigs in the group. Our previous study[1] indicated that social structure was associated with development of tail biting in nursery pigs. However, it is not clear whether social preference plays a role in development of tail biting. This study was designed to investigate: 1) social preferences of tail biters, pigs victimized by tail biting, and other pigs (neither biters nor victims), and 2) association between social preference and tail-biting incidence in growing pigs, with an ultimate goal of evaluating whether social preference is associated with development of tail biting.

**Materials and Methods** Pigs (n = 96, initial weight = 25.0 ± 3.0 kg; Landrace × Yorkshire × Duroc) with intact tails were housed in 12 pens of 8 pigs (4 barrows and 4 gilts) in a confinement barn. Pigs were assigned to 3 treatment groups based on their litter origin: littermates (pigs in a pen were farrowed and nursed by the same sow), half-group littermates (pigs in a pen were farrowed and nursed by two sows with equal number of pigs from each sow), and non-littermates (pigs in a pen were farrowed and nursed by 8 different sows). Four pens of each litter origin treatment were studied for 14 weeks. Tail injury was assessed once weekly in addition to during tail biting outbreaks to identify pigs victimized by tail biting (pigs with visible blood on the tail). Behavior of pigs was video-recorded for one day between 0900 h and 1500 h at 4-week intervals starting one week after study initiation. Video-recordings were viewed continuously to identify tail-biting events and tail biters (bit the tail of another pig causing reactions of the recipient) in each pen. For social network analysis, video-recordings were scanned at 10-min intervals to register pigs that were lying together (1) or not (0) in binary matrices. Half-weight association index[2] was used for social network construction[3]. Social network analysis was performed using the UCINET software. Comparisons among litter origin treatments were conducted using the Glimmix Procedure of SAS with Adjust Tukey Test for multiple comparisons of least square means.

**Results** Fifty nine percent of pigs in the littermate treatment were identified as victims of tail biting, which was higher than victimized pigs in other treatments (34% for half-group of littermates and 22% for non-littermates; P = 0.02). Pigs assigned to the littermate treatment (Table 1) formed more social ties between tail biters and victims (P = 0.03), and fewer social ties between biters and other pigs (P = 0.01) and between victims and other pigs (P = 0.02) than non-littermates.

**Table 1** Effect of litter origin on social ties (social preference) among growing-finishing pigs

Item	Litter Origin			Pooled SE	P <
	Non-littermates	Half littermates	Littermates		
Number of pens	4	4	4		
Average number of pigs in each category (pigs/pen, means ± SD )					
Biters	2.3±1.26	2.5±1.29	3.0±1.41		
Victims	1.5±1.00	2.0±1.41	3.0±1.41		
Others	4.2±1.50	3.5±2.52	2.0±1.83		
<i>Number of social ties</i>					
Between biters	0.31	0.62	0.55	0.196	0.44
Between biters and victims	0.74 <sup>a</sup>	1.48 <sup>ab</sup>	1.92 <sup>b</sup>	0.304	0.03
Between biters and others	2.34 <sup>a</sup>	1.14 <sup>b</sup>	0.72 <sup>b</sup>	0.265	0.01
Between victims and others	3.78 <sup>a</sup>	2.60 <sup>ab</sup>	2.04 <sup>b</sup>	0.402	0.02

<sup>ab</sup>Lsmeans within a row without a common superscript differ (P < 0.05)

**Discussion** Compared with pigs in the non-littermate treatment, pigs in the littermate treatment had more tail injuries caused by tail-biting, which coincided with more social ties between tail biters and victimized pigs. This suggests that social preference of tail biters and victimized pigs may be associated with development of tail biting.

**Conclusions** More social ties between tail biters and victims, and fewer social ties between victims and other pigs may predispose littermates to development of tail biting.

## References

- Li, Y. Z., H. Zhang, L. J. Johnston, and W. Martin. 2018. Understanding tail-biting in pigs through social network analysis. *Animals*. 8, 13; doi:10.3390/ani8010013.
- Durrell, J., L. I. A. Sneddon, N. E. O'Connell, and H. Whitehead. 2004. Do pigs form preferential associations? *Appl. Anim. Behav. Sci.* 89: 41-52.
- Farine, R. A., and H. Whitehead. 2015. Constructing, conducting, and interpreting animal social network analysis. *J. Anim. Eco.* 84: 1144-1163.

### Stakeholder groups' perceptions of the primary causes of poor dairy cow welfare and dairy cow culling

J. F. Mee<sup>1</sup>, J. Marchewka<sup>2</sup> and L. Boyle<sup>1</sup>

<sup>1</sup>Teagasc, Moorepark Research Centre, Ireland; <sup>2</sup>Institute of Genetics and Animal Breeding, Warsaw, Poland

Email: [john.mee@teagasc.ie](mailto:john.mee@teagasc.ie)

**Introduction** Dairy cows in pasture-based systems of milk production generally have better welfare than those in confinement systems (Boyle and Rutter, 2013, Mee, 2012). However, abolition of the EU milk quota (2015) and subsequent intensification in the EU dairy industry may pose threats to cow welfare in pasture-based systems. ProWelCow (DAFM RSF - A 14/S/890), a nationally funded research project, was set up to investigate risks and strategies to protect and improve the welfare of Irish dairy cows. The task reported here aimed at identifying areas of consensus and disagreement between stakeholders on issues related to dairy cow welfare.

**Methods** A questionnaire of approximately 40 questions was developed, piloted and modified accordingly in conjunction with Teagasc dairy research and specialist advisory staff. The survey was conducted with dairy farmers (F; n=115) at two national farming events and with cattle veterinarians (V; n=60) at the Cattle Association of Veterinary Ireland Conference by interview. The survey was distributed amongst Teagasc dairy advisors (A; n=48) at the beginning of an in-service training day where they were asked to complete the survey themselves. The 223 respondents were asked 1) Do you perceive that expansion in the dairy industry poses concerns for dairy cow welfare (yes/no); 2) identify the main causes of poor welfare in cows from the following list: lameness, poor body condition score, social stress due to overcrowding, mastitis, metabolic disorders, infectious diseases, cold stress and calving difficulties and 3) number in order of importance the main reasons for culling from the following list: infertility, lameness, mastitis/high SCC and other. The results are expressed as a % of each group surveyed. A Chi-Square Fisher test was used to investigate whether distributions of response frequencies differed between stakeholder groups using PROC FREQ in SAS.

**Results** A high proportion (c. 80%) of respondents in all groups agreed that expansion poses challenges for cow welfare ( $p>0.05$ ). The majority of farmers (22.6%) chose poor body condition as the 1<sup>o</sup> welfare issue; advisors (10.4%) and vets (8.3%) ( $p<0.001$ ). The majority of advisors (43.8%) chose social stress; different to farmers (14.8%,  $p<0.05$ ) but not to vets (30.0%) ( $p>0.05$ ). The highest proportion of vets selected lameness as the 1<sup>o</sup> welfare issue (28.3%); differed from advisors (2.1%) and farmers (13.0%) ( $p = 0.001$ ). The main reason for culling cows was infertility, followed by lameness and mastitis/high SCC. Vets ( $p=0.01$ ) and advisors ( $p=0.021$ ) perceived infertility as the primary reason for culling more often than farmers.

**Conclusions** There was a lack of consensus regarding the importance of lameness and poor BCS between stakeholders. This was surprising, and worrying, but probably reflects the differing focus and areas of expertise between the three stakeholder groups. Given these results, greater cross-dialogue between stakeholder groups is required.

#### References

- Boyle, L.A. and Rutter, S.M. (2013). Comparison of the welfare of dairy cows in 'pasture based' and 'zero grazed' milk production systems. Profitable and sustainable grazing systems – moving forward with science. Joint BGS and BSAS conference, Malvern, UK pp. 11-14.
- Mee, J.F., (2012). Reproductive issues arising from different management systems in the dairy industry. *Reproduction in Domestic Animals*, 47:42-50.

### Problems and solutions associated with testing a novel tail-affixed calving biosensor in dairy cows

J. F. Mee<sup>1</sup>, L. English<sup>2</sup> and J. P. Murphy<sup>1</sup>

<sup>1</sup>Teagasc, Moorepark Research Centre, Ireland; <sup>2</sup>Animal Sensing Limited, Tipperary Town, Ireland

Email: john.mee@teagasc.ie

**Introduction** Calving is an unpredictable event. Ideally, farmers would like to be able to predict to within a few hours when a cow is going to calve. But, both the signs of impending calving and the ability of the observer to detect and interpret them are highly variable. Prediction of the onset of calving would potentially prevent dystocia and stillbirth at unobserved calvings and facilitate prompt colostrum feeding, especially for heifer calves, and calf removal, particularly in paratuberculosis-infected herds. More than a dozen indicators of impending parturition have been tested to develop commercial calving alarms. Currently available devices predict onset of calving from body temperature changes, abdominal contractions, tail elevation, restlessness, vulval separation or detection of ambient light or temperature. Some approaches only predict the day of calving (e.g. progesterone decline precalving) while others attempt to predict the hour of calving. One area which shows potential promise is detection of tail elevation precalving as this has been shown to be closely and uniquely associated with calving in cows (Miedema, 2009). Ideally calving alarms would detect the onset of stage two of calving (when the farmer can possibly intervene) and differentiate between eutocia and potential dystocia. Hence, the objective of this study was pilot-test a new biosensor to predict the onset of stage two of calving in dairy cows. The study was designed to detect any problems associated with *in vivo* testing of this pre-commercial prototype and to collect preliminary data from calvings to train the predictive algorithms.

**Materials and methods** The prototype device consisted of a tail-mounted sensor and a base station. The activity monitor was developed containing an accelerometer and other gravitational measurement devices. It was attached to the upper side of the cow's tail approximately 6 cm below the anus using a self-adhesive blue bandage wrap. Three behavioural changes were monitored: (1) tail raise frequency and duration (2) angle of tail raise and (3) bouts of standing/lying down. The sensor readings from the sensors on the cow's tail were communicated every 2 seconds via radio frequency to the base station where they were stored on micro-SD cards. The base station was connected to the GSM network over a range of c. 30m indoors, with a capacity for up to 20 tail units. The device was tested on 20 Holstein-Friesian cows on two farms. The device was on the cows for between 1 and 4 days precalving. The time of calving was established by 24 h staff supervision and CCTV.

**Results** Of the 20 cows, 12 calvings were monitored, (6 primiparae, 6 pluriparae); 5 unassisted, 6 easily assisted and 1 difficult. The reasons for the incomplete recordings were: the GUI (graphical user interface) format data could not be disaggregated enough to define trends in behavioural change (2 calvings), signal was poor, resulting in data not being received, or gaps in the data rendering it unusable (2), cows pulled the devices from each other's tails (2), laptop in hibernation failed to record the data being sent from the monitor (1) and antenna snapped off the receiver base (1). In recorded calvings, prolonged elevation of the tail (>30-45 degrees for >20 seconds and 4 repetitions within 60 minutes), either alone or in combination with an abnormal standing pattern (within a 30 min. period) were observed within 4 hours of all calvings (unassisted calvings 2 - 3.3h; assisted calvings 45 mins - 3h). However, some practical issues arose during this study with the tail-mounted sensor. Cows dislodged some devices from each other's tails. Further testing revealed this was due to the blue colour of the bandage; when this was replaced with a black bandage this problem stopped. Additionally, oedema formed in the tail at device attachment in some cows after three days of attachment. This was resolved by not placing the device on cows until they were closer to the point of calving and by not over-tightening the bandage wrap.

**Conclusions** It is concluded that prolonged tail elevation combined with increased restlessness was predictive of imminent calving. The monitor was able to detect and record the pattern of calving behaviours and the algorithm was able to detect distinct onset of calving-specific behavioural change up to four hours before birth. Thus this prototype device shows potential to detect the onset of stage two of calving. Further study in a larger population of animals with this device is required to confirm these preliminary results.

### References

Miedema, H. (2009) Investigating the use of behavioural accelerometer and heart rate measurements to predict calving in dairy cows. Doctoral Dissertation, University of Edinburgh, UK.

### Tail-biting in pigs: change in feeding behaviours during a tail-biting outbreak

C. Ollagnier

Agroscope, Swine Production Research Group, Posieux, Switzerland

Email: catherine.ollagnier@agroscope.admin.ch

**Introduction** Since 2008, tail docking is prohibited in EU and in Switzerland but only two of the European countries (Sweden and Finland) have actually implemented it. Tail biting is triggered by lack of enrichment material, bad environmental conditions, unbalanced diet, and disease (Sonoda et al. 2013). The objective of this retrospective data analysis was to assess changes in feeding behaviour of group housed pigs fed a protein restricted diet before during and after a tail biting outbreak.

**Materials and Methods** Seventy-one pigs ( $117.0 \pm 10.7$  day old;  $47.8 \pm 9.7$  kg) had restricted (80% of assumed ad libitum intake) access to the grower diet. The diet was formulated to contain 80% dietary crude protein (CP) and essential amino acid (EAA) of the Swiss feeding recommendation. Pigs were housed in a 78 m<sup>2</sup> pen with straw in racks and woodchips on the floor. The pen was split into 4 subunits, with 13.39 m<sup>2</sup> plain resting area and 6 m<sup>2</sup> of slatted floor in each. The pen was equipped with 4 automatic feeders, which allowed measuring the individual feed intake per visit, the number of visit per day and the time spent at each visit. Pigs had *ad-libitum* access to water through nipple drinker. Two months after the beginning of the trial, around 75% of the pigs presented tail lesions. The tail-biting outbreak was retrospectively divided into 3 phases (Taylor et al. 2010): the **pre-injury phase (A)**, before tail damage appears, the **acute phase (B)**, once the phenomenon was discovered) and the **recovery phase (C)**, after the tail biting initiator was removed and *ad libitum* feeding was restored). Each phase lasted 7 days. Total feed intake, average feed intake per visit, maximal consumption per visit, minimum consumption per visit, numbers of visit, and total time spent eating were calculated per pig and per day. The feed efficiency and daily gain were summarized per phase and per pig. For “total feed intake” and “total time spent at the feeder” traits, body weight or total feed intake were respectively included as covariate in the model. Comparisons between phases were performed with R in repeated measures ANOVA (“emmeans and “lme4” packages).

**Results** Except for average feed intake per visit, feeding behavior traits differed ( $P < 0.01$ ) among the phases (Table 1). The daily feed intake reported for a standardized bodyweight (48.3 kg) was reduced ( $P < 0.001$ ) in the A and B compared to the C phases, which concurs with the restoration of *ad libitum* feeding in phase C. In accordance, average daily gain was lower ( $P < 0.001$ ) in phase A (0.66 kg/d) and B (0.59 kg/d) compared to C (1.34 kg/d). Mean consumption time adjusted for a defined feed intake (2100 g) was longer in phase A compared to phases B and C, meaning that pigs learn to eat faster and this independently of age. This behavior persisted even when feed was offered *ad libitum*. Feed efficiency was lower ( $P < 0.05$ ) in phases A and B compared to phase C. One may think this is a consequence of the stress generated by the tail-biting outbreak. Number of visits to the feeder differed ( $P < 0.05$ ) among phases, decreasing from phase A to B and increasing from B to C, but not reaching a higher level as in phase A.

**Table 1** Differences in average feed intake and feeding behaviour traits among the pre-injury (A), the acute (B) and the recovery phase (C)

		Differences in estimated mean <sup>1</sup>	P-value
Daily feed intake, g/d	A-B	-6.78	0.961
	B-C	-656.3	<0.001
	A-C	-649.2	<0.001
Feed efficiency	A-B	0.071	0.338
	B-C	-0.190	<0.001
	A-C	-0.119	0.050
Mean consumption time, s	A-B	192.2	<0.001
	B-C	-26.4	0.728
	A-C	165.9	<0.001
Number of visits	A-B	2.35	<0.001
	B-C	-0.98	0.006
	A-C	1.37	<0.001

<sup>1</sup> e.g.: A-B = estimated mean phase A- estimated mean phase B

**Conclusions** Total feeding time, number of visits and feed efficiency were reduced ( $P < 0.05$ ) during the outbreak of tail-biting. These finding could be an indicator of a putative increased level of stress. Restoring *ad libitum* feeding and removing the tail-biting initiator led to the end of the outbreak. Thus, one can conclude that feeding behaviour traits may be a potential forerunner indicator of tail-biting outbreaks.

### References

Sonoda, L. T., M. Fels, M. Oczak, E. Vranken, G. Ismayilova, M. Guarino, S. Viazzi, C. Bahr, D. Berckmans, and J. Hartung. 2013. 'Tail biting in pigs--causes and management intervention strategies to reduce the behavioural disorder. A review', Berl Munch Tierarztl Wochenschr, 126: 104-12.  
Taylor, N. R., D. C. Main, M. Mendl, and S. A. Edwards. 2010. 'Tail-biting: a new perspective', Vet J, 186: 137-47.

### Toward a homeostatic view of inflammation: The transition dairy cow example

B. Bradford and T. Swartz

Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS, USA

Email: [bbradfor@ksu.edu](mailto:bbradfor@ksu.edu)

**Introduction** Systemic inflammation, as quantified by circulating concentrations of positive acute phase proteins and inflammatory oxylipids, seems to occur in postpartum dams across species. This inflammatory state has often been interpreted to indicate poor health status during this period, and in many cases, potential adaptive roles of inflammatory signaling have not been considered.

**Materials and Methods** Several studies were conducted to either artificially enhance or to suppress inflammatory signaling in postpartum Holstein cows. Daily subcutaneous injection of 1.5 or 3.0 µg/kg body weight of bovine tumor necrosis factor α (TNFα) for the first 7 days postpartum was used to induce a subacute inflammatory state relative to control postpartum cows. Conversely, sodium salicylate (~125 g/day) or meloxicam (1 µg/kg body weight) were as non-steroidal anti-inflammatory drugs (NSAID).

**Results** Several observations from these studies point to homeostatic control of inflammatory tone in healthy cows, which may be a mechanism to keep downstream effects under control. For starters, removal of sodium salicylate treatment a week after parturition lead to a dramatic overshoot in inflammatory oxylipids in the subsequent week. Secondly, continuous infusion of TNFα into a subcutaneous adipose depot for 7 days led to a significant increase in the anti-inflammatory cytokine interleukin-10 in the contralateral adipose tissue. These counter-intuitive responses may be due to protection of an inflammatory set-point, potentially involving T cells, eosinophils, and macrophages in adipose tissue.

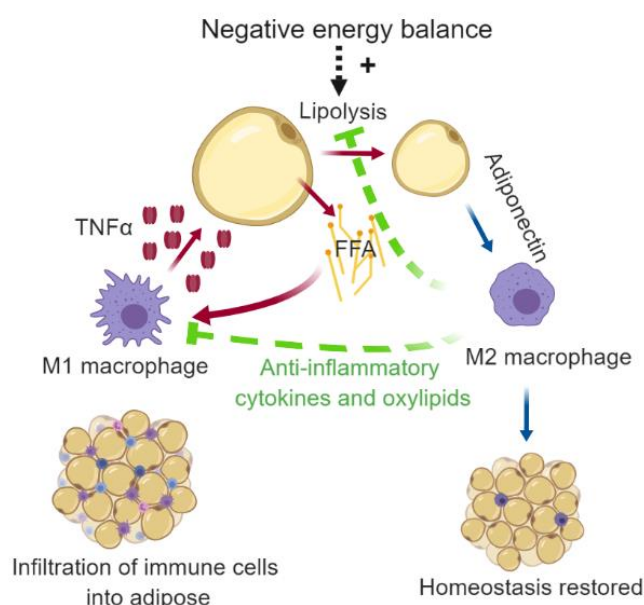
Recent evidence also suggests that peripartum inflammatory changes influence whole-body nutrient flux of dairy cows over the course of days and months. Inflammatory mediators can suppress appetite, even at levels that do not induce acute responses (e.g., fever), thereby decreasing nutrient availability. Pro-inflammatory signals generally promote lipolysis and other catabolic processes, consistent with metabolic changes required during disease and also with the extreme losses in body weight of unhealthy postpartum cows.

On the other hand, inhibition of inflammatory signaling with non-steroidal anti-inflammatory drug (NSAID) treatment suppresses hepatic gluconeogenesis, leading to hypoglycemia in some cases. Over the long-term, though, peripartum NSAID treatment substantially increases peak and whole-lactation milk synthesis by multiparous cows, although these mechanisms are not understood.

**Conclusions** Inflammatory regulation of nutrient flux may provide a homeorhetic mechanism to aid cows in adapting to the rapid changes in metabolic demand at the onset of lactation, but excessive systemic inflammation has negative effects on metabolic homeostasis through inhibition of appetite and promotion of immune cell activity. Overlapping regulation of immune responses and metabolism by inflammatory mediators, therefore, may provide a mechanistic underpinning for links between infectious and metabolic diseases in transition dairy cows, and points to novel approaches to management of this challenging phase of the production cycle.

### References

- Farney JK, Mamedova LK, Coetzee JF, KuKanich B, Sordillo LM, Stoakes SK, Minton JE, Hollis LC and Bradford BJ 2013. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. *Am J Physiol Regul Integr Comp Physiol* 305, R110–R117.
- Martel CA, Mamedova LK, Minton JE, Jones ML, Carroll JA and Bradford BJ 2014. Continuous low-dose infusion of tumor necrosis factor alpha in adipose tissue elevates adipose tissue interleukin 10 abundance and fails to alter metabolism in lactating dairy cows. *J Dairy Sci* 97, 4897–4906.
- Montgomery SR, Mamedova LK, Zachut M, Kra G, Häussler S, Vaughn M, Gonzalez J and Bradford BJ 2019. Effects of sodium salicylate on glucose kinetics and insulin signaling in postpartum dairy cows. *J Dairy Sci* 102, 1617–1629.
- Yuan K, Farney JK, Mamedova LK, Sordillo LM and Bradford BJ 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. *PLoS ONE* 8, e80316.



**Figure 1** Adipocyte interactions with resident immune cells likely regulate both lipolytic flux and influence whole-body inflammatory tone in postpartum dairy cows. Anti-inflammatory and resolving signals from adipose tissue may be responsible for the recovery of homeostasis within the first week or so after parturition in healthy cows.

### **Innovative insights in the interaction between metabolic stress and reproduction in high yielding dairy cows**

G. Opsomer

Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium

Email: [Geert.Opsomer@UGent.be](mailto:Geert.Opsomer@UGent.be)

In order to start lactation, dairy cows have to calve. The latter causes reproductive performance of a dairy herd to be the general driving force for milk production. Due to enduring and successful selection in combination with further optimization of the overall management, dairy farmers have been successful in increasing milk yield of our modern dairy cows. Daily milk yields of over 60 liters and 305-day productions of over 11.000 are rather common nowadays. The steep increase in milk production has for long time been felt to be associated with a significant decline in the reproductive capacity of the animals. Whether this is true or not is very difficult to scientifically research since herd management is a major confounding factor. However, most problems are stated to occur in adult, milk producing animals while reproductive capacity of nulliparous heifers seems relatively unaffected. The latter suggests a deleterious impact of the metabolic status related to the level of milk production.

Successful fertility necessitates easy calving and subsequent uterine involution, early resumption of ovarian activity, successful detection of the correct moment of insemination, easy fertilization and instant growth and development of the embryo, and full maintenance of pregnancy. In order to reach an economically sound calving interval, all these events should happen when the cow is confronted with the deleterious impact of metabolic stress. Metabolic stressors associated with the initiation and maintenance of lactation are known to perturb the finely tuned biological processes of the reproductive axis that are necessary for pregnancy establishment and maintenance.

Modern high yielding dairy cows have been said to have a higher risk to suffer from postpartal uterine diseases. Especially the incidence of subclinical endometritis has been proven to be up to 40% and even more than 25% at insemination, the latter being significantly associated with lower pregnancy results. Managing the inflammatory status of the cow around the moment of calving aiming for a quick immunological response that is sufficient to effectively clean the uterus following parturition, is a main challenge for modern dairy farmers. Besides the importance of calving ease and nutritional aspects to avoid overconditioned cows at calving and an overwhelming negative energy balance, recent studies suggest that the need for glucose to produce milk is that overwhelming in modern dairy cows, that it dominates the function and normal activity of immune cells. On the other hand, at least some cows seem to be confronted with an hyperinflammatory state in the peripartum period. This exorbitant inflammatory status starts in some cows already before calving and is further characterized by its prolonged duration, leading to almost 25% of cows suffering from subclinical endometritis at the moment of insemination.

Resumption of ovarian activity is the next challenge for the cow. This seems to be associated with the duration of the negative energy balance and is therefore generally retarded in high yielding animals. Besides its association with the negative energy balance, managerial factors like nutrition and genetics are known to be involved. Specific ovarian disturbances leading to an extended interval to first ovulation seem to be more prevalent in modern high yielding cows, although there is no general consensus concerning their pathogenesis.

A significant reduction in heat expression by the modern high yielding cow has been named a next significant challenge for the modern dairy farmer. Cows daily producing more than 40 liters of milk are more difficult to detect in heat and hence to inseminate at the correct moment to guarantee an adequate level of fertilization. Multiple underlying reasons have been proposed although without general consensus. Finally, different strategies from timed artificial insemination to the use of sensors to predict the ideal moment of insemination are currently applied.

Fertilization has so far not been indicated as a major problem in modern dairy cattle reproduction. When insemination is performed at the correct moment of the cycle, up to 85-90% of the oocytes will be fertilized. Main challenge however is the sufficient elongation of the young embryo in order to be able to reach enough endometrial cells and to produce a sufficient amount of interferon-tau to interrupt prostaglandin secretion. In up to 30% of the cases, embryos will die and cows will come back in heat. Reasons for this high number of embryo mortality are multiple among which, insufficient support of progesterone both before as well as after fertilization, low oocyte quality following confrontation with toxic metabolites and inflammatory mediators during final maturation, and a deleterious uterine environment are known to be the most important.

Currently, modern research is ongoing to study the effect of the microbiome present in the female genital tract including the uterus, on fertility results. Although the presence of bacteria in clinically health uteri seems to be rather low, differences in the abundance of specific phyla might be associated with the presence of (sub)clinical endometritis and hence with pregnancy results. Furthermore, in dairy cattle prenatal programming of postnatal reproductive capacity might be a next interesting topic to study, since dairy animals are still growing when pregnant as a young animal and produce large amounts of milk when pregnant as an adult.

### **Mechanisms and impact of inflammatory diseases on reproduction in dairy cows**

J. E. P. Santos<sup>1</sup> and E. S. Ribeiro<sup>2</sup>

Department of Animal Sciences, University of Florida, Gainesville, USA; <sup>2</sup>Department of Animal Biosciences, University of Guelph, Guelph, Canada

Email: Jepsantos@ufl.edu

Approximately 40% of the dairy cows develop one or more clinical diseases in the first 4 to 8 weeks of lactation and they represent 75 to 80% of all first diagnosis of disease in a given lactation. Many of the diseases that affect dairy cows are of inflammatory nature and have long-lasting impacts on reproduction. A conserved mechanism during disease is sickness behavior that cause hypophagia and consequent reduced nutrient intake. Of the important diseases that affect dairy cows, uterine and mammary gland inflammation are among the most common and both metritis and mastitis result in hyperthermia and systemic reactions that result in an acute phase response. Activation of the immune system and acute phase response is costly and alters nutrient partition to repair tissue and combat infection at the expense of tissue accretion, production, and reproduction. In dairy cows, activation of the immune system requires large quantities of glucose, which is in short supply in the first days postpartum. Induced inflammation extensively increases hepatic uptake of amino acids, which limits supply to other peripheral tissues, including the mammary gland. The alterations in nutrient partition induced by disease exacerbate the negative nutrient balance that cows undergo in early lactation. As consequence of negative nutrient balance, the uncoupling of growth-hormone and insulin-like growth factor 1 is extended and splanchnic metabolic signals that communicate nutritional status with hypothalamic centers are depressed. These changes compromise resumption ovarian follicular activity resulting in increased prevalence of anovular cows at the end of the voluntary waiting period. Acute disease compromises oocyte competence either because of hyperthermia or translocation of pathogen-associated molecular patterns that induce apoptosis of follicular cells and hastened progression of oocyte maturation. It's been suggested that acute uterine diseases might reduce the ovarian reserve and recent work with induced endometritis has shown compromised early embryonic development in dairy cows subjected to *in vitro* embryo production. Uterine disease causes tissue damage that leaves inflammatory signatures on reproductive tissues even after resolution of the disease. Inflammatory disease before the first postpartum insemination reduced fertilization and embryo development to the morula stage in lactating dairy cows, and impaired early conceptus development in the peri-implantation period with reduced secretion of interferon- $\tau$  into the uterine lumen. Concepti from cows diagnosed with disease had inflammation-like changes in the transcriptome, thereby suggesting that either local or systemic effects of disease in early lactation leave a legacy of impacts on the reproductive tract that eventually increase the risk of pregnancy loss. Controlling peripartum diseases is paramount for proper reproduction in dairy cows and that involves integration of multiple aspects of dairy cow management.

### Interaction between inflammation and metabolism in periparturient dairy cows

E. Trevisi, A. Minuti and M. Mezzetti

Department of Animal Sciences, Food and Nutrition (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy

Email: [erminio.trevisi@unicatt.it](mailto:erminio.trevisi@unicatt.it)

Immune system is made of a variety of cells, molecules and biological processes that interact each other to prevent or counteract biotic (i.e. microbial invasions) and abiotic (i.e. trauma, poisoning) stressors. Innate immunity is the primary defense line. Its activation is accompanied from the release of cytokines that regulate the inflammatory response against harmful stimuli. In normal condition, inflammatory processes occur at local level, allowing the recognition of foreign molecules, the elimination of existing sources of cellular injuries and the restoration of the normal functions of tissues once problem has been solved. In the last decades, severe alterations of innate immune system have been reported during the transition period (TP) of dairy cows. Bovine polymorphonuclear cells (PMN) have an altered abundance in mRNA transcripts encoding for functional genes between -1 and 2 weeks from calving, in comparison to the level found at 4 weeks after calving. A reduced immune competence has been widely assumed as the main phenomena affecting those cells in TP. In fact, PMN of transition cows have a lower production of reactive oxygen metabolites due to an impaired myeloperoxidase activity, and their chemotaxis and phagocytosis are impaired as well. Nevertheless, immunosuppression could not fully account for typical phenomena affecting bovine immune system during peripartum. In fact, inflammatory events occurring around calving suggest innate immune system to provide an effective response against stressors. Such inflammations commonly assume a systemic connotation in transition cows, suggesting an impaired capacity of immune system to restore homeostasis. Systemic inflammations imply an impairment of hepatic function driven by the acute phase response. During acute phase, the liver produces more  $\alpha$ -globulins, known as positive acute phase proteins (APP; i.e. haptoglobin, ceruloplasmin and serum amyloid alpha). Conversely, it reduces the syntheses of other proteins (i.e. albumin, retinol binding protein, paraoxonase and lipoproteins) known as negative APP and sequester minerals (as zinc and iron) from the blood. Systemic inflammation could induce fever and induce an anorexic stimulus as a side effect of the massive concentrations of cytokines circulating in the body, and an oxidative stress could occur due to impaired capacity of the liver to manage antioxidant compounds. Nevertheless, systemic inflammations are not reflected from any clinical symptoms in most of the animals. Despite that, systemic inflammations around calving could induce severe consequences in early lactation. Such consequences include an altered control of appetite, an increased likelihood in developing mastitis and a delayed resumption of ovarian function. Immune alterations could depend on a specific adaptation of immune cells to the sudden changes occurring in TP (i.e. alterations in endocrine asset, limitations of maternal immune responses against the allogeneic conceptus, alterations in energy metabolism with sudden utilization of body reserves and in reduction-oxidation balance). When peripartal alterations exceed the control of homeorhetic and homeostatic mechanisms a physiological imbalance (PI) condition could occur, aggravating the immune dysfunction around calving and increasing the duration of inflammatory processes. Genotype could also have a contribution in regulating inflammatory processes, as highly selected dairy cows seem to be less capable than autochthonous breeds to dampen massive inflammatory responses occurring in early lactation. PI conditions could arise from the repeated exposure to stressing events in peripartum. Among the most relevant stressing factors a frequent regrouping of the animals, an inadequate environment at calving time (i.e. overcrowding, climatic stress), nutritional upsets (i.e. unbalanced diets or feeds containing contaminants as mycotoxins) and a suboptimal physiological status in late gestating animals (i.e. excessive accumulation of body fat) could be listed. Sudden changes of diet occurring in peripartum are also involved in the development of inflammations. In fact, altered rumen and/or gut permeability that accompanies diet changes could allow the absorption of immunogenic compounds (i.e. lipopolysaccharides, ammines). A wide range of anti-inflammatory strategies has been listed to reduce negative effects of uncontrolled inflammations in TP. The post partal administration of nonsteroidal anti-inflammatory agents with mild side effects (i.e. aspirin) has been proposed to improve both performance and metabolism of cows. Wider nutritional strategies to optimize dairy cow's immunity during TP should be focused on reducing the PI degree related to calving, as this condition could be referred as a common denominator between immune dysfunction and diseases. In example, feeding cows with excessive amounts of energy during dry period could increase the deposition of body fat, inducing a severe mobilization of nonesterified fatty acids in early lactation. In these conditions, the severity of the acute phase response, as well as the likelihood of metabolic diseases in early lactation are known to increase. Moreover, nutrients as fatty acids are directly involved in immune modulation. Omega-3 and omega-6 fatty acids are precursors of oxylipids exerting both pro and antiinflammatory actions. An increased availability of dietary omega-3 fatty acids from late pregnancy to early lactation could improve the omega-3/omega-6 balance in the body, improving the immune regulation. Conjugated linoleic acid (CLA) is another immune modulator. The administration of cis-9 trans-11 and trans-10 cis-12 CLA isomers around calving reduce the inflammation and the negative energy balance in early lactation. Finally, plant extracts (i.e. *Aloe arborescens*, *Echinacea spp.*) and specific nutrients as methionine and choline, could further provide an aid in modulating immune system around calving, sorting beneficial effects on health and performances. In a wider perspective, the nutritional optimization could mitigate the immune alterations of transition cows. Despite that, minimize the occurrence of stressing events during the whole peripartal period could be the best strategy to alleviate immune dysfunctions and prevent overexuberant inflammations around calving.





### Ketosis in dairy cows: Prevention and therapy

S. Dänicke

Institute of Animal Nutrition, Braunschweig, Germany

Email: [sven.daenicke@fli.de](mailto:sven.daenicke@fli.de)

**Introduction** Ketosis in dairy cows is characterized by increased levels of circulating ketone bodies, particularly of beta-hydroxybutyrate (BHB), as a result of the inability of the liver to metabolize an excess of non-esterified fatty acids (NEFA) which is typically observed shortly after calving. In this period the energy intake of the cow increases more slowly than the milk energy output does ultimately resulting in a negative energy balance (NEB) and a stimulated lipolysis. Thus, the p.p. lipomobilization syndrome is accompanied by hyperketonaemia and hepatolipidosis (e.g. [1]). Based on the absence or presence of clinical symptoms in combination with the levels of BHB in blood circulation the hyperketonaemia is classified as either subclinical or clinical ketosis irrespective of pathogenesis. Most authors define a subclinical ketosis at BHB concentrations between  $\geq 1.2$  and  $\leq 2.0$  mMol/L although these thresholds should be considered a convention (e.g. [2-4]). Clinical signs are often paralleled by BHB concentrations of  $>2.0$  mMol/L although they might be absent even at higher BHB levels. Considering this classification it becomes clear that a medical treatment is mostly confined to clinical cases while subclinical cases usually are not discovered and consequently not treated. Preventive measures need to be implemented in view of the significant incidence of subclinical ketosis, its adverse impact on dry matter intake and milk yield, and its association with clinical ketosis and other production diseases, such as displaced abomasum, metritis and mastitis [2] but also with vaccination success ([5]). Based on the fact that a subclinical ketosis is at high risk to derail into a clinical ketosis prevention of the subclinical form concurrently reduces the incidence of clinical cases and should be the method of choice.

**Prevention** Preventive measures can be derived from factors putting cows at risk of development of a ketosis. Thus, energy intake shortly after calving should be stimulated while milk energy output could be decreased ultimately resulting in a less pronounced NEB. Energy intake is usually stimulated by gradual increase of the energy concentration of the ration within a period of approximately 2 weeks by means of increasing concentrate feed proportions. At the same time, high-quality roughage needs to be offered ensuring both a high energy concentration [6] and sufficient physically effective neutral detergent fibre (peNDF) (e.g. [7]). The latter is particularly important to stimulate rumination and to avoid ruminal disorders such as subacute ruminal acidosis (SARA). Other measures to improve the energy supply include optimization of the dietary starch content resistant to rumen degradation in a way that such an amount of ingested starch reaches the small intestine which can be digested and absorbed without flooding the hindgut by undegraded starch. As bovine gluconeogenesis relies on rumen originating propionate feedstuffs consisting of gluconeogenesis its precursors such as propylene glycol are widely used around calving, either as a part of the ration or as drench, in order to provide substrates for gluconeogenesis whereby ketogenic pathways of metabolic intermediates are downregulated. Not only an increased energy supply has the potential to decrease the magnitude of the NEB but also a decreased milk energy output. However, a decreased milk energy output during the first days after parturition by milking the cows only once a day is less popular because of long-persisting adverse effects on milk yield although this method is the most effective way to minimize p.p. NEB. Therefore, alternatives for decreasing the milk energy output are aimed at decreasing the energy concentration of the milk and at the same time maintaining the milk yield. The milk fat content can effectively be reduced by feeding conjugated linoleic acids (CLA) which has a marked effect on the energy content of milk due to the high gross energy concentration of fat compared to carbohydrates and proteins. However, literature results are inconsistent regarding the net effect of feeding CLA on NEB showing in some cases a parallel increase in milk yield or a decrease in dry matter intake (DMI).

Besides energy intake and milk energy output, the extent of mobilization of body reserves also influences the magnitude of NEB. However, particularly an excessive lipomobilization is known to trigger ketogenesis. Cows overconditioned at parturition are at higher risk for such a condition. Body condition score (BCS) is a suitable indicator for production, reproduction and health of cows. It is simple to determine and particularly the loss of body condition after calving, expressed in BCS points, is related to NEB [8]. It was suggested that for many production and health traits the association to BCS was non-linear [8] which might hinder its usefulness as a predictor for all nutritional and metabolic states. Nevertheless, the optimal BCS according to a 5-point-scale ranges between 3.0 to 3.25; a lower BCS is associated to a compromised production and reproduction whereas a BCS higher than 3.5 is related to a reduced dry matter intake shortly after parturition and to an increased susceptibility to metabolic diseases, most notably to ketosis [8]. Thus, avoiding a too high BCS prior to calving is one of the key elements in preventive feeding strategies. Based on the common practice to offer diets as total mixed rations (TMR) for ad libitum consumption through the whole production cycle it is not always feasible to feed individual cows or groups of cows according to a target BCS. Grouping might even become questionable when the herd is too small. An alternative might be offering partial mixed rations consisting of roughage and a small portion of concentrate feed while most of the concentrate feed is assigned individually according to actual performance and/or BCS through transponder regulated automatic feeders. Although such a way of feeding is close to precision feeding the technical and personal expenditure still needs to be optimized.

**Therapy** Therapeutic measures that are used to treat a clinical ketosis might include dextrose infusion, treatment with dexamethasone, insulin, a butaphophan-cyanocobalamin combination product, and further measures discussed above; particularly oral propylene glycol treatment (for review see [9]).

### References

- [1] Dänicke, S. *et al.*, Res Vet Sci, 2018. **116**: p. 15-27. [2] Suthar, V.S. *et al.*, J Dairy Sci, 2013. **96**(5): p. 2925-38. [3] Duffield, T.F. *et al.*, J Dairy Sci, 2009. **92**(2): p. 571-580. [4] Dirksen, G. *et al.*, Parey-Verlag, Berlin-Wien, 2002. [5] Drong, C. *et al.*, J Anim Physiol Anim Nutr, 2017. **101**(4): p. 791-806. [6] Schmitz, R. *et al.*, Arch Anim Nutr, 2018. **72**(2): p. 100-120. [7] Zebeli, Q. *et al.*, Livest Sci, 2010. **127**(1): p. 1-10. [8] Roche, J.R. *et al.*, J Dairy Sci, 2009. **92**(12): p. 5769-5801. [9] Gordon, J.L. *et al.*, Vet Clin North Am Food Anim Pract, 2013. **29**(2): p. 433-45.

### The effect of monensin on dairy cows in relation to farm or breed

P. Karis, K. Ling, H. Jaakson, L. Post and M. Ots

Estonian University of Life Sciences, The Chair of Animal Nutrition, Tartu, Estonia

Email: Priit.Karis@emu.ee

**Introduction** Ketosis is a common health problem for high-producing dairy cows at the beginning of lactation. The prevalence of subclinical ketosis in Europe is on average 25 % and it is costly for the farmers as the price is calculated to be 294 € per incident (Raboisson et al 2015). In European Union it is allowed to use monensin as a continuous-release intraruminal device for the reduction of incidence of ketosis in the peri-parturient dairy cow and heifer. Monensin is an antimicrobial agent that attaches to the cell membrane of mainly Gram-positive bacteria and interferes with their ion balance. The shift in the population of ruminal bacteria results in increased production of propionate, which is the main precursor for glucose production in ruminants. Our aim was to study the effect of monensin on high-yielding dairy cows on two farms with different breeds.

**Materials and Methods** The study was performed on two commercial farms (A, B) with multiparous cows fed grass silage based TMR. Annual milk yield per cow was 11400 kg on Farm A and 12400 kg on farm B. Rations metabolizable energy and protein content were as follows: close-up: Farm A – 10,4 MJ/kg, 85 g/kg; Farm B – 9,65 MJ/kg, 81 g/kg; lactation: Farm A - 11,9 MJ/kg, 102 g/kg; Farm B - 11,9 MJ/kg, 113 g/kg. On farm A 223 Estonian Holstein (EH) (Groups: Experimental – Mon, n=98; Control – Con, n=125) and on farm B 30 crossbreeds of EH and Estonian Red cows (Mon, n=15; Con, n=14) were enrolled. Monensin continuous-release device (Kexxtone; Elanco®, Bad Homburg, Germany) was administered according to the manufacturer's recommendations three weeks before calving to Mon cows on both farms. The average body condition score (BCS) at monensin administration was 3.4±0.40 on farm A and 3.5±0.32 on farm B. Blood samples were taken on days 1, 7, 14, 21, 28, 42 relative to calving and analysed spectrophotometrically for NEFA and BHB. Mixed linear model was fitted separately for either farm in program "R" considering the fixed effects of time, monensin, BCS, the interaction of time and monensin and the interaction of time and BCS, and the random effects of animal.

**Results** Time was a significant factor on both farms for both metabolites due to the dynamic use of body lipids postpartum. On Farm A monensin influenced the concentration of NEFA and the metabolite differed between groups on d21 (P=0.002). Thus, Mon cows used less body reserves at the beginning of lactation than Con cows. This brought along a noticeably lower BHB concentration in group Mon from d1 to d28 and overall and time specific monensin effect on BHB on farm A. Subclinical ketosis occurrence was also lower in Mon vs Con, 9% vs 3%, respectively. On the other hand, monensin effect was recorded neither on NEFA nor on BHB on farm B. The values of NEFA and BHB were numerically lower on farm B, except at calving, indicating a lower use of body lipids. BCS and its interaction model estimates (data not shown) for NEFA were greater compared to monensin on both farms. However, BCS influenced BHB on farm A, but not on farm B.

**Table 1** The dynamics of non-esterified fatty acids (NEFA) and  $\beta$ -hydroxybutyrate (BHB) concentrations in cows either treated with monensin (Mon) or not (Con) on two farms. Values are expressed as LSM. \* Indicate a difference within a farm (P<0.05).

		Day relative to calving						P-value					
Item	Group	1	7	14	21	28	42	SEM	Time	Monensin	BCS	Time x Monensin	Time x BCS
NEFA													
Farm A	Con	0.50	0.76	0.66	0.47*	0.40	0.21	0.04	<0.001	0.034	<0.001	0.066	0.006
	Mon	0.47	0.66	0.62	0.37*	0.36	0.21						
Farm B	Con	0.77	0.47	0.18	0.11	0.31	0.13	0.07	<0.001	0.367	0.008	0.434	0.032
	Mon	0.60	0.29	0.21	0.12	0.12	0.13						
BHB													
Farm A	Con	0.61*	0.82*	0.83*	0.77*	0.80*	0.53	0.05	<0.001	<0.001	0.002	0.004	0.117
	Mon	0.54*	0.61*	0.65*	0.64*	0.61*	0.55						
Farm B	Con	0.71	0.48	0.32	0.42	0.53	0.45	0.07	<0.001	0.694	0.457	0.420	0.908
	Mon	0.70	0.54	0.32	0.42	0.52	0.45						

**Conclusions** Breed or farm specific factors alter the effect of monensin. The use of monensin on farms with a low adipose tissue mobilization may not be profitable. Managing BCS on farm has a greater effect on body lipid mobilization than the use of monensin.

#### References

Raboisson, D., Mounié, M., Khenifar, E., Maigné, E. 2015. The economic impact of subclinical ketosis at the farm level: tackling the challenge of over-estimation due to multiple interactions. Prev. Vet. Med. 122:417–425.

**Ketone bodies in milk of Polish Holstein-Friesian cows in early lactation – a big data study**Z. M. Kowalski<sup>1</sup>, M. Sabatowicz<sup>1</sup>, W. Jagusiak<sup>2</sup>, P. Gorka<sup>1</sup> and A. Otwinowska-Mindur<sup>2</sup><sup>1</sup>Department of Animal Nutrition and Dietetics, University of Agriculture in Krakow, Poland; <sup>2</sup>Department of Animal Breeding, University of Agriculture in Krakow, Poland

Email: rzkowals@cyf-kr.edu.pl

**Introduction** The system of monitoring of subclinical ketosis (SCK) in Polish dairy herds was introduced into the practice on April 1, 2013. Briefly, this system is based on the concentration of milk ketone bodies (□ hydroxybutyrate (BHB) and acetone (ACE)), determined by FTIR method in test-day (TD) milk samples. Monthly, it considers about 90,000 milk recorded cows, which are within 6-60 days in milk (DIM). Since the start of a system, a big dataset has been collected on ketone bodies concentration in milk. In this study we present the data for Polish Holstein-Friesian cows, which comprise about 90% of milk recorded cows in Poland.

**Materials and Methods** The dataset was provided by Polish Federation of Cattle Breeders and Dairy Farmers. The dataset consisted of 3,857,160 TD milk samples collected in about 19,900 herds. The data were limited to a period from April 1., 2013 to March 31, 2017. The number of cows per herd ranged between 1 and 992, the average being 38. The number of samples per cow per lactation ranged from 1 to 2. Data were divided for primi- and multiparous cows and for two lactation stages (LS), i.e. 6-21 (1,186,181 milk samples) and 22-60 (2,670,979) DIM. Milk fat (F) and protein (P) were determined using the MilkoScanTM FT+ automatic milk analyzer (FOSS, Hillerod, Denmark). Milk BHB and ACE were determined by FTIR using the CombiFoss FT+ infrared spectrometer (FOSS, Hillerod, Denmark). To classify a cow as ketotic we used 3 criterions: ACE ≥ 0.150 mmol/L (A); BHB ≥ 0.100 mmol/L (B); ACE ≥ 0.150 mmol/L or BHB ≥ 0.100 mmol/L (C). Additionally, the Pearson's correlation coefficients between BHB, ACE, F, P, and F/P were calculated using the CORR procedure of SAS (2014).

**Results** According to the criterion (A, B, C), 18.9, 29.3, and 31.8% of milk samples were classified as originating from ketotic cows. Irrespective of criterion, the percentage of ketotic cows was higher in 6-21 than 22-60 DIM (Table 1). The difference between LS in percentage of ketotic cows was greater for primiparous cows. Although milk fat content, as well as F/P were higher for ketotic than non-ketotic cows (Table 2), the correlation coefficients between BHB or ACE and F or F/P were less than 0.45. The correlation coefficients between BHB or ACE and milk protein content were much lower (close to 0). BHB and ACE were highly correlated ( $r_2 = \sim 0.70$ ). However, the ratio of ACE to BHB differed between LS. It was much higher in milk samples collected within 6-21 DIM, especially in milk samples originated from ketotic cows.

**Table 1** Percentage of milk samples classified as originated from ketotic cows based on different criterions for ketosis

	All milk samples N / %	Criterion for ketosis		
		A <sup>1</sup>	B <sup>2</sup>	C <sup>3</sup>
All milk samples		%	%	%
6 – 60 DIM	3 857 160	18.9	29.3	31.8
6 – 21 DIM	1 186 181	30.9	37.5	42.2
22 – 60 DIM	2 670 979	13.6	25.7	27.2
Milk samples of primiparous cows				
6 – 60 DIM	32.9 % <sup>4</sup>	20.2	26.4	29.9
6 – 21 DIM	30.8 % <sup>5</sup>	35.0	37.0	43.4
22 – 60 DIM	69.2 % <sup>5</sup>	13.7	21.7	23.9
Milk samples of multiparous cows				
6 – 60 DIM	67.1 % <sup>4</sup>	18.2	30.7	32.8
6 – 21 DIM	30.8 % <sup>5</sup>	29.0	37.7	41.7
22 – 60 DIM	69.2 % <sup>5</sup>	13.5	27.6	28.8

<sup>1</sup>Milk samples with milk ACE ≥ 0.150 mmol/L<sup>2</sup>Milk samples with milk BHB ≥ 0.100 mmol/L<sup>3</sup>Milk samples with milk ACE ≥ 0.150 mmol/L or milk BHB ≥ 0.100 mmol/L<sup>4</sup>% of all milk samples<sup>5</sup>% within parity class**Table 2** Milk ACE, BHB, F, P, and F/P in milk samples originated from non-ketotic<sup>1</sup> and ketotic<sup>2</sup> cows (criterion C)

	All	Non-ketotic	Ketotic
ACE, mmol/L			
6 – 60 DIM	0.098	0.036	0.229
6 – 21 DIM	0.143	0.043	0.279
22 – 60 DIM	0.077	0.034	0.194
BHB, mmol/L			
6 – 60 DIM	0.081	0.035	0.178
6 – 21 DIM	0.095	0.036	0.175
22 – 60 DIM	0.074	0.035	0.180
ACE/BHB			
6 – 60 DIM	1.21	1.03	1.29
6 – 21 DIM	1.51	1.19	1.59
22 – 60 DIM	1.04	0.97	1.08
F, % <sup>3</sup>	4.03	3.74	4.65
P, % <sup>3</sup>	3.04	3.03	3.07
F/P <sup>3</sup>	1.33	1.24	1.53

<sup>1</sup>ACE ≤ 0.150 mmol/L or BHB ≤ 0.100 mmol/L<sup>2</sup>ACE ≥ 0.150 mmol/L or BHB ≥ 0.100 mmol/L<sup>3</sup>6-60 DIM

**Conclusions** The results of a present study show that the prevalence of SCK in Poland is high. Especially high prevalence of SCK within 6-21 DIM in primiparous cows should be considered in the heifer rearing programmes. Our big data study confirms poor usefulness of determination of milk fat content or milk fat to protein ratio, and especially milk protein content in monitoring of SCK. Different ACE to BHB ratio in milk samples in 6-21 and 22-60 needs further studies.

### Do *Fusarium* toxin contaminated diets have an impact on dairy cow health and performance?

S. Carp - van Dijken, G. Hop, G. Counotte, K. van den Broek, K. Junker and A. Velthuis

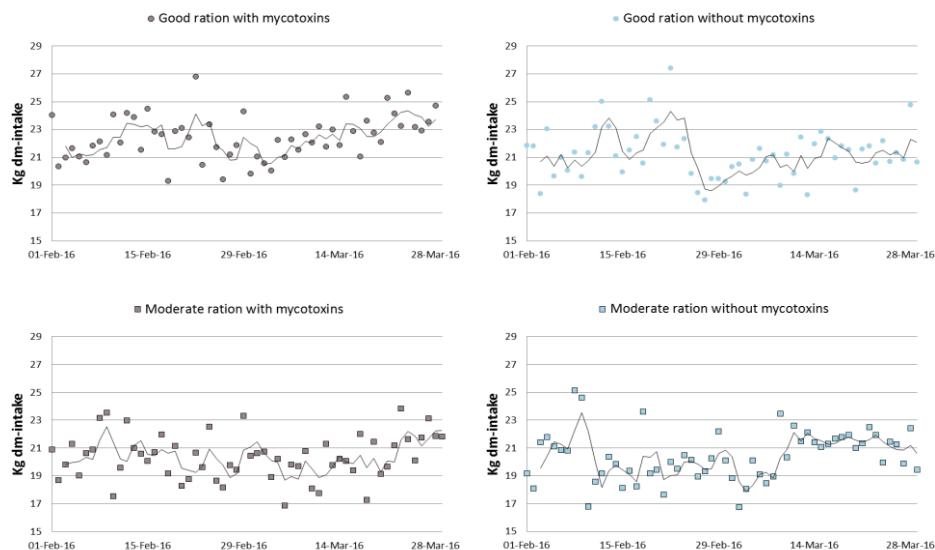
GD Animal Health, Deventer, The Netherlands

Email: s.v.dijken@gddiergezondheid.nl

**Introduction** Mycotoxins are metabolic products of fungi and are regularly found in silage. In Western Europe mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) are found most in feed, often simultaneously and especially in maize silage. DON and ZEA are produced by the *Fusarium* species of fungi and are generally field-derived mycotoxins, which development is strongly influenced by weather conditions. Ruminants are regarded as less susceptible to mycotoxins than monogastric animals, mainly due to the degradation of mycotoxins in the rumen. However, in literature several causes are mentioned which could undermine the resistance of ruminants to mycotoxins. The presence of mycotoxins and a lower quality of roughage are often simultaneously observed, which makes it difficult to investigate if cow health problems that farmers encounter are related to either mycotoxins or a moderate roughage quality. This study aimed to determine the direct effects of intake of the mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) through naturally contaminated maize on dairy cow health and performance during an eight-week period. This study specifically looked at the effects of DON and ZEA through two different silage qualities, using an experimental block design.

**Materials and Methods** After a four-week period of acclimatization, 32 high-yielding, pluriparous (parity 2 or 3) Holstein Friesian dairy cows in the first 70 days of lactation and free of antibodies against IBR, BVDV, neospora and paratuberculosis were divided into four groups. They received a total mixed ration based on good quality silage (good ration: GR) or moderate quality silage (moderate ration: MR) with mycotoxins (MT+) or without (MT-) for eight weeks. On average, MT+ rations contained 789 µg DON and 460 µg ZEA per kg ration. The following parameters were collected and analyzed: parameters related to the overall animal health, production characteristics, rumen function and digestion, energy and protein supply, kidney damage and function, liver damage and function, resilience and inflammation, fertility, and pathologic findings. Descriptive statistics and rank-sum tests were used to test for statistically significant differences among the four groups.

**Results** Blood and milk research, clinical examinations of the animals and necropsy of the GRMT+ and MRMT+ cows did not reveal systematic effects of DON and ZEA on cow health and performance. Some parameters differed significantly between the MT+ and MT- groups, e.g. milk production, protein, fat and lactose content and various blood parameters. However, these parameters differed throughout the entire trial period and are, therefore, expected to be caused by the group composition. A few parameters were significantly different between the GR- and MR-groups, e.g. a lower feed intake and a less efficient digestion of feed.



**Conclusions and recommendations** In this study the composition of the ration and the feed quality appeared to have more influence on cow health and performance compared to the presence of the mycotoxins DON and ZEA in the rations. Since this study did not reveal any direct effect of the mycotoxins DON and ZEA on cow health, in case of animal health problems combined with mycotoxins in silage it is recommended to first look at improving the ration and other management factors before pointing at the mycotoxins DON and ZEA as cause for the experienced problems.

#### References

- Dänicke, S., et al 2005. Effects of *Fusarium* toxin-contaminated wheat grain on nutrient turnover, microbial protein synthesis and metabolism of deoxynivalenol and zearalenone in the rumen of dairy cows. *Journal of Animal Physiology and Animal Nutrition* 89(9-10):303-315.
- Driehuis, F et al, 2008a. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *Journal of dairy science* 91(11):4261-4271.
- Driehuis, F., M. C. Spanjer, J. M. Scholten, and M. C. Te Giffel. 2008b. Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. *Food additives & contaminants. Part B, Surveillance* 1(1):41-50.

### Effects of pre-transport diet, transport duration and type of vehicle on metabolism and immunity of young veal calves

F. Marcato<sup>1,2</sup>, H. van den Brand<sup>1</sup>, B. Kemp<sup>1</sup>, B. Engel<sup>3</sup>, M. Wolthuis-Fillerup<sup>2</sup> and K. van Reenen<sup>2</sup>

<sup>1</sup>Adaptation Physiology Group, Department of Animal Sciences, Wageningen University & Research, The Netherlands; <sup>2</sup>Livestock Research, Wageningen University & Research, The Netherlands; <sup>3</sup>Biometris, Wageningen University & Research, The Netherlands  
Email: francesca.marcato@wur.nl

**Introduction** Transport is one of the largest challenges faced by veal calves. The first transport occurs when calves are transported from the dairy farms to a collection center at 14-20 days of age. Then, with a second transport, calves are brought to the veal farms. During transport and at the collection center, calves are mixed and exposed to new environmental conditions and microorganisms. Consequently, transport is associated with a high level of stress and an increase in infectious disease incidence. As a consequence of stress, the metabolism, immunity and health of calves might be compromised. Previous studies explored effects of transport on hematological, metabolic and immunological variables of calves. However, these studies focused just on one single factor (e.g. transport duration or pre-transport nutrition) and not on a combination of multiple factors associated with transport. The current research aimed to investigate effects of a pre-transport diet, transport duration and type of vehicle on metabolic and immunological variables of young calves upon arrival at the veal farm.

**Materials and Methods** The experiment was set up as a  $2 \times 2 \times 2$  factorial arrangement with 3 factors: 1) provision of breeding milk or electrolytes prior to transport to the veal farm; 2) transport duration (6 or 18 h) and 3) type of vehicle (open truck or conditioned truck). This resulted in 8 treatments. A total of 368 Holstein-Friesian and cross-bred calves ( $18 \pm 4$  days;  $45.3 \pm 3.3$  kg body weight (BW)) were randomly assigned to the different treatments and transported in two consecutive batches from a collection center to a veal farm. Blood samples were collected from calves before transport, immediately after transport (0 h) and 4 h, 24 h, 48 h, wk 1, wk 3 and wk 5 post-transport. Blood was analyzed for glucose, urea, lactate, non-esterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate ( $\beta$ -HB), creatine kinase (CK), albumin, total protein, osmolality, calcium, sodium, magnesium, cortisol, bilirubin, haptoglobin, IgG, IgM. Body weight, rectal temperature (RT) and skin elasticity were determined prior and post-transport. Data on immediate post-transport and differences between pre- and post-transport values were analyzed with PROC MIXED and GLIMMIX procedures used for continuous data and proportional data, respectively. The model included batch, position in the truck, pre-transport diet, transport duration and type of vehicle. Three-way and two-way interactions between pre-transport diet, transport duration and type of vehicle were also specified in the model. Data determined over time were analyzed with the same procedures with time as additional fixed effect and also including two-way interactions between time and pre-transport diet, transport duration, and type of vehicle.

**Results** Immediately post-transport, calves fed with milk or electrolytes and transported for 18 h did not differ in plasma glucose concentrations or serum NEFA concentrations. However, after 6 h transport, calves fed with milk had higher plasma glucose and lower serum NEFA concentrations ( $4.71 \pm 0.14$  mmol/l and  $586.5 \pm 54.8$   $\mu$ mol/l, respectively) than calves fed with electrolytes ( $3.56 \pm 0.14$  mmol/l and  $916 \pm 54.8$   $\mu$ mol/l, respectively) ( $P < 0.01$ ). Plasma urea concentration did not differ in milk-fed or electrolyte-fed calves transported for 6 h. In contrast, calves fed with milk had lower urea concentrations ( $5.40 \pm 0.42$  mmol/l) than calves fed with electrolytes ( $7.38 \pm 0.42$  mmol/l) after 18 h transport ( $P = 0.04$ ). In addition, after 6 h transport, milk-fed calves gained weight ( $\Delta = 0.41$  kg), whereas electrolyte-fed calves lost weight ( $\Delta = -0.16$  kg) ( $P = 0.03$ ). After 18 h transport, both milk-fed and electrolyte-fed calves showed BW losses ( $\Delta = -0.67$  kg,  $\Delta = -0.74$  kg, respectively). Calves fed with either milk or electrolytes and transported for 18 h had an increase in plasma bilirubin concentrations upon arrival at the veal farm ( $\Delta = 1.83$   $\mu$ mol/l and  $\Delta = 1.50$   $\mu$ mol/l, respectively). The increase in bilirubin concentrations was also found in calves fed with electrolytes after 6 h transport ( $\Delta = 3.64$   $\mu$ mol/l), whereas calves fed with milk and transported for 6 h showed a decrease in bilirubin concentration in the immediate post-transport ( $\Delta = -3.75$   $\mu$ mol/l) ( $P = 0.03$ ). An interaction between transport duration and time was found for most of the blood metabolites. Concentrations of NEFA and  $\beta$ -HB reached the maximum values at 0 h and then they decreased until wk 5 post-transport. At 0 h, the increase in NEFA and  $\beta$ -HB concentrations was less pronounced in calves transported for 6 h ( $746.1 \pm 20.6$   $\mu$ mol/l and  $0.38 \pm 0.06$  mmol/l, respectively) than in calves transported for 18 h ( $850.6 \pm 20.6$   $\mu$ mol/l and  $0.50 \pm 0.06$  mmol/l) ( $P < 0.001$ ). Upon arrival at the veal farm, calves transported for 18 h showed a higher amount of serum cortisol ( $11.63 \pm 0.64$  ng/ml), whereas calves transported for 6 h showed a lower amount of cortisol ( $7.95 \pm 0.64$  ng/ml) ( $P = 0.012$ ). After 0 h, concentrations of cortisol decreased until wk 5 in calves transported for both 6 h or 18 h.

**Conclusions** The results of this study showed that feeding milk at the collection centre and a short transport duration exert the most beneficial effects on calf metabolic and immune data. In addition, this study showed that metabolic and immunological changes occur on a short term after transport and they do not persist in the longer term. However, effects of health and performance upon slaughter age need to be evaluated.



### Effect of energy restriction during a one-phase dry period on the productive, metabolic and endocrine status of dairy cows

M. Elsabagh<sup>1,2</sup>, T. Hasunuma<sup>3</sup>, K. Kawashima<sup>4</sup>, S. Sato<sup>5</sup>, S. Kushibiki<sup>6</sup>, T. Obitsu<sup>1</sup> and T. Sugino<sup>1</sup>

<sup>1</sup>Graduate School of Biosphere Science, Hiroshima University, Japan; <sup>2</sup>Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt; <sup>3</sup>Toyama Prefectural Livestock Research Center, Toyama, Japan; <sup>4</sup>Chiba Prefectural Livestock Research Center, Chiba, Japan; <sup>5</sup>Faculty of Agriculture, Iwate University, Japan; <sup>6</sup>National Institute of Livestock and Grassland Science, Tsukuba, Japan

Email: mabrouk.elsabagh@vet.kfs.edu.eg

**Introduction** Modern dairy cows possess high genetic merit for milk yield, but not for dry matter intake (DMI) and health traits (Weigel, 2015). The stress of the high production increases metabolic disorders incidence, particularly during the transition period (3 weeks prepartum to 3 weeks postpartum) where major metabolic and endocrine changes occur as cow progresses from pregnancy into lactation (Sundrum, 2015). In early lactation, cows are in a state of negative energy balance (NEB) because the DMI does not meet the increasing energy demand for milk production. To provide additional energy, the NEB induces lipid mobilization that elevates non-esterified fatty acids (NEFA) and  $\beta$ -hydroxybutyric acid (BHBA) levels in the blood. With further NEB, excessive fat infiltration occurs in the liver compromising liver functions and progressing to fatty liver and ketosis that may be complicated by various metabolic and productive diseases leading to major economic losses in the dairy industry (Sundrum, 2015).

Limited prepartum energy intake has been indicated to improve the postpartum DMI and energy balance. However, most research focused on feeding management during the far-off and close-up periods independently of each other. Feeding diets of low energy density during the whole dry period confer more benefits to cow's health and performance (Beever, 2006; Wang et al., 2017). However, little information is available on the one-phase dry period feeding and more research is needed to further confirm the benefits of this feeding strategy. Therefore, this study aimed to evaluate the production, metabolic health and endocrine status of multiparous high producing dairy cows fed two diets of differing energy levels in a one-phase dry period of 6 weeks.

**Materials and Methods** Twenty healthy multiparous Holstein dairy cows (body weight (BW):  $698.4 \pm 16.9$  kg, body condition score (BCS):  $3.13 \pm 0.10$ ) in the dry period were randomly divided into two groups and fed either high or low energy diet for 6 weeks prepartum: 110 or 90% of metabolizable energy (ME) requirements, respectively. Thereafter, cows were fed a diet of the same energy for lactation ( $NE_L: 1.52$  Mcal/kg of DM) for 8 weeks postpartum. Cows were fed twice a day (06:00 and 18:00) during the dry period and *ad libitum* during the lactation period and were milked twice a day. Changes in DMI, BW, BCS and milk yield and composition were monitored during the experimental period. Blood samples were collected at -3, 0, 2, 4, and 6 weeks relative to the calving time, and were assayed for metabolites and hormones. Data were analyzed using the MIXED procedure of SAS 9.4 and significance was declared at  $P \leq 0.05$  using the Student's t-test.

**Results** The prepartum DMI was greater in the high than in the low ME group (11.9 vs 10.4 kg/d,  $P < 0.0001$ ) but the low ME group surpassed the high ME one in the DMI from the 1<sup>st</sup> to 3<sup>rd</sup> week postpartum (22.0 vs 18.7 kg/d,  $P = 0.002$ ) with no change thereafter. The BW did not change, but the BCS tended to decrease in the low ME group from the 1<sup>st</sup> to 3<sup>rd</sup> week postpartum (2.97 vs 3.08,  $P = 0.101$ ). Milk yield did not differ between the high (42.1 kg/d) and the low (43.2 kg/d) ME diets from the 1<sup>st</sup> to the 8<sup>th</sup> week postpartum. Milk protein was higher (3.47 vs 3.24%,  $P = 0.027$ ) but milk fat tended to be lower (4.21 vs 4.63%,  $P = 0.110$ ) in the low ME group from the 1<sup>st</sup> to 3<sup>rd</sup> week postpartum whereas the solid-not fat was greater (8.75 vs 8.56%,  $P = 0.001$ ) in the low ME group from the 1<sup>st</sup> to the 8<sup>th</sup> week postpartum compared to the high ME group. The feed efficiency ratio (FER) tended to be lower (1.20 vs 1.32,  $P = 0.110$ ) in the low ME group from the 1<sup>st</sup> to 3<sup>rd</sup> week postpartum with no change thereafter. A remarkable increase in milk fat (5.29 vs 3.98%,  $P = 0.001$ ) and FER (1.51 vs 1.14,  $P = 0.05$ ) was observed at the 2<sup>nd</sup> week postpartum in the high vs the low energy group. There were no noticeable changes between the two groups in plasma growth hormone, insulin, glucagon and insulin-like growth factor-1 concentrations in both pre- and postpartum. Similarly, there were no significant differences in plasma metabolites (glucose, NEFA, BHBA, triglycerides and total cholesterol) and liver enzymes (Aspartate aminotransferase and Gamma-glutamyl transferase) concentrations, except an increase in serum albumin (4.07 vs 3.88 g/dL,  $P = 0.001$ ) and blood urea nitrogen (BUN: 12.44 vs 10.9 mg/dL,  $P = 0.030$ ) in the low energy group compared to the high energy one during the period from the 4<sup>th</sup> to the 8<sup>th</sup> week postpartum. However, the circulating albumin and BUN levels in the low ME group were within the normal range for healthy cows.

**Conclusions** In conclusion, prepartum limited energy feeding in a one-phase dry period for 6 weeks could improve the postpartum DMI and energy balance without compromising the transition cow's metabolic health and performance.

#### References

- Beever, DE. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. *Anim Reprod Sci* 96, 212-226.
- Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. *Animals* 5, 978-1020.
- Wang, Y., Hou, Q., Cai, G., Hu, Z., Shi, K., Yan, Z., Lin, X. and Wang, Z. 2017. Effects of dietary energy density in the dry period on the production performance and metabolism of dairy cows. *Adv Biosci Biotechnol* 8, 104-126.
- Weigel, KA. 2015. Genetic improvement programs for dairy cattle. In: *Molecular and quantitative Animal Genetics*. Hasan Khatib (ed.). John Wiley & Sons, Inc., Hoboken, New Jersey.

# Effect of over-conditioning around calving on the mRNA abundance of genes related to the mitochondrial protein import machinery in the liver of dairy cows

M. H. Ghaffari<sup>1</sup>, M. T. Alaedin<sup>1</sup>, K. Schuh<sup>1,2</sup>, H. Sadri<sup>3</sup>, C. Koch<sup>4</sup>, D. Fritzen<sup>2</sup> and H. Sauerwein<sup>1</sup>

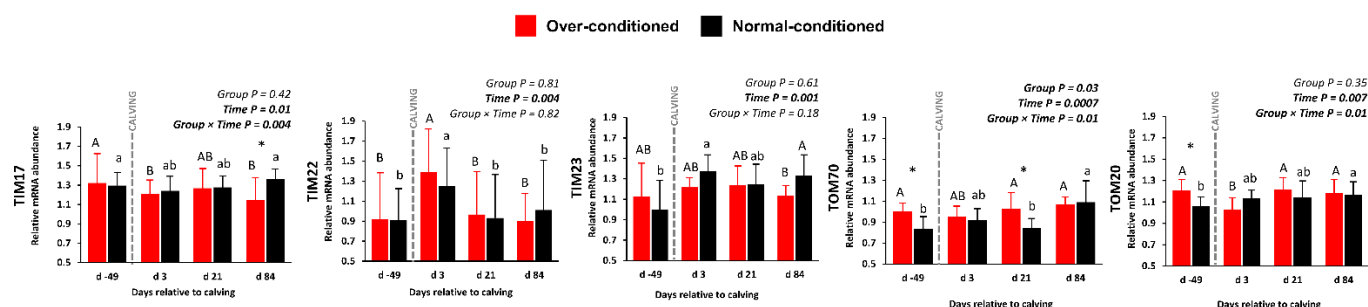
<sup>1</sup>Institute of Animal Science, University of Bonn, Germany; <sup>2</sup>Department of Life Sciences and Engineering, Animal Nutrition and Hygiene Unit, University of Applied Sciences, Bingen, Germany; <sup>3</sup>Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Iran; <sup>4</sup>Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany

Email: mhossein@uni-bonn.de

**Introduction** Mitochondria contain more than 1,000 different proteins, but the majority of them (99%) is encoded by nuclear genes and not by the mitochondrial genome. The nuclear-encoded proteins are transferred from the cytosol into the mitochondria via the mitochondrial protein import machinery (MPIM). Translocation of precursor proteins into the mitochondrial matrix occurs through assembled protein complexes in the outer (TOM complex) and inner (TIM complex) membranes<sup>1</sup>. The MPIM is regulated at multiple levels in response to e.g., energetic status and cellular stress conditions (1). During the transition from late gestation to lactation comprehensive metabolic and endocrine changes occur in dairy cows. Cows with high body condition score (BCS) are less successful in adapting their metabolism to lactation and are at greater risk for developing production diseases. With this backgrounds, our objectives were: (A) to assess the hepatic mRNA abundance of key components of MPIM including TOM complex (TOM70 and TOM20) and TIM complex (TIM17, TIM22, and TIM23) in cows during the transition period, and (B) to compare the mRNA abundance of these genes in over versus normal-conditioned cows during this time.

**Materials and Methods** Fifteen weeks antepartum, multiparous Holstein cows were allocated to two groups that were fed differently to reach either high- [(HBCS; 7.2 NEL MJ/kg dry matter (DM)] or normal BCS (NBCS; 6.8 NEL MJ/kg DM) at dry off. The targeted BCS and backfat thickness (BFT) at dry-off (HBCS: >3.75 and >1.4 cm; NBCS: <3.5 and <1.2 cm) were reached. Thereafter, both groups were fed identical diets. The allocation was also based on differences in BCS in the previous and the ongoing lactation as described earlier<sup>2</sup>. Liver biopsies (n = 10/group) were collected at d -49, +3, +21, +84 relative to calving. Total RNA was extracted using the Qiagen reagent (Qiagen, Hilden, Germany) and cDNA was synthesized. Expression of the targeted genes in liver was assayed by RT-qPCR on a 96.96 gene expression chip for use in the BioMark HD instrument (both from Fluidigm, San Francisco, USA) in accordance with the MIQE guidelines<sup>3</sup>. The mRNA data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The model included the random effect of cow and the fixed effects of group, time, and their interaction (group × time).

**Results** As shown in Figure 1, the mRNA abundance of TIM17 in the liver at d +84 was lower in HBCS compared to NBCS cows ( $P < 0.05$ ), whereas TIM22- and TIM23-mRNA were affected only by time and no group or group × time interaction was observed. The mRNA abundance of the two major receptor subunits of the TOM complex (TOM20 and TOM70) were affected by group and time (Figure 1). The mRNA abundance of TOM70 at d -49 and d +21 and TOM20 at d -49 in liver was greater in HBCS compared to NBCS cows ( $P < 0.05$ ).



**Figure 1** The mRNA abundance of genes related to the mitochondrial protein import machinery in the liver of normal- (NBCS) and over-conditioned (HBCS) cows (n = 10 per group). Different letters indicate differences ( $P < 0.05$ ) between the time points in the HBCS (A, B), and in the NBCS (a, b) group, respectively. Asterisks indicate a significant difference ( $P < 0.05$ ) between HBCS and NBCS at a given time-point.

**Conclusions** We observed for the first time that the transition from late pregnancy to early lactation as well as over-conditioning around calving leads to regulation of the genes involved in the MPIM in the liver of cows. The results suggest that the TOM complex, which functions as import receptors may be more stimulated in over-conditioned cows as indicated by the changes in the mRNA abundance of TOM20 and TOM70.

## References

- (1) Harbauer, A.B. et al. 2014. Cell Metabolism. 19: 357-372.
- (2) Schuh et al., 2019. Animal. 7:1-11.
- (3) Bustin, S.A., et al. 2009. Clin. Chem. 55: 611-622.



## Effect of over-conditioning around calving on carnitine palmitoyltransferase gene expression in the liver and skeletal muscle of dairy cows

M. H. Ghaffari<sup>1</sup>, M. T. Alaedin<sup>1</sup>, K. Schuh<sup>1,2</sup>, I. Hofs<sup>1</sup>, D. Fritzen<sup>2</sup>, C. Koch<sup>3</sup>, H. Sadri<sup>4</sup> and H. Sauerwein<sup>1</sup>

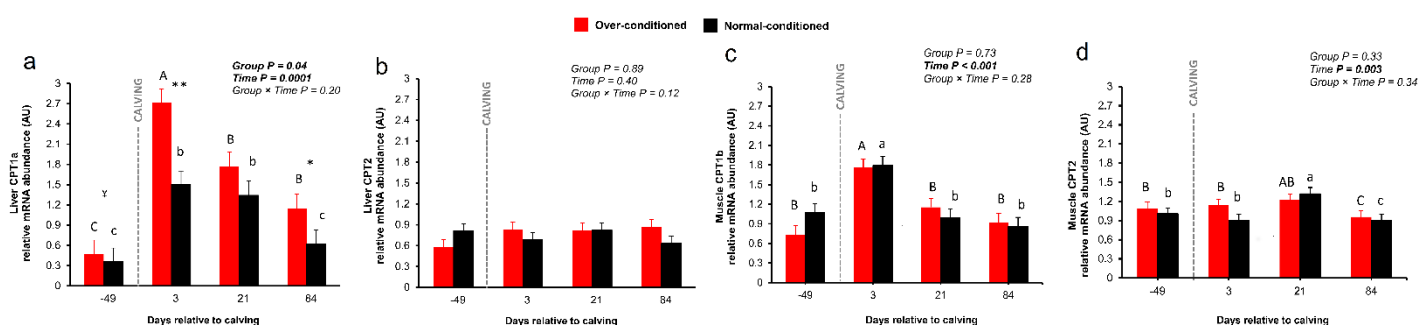
<sup>1</sup>Institute of Animal Science, University of Bonn, Germany; <sup>2</sup>Department of Life Sciences and Engineering, Animal Nutrition and Hygiene Unit, University of Applied Sciences, Bingen, Germany; <sup>3</sup>Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler an der Alsenz, Germany; <sup>4</sup>Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Iran

Email: mhossein@uni-bonn.de

**Introduction** The adaptation to the metabolic changes in early lactation carries some risks for metabolic disorders postpartum (pp) in dairy cows<sup>1</sup>. For generating energy, long-chain fatty acids (FA) need to pass into the mitochondrial matrix through a carnitine-dependent transport shuttle. Incomplete oxidation of long-chain FA due to impaired activity of carnitine palmitoyltransferase (CPT) or to depletion of TCA cycle intermediates may increase the levels of long-chain acylcarnitines (AcylCN)<sup>2</sup>, which indicate defects in FA oxidation (FAO)<sup>3</sup>. Likewise, in over-conditioned dairy cows, long-chain AcylCN increases around parturition and support that excessive lipolysis and incomplete FAO occurs in these animals. With this background, our objectives were: (A) to assess the mRNA abundance of carnitine acyltransferases, CPT1a (liver), CPT1b (muscle) and CPT2 (liver and muscle) in dairy cows during the transition from late gestation to lactation, and (B) to compare the mRNA abundance of these genes in over versus normal-conditioned cows during this time.

**Materials and Methods** Fifteen weeks antepartum, multiparous Holstein cows were allocated to two groups that were fed differently to reach either high- [(HBCS; 7.2 NEL MJ/kg dry matter (DM)] or normal BCS (NBCS; 6.8 NEL MJ/kg DM) at dry off. The targeted BCS and backfat thickness (BFT) at dry-off (HBCS: >3.75 and >1.4 cm; NBCS: <3.5 and <1.2 cm) were reached. Thereafter, both groups were fed identical diets. The allocation was also based on differences in BCS in the previous and the ongoing lactation as described earlier<sup>4</sup>. Liver (n = 10/group) and skeletal muscle (M. semitendinosus, n = 18/group) biopsies were collected at d -49, +3, +21, +84 relative to calving. Total RNA was extracted from the tissue samples and cDNA was synthesized. Expression of target genes in liver was assayed by RT-qPCR using microfluidics integrated fluidic circuit chips (Fluidigm, San Francisco, CA, USA) and in skeletal muscle using an Mx3000P cycler (Agilent, Santa Clara, CA) in accordance with MIQE guidelines<sup>5</sup>. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model included the random effect of cow and the fixed effect of the main factors group, time, and their interaction (group × time).

**Results** The mRNA abundance of CPT1a in the liver was greater (at d 3 and d 84 pp) in HBCS compared to NBCS cows ( $P < 0.05$ , Figure 1a), whereas those of CPT2 was not affected by group and time (Figure 1b). No differences in the mRNA abundance of CPT1b and CPT2 in skeletal muscle were observed between the groups (Figure 1c, d), but they were changed over time. The mRNA abundance of CPT1a and CPT1b increased from day -49 to 3 pp ( $P < 0.001$ ), but decreased thereafter in both tissues. The mRNA abundance of CPT2 in muscle increased on day 21 pp.



**Figure 1** The mRNA abundance of CPT1 and CPT2 in the liver and skeletal muscle of normal- (NBCS) and over-conditioned (HBCS) cows. Different letters indicate differences ( $P < 0.05$ ) between the time points in the HBCS (A, B), and in the NBCS (a, b) group, respectively. Symbols indicate differences ( $0.05 < P \leq 0.10$ ; \*  $P < 0.05$ ) between HBCS and NBCS at a given time-point.

**Conclusions** Up-regulation of the mRNA abundance of CPT1a in the liver with a greater extent in HBCS cows and of CPT1b in muscle from the day -21 to day 1 may suggest a physiological increase in the capacity of long-chain fatty acyl-CoA entry into liver and muscle mitochondria around parturition. However, the observed upregulation of CPT1 expression was not accompanied by the increase of CPT2 expression. Thus, these data may suggest that post-CPT1 events, including deficiency or impaired in CPT2 function and TCA cycle flux may causally contribute to the accumulation of AcylCN in serum around parturition.

## References

- Drackley, J. K., et al. 2005. Ital. J. Anim. Sci. 4: 323-344.
- Mihalik, S. J., et al. 2010. Obesity. 18: 1695-1700.
- Ramos-Roman, M. A., et al. 2012. Metabolism. 61: 202-212.
- Schuh, K., et al., 2019. Animal. 7:1-11.
- Bustin, S.A., et al. 2009. Clin. Chem. 55: 611-622.

### Impact of essential fatty acids and conjugated linoleic acid on acute phase response and antioxidative status of transition dairy cows fed an n-3 fatty acid reduced diet

M. Gnott<sup>1</sup>, L. Vogel<sup>1</sup>, A. Starke<sup>2</sup>, A. Tröscher<sup>3</sup>, E. Trevisi<sup>4</sup>, U. Bernabucci<sup>5</sup>, T. Stefaniak<sup>6</sup> and H. M. Hammon<sup>1</sup>

<sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; <sup>2</sup>University of Leipzig, Clinic for Cattle and Swine, Leipzig, Germany; <sup>3</sup>BASF SE, Limburgerhof, Germany; <sup>4</sup>Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy; <sup>5</sup>Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia, Viterbo, Italy;

<sup>6</sup>Faculty of Veterinary Medicine, University of Environmental and Life Science, Wrocław, Poland

Email: hammon@fbn-dummerstorf.de

**Introduction** Modern dairy cow rations are commonly based on corn silage (CS), which results in a reduced availability of essential fatty acids (EFA), especially  $\alpha$ -linolenic acid (ALA), and conjugated linoleic acid (CLA) (Weber et al., 2016). Furthermore, the increased metabolism and physiological inflammatory processes around parturition expose dairy cows to greater oxidative stress (Bernabucci et al., 2005). EFA and CLA are known for their health promoting and antioxidative properties and an imbalanced or reduced intake may contribute to a more severe inflammatory response in early lactation. The present study investigated the effects of abomasal supplementation of EFA, in particular ALA and CLA, on the acute phase response (APP) in blood plasma and hepatic tissue and the antioxidative status in plasma of dairy cows that were fed a CS based diet with a low-fat content from late to early lactation.

**Materials and Methods** Thirty-eight lactating rumen-fistulated Holstein-Friesian cows (11,000 kg milk/305 d in 2<sup>nd</sup> lactation) were investigated in 5 blocks and received a corn silage-based total mixed ration during lactation [wk 22-6 before and wk 0-8 after calving, 7.1 MJ NEL/kg dry matter (DM)] and dry period (wk 6-0 before calving, 6.5 MJ NEL/kg DM). The diets were fed *ad libitum*. From wk 9 antepartum (ap) until wk 9 postpartum (pp) cows were daily treated by abomasal infusion either with coconut oil (CNTR, 76g/d; n = 9), linseed and safflower oil (EFA, 78 and 4g/d; n = 9), Lutalin® (CLA, c9, t11 and t10, c12 in equal amounts, 10 g/d; n = 10) or the combination of EFA+CLA (n = 10). The dose was halved during dry period. Blood samples for analysis of haptoglobin (HP), interleukin 6 and 1 $\beta$  (IL6, IL1 $\beta$ ), and paraoxonase (PON) were taken on d 63, 42, 35, 28, 21, 10 ap and weekly up to 56 d pp. Plasma fibrinogen (FGA) was measured on d 63, 42, 21 ap and 1, 14, 28, 42, 56 d pp. Plasma concentrations of thiol groups (SH), reactive oxygen metabolites (ROM), biological antioxidant potential (BAP) and glutathione peroxidase activity (GPX) were measured on d -42, 1, 28, and 56 relative to calving. Liver tissue samples were taken on d 63 and 21 ap and on d 1, 28, and 63 pp to measure HP, serum amyloid A (SAA), FGA, and C-reactive protein (CRP) mRNA abundance. The data were analysed by repeated measurements ANOVA of SAS with the fixed effects of treatment (EFA, CLA and their interaction), time, treatment  $\times$  time and block. Calving interval and milk yield, from 2<sup>nd</sup> lactation, were used as covariates.

**Results** Plasma concentration of HP peaked around calving in all groups ( $P < 0.001$ ). Before calving HP was lowered by EFA treatment ( $P < 0.05$ ) and concentrations were lower ( $P < 0.05$ ) in EFA+CLA than in CNTR on d -21 ap. Plasma concentration of FGA slightly dropped after onset of CLA treatment (EFA  $\times$  CLA, and CLA  $\times$  time,  $P < 0.05$ ). In all groups plasma except EFA plasma FGA peaked at d 1 pp and FGA concentration in EFA and EFA+CLA were higher ( $P < 0.05$ ) than in CLA on d 14 pp. Plasma concentrations of IL-1 $\beta$  and IL-6 decreased ( $P < 0.001$ ) after calving and IL-1 $\beta$  was lower in CLA treatments after calving (CLA  $\times$  time,  $P < 0.01$ ). IL-1 $\beta$  was greater ( $P < 0.05$ ) in EFA than CTRL on d 35 ap and d 56 pp and greater ( $P < 0.05$ ) than in EFA+CLA on d 42 and 56 pp. Plasma concentration of PON tended ( $P = 0.08$ ) to be affected by EFA treatment, it declined ( $P < 0.001$ ) around calving and increased more in EFA treatments (EFA  $\times$  time:  $P < 0.05$ ) pp. On d 49 pp PON was greater ( $P < 0.05$ ) in EFA+CLA than in CNTR. Plasma activity of GPX peaked ( $P < 0.001$ ) in all groups around calving. Plasma SH concentration was highest on d 1 ( $P < 0.05$ ), decreased towards d 28 pp, but increased in EFA on d 56 pp (EFA  $\times$  CLA:  $P = 0.8$ ). Plasma BAP concentration was lower pp than ap ( $P < 0.001$ ) and was reduced by EFA treatment ( $P < 0.05$ ). Highest plasma ROM concentration was observed around parturition ( $P < 0.05$ ), ROM decreased thereafter but increased again in EFA+CLA on d 56 pp. Abundance of HP, SAA, CRP and FGA mRNA increased ( $P < 0.001$ ) around calving but revealed no differences between groups.

**Conclusions** The acute phase response and indicators of oxidative stress showed time changes that are typical for periparturient cows, but the inflammatory and oxidative status seemed to be more severe affected by the EFA treatment. The pattern of hepatic gene expression of APP matched the results of the plasma concentrations but EFA and CLA treatments had only minor effects on respective gene expression in the liver.

#### References

BERNABUCCI, C. et al. (2005) J. Dairy Sci. 88, 2017–2026

WEBER, C. et al. (2016) ISEP, EAAP Publication Vol. 137: 245–246, Krakow 2016

### Dynamics of metabolic oxidation in late-pregnant and early lactating dairy cows supplemented with conjugated linoleic acid or $\alpha$ -linolenic acid using measurements obtained in respiration chambers

L. Vogel<sup>1</sup>, M. Gnott<sup>1</sup>, A. Tröschner<sup>2</sup>, M. Derno<sup>1</sup>, B. Kuhla<sup>1</sup>, A. Starke<sup>3</sup> and H. M. Hammon<sup>1</sup>

<sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; <sup>2</sup>BASF SE, Lampertheim, Germany; <sup>3</sup>Clinic for Ruminants and Swine, Faculty of Veterinary Medicine, University of Leipzig, Germany

Email: hammon@fbn-dummerstorf.de

**Introduction** The increasing milk yield in dairy production changes the nutritional management from pasture to an intense use of corn silage and concentrate-rich diets with a high energy density resulting in a low supply of essential fatty acids (EFA), i. e.  $\alpha$ -linolenic acid (ALA), and conjugated linoleic acid (CLA; Weber *et al.*, 2016). First results of the present study indicated an improved energy balance (EB) in CLA treated cows due to reduced milk fat output during lactation (Vogel *et al.*, 2018). However, CLA and EFA treatment can also affect dry matter intake (DMI) in dairy cows (Schäfers *et al.*, 2017; Moallem, 2018). The technique of indirect calorimetry provides continuous measurements of feeding behavior, substrate oxidation of macronutrients and energy expenditure, and can further reflect relationships between DMI and energy metabolism. The aim of the present study was to determine dynamic changes in feeding pattern and metabolic oxidation of late gestation and early lactating cows supplemented with CLA and ALA.

**Materials and Methods** Rumen cannulated German Holstein cows (n=38; 11,000 kg milk/305 d in 2<sup>nd</sup> lactation) received a corn silage-based total mixed ration *ad libitum* during dry period [10.7 MJ ME/kg dry matter (DM)] and lactation (11.5 MJ ME/kg DM). From wk 9 antepartum (ap) until wk 8 postpartum (pp), cows were abomasally supplemented twice daily either with coconut oil (CNTR, 76 g/d; n=9), linseed and safflower oil (EFA, 78 and 4 g/d; n=9), Lutalin® (CLA, c9, t11 and t10, c12 in equal amounts, each 10 g/d; n=10) or the combination of EFA+CLA (n=10). During the dry period (wk 6-1 ap), dosages of the supplements were halved. In wk 4 ap and wk 3 pp dynamics of feed and water intake, as well gas exchange of O<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub> were measured in open-circuit respiration chambers for 24 h in 6 min intervals. Based on these measurements meal size (kg DM/event), meal frequency (events/day), eating rate (kg DM/min), as well as metabolic heat production (HP), EB, metabolic respiratory quotient (RQ), carbohydrate oxidation (COx) and fat oxidation (FOx) were calculated by equations according to Derno *et al.* (2013). HP, COx and RQ averaged individual peri-prandial rises (APR) and postprandial falls (APF) were calculated as  $\Delta/\text{time}$ . Peri-prandial APF and post-prandial APR were calculated for FOx. Gastrointestinal passage rate was determined as mean retention time (MRT) by intraruminally supplementation of 15 g titanium dioxide and feces sampling 6 times on the following 4 days at 0700 and 1730 h. The data were analyzed by repeated measurements ANOVA of SAS with the fixed effects of treatment (EFA, CLA and their interaction), time and treatment  $\times$  time. Calving interval and projected milk yield from 2<sup>nd</sup> lactation served as covariates. Cross-correlation analysis to determine lag times of COx, FOx, RQ and HP after meal were estimated using Proc Timeseries of SAS.

**Results** Average daily DMI, meal size and water intake were higher pp than ap ( $P<0.05$ ), but pp DMI tended to be lower in CLA than in CNTR ( $P=0.09$ ). From ap to pp meal frequency was reduced in CLA cows ( $P<0.05$ ) and only increased in CNTR ( $P<0.05$ ). Meal duration remained constant in CLA but declined in non-CLA from ap to pp ( $P=0.05$ ). Eating rate increased more in EFA than in non-EFA ( $P<0.05$ ) cows from ap to pp and remained constant in the CLA group. MRT and CH<sub>4</sub>/DMI were lower pp than ap ( $P<0.001$ ) without group differences. The EB tended to be higher ( $P=0.06$ ) and pp milk energy was lower ( $P<0.001$ ) in CLA cows, but milk yield did not differ. Daily HP was greater pp than ap ( $P<0.01$ ) and pp tendentially higher in EFA+CLA than CLA ( $P=0.07$ ). HP APR decreased in non-EFA from ap to pp and remained constant in EFA ( $P=0.05$ ), but APR/meal size decreased in all groups pp ( $P<0.05$ ). HP APF tended to be higher in EFA+CLA than in EFA ( $P=0.08$ ) but there are no time or treatment differences in APF/meal size. Daily COx was higher ap than pp ( $P<0.001$ ). COx APR increases from ap to pp ( $P<0.001$ ) with a higher increase in EFA than in non-EFA ( $P<0.01$ ), whereas APR/meal size showed no time or treatment effects. COx APF and APF/meal size increased pp ( $P<0.05$ ). COx APF/meal size was lower in EFA+CLA than in EFA pp ( $P<0.01$ ). The lag time of COx slightly increased pp ( $P=0.01$ ). Daily FOx was lower ap than pp ( $P<0.01$ ). FOx APF and APR, but not APF/meal size and APR/meal size increased pp ( $P<0.05$ ). FOx APR/meal size was lower in EFA+CLA than in EFA pp ( $P=0.05$ ). Lag time of FOx tended to be higher in EFA than in CNTR pp ( $P=0.1$ ). RQ was lower pp than ap ( $P<0.001$ ). RQ APR/meal size showed no differences, but pp RQ APF/meal size was lower in EFA+CLA than in EFA ( $P<0.01$ ).

**Conclusions** The trend for a higher EB with CLA supplementation seemed to be opposed in CLA cows by lower DMI and in EFA+CLA cows by greater HP. Feeding pattern indicate a reduction in the sense of hunger in CLA cows. MRT depend on DMI and further affect methane yield and maybe feed digestibility. In early lactating cows FOx instead of COx seemed to be preferred and rates depended on meal size. Higher HP, smaller COx and RQ APF/meal size, as well FOx APR/meal size in EFA+CLA indicate more intermediate substrate metabolism due to less need for energy supply.

#### References

- Derno, M. *et al.* (2013) J. Dairy Sci. 96:971–980 DOI: 10.3168/jds.2012-5727  
Moallem, U. (2018) J. Dairy Sci. 101:1–21 DOI: 10.3168/jds.2018-14772  
Schäfers, S. *et al.* (2017) J. Dairy Sci. 100:3193–3208 DOI: 10.3168/jds.2016-11882  
Vogel, L. *et al.* (2018) Proc. Soc. Nutr. Physiol. 27:181  
Weber, C. *et al.* (2016) ISEP, EAAP Publication Vol. 137:245-246

### Risk factors for dairy cow herds to be classified as “ketotic”

M. Sabatowicz<sup>1</sup>, Z. M. Kowalski<sup>1</sup>, W. Jagusiak<sup>2</sup> and P. Gorka<sup>1</sup>

<sup>1</sup>Department of Animal Nutrition and Dietetics, University of Agriculture in Krakow, Poland; <sup>2</sup>Department of Animal Breeding, University of Agriculture in Krakow, Poland

Email: rzkowals@cyf-kr.edu.pl

**Introduction** In the system of monitoring of subclinical ketosis (SCK) in Polish dairy herds, the farmers are informed about the risk of ketosis in the herd. A system is based on the concentration of ketone bodies (□ hydroxybutyrate and acetone) in milk samples of cows which are determined by FTIR method in test-day (TD) milk samples. For each herd, so called probable attendance of subclinical ketosis (PFSK) is being calculated, based on the percentage of ketotic cows in the herd, sensitivity and specificity of a model used to detect the ketotic cow as well as a herd size. The herd is classified as “ketotic” if PFSK is equal to or higher than 10%. In most studies published so far, the risk factors for SCK have been limited to individual cows. We hypothesize that there are the factors which increase the risk of the herd to be classified as “ketotic” (i.e. with PFSK over 10%). Thus, the aim of our study was to determine risk factors of the increased number of ketotic cows in the herd.

**Materials and Methods** To conduct this study two datasets were used. The dataset 1 was provided from the Polish Federation of Cattle Breeders and Dairy Farmers. It consisted of data collected in a period of 3 months, from May 1 to August 31, 2014 from 15,859. The variables were: PFSK of herd, parity of cow, days in milk (DIM) of cow, previous lactation length of cow, herd size, average daily milk yield of herd, age of first calving of primiparous cow, month of a year (Table 1). The dataset 2 consisted of the answers of owners or managers of the farm to 10 questions regarding feeding of cows, their grouping, control of BCS, as well as farmers'/managers' education and farm modernization (Table 1). Of total number of milk recorded herds in Poland (~19,000), the answers from 15,859 herds were collected. Generalized logistic mixed model of factors associated with dependent variable Y (PFSK: non-ketotic herd when < 10% or ketotic herd when ≥ 10%) was used. Variables were tested for inclusion in the model by stepwise selection procedure (P-value for retention P < 0.05). Multivariate multinomial logistic regression compared the odds ratio (OR) of a herd being yes or no, for various risk factors.

**Results** Among variables studied, the season (month), herd size, average daily milk yield, housing system (tie-stall vs. free stall), grazing (yes vs. no), dry cow grouping and farmers/manager education were the most important in the model. Bigger farms, with higher milk yield, managed by educated farmer was associated with lower risk of the herd being classified as ketotic. As expected, the grazing, tie-stall housing and lack of dry cow group increases the risk of classifying the herd as ketotic.

**Table 1** Risk factors to classify the dairy cow herds as ketotic (PFSK ≥ 10%)

Risk factors	Variable	Estimate	SE	OR	P-value
Intercept	Continuous	-2.58	0.07		<.0001
Season	June vs. May	-0.07	0.05	0.94	0.1572
	July vs. May	0.24	0.04	1.27	<.0001
	August vs. May	0.00	0.05	1.00	0.9376
	August vs. May	0.00	0.05	1.00	0.9376
Herd size, n cows	21-50 vs. ≤20	0.33	0.04	1.39	<.0001
	51-100 vs. ≤20	0.20	0.07	1.22	0.0071
	101-200 vs. ≤20	-0.64	0.17	0.53	0.0002
	201-500 vs. ≤20	-1.46	0.46	0.23	0.0014
	501-1000 vs. ≤20	-1.07	1.01	0.34	0.2890
	>1000 vs. ≤20	-	-	-	-
	>1000 vs. ≤20	-	-	-	-
Daily milk yield, kg	16.1-20 vs. ≤16	0.11	0.07	1.11	0.1178
	20.1-24 vs. ≤16	0.01	0.07	1.01	0.8500
	24.1-28 vs. ≤16	-0.26	0.07	0.77	0.0003
	28.1-32 vs. ≤16	-0.66	0.09	0.52	<.0001
	32.1-36 vs. ≤16	-1.06	0.18	0.35	<.0001
	≥ 36.1 vs. ≤16	-1.06	0.42	0.35	0.0114
Housing system	Tie-stalls vs. Free	-0.30	0.07	0.74	<.0001
	Tie-stalls 2 vs. Free	-0.17	0.09	0.85	0.0614
Grazing	No vs. Yes	0.42	0.04	1.52	<.0001
Dry cow grouping	One vs. No	-0.20	0.04	0.82	<.0001
	Two or more vs. No	-0.20	0.07	0.82	0.0057
Farmer agricultural education	No vs. Yes	-0.17	0.04	0.84	<.0001

**Conclusions** The results of a study show that high milk yield is not a risk factor for classifying the dairy cow herd as ketotic. Conversely, the small size of the farm and the low milk yield increase the risk of classifying the herd to the group endangered with ketosis. In the first such study, we also showed that some management aspects increase or decrease of such risk.

## The role of altered immune function during the dry period in promoting the development of ketosis in early lactation

M. Mezzetti<sup>1</sup>, A. Minuti<sup>1</sup>, F. Piccioli-Cappelli<sup>1</sup>, M. Amadori<sup>2</sup>, M. Bionaz<sup>3</sup> and E. Trevisi<sup>1</sup>

<sup>1</sup>Department of Animal Sciences, Food and Nutrition (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy; <sup>2</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Laboratory of Cellular Immunology, Brescia, Italy; <sup>3</sup>Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, USA  
Email: Matteo.Mezzetti@unicatt.it

**Introduction** Ketosis impairs white blood cell functions and increase the prevalence of diseases. Ketosis mainly occurs after calving, but physiological conditions in late pregnancy seem to favour its appearance. Our study investigated changes occurring in the immune system prior to disease onset to elucidate their role in the occurrence of ketosis.

**Materials and Methods** Thirteen Holstein dairy cows were housed in tied stalls and retrospectively divided into 2 groups based on their plasma concentration of beta-Hydroxybutyric acid (BHB) after calving. BHB concentration lower than 1.4 mMol/L were found in seven cows (CTR) and concentration higher than 1.4 mMol/L were found in six cows (KET). From -48 to 35 days from calving (DFC), the dry matter intake, rumination time, and milk yield (MY) were measured, and blood samples were collected regularly to assess the hematocrit profile and to perform two *ex vivo* whole blood stimulation assay (WBA) with lipopolysaccharides (LPS) and with *Mycobacterium avium*. Data were submitted for ANOVA testing using a mixed model for repeated measurements (Mixed Procedure, SAS Instruments, Inc.), including the BHB group (Hs) and time (t) and their interactions (Hs\*t) as fixed effects.

**Results** KET cows prior to calving had higher plasma concentrations of interleukin 1 beta (IL1B; Figure 1.d), myeloperoxidase (Figure 1.e), and oxidant species (reactive oxygen metabolites, nitric oxide and nitrate;  $p < 0.05$ ) and greater interferon gamma (IFNG) responses to *Mycobacterium avium* ( $p < 0.01$ ), higher blood concentrations of gamma glutamyl transferase (GGT; Figure 1.b), and lower plasma concentrations of phosphorus, sodium, potassium and zinc ( $p < 0.05$ ). Higher levels of NEFA, BHB, and glucose were also detected in KET cows during the dry period ( $p = 0.01$ ). After calving, KET cows had a lower DMI and MY during the fifth week of lactation ( $p < 0.05$ ). In early lactation, KET cows compared with CTR had reduced plasma glucose ( $p < 0.05$  at 3 DFC) and retinol (Figure 1.i) while they had higher blood concentrations of NEFA and BHB ( $p < 0.01$ ), glutamate-oxaloacetate transaminase, GGT and bilirubin (Figure 1.a-b-c), haptoglobin, ceruloplasmin and serum amyloid alpha (Figure 1.f-g-h). The WBC of the KET cows had a modified response to an *ex vivo* stimulation assay with bacterial LPS (Table 1), with a lower production of IL1B since -21 DFC and a greater production of interleukin 6 (IL6) and lactate after calving.

Item	Hs <sup>1</sup>	Days from calving					SE <sup>2</sup>	Effect	P-Value
		-48	-21	-3	7	28			
IL1B	CTR	25.3	30.6	41.0	46.7	20.3	0.24	Hs <sup>1</sup>	<0.01
	KET	7.3	7.3	12.3	10.7	31.3	0.26	t <sup>3</sup>	0.02
	P-Value		**	**	**			Hs*t <sup>4</sup>	<0.01
IL6	CTR	1.8	1.6	1.7	2.1	1.9	0.10	Hs <sup>1</sup>	0.04
	KET	2.0	1.7	2.1	2.5	3.7	0.11	t <sup>3</sup>	<0.01
	P-Value				+	**		Hs*t <sup>4</sup>	0.01
D-lactic acid	CTR	1.04	1.05	1.12	1.03	1.01	0.02	Hs <sup>2</sup>	0.16
	KET	1.03	1.08	1.08	1.09	1.17	0.03	t <sup>3</sup>	0.10
	P-Value					**		Hs*t <sup>4</sup>	<0.01
L-lactic acid	CTR	1.02	1.05	1.05	1.04	1.04	0.01	Hs <sup>2</sup>	0.01
	KET	1.03	1.06	1.05	1.10	1.14	0.01	t <sup>3</sup>	<0.01
	P-Value			**	**			Hs*t <sup>4</sup>	<0.01

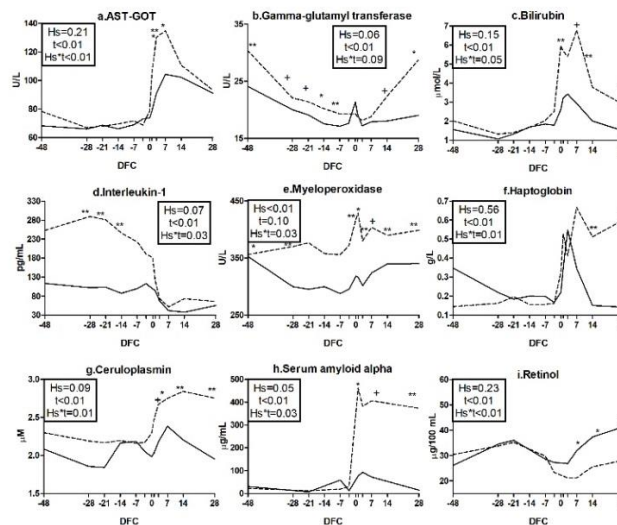
<sup>1</sup>Health status (CTR is control and KET is ketosis).

<sup>2</sup>Standard error.

<sup>3</sup>Time.

<sup>4</sup>Health status x time interaction (\*\* is  $P < 0.01$ ; + is  $P < 0.1$ ).

**Table 1** Fold changes of cytokines (IL1B and IL6) and lactic acid production during WBA with LPS in control dairy cows or cows that showed ketosis after calving. Values are expressed as fold change with respect to baseline.



**Figure 1** Plasma concentrations of aspartate amino transferase (a), GGT (b), bilirubin (c), IL1B (d), myeloperoxidase (e), haptoglobin (f), ceruloplasmin (g), serum amyloid alpha (h) and retinol (i) in CTR (solid line) or KET (dotted line) dairy cows during transition period. \*\* is  $P < 0.01$ ; \* is  $P < 0.05$ ; + is  $P < 0.1$ .

**Conclusions** Ketosis was preceded during the dry period by oxidative stress, high plasma levels of IL1B and nitrates, reduced liver and renal functions, reduced plasma mineral concentrations, and activation of the immune system. Increased production of IFNG by WBC may have affected peroxisome proliferation activated receptor-gamma expression and reduced the insulin sensitivity. The latter might explain the larger prepartal concentrations of NEFA and BHB in KET cows. The anorexic effect of IL1B, NEFA, BHB and the high circulating concentrations of glucose reduced the DMI around calving, impairing the MY and boosted the mobilization of lipid sources. These conditions appear to have influenced the greater acute-phase response after calving, which compromised the liver function to some degree. A possible endotoxin tolerance of WBC in KET cows could account for the reduced production of IL1B and increased production of lactate and IL6 found at stimulation with LPS. Our data suggest a role for the activation of immune system during the dry period in promoting ketosis in the early postpartum period.

### Testing dry cows for risk of postpartum diseases: two-year results from a routine laboratory

S. van der Drift and S. Carp – van Dijken  
GD Animal Health, Deventer, The Netherlands  
Email: [s.v.d.drift@gddiergezondheid.nl](mailto:s.v.d.drift@gddiergezondheid.nl)

**Introduction** Increased NEFA and BHBA concentrations in the (late) dry period are associated with an increased risk for postpartum diseases and culling (Chapinal et al., 2011; Roberts et al., 2012). Since July 2016, dairy farmers can send serum samples of a set of 4 to 10 dry cows between 21 and 2 days before calving to the routine laboratory of GD Animal Health in the Netherlands. Samples are analyzed for NEFA, BHBA, urea, haptoglobin, and magnesium concentrations and can be used to evaluate dry cow management. Magnesium analysis was part of the test panel only from April 2018. This objective of this research was to present the results from two years of testing close-up dry cows based on samples sent to the GD Animal Health routine laboratory from 2016 to 2018.

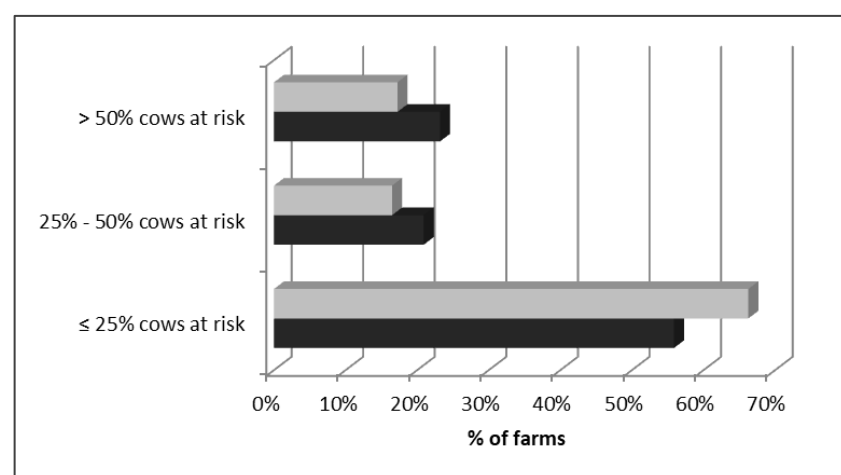
**Materials and Methods** Results from sets of 4 to 10 serum samples that were sent to the GD routine laboratory for the specific dry cow test panel between July 2016 and July 2018 with complete results for all parameters were included in the analysis (3228 samples; 655 sets of 4 to 10 samples). Results in the datafile may (occasionally) originate from cows that were sampled outside the targeted interval of 21 to 2 days before calving. Descriptive statistics were performed on serum results using Statistix. Cows with NEFA concentrations  $\geq 0.40$  mmol/L and/or BHBA  $\geq 0.80$  mmol/L were indicated in the lab tests result to be at an increased risk for postpartum disease due to negative energy balance. Cows with urea concentrations  $\leq 3.3$  and  $\geq 6.6$  mmol/L were indicated to have a suboptimal protein supply. At haptoglobin concentrations  $\geq 0.30$  g/L, cows were indicated to be at an increased risk for postpartum disease. For magnesium concentrations, a cut-off value of  $\leq 0.78$  mmol/L was used to indicate increased risk for hypocalcaemia.

**Results** Descriptive statistics for concentrations of trace elements in serum of dry cows are shown in Table 1. The percentage of farms in the different categories of proportions of cows at risk are shown in Figure 1.

**Table 1** Descriptive statistics for concentrations of NEFA, BHBA, urea, haptoglobin, and magnesium in serum of 3228 cows (655 sets of 4 to 10 samples) sent to the GD Animal Health routine laboratory from July 2016 to July 2018 for the dry cow test panel (advise GD Animal Health is to sample cows between 21 and 2 days before parturition).

Indicator	Mean ( $\pm$ SD)	Median	Min - Max	25 <sup>th</sup> – 75 <sup>th</sup> perc.	Cut-off value	% of cows above/below cut-off
NEFA (mmol/L)	0.25 ( $\pm$ 0.20)	0.19	0.05 – 2.03	0.14 – 0.28	$\geq 0.40$	13%
BHBA (mmol/L)	0.6 ( $\pm$ 0.2)	0.6	0.1 – 5.2	0.5 – 0.7	$\geq 0.80$	23%
Urea (mmol/L)	4.3 ( $\pm$ 1.3)	4.2	2.0 – 20.4	3.5 – 5.1	$\leq 3.3$ and $\geq 6.6$	22% and 5%
Haptoglobin (g/L)	0.13 ( $\pm$ 0.23)	0.07	0.03 – 2.90	0.06 – 0.10	$\geq 0.30$	6%
Magnesium* (mmol/L)	0.99 ( $\pm$ 0.10)	0.98	0.64 – 1.36	0.93 – 1.05	$\leq 0.78$	3%

\*Results for magnesium are based on samples of 461 cows (added to the test panel from April 2018)



**Figure 1** Percentage of farms with  $\leq 25\%$  cows at an increased risk, 25% - 50% cows at an increased risk, or  $> 50\%$  cows at an increased risk for postpartum health problems based on NEFA and BHBA concentrations (energy balance; black bars) or urea concentrations (protein supply; grey bars) from 3228 serum samples sent to the GD routine laboratory for the dry cow test panel.

**Conclusions** Testing cows for metabolic indicators in the dry period can be helpful for farmers and their (veterinary) advisors in the field to evaluate dry cow management and indicate cows at risk for postpartum diseases. Over 40% of farms participating in the dry cow test had  $> 25\%$  of cows at an increased risk for postpartum diseases.

### References

- Chapinal N, Carson M, Duffield TF, Capel M, Godden S, Overton M, Santos JE, LeBlanc SJ, 2011. The association of serum metabolites with clinical disease during the transition period. *J Dairy Sci.* 94(10):4897-903.  
Roberts T, Chapinal N, Leblanc SJ, Kelton DF, Dubuc J, Duffield TF, 2012. Metabolic parameters in transition cows as indicators for early-lactation culling risk. *J Dairy Sci.* 95(6):3057-63.

## Feed restriction in late gestation and early lactation affects body weight and colostrum and milk production in Iranian fat-tailed ewes

M. Zarrin<sup>1</sup>, M. Sanginabadi<sup>1</sup>, M. Nouri<sup>1</sup>, A. Ahmadpour<sup>2</sup> and R. M. Bruckmaier<sup>3</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Yasouj University, Iran; <sup>2</sup>Department of Biological Engineering, Utah State University, UT, USA; <sup>3</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland

Email: zarrin1357@gmail.com

**Introduction** During conditions of feed scarcity, pregnant animal's adaptation, and nutrient partitioning may become insufficient, resulting in reduced birth weight and performance. Mammary system development which mostly occurs in the last four weeks of gestation and providing nutritional demands for the onset of lactation make this period the most crucial period of ewes' productive life. Hence, any nutritional and management deficiencies during such period suppress lamb growth. More than 98% of sheep breeds in Iran are fat-tailed and are reared in sub-rural and nomadic systems which depend on continuous change of the location to allow adequate feed availability. Especially in tropical and sub-tropical areas nomadism leads to additional energy consumption for grazing and pasture change. To investigate consequences of a comparable nutrient loss, the effects of a feed restriction during late gestation and early lactation on the performance of fat-tailed ewes and their lambs was studied.

**Materials and Methods** Eighteen multiparous Torky-Ghashghaei ewes (BW: 53 ± 1.8 kg) in late gestation were divided randomly into control (Control; n = 9) and restriction (Rest; n = 9) groups. After 14 days of adaptation, both groups were fed a balanced ration freely for one week. The Rest group was offered a restricted feed for three weeks which increased weekly from 50-60 %, 60-70 %, to 70-80 % compared to the Control. All ewes were fed ad libitum during the fifth week of experiment. This schedule was repeated after parturition for both groups. Body weight was recorded weekly. Colostrum samples were collected daily in the morning and evening during 72 h after lambing, and consequently, milk was sampled and recorded daily. Variations between groups within each time point and differences between time points within groups for animal body weight, milk yield, milk constituents, and energy contents of the milk were tested for significance ( $P \leq 0.05$ ) and tendency ( $P < 0.1$ ) applying MIXED procedure of SAS. Data of birth weight of the lambs and total 72 h colostrum yield were evaluated by GLM procedure of SAS (Release 9.2; SAS Institute Inc., Cary, NC).

**Results** Feed restriction during late gestation affected the body weight of the ewes; the highest loss was related to 50-60% Rest ( $P < 0.05$ ). The body weight did not differ between the two groups immediately after lambing, while more weight loss was related to weeks 4, and 5 post-partum in Rest ( $P < 0.05$ ). Feed restriction did not affect the birth weight of the lambs ( $P = 0.36$ ; Table 1). Feed restriction during late gestation reduced total 72 h colostrum yield by approximately 30% in the Rest compared to the Control group (4.45 ± 0.45 kg in Control vs. 3.16 ± 0.34 kg in Rest;  $P < 0.05$ ). The average daily milk yield during the first five weeks of lactation was lower in Rest group compared to Control ( $P < 0.01$ ), except the fifth week of lactation which showed a tendency to be higher in Control compared to Rest ( $P = 0.08$ ); the lowest daily milk yield was recorded at 50-60% feed restriction. Lambs in Rest group consumed less milk than lambs in the Control during the experimental period ( $P < 0.05$ ). Feed restriction had no impact on milk fat, protein, lactose, and solid none fat (SNF) percentage. However, concomitantly with the lower milk yield, milk fat, protein, lactose, and SNF yield were lower in Rest rather than Control during the five weeks of lactation ( $P < 0.05$ ). Maternal feed restriction during early lactation suppressed their lambs' weight gain compared to Control ( $P < 0.05$ ) from the second week until the end of the experiment. Energy value of the milk (kJ/kg) did not change due to feeding restriction during the five weeks of early lactation.

**Table 1** Birth weights of lambs, total 72 h colostrum yield post-partum and daily milk yield during the first 5 weeks of lactation in control (Control; n=9) and restricted (Rest; n=9) ewes. Data are presented as LS means ± SEM.

Variables	Feeding Level		P Value, Groups	P Value, Weeks	P value, G*W
	Control	Restriction			
Birth weight of lambs, kg	4.80 ± 0.37	4.35 ± 0.36	0.36		
Colostrum yield, (72 h, kg)	4.45 ± 0.45	3.16 ± 0.34	0.05		
Milk yield, kg			< 0.01	< 0.0001	< 0.0001
Week 1 (Ad libitum)	1.28 ± 0.07 <sup>a*</sup>	0.89 ± 0.09 <sup>A</sup>			
Week 2 (50-60%)	1.29 ± 0.09 <sup>a*</sup>	0.50 ± 0.05 <sup>D</sup>			
Week 3 (60-70%)	1.19 ± 0.08 <sup>ab*</sup>	0.57 ± 0.06 <sup>CD</sup>			
Week 4 (70-80%)	1.14 ± 0.08 <sup>b*</sup>	0.66 ± 0.07 <sup>C</sup>			
Week 5 (Ad libitum)	1.02 ± 0.13 <sup>b#</sup>	0.76 ± 0.08 <sup>B</sup>			

<sup>a,b</sup> or <sup>A,B,C</sup>: Each column of milk yield without common letters are significantly different ( $P < 0.05$ ) in Control and Rest group, respectively. Differences ( $P \leq 0.05$ ) and tendency to a difference ( $P < 0.1$ ) between each row (time point) are indicated with (\*) and (#), respectively.

**Conclusions** In conclusion, feed scarcity in tropical regions particularly during late gestation and early lactation could affect offspring's postnatal growth rates through an adverse effect on colostrum and milk production. However, feed availability during this critical period could be a strategy to be considered to compensate for the energy loss through nomadic pasture systems.



### Regulation of the endocannabinoid system in the hypothalamus of late and early lactating dairy cows

B. Kuhla<sup>1</sup> and A. Kuhla<sup>2</sup>

<sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Institute of Nutritional Physiology “Oskar Kellner” Dummerstorf, Germany;

<sup>2</sup>Institute for Experimental Surgery, Rostock University Medical Center, Rostock, Germany

Email: b.kuhla@fbn-dummerstorf.de

**Introduction** The endocannabinoid system of the hypothalamus participates in the control of feed intake and energy metabolism, e.g. by increasing orexigenic signaling and carbohydrate oxidation or inhibiting the hypothalamic-pituitary-adrenal axis (Quarta et al., 2011; Chapman et al., 2012; Luce et al., 2014)). Most mammals increase feed intake and metabolic rate after parturition to cope with the increased energy and nutrient requirements for milk production. However, the regulation of the hypothalamic endocannabinoid system in early lactation has not been investigated yet. N-arachidonylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), two abundant endocannabinoids are synthesized from phospholipids. While the N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) catalyses the last step in the formation of AEA, 2-AG is formed by the hydrolysis of diacylglycerols catalysed by diacylglycerol lipase- $\alpha$  (DAGL- $\alpha$ ). On the other hand, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) degrade AEA and 2-AG, respectively. Both, AEA and 2-AG may bind to the endocannabinoid receptor 1 (CB1) to trigger signal transduction. The aim of the present study was to investigate the expression of the endocannabinoid tone regulating system in the hypothalamus of early and late lactating cows.

**Materials and Methods** Ten early lactating German Holstein dairy cows between 11 and 66 days postpartum in 3<sup>rd</sup> to 5<sup>th</sup> lactation and nine non-pregnant cows between 180 and 319 days of late 2<sup>nd</sup> to 4<sup>th</sup> lactation were killed in the institutional slaughter house. All cows were fed a similar total mixed ration (TMR) consisting of grass silage, corn silage, barley straw, hay, corn meal, extracted rapeseed and soybean meal, soy oil, wheat, sugar beet pulp, mineral feed and limestone. The net energy content (NEL) amounted to 6.5 MJ/kg of dry matter (DM) before and 7.0 MJ/kg DM after parturition. A blood sample was taken after captive bolt stunning into an EDTA containing tube to analyse plasma glucose, non-esterified fatty acid and beta-hydroxybutyrate concentrations. From these the physiological imbalance (PI) index as an estimate of energy balance was calculated (Moyes et al., 2013). The hypothalamus was isolated and fixed in phosphate buffered 4% formaldehyde solution at 4°C for 4-5 weeks. Brain tissue was dehydrated in increasing ethanol concentrations and embedded in paraffin. Tissue was sliced (4  $\mu$ m) and slices were pretreated with citrate buffer and peroxidase block. After protein blocking slices were incubated with antibodies against CB1, DAGL- $\alpha$ , MAGL, FAAH or NAPE-PLD followed by incubation with secondary conjugated antibodies. HRP conjugated immunoreactivity was visualized via 3,3'-diaminobenzidine and AP conjugation with Permanent Red precipitation. Staining intensities were analysed by the IHC profiler tool of ImageJ software and statistically evaluated using Student's t-test.

**Results** The PI index was  $-20 \pm 3.8$  and  $-60.2 \pm 10.1$  for late and early lactating cows, respectively ( $P < 0.05$ ). Substantial NAPE-PLD immunoreactivity was found in the sub-epithelial space and parvocellular cells of the paraventricular nucleus (PVN) and was greater in early than late lactating cows ( $P < 0.05$ ). No NAPE-PLD reactivity was found in the arcuate nucleus (ARC). The abundance of FAAH controlling the degradation of AEA abundances were 33 - 50% lower in the PVN and ARC of early relative to late lactating cows ( $P < 0.05$ ). Strong immunoreactivity for DAGL- $\alpha$  was found in magnocellular neurons of the PVN, but also in a few neurons of the ARC. However, PVN and ARC DAGL- $\alpha$  abundances did not differ between late and early lactating cows. On the other hand, immunoreactivity for the 2-AG-degrading enzyme MAGL was found in magnocellular and parvocellular portions of the PVN and its expression was 152% in early relative to late lactating cows ( $P < 0.05$ ). Contrary to the regulation of EC synthesizing and degrading enzymes, CB1 immunoreactivity of neurons located in the PVN and the ARC of the hypothalamus did not differ between early and late lactating cows.

**Conclusions** In conclusion, the hypothalamic endocannabinoid system is regulated to increase the local AEA but to reduce the local 2-AG tone. Increased hypothalamic AEA levels point to an activated orexigenic signaling, peripheral carbohydrate oxidation and an inhibited hypothalamus-pituitary-adrenal axis. Reduced hypothalamic 2-AG levels are suggested to retard feed intake and support fatty acid oxidation during the early lactation period. Thus, the hypothalamic endocannabinoid system supports the increase in nutrient and energy requirements for milk synthesis in early lactation.

#### References

- Chapman CD, Dono LM, French MC, Weinberg ZY, Schuette LM, Currie PJ: Paraventricular nucleus anandamide signaling alters eating and substrate oxidation. *Neuroreport*. 2012;23:425-429.
- Luce V, Fernandez Solari J, Rettori V, De Laurentiis A: The inhibitory effect of anandamide on oxytocin and vasopressin secretion from neurohypophysis is mediated by nitric oxide. *Regul Pept* 2014;188 31-39.
- Moyes KM, Larsen T, Ingvarsten KL: Generation of an index for physiological imbalance and its use as a predictor of primary disease in dairy cows during early lactation. *J Dairy Sci* 2013;96:2161-2170.
- Quarta C, Mazza R, Obici S, Pasquali R, Pagotto U: Energy balance regulation by endocannabinoids at central and peripheral levels. *Trends Mol Med* 2011;17:518-526.



### Evaluation and reduction of influence of stress in fresh dairy cows

G. Lošytė, M. Televičius, D. Malašauskienė and R. Antanaitis

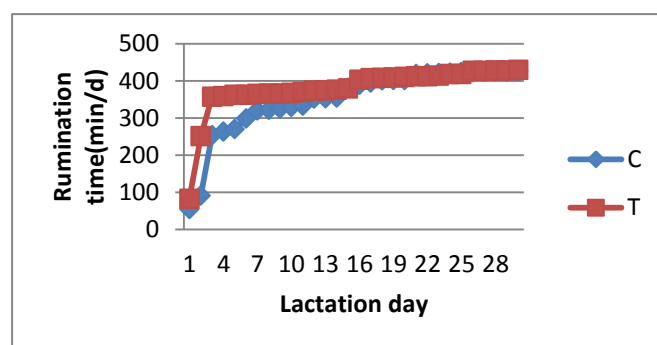
Large Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Email: Ramunas.Antanaitis@ismuni.lt

**Introduction** Cortisol is known to cause immunosuppression and therefore, increase the risk of infection. High circulating cortisol concentrations were reported to be linked with reduced milk yields (Negrao and Marnet 2003). Stress stimulates the release of adrenocorticotrophic hormone from the pituitary gland which promotes the production of the steroid hormone cortisol from the cortex of the adrenal glands. This steroid hormone is more important in altering the body's metabolism (ie. raising plasma glucose) under conditions of longer term, ongoing (chronic), rather than acute stress (Berci 2002). The aim of this study was to evaluate the impact of stress on the health of fresh-lactation cows. Also to evaluate the effect of NSAIDs on the stress indicator cortisol and somatic cell count (SCC). And to evaluate the effect of stress on the health of fresh-lactation cows from 1 day to 30 days after calving, using the computer herd management program.

**Materials and Methods** There was 600 lactating Lithuanian Black and White cows. Each month there were 40 – 50 calving cows. According to the analogue principle (according to the age of breeding, etc.), 30 cows up to 1 hour after calving were sampled for research. Selected cows were divided into two groups of 15. The testing group of cows (TG), (n=15), were given a subcutaneous injection of Rimadyl Cattle® (50 mg/ml of carprofen) dose were 1.4 mg per 1 kg BW, up to 1 hour after calving. The control group of cows (CG), (n=15) at the same time were given an injection of 0.9 % NaCl. Before the injection of medication, 24 and 48 hours after administration of the drug blood samples were taken to determine plasma cortisol concentration. 1 hour and 30days after calving milk samples were also taken, to determine somatic cell count and milk composition. To assess the effect of stress on the health of fresh-dairy cows, from day 1 to 30 after calving, illness, that usually occurs in post-calving period have been recorded, and ruminating time was also recorded using the computer herd management program.

**Results and conclusions** In control group cows during the period after calving, the incidence of hypocalcaemia was 30%, endometritis – 25%, metritis – 10%. After the use of NSAID hypocalcaemia was reduced to 12%, endometritis to 10% and metritis to 5%. NSAIDs (carprofen 50 mg / ml) have statistically significantly reduced cortisol plasma concentrations within 48 hours. from the use of the drug. NSAIDs did not have an impact on SCC. It was found that SCC was higher than control groups 1 and 30 days after calving, but did not exceed the physiological limits. Carprofen did not have an impact on milk urea and milk protein to fat ratio, although it changed less in test group than control group. The use of NSAID statistically significantly increased the average ruminating time from the third to the eleventh day after calving.



**Figure 1** Change in the duration of rumination during the study. C – control group. T – test group.

**Table 1** Cortisol serum concentrations change during the study. SE – standard deviation. T – testing group. C – control group

Time (hour)	Group	Average, ug/dl	SE, ug/dl	Minimum	Maximum
2	T	2.727	0.910593	0.44	10.5
	C	1.6	0.271277	0.21	3.24
24	T	1.306	0.262167	0.29	2.95
	C	1.626	0.300988	0.48	3.81
48	T	0.754	0.258028	0.24	2.54
	C	0.961	0.198346	0.27	1.96

### References

Negrão, J. A., & Marnet, P. G. (2003). Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. *Small Ruminant Research*, 47(1), 69-75.

Berci I DVM. PhD. Neuroimmune Biology. Vol 1. 2002. pp. 3-45. Elsevier Science. 2001.

## Intensive feeding alters sphingolipid metabolism in Holstein bulls

Á. Kenéz<sup>1</sup>, S. Bäßler<sup>2</sup>, C. Koch<sup>3</sup>, T. Scheu<sup>3</sup> and K. Huber<sup>2</sup>

<sup>1</sup>Department of Infectious Diseases and Public Health, City University of Hong Kong, Hong Kong; <sup>2</sup>Institute of Animal Science, University of Hohenheim, Stuttgart, Germany; <sup>3</sup>Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler an der Alsenz, Germany

Email: akos.kenez@cityu.edu.hk

**Introduction** An intensive nutritional regimen, particularly a high concentrate to roughage ratio, is common practice for fattening Holstein bulls to shorten the fattening period by ensuring a more rapid body weight gain. However, intensive fattening was shown to increase body fat deposition and to alter tissue fatty acid profiles, particularly increasing the omega-6/omega-3 polyunsaturated fatty acid (PUFA) ratio (Nogalski et al., 2014). This change in PUFA ratio was linked to an increased activity of inflammatory processes in various species, including Holstein cattle (Greco et al., 2015). Further, inflammation was also shown to be modulated by sphingolipid metabolism, particularly through specific sphingolipids exerting a signaling function and driving cytokine production of immune cells (Maceyka and Spiegel, 2014). Hence, our overall working hypothesis was that a high-energy diet and the enhanced body weight gain due to intensive fattening might have an impact on health status though altering metabolism. Specifically, the present study aimed to quantify specific sphingolipid species in blood plasma, liver, skeletal muscle and adipose tissue of Holstein bulls fed an intensive diet.

**Materials and Methods** Holstein bulls intended for beef production were randomly assigned to an intensive (n=15) or a control nutritional regimen (n=15). Diets were based on corn- and grass-silage and the intensive group received additionally 6 kg/day/animal concentrate feed for the last 8 months of the fattening period. Bulls were slaughtered at the age of 20 months and blood plasma, liver, skeletal muscle and subcutaneous adipose tissue samples were collected. Samples were subjected to targeted liquid chromatography – tandem mass spectrometry analyses: plasma samples were analyzed by the AbsoluteIDQ® p180 kit (Biocrates Life Sciences, Innsbruck, Austria), while the tissue samples were analyzed in the laboratories of The Metabolomics Innovation Centre (Edmonton, Canada). A subset of specific sphingomyelin (SM) species, selected from a larger metabolomics data set, was used in the present study; these include SM C16:0, SM C16:1, SM C17:0, SM C18:0, SM C18:1, SM C24:0, SM C24:1, SM C26:0 and SM C26:1. Concentrations of the 9 SM in the plasma and in the three tissues were compared between intensively fed and control bulls by t-test. Level of significance was set at  $P < 0.05$ .

**Results** Final body weight was  $806.5 \pm 9.4$  and  $712.1 \pm 11.5$  (mean  $\pm$  SEM) in the intensively fed and control groups, respectively. Concentrations of SM were affected by the diet in the plasma and in all three tissues. Intensively fed bulls had lower concentrations of SM C17:0 in plasma, in muscle and in adipose tissue, SM C18:0 in plasma and muscle, SM C18:1 in plasma, SM C24:0 in plasma, SM C26:0 in plasma and liver, and greater concentrations of SM C16:0 in adipose tissue, SM C18:0 in adipose tissue and SM C18:1 in liver and adipose tissue. Exact mean values of concentrations with respective P values are shown in Table 1.

**Table 1** Concentrations of sphingomyelins (SM) in blood plasma, liver, skeletal muscle and adipose tissue of Holstein bulls receiving an intensive nutritional regimen or a control diet (mean  $\pm$  SEM, n=15). Significant P values (t-test) are shown in bold.

	Plasma			Liver			Muscle			Adipose tissue		
	Intensive	Control	<i>P value</i>	Intensive	Control	<i>P value</i>	Intensive	Control	<i>P value</i>	Intensive	Control	<i>P value</i>
	Mean $\pm$ SEM	Mean $\pm$ SEM		Mean $\pm$ SEM	Mean $\pm$ SEM		Mean $\pm$ SEM	Mean $\pm$ SEM		Mean $\pm$ SEM	Mean $\pm$ SEM	
SM C16:0	70.25 $\pm$ 3.10	74.51 $\pm$ 3.42	<i>0.36</i>	30.77 $\pm$ 1.00	28.97 $\pm$ 1.08	<i>0.23</i>	4.10 $\pm$ 0.26	4.59 $\pm$ 0.17	<i>0.13</i>	20.40 $\pm$ 0.96	17.61 $\pm$ 0.77	<b>0.03</b>
SM C16:1	7.63 $\pm$ 0.48	8.46 $\pm$ 0.42	<i>0.21</i>	1.80 $\pm$ 0.13	1.83 $\pm$ 0.16	<i>0.91</i>	0.23 $\pm$ 0.02	0.26 $\pm$ 0.01	<i>0.16</i>	0.49 $\pm$ 0.03	0.50 $\pm$ 0.04	<i>0.93</i>
SM C17:0	6.18 $\pm$ 0.30	8.20 $\pm$ 0.46	<b>&lt;0.01</b>	32.56 $\pm$ 1.08	34.87 $\pm$ 0.88	<i>0.11</i>	15.71 $\pm$ 0.57	20.39 $\pm$ 0.59	<b>&lt;0.01</b>	12.22 $\pm$ 0.78	14.25 $\pm$ 0.50	<b>0.03</b>
SM C18:0	9.55 $\pm$ 0.51	11.70 $\pm$ 0.60	<b>0.01</b>	8.37 $\pm$ 0.27	8.22 $\pm$ 0.23	<i>0.68</i>	14.28 $\pm$ 0.50	15.86 $\pm$ 0.44	<b>0.03</b>	13.91 $\pm$ 0.72	11.88 $\pm$ 0.49	<b>0.03</b>
SM C18:1	4.00 $\pm$ 0.24	4.84 $\pm$ 0.25	<b>0.02</b>	4.98 $\pm$ 0.23	4.01 $\pm$ 0.23	<b>0.01</b>	5.82 $\pm$ 0.18	6.06 $\pm$ 0.30	<i>0.49</i>	2.37 $\pm$ 0.16	1.98 $\pm$ 0.11	<b>0.05</b>
SM C24:0	18.23 $\pm$ 1.22	31.73 $\pm$ 1.55	<b>&lt;0.01</b>	13.08 $\pm$ 0.60	14.59 $\pm$ 1.36	<i>0.32</i>	3.70 $\pm$ 0.18	3.75 $\pm$ 0.13	<i>0.84</i>	12.59 $\pm$ 0.65	11.34 $\pm$ 0.46	<i>0.12</i>
SM C24:1	6.63 $\pm$ 0.37	6.99 $\pm$ 0.32	<i>0.46</i>	2.05 $\pm$ 0.14	2.33 $\pm$ 0.28	<i>0.38</i>	1.02 $\pm$ 0.05	1.14 $\pm$ 0.06	<i>0.12</i>	2.70 $\pm$ 0.16	2.57 $\pm$ 0.09	<i>0.48</i>
SM C26:0	0.28 $\pm$ 0.02	0.36 $\pm$ 0.03	<b>0.05</b>	1.05 $\pm$ 0.10	1.51 $\pm$ 0.17	<b>0.03</b>	0.09 $\pm$ 0.01	0.10 $\pm$ 0.01	<i>0.30</i>	0.46 $\pm$ 0.08	0.41 $\pm$ 0.02	<i>0.48</i>
SM C26:1	0.22 $\pm$ 0.03	0.15 $\pm$ 0.04	<i>0.19</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	<i>0.81</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	<i>1.00</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	<i>0.29</i>

**Conclusions** Decreased SM concentrations observed in intensively fed bulls can reflect a higher activity of SM hydrolytic pathways, potentially triggered by pro-inflammatory signals. This is in line with lower SM concentrations found in transition cows, particularly after prepartum overconditioning, which was discussed to be an effect of an increased ceramide production from SM due to inflammatory factors (Rico et al., 2017). However, the exact role of different SM species is not clear yet and future research is warranted to understand the (patho-)physiological meaning of different SM patterns seen across blood plasma and different tissues.

## References

- Greco LF, Neto JTN, Pedrico A, Ferrazza RA, Lima FS, Bisinotto RS, Martinez N, Garcia M, Ribeiro ES, Gomes GC, Shin JH, Ballou MA, Thatcher WW, Staples CR and Santos JEP 2015. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on performance and inflammatory responses to a lipopolysaccharide challenge in lactating Holstein cows. *J Dairy Sci* 98, 602.
- Maceyka M and Spiegel S 2014. Sphingolipid metabolites in inflammatory disease. *Nature* 510, 58.
- Nogalski Z, Wielgosz-Groth Z, Purwin C, Nogalska A, Sobczuk-Szul M, Winarski R and Pogorzelska P 2014. The Effect of Slaughter Weight and Fattening Intensity on Changes in Carcass Fatness in Young Holstein-Friesian Bulls. *Ital J Anim Sci* 13, 2824.
- Rico JE, Saed Samii S, Mathews AT, Lovett J, Haughey NJ and McFadden JW 2017. Temporal changes in sphingolipids and systemic insulin sensitivity during the transition from gestation to lactation. *PLOS ONE* 12, e0176787.

### Effects of physiological stress during pregnancy on adaptation processes in imported cows during postpartum and dry periods

I. Ventsova<sup>1</sup>, A. Vostroilov<sup>1</sup> and V. Safonov<sup>2</sup>

<sup>1</sup>Voronezh State Agricultural University N.A. Emperor Peter the Great, Voronezh, Russia; <sup>2</sup>Vernadsky Institute of Geochemistry and Analytical Chemistry, Moscow, Russia

Email: sineva\_2020@list.ru

**Introduction** It is well known that stress reactions in the body are accompanied by a combination of general stereotypical responses and functional changes, which also include significant biochemical changes in the blood of animals. One of the manifestations of stress reactions of the body is the physiological stress during pregnancy and parturition. It is these periods, as well as the early postpartum period, which are characterized by the most dramatic changes in the metabolism in the bodies of animals. The purpose of this study was to analyze the indicators of lipid and carbohydrate exchanges during dry periods and after parturition in domestic and imported cows in order to monitor their adaptation to the conditions of farms of the Central Black Earth Region of the Russian Federation.

**Materials and methods** Studies were performed on heifers and first-calf cows of the Holstein breed. All animals were divided into two groups: 1 - heifers and first-calf cows of German selection, 2 - heifers and first-calf cows of domestic selection belonging to the household of Liskinsky district of the Voronezh region. Their blood and serum were tested for levels of cholesterol, total lipids, glucose, lactate and pyruvate.

**Results** A month before calving, the level of cholesterol in the blood of cows in both groups was lower than the physiological norm by 16.4-27.2%. The content of total lipids in their blood was close to the lower limit of normal. Such low levels of lipid metabolism may indicate the activation of catabolism processes aimed at enhanced growth of the fetus and the preparation of the mother's body for delivery act.

A similar trend is observed in carbohydrate metabolism. Thus, the blood glucose in cows was lower than the normal content by 27.7-31.8%, lactate - by 24.5-28.3%, and the level of pyruvate was close to the lower limit of the physiological norm.

**Table 1** Indicators of lipid and carbohydrate metabolism in cows during pre-and postpartum periods.

Indicators	Observation periods					
	1 - 1.5 months before calving		2 weeks before calving		1-3 days after calving	
	1 group	2 group	1 group	2 group	1 group	2 group
Cholesterol, mM / L	3.61±0.14	3.42±0.27	3.55±0.22	3.90±0.11	2.31±0.21***	3.35±0.18***
Total lipids, g / L	3.93±0.08	3.45±0.20	3.28±0.27	3.25±0.13	3.06±0.08	3.35±0.21
Glucose, mM / L	1.60±0.08	1.51±0.05	2.59±0.05***	2.54±0.04***	1.55±0.33	1.66±0.30
Lactate, mM / L	0.80±0.08	0.76±0.03	1.90±0.17***	1.95±0.16***	0.93±0.07**	0.90±0.21**
Pyruvate, mkM / L	131.40±5.59	117.80±5.18	337.40±15.77***	334.80±4.12***	298.30±66.55	183.60±7.31***

Note: \*\* - P<0.01; \*\*\* - P<0.001

Two weeks before birth, the lipid metabolism indices remain almost at the same level, which can be associated not only with participation of energy exchange, but also with their inclusion in the synthesis of steroid hormones, which prepare the body for labor and are therefore their direct inducer.

During the first three days after calving, the cholesterol content is further reduced by 34.6-35.1% compared with the prenatal indicators. This may indicate its active inclusion in the synthesis of estrogens, which are among the inducers of the parturitional act, but also ensure the subsequent involution of genital organs.

The content of glucose and lactate in the first days after parturition is significantly reduced by 34.8-59.7%, respectively, which may be caused by the restoration of the carbohydrate "depot" of the body, that is, increased glucose resynthesis of glycogen.

**Conclusions** Since there were no particularly significant differences in the blood indices that were analyzed between the groups of Russian and German cows, it can be concluded that the adaptation processes of the imported cattle to their new living conditions can proceed safely and that such cattle is sufficiently resistant to the climatic zone of the Central Black Earth Region of the Russian Federation.

### Effects of an intramammary LPS challenge on inflammatory and metabolic responses in early lactating cows supplemented with conjugated linoleic acid

J. J. Gross<sup>1</sup>, L. Grossen-Rösti<sup>1</sup>, R. Héritier<sup>1</sup>, A. Tröscher<sup>2</sup> and R. M. Bruckmaier<sup>1</sup>

<sup>1</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland; <sup>2</sup>BASF SE, Lampertheim, Germany

Email: josef.gross@vetsuisse.unibe.ch

**Introduction** The trans-10, cis-12 isomer of conjugated linoleic acid (CLA) causing a milk fat depression was shown to allow nutrient repartitioning despite an energy deficiency in early lactation and also to spare glucose. The “abundant” nutrients, in particular glucose, might improve the metabolic status and benefit the immune system. In the present study we investigated the potential nutrient sparing effects of CLA in early lactating dairy cows with low plasma glucose concentrations exposed to an intramammary LPS challenge. We hypothesized that CLA supplementation affects the metabolic and immune responses to LPS.

**Material and Methods** Fifteen multiparous Holstein dairy cows (parity:  $2.8 \pm 0.9$ , milk yield in previous lactation:  $7,376 \pm 1,247$  kg, mean  $\pm$  SD) were studied in week 4 after parturition (p.p.). Based on parity and milk yield in the previous lactation, cows were assigned to a group with CLA supplementation (CLA; n = 8) and one without CLA supplementation (CON; n = 7) 5 week before expected calving. Cows were supplemented daily either with 70 g of lipid-encapsulated CLA (6.8 g t10,c12 and 6.6 g of c9,t11 CLA isomer) or 56 g of control fat. In week 4 p.p., an intramammary LPS challenge (applied to one quarter) was performed in all cows as described earlier (Wellnitz et al., 2011) to induce an inflammatory response. Milk (LPS and control quarter) and blood samples were taken every 30 and 60 min, respectively. In parallel to the blood samples, all cows were clinically examined. Plasma was analyzed for concentrations of glucose, free fatty acids (FFA), beta-hydroxybutyrate (BHB), cortisol, insulin, and glucagon. In milk, SCC and activity of lactate dehydrogenase (LDH) were determined. Performance and metabolic data were compared using the GLM procedure of SAS with group as fixed effect and initial values of the respective parameters as covariates. Metabolic, clinical, and endocrine responses of CLA and CON during the LPS challenge period were compared by using mixed models with time, group and the time  $\times$  group interaction as fixed effects and the individual cow as repeated subject. Differences between CLA and CON groups were assessed by the Bonferroni corrected t-tests at  $P < 0.05$ .

**Results** In week 4 p.p., no differences in performance parameters were detected between CLA and CON, except with lower plasma glucose concentration in CLA ( $P < 0.05$ ). Between 2 and 3 h after LPS stimulation, rectal temperature, plasma cortisol concentration, LDH activity and SCC in milk began to rise. During the LPS stimulation, CLA tended to have higher rectal temperature compared with CON ( $P = 0.08$ ). However, the increase of body temperature in CLA started earlier, the difference between peak and basal temperature was higher, and the decline thereafter occurred earlier in CLA compared with CON ( $P < 0.05$ ). The increase from basal to peak glucose concentrations following LPS stimulation was higher in CLA compared with CON ( $P < 0.05$ ). Insulin concentration started to increase after 3 h relative to the LPS injection in both CLA and CON ( $P = 0.51$ ). Concomitant with glucose and insulin, plasma glucagon concentration increased. Whereas after 6h glucagon concentration started to decline again in CLA, it further increased in CON. During the first 2 h after LPS administration in each group, plasma BHB concentration remained similar within CLA and CON. Thereafter, BHB concentration declined to a higher extent in CLA and approached similar concentrations to CON from 5 h after LPS injection.

**Conclusions** CLA supplementation of early lactating cows exposed to an intramammary LPS challenge affected local and systemic immune responses. Cows supplemented with CLA provided more glucose and preferentially used BHB as an energy source during the immune response. The more intense metabolic and more concentrated endocrine responses support an immunomodulatory effect of CLA supplementation.

#### References

Wellnitz, O., Arnold, E. T. and Bruckmaier, R. M. (2011): Lipopolysaccharide and lipoteichoic acid induce different immune responses in the bovine mammary gland. J. Dairy Sci. 94: 5405-5412

### Recovery of milk somatic cell count and performance after an intramammary LPS challenge is dependent on the metabolic status of dairy cows

J. J. Gross, L. Grossen-Rösti, S. K. Wall, O. Wellnitz and R. M. Bruckmaier

Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland

Email: josef.gross@vetsuisse.unibe.ch

**Introduction** Infections of the mammary gland in dairy cows are commonly accompanied by reduced milk production, feed intake, and poor milk quality. The recovery of the blood-milk barrier functionality after mastitis is crucial to prevent the unintentional exchange of blood and milk components. The metabolic status of early lactating cows is known to affect immune response to pathogens and imposed immune challenges. We investigated to which extent the metabolic status prior to an intramammary LPS challenge (LPS-CH) impacts immune response, milk production and feed intake, and further affects the recovery hereof.

**Materials and Methods** For 15 Holstein cows, weekly blood sampling and daily recording of dry matter intake (DMI), milk yield, milk composition, and body weight (to calculate energy balance) started 3 wk a.p.. In wk 4 p.p., cows underwent a LPS-CH (50 µg LPS into 1 quarter) with frequent blood and milk sampling. Plasma was analyzed for glucose, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). In milk, serum albumin (SA), immunoglobulin (Ig) G concentration, somatic cell count (SCC) and lactate dehydrogenase (LDH) activity were determined. DMI and milk yield were recorded for a further 6 d. Milk from the LPS treated quarter was sampled for 8 d after the challenge.

**Results** Based on glucose concentrations in wk 1-4 p.p. prior to LPS-CH, cows were retrospectively grouped into a high (HG, n=7) and low glucose group (LG, n=8). Data were evaluated using mixed models with time, group, time×group interaction as fixed effects and cow as repeated subject. Glucose was lower ( $3.17 \pm 0.06$  vs.  $3.68 \pm 0.12$  mmol/L) and BHB higher ( $0.99 \pm 0.16$  vs.  $0.71 \pm 0.08$  mmol/L) in LG compared to HG before LPS-CH ( $P < 0.05$ ), while DMI ( $18.6 \pm 1.0$  vs.  $19.0 \pm 0.7$  kg/d), energy balance ( $-35.1 \pm 8.1$  vs.  $-26.3 \pm 8.2$  MJ NEL/d) and SCC ( $2.1 \pm 1.5$  vs.  $3.0 \pm 2.9$  [log10/mL]) did not differ. During LPS-CH, SCC (HG:  $4.7 \pm 0.1$  to  $7.0 \pm 0.3$ , LG:  $4.7 \pm 0.1$  to  $7.2 \pm 0.2$  at 8 h after the LPS application [log10/mL]) and LDH (HG:  $57.9 \pm 7.4$  to  $2,427.4 \pm 537.6$ , LG:  $62.8 \pm 9.0$  to  $1,774.3 \pm 322.7$  U/L at 8 h after LPS application) increased similarly in HG and LG ( $P > 0.05$ ), body temperature increased less in HG ( $40.5 \pm 0.4$  vs.  $41.5 \pm 0.2^\circ\text{C}$  after 5 h), BHB ( $0.72 \pm 0.12$  vs.  $0.64 \pm 0.06$  mmol/L) and NEFA ( $0.24 \pm 0.06$  vs.  $0.14 \pm 0.04$  mmol/L) were higher in LG compared to HG ( $P < 0.05$ ). DMI declined in both groups at LPS-CH, but recovered earlier to pre-challenge values in HG (day 1 vs. day 2;  $P < 0.05$ ). Milk yield recovered within 2 d after the LPS-CH with no differences in morning milkings, whereas evening milk yield increased faster in HG (day 1 vs. day 2 relative to the LPS challenge day;  $P < 0.05$ ). During 8 d after LPS-CH, SCC ( $5.9 \pm 0.1$  vs.  $6.0 \pm 0.1$  [log10/mL]), LDH ( $401 \pm 95$  vs.  $571 \pm 98$  U/L), IgG ( $0.39 \pm 0.06$  vs.  $0.47 \pm 0.08$  mg/mL;  $P < 0.05$ ) and SA ( $0.21 \pm 0.06$  vs.  $0.33 \pm 0.07$  mg/mL;  $P = 0.07$ ) in milk were lower in HG compared to LG.

**Conclusions** In conclusion, the metabolic status of cows affects metabolic responses during a LPS-CH as well as the recovery of udder health and performance thereafter.

### Prevalence of subclinical ketosis and production diseases during early lactation in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand and Eastern Europe

N. Brunner<sup>1</sup>, S. Groeger<sup>1</sup>, J. Canelas Raposo<sup>1</sup>, R. M. Bruckmaier<sup>2</sup> and J. J. Gross<sup>2</sup>

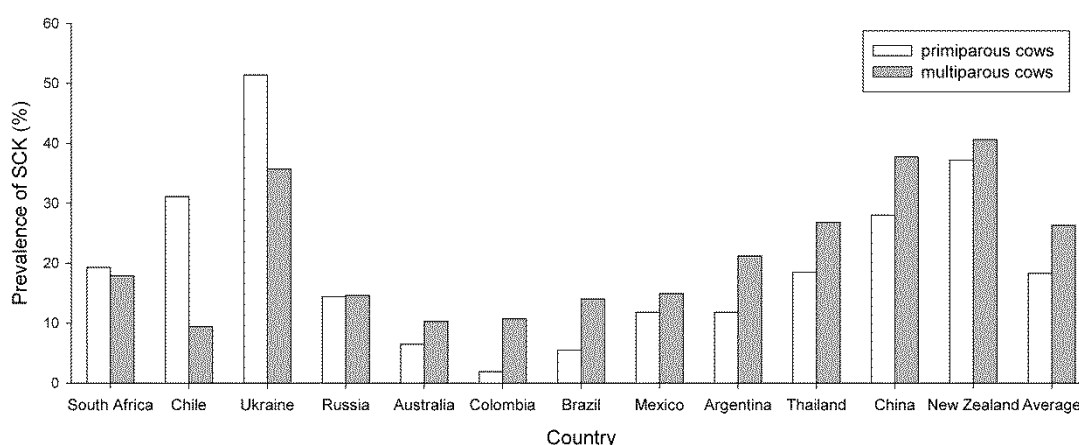
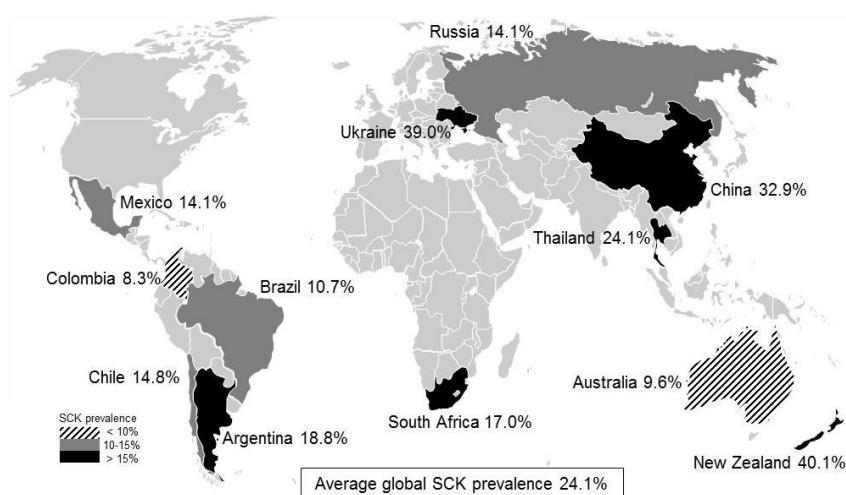
<sup>1</sup>Bayer Animal Health GmbH, Leverkusen, Germany; <sup>2</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland  
Email: nikolaus.brunner@bayer.com

**Introduction** Subclinical ketosis (SCK) and other periparturient diseases considerably account for economic and welfare losses in dairy cows. The majority of scientific reports investigating the prevalence of SCK and production diseases in early lactation are predicated on empirical studies conducted in countries of Western Europe and North America. The objective of the present study was to survey the prevalence of SCK and production-related clinical diseases in early lactating dairy cows in various countries across the world beyond North America and Western Europe.

**Materials and Methods** Twelve countries of South and Central America (Argentina, Brazil, Chile, Colombia, Mexico), Africa (South Africa), Asia (Thailand, China), Eastern Europe (Russia, Ukraine), Australia and New Zealand were assessed, and data from a total of 8 902 cows kept at 541 commercial dairy farms were obtained. A minimum of five cows per farm was investigated once after parturition up to day 21 of lactation. Plasma concentration of  $\beta$ -hydroxybutyrate (BHBA) was measured and the presence of production-related diseases such as retained placenta, milk fever, metritis, mastitis, displaced abomasum, claw disease and lameness, and clinical ketosis was recorded. The threshold for classifying cows with SCK was set at a BHBA concentration of 1.2 mmol/l.

**Results** More than 95% of all cows were examined in their second week of lactation. Across all investigated countries, the SCK prevalence was 24.1%, ranging from 8.3% (Colombia) up to 40.1% (New Zealand). The prevalence of production-related diseases detected during the first 21 days of lactation was relatively low (retained placenta 4.0%, milk fever 4.3%; metritis 5.3%, mastitis 3.4%; displaced abomasum 0.3%; claw diseases and lameness 1.7%; clinical ketosis 0.7%). Calculated odds ratios (OR) did not indicate an elevated risk for production diseases in cows with SCK.

► **Figure 1** Prevalence of subclinical ketosis (SCK, blood BHBA concentrations  $\geq 1.2$  mmol/L) in early lactating dairy cows studied in 12 countries



▲ **Figure 2** Occurrence of subclinical ketosis (SCK, blood BHBA concentrations  $\geq 1.2$  mmol/L) in primiparous and multiparous cows during early lactation in different countries worldwide

**Conclusions** Despite differences in production systems across countries and variation between individual farms within a region, the present data on SCK prevalence agree with observations in Western European and North American dairy herds. At the time of sampling and clinical examination, which was early in lactation, the occurrence of common production diseases was minor. Results might have been different at a later stage of lactation.

### Is there an association between methane emission and lymphocyte response in periparturient cows?

S. Meese<sup>1,6</sup>, S. E. Ulbrich<sup>1</sup>, H. Bollwein<sup>2</sup>, R. Bruckmaier<sup>3</sup>, O. Wellnitz<sup>3</sup>, M. Kreuzer<sup>1</sup>, M. Röntgen<sup>4</sup>, U. Gimsa<sup>4</sup> and A. Schwarm<sup>5</sup>

<sup>1</sup>ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland; <sup>2</sup>Vetsuisse Faculty, University of Zurich, Switzerland;

<sup>3</sup>Vetsuisse Faculty, University of Berne, Switzerland; <sup>4</sup>Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany;

<sup>5</sup>Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, Ås, Norway; <sup>6</sup>AgroVet-Strickhof, Lindau, Switzerland

Email: [susanne-meese@ethz.ch](mailto:susanne-meese@ethz.ch)

**Introduction** Lactation is an energy consuming process and might be maintained at the expense of other processes, e.g. maternal immune function. Disease susceptibility is often associated with limitations in energy availability in lactating dairy cows (Mallard et al. 1998, Dänicke et al., 2018). The host defence largely differs among cows, especially in early lactation, as a result of metabolic and endocrine changes (Meese et al. 2018; Wang et al. 2018). The primary cause of a reduced proliferation might be immune cell cycle abortion after activation. The aim of the present study was to investigate interrelations of the peripheral blood mononuclear cell (PBMC) response with metabolic and digestive traits during the transition period in high-yielding dairy cows kept under equal feeding and housing conditions.

**Materials and Methods** Holstein cows (n=17) in transition from their second to fifth lactation were studied 14 ± 6 days before and 11 ± 3 days after calving. Cows were kept in a tiestall and were restricted in dry matter intake (DMI) before and had *ad libitum* feed access after calving. Zootechnical parameters (body weight, back fat thickness) as well as milk yield, plasma glucose, non-esterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate (BHB) and cortisol concentrations were assessed. Additional, gaseous exchange of cows was measured individually for 2 days in respiration chambers to quantify CH<sub>4</sub> production, heat production and resting metabolic rate (RMR). Cows were clinically healthy and based on the proliferation index (PI) of PBMC after calving classified as low (L), medium (M) and high (H) immune responders. The PI is the ratio of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide-reducing activity of stimulated and non-stimulated PBMC after 72 h. The activation index (AI) is the ratio of oxygen consumed by stimulated and non-stimulated PBMC after 24 h. Repeated measurement analyses of variance was performed with the Mixed procedure (SAS 9.3) with time (before/after calving) and group (L, M, H) and time × group as fixed effects. Multiple comparisons were performed by the Tukey-Kramer test.

**Results** After calving, an increase ( $P < 0.001$ ) in DMI (from 11 to 22 kg), RMR and heat production was observed, but CH<sub>4</sub> yield (per DMI), plasma glucose and cortisol decreased ( $P < 0.05$ ) and no effect on body reserve mobilization (backfat thickness, NEFA and BHB) was observed. After calving, methane yield (per unit DMI) decreased ( $P < 0.01$ ) in low responder cows and CH<sub>4</sub> emission per unit of body weight increased in all groups, but least in L cows after calving. Efficiency of milk production (milk/body weight) and feed efficiency (milk/intake) did not significantly differ among groups. Methane emission intensity (per unit milk) was lower in L than M and H cows. The AI of PBMC was not affected by group or time, but there was a trend for an interaction between group and time.

**Conclusions** Cows markedly differed in proliferative response of the lymphocytes despite their comparable activation and metabolic as well as endocrine status. However, changes in proliferation coincided with changes in enteric CH<sub>4</sub> formation. Cows with a low PI in early lactation had 20% lower CH<sub>4</sub> yields and 25% lower CH<sub>4</sub> emission intensities than cows with a medium and high PI. Because the level of CH<sub>4</sub> emissions is an indicator of rumen fermentation intensity, results suggest that L cows might exhibit a lower fermentation intensity in early lactation. As a consequence, L cows might gain less energy to sustain the shift from lymphocyte activation to proliferation, which might increase disease susceptibility, because energy is spent without achieving host defense.

**References** Danicke, S., Meyer U., Kersten S., and J. Frahm, 2018. Animal models to study the impact of nutrition on the immune system of the transition cow. *Research in Veterinary Science*. 116:15–27.

Mallard, B.A., Dekkers, J.C., Ireland, M.J., Leslie, K.E., Sharif, S., Vankampen, C.L., Wagter, L., Wilkie, B.N., 1998. Alteration in immune responsiveness during the periparturient period and its ramification on dairy cow and calf health. *Journal of Dairy Science*, 81: 585–595.

Meese, S., Gimsa, U., Röntgen, M., Weitzel, J.M., Tuchscherer, A., Miersch, C., Vernunft, A., Viergutz, T., Kreuzer, M. and A. Schwarm, 2018. Effect of immune modulators and lactation number on in vitro proliferation of lymphocytes from non-pregnant dairy heifers and cows. *Canadian Journal of Animal Science* 98: 898–901.

Wang, S., Meese, S., Ulbrich, S.E., Bollwein, H., Röntgen, M., Gimsa U. and A. Schwarm, 2018. Effect of immune modulators on in vitro activation and proliferation of peripheral blood mononuclear cells from multiparous Holstein cows periparturient. *Journal of Animal Physiology and Animal Nutrition* 102: 1515–1520.

**Acknowledgements** The study was supported by the German Research Foundation (SCHW 1485/3-1).

### Breeding for Health, Welfare and Efficiency: Help Wanted

C. F. Baes<sup>1,2</sup>, G. de Oliveira<sup>1</sup>, H. Emamgholi-Begli<sup>1</sup>, E. Abdalla<sup>1</sup> and B. J. Wood<sup>1,3</sup>

<sup>1</sup>University of Guelph, Department of Animal Biosciences, Guelph, Ontario, Canada; <sup>2</sup>Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland; <sup>3</sup>Hybrid Turkeys, Riverbend Drive, Kitchener Ontario, Canada

Email: [christine.baes@vetsuisse.unibe.ch](mailto:christine.baes@vetsuisse.unibe.ch)

**Introduction** Genetic improvement of health, welfare, efficiency, and fertility traits is challenging due to expensive and fuzzy phenotypes, the polygenic nature of the traits, antagonistic genetic correlations to production traits and low heritabilities. Nevertheless, many organizations have introduced large-scale genetic evaluations for such traits in routine selection indexes. Medium (50K; 54,609 SNP) and high-density (HD; 777,962 SNP) arrays can be applied in genomic selection strategies to improve breeding value accuracy, and also in genome-wide association studies (GWAS) to identify causative mutations responsible for economically important traits. Genomic information is particularly helpful when traits have low heritability. Here we describe examples of health, welfare, efficiency, and fertility traits taken from large-scale genetic and genomic analyses in two species (dairy and turkey), and we identify areas of potential improvement in terms of trait definition and performance testing.

**Materials and Methods:** Dairy data included phenotypic information on 62,498 first-lactation Holstein dairy cattle on 29 of 57 traits currently recorded by the Canadian Dairy Network (available data included fertility records, health records, production records, and type records for all animals, and efficiency records of approximately 2,000 animals). Bivariate and multi-variate genetic correlations between individual traits were estimated using a Markov Chain Monte Carlo Gibbs sampling approach. Traits with high correlations between one another ( $>0.80$ ), with little or no economic value, or with potential for improvement in their definition were noted.

Turkey data included phenotypic information on 25,000 purebred, genotyped turkeys from three distinct lines on 17 traits currently recorded by Hybrid Turkeys (available data included fertility and reproduction records, broodiness, mobility records, meat production records, and feed-efficiency records). Genetic parameters and breeding values were estimated using a restricted maximum likelihood approach, and single-step genomic best linear prediction was implemented using a combined pedigree-genomic relationship matrix. Egg-laying behaviour traits were analysed using a multivariate animal model. Traits with high correlations between one another ( $>0.80$ ), with little or no economic value, or with potential for improvement in their definition were noted.

**Results** General tendencies between trait groups confirm that a number of moderate unfavourable correlations ( $\pm 0.20$  or higher) exist between economically important trait complexes and health, welfare, and fertility traits. For example, production traits are negatively correlated with fertility traits in the dairy dataset; unfavourable correlations were observed between egg-laying behaviour (broodiness) and egg production in turkeys. A number of trait complexes were identified in which “closer-to-biology” phenotypes could provide clear improvements to routine genetic and genomic selection programs.

**Conclusions** While conventional variance component estimation methods have underpinned the genomic component of some traits of economic interest in dairy and turkey, performance testing for health, welfare, efficiency, and fertility traits remains an elusive goal for breeding programs. Although these results are encouraging, there is much to be done in terms of trait definition and obtaining better measures of physiological parameters for wide-scale application in breeding programs. Close collaboration between veterinarians, physiologists, and geneticists is necessary to attain meaningful advancement in such areas.



### Combining multiple omics data types to breed healthier animals

H. D. Daetwyler<sup>1,2</sup>, R. Xiang<sup>1,3</sup>, I. M. MacLeod<sup>1</sup>, T. D. W. Luke<sup>1,2</sup>, P. N. Ho<sup>1</sup>, T. T. T. Nguyen<sup>1</sup>, M. Wang<sup>1,2</sup>, C. P. Prowse-Wilkins<sup>1,3</sup>, C. J. Van der Jagt<sup>1</sup>, Z. Liu<sup>1</sup>, B. Sunduimijid<sup>1</sup>, A. Benedet<sup>4</sup>, C. Phyn<sup>5</sup>, B. J. Hayes<sup>6</sup>, S. J. Rochfort<sup>1,2</sup>, V. Bonfatti<sup>7</sup>, A. J. Chamberlain<sup>1</sup>, M. E. Goddard<sup>1,3</sup> and J. E. Pryce<sup>1,2</sup>

<sup>1</sup>Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Australia; <sup>2</sup>School of Applied Systems Biology, La Trobe University, Bundoora, Australia; <sup>3</sup>Faculty of Veterinary & Agricultural Science, The University of Melbourne, Parkville, Australia; <sup>4</sup>Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Legnaro, Italy; <sup>5</sup>DairyNZ Ltd, Hamilton, New Zealand; <sup>6</sup>Centre for Animal Science, The University of Queensland, St Lucia, Australia; <sup>7</sup>Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy

Email: [hans.daetwyler@ecodev.vic.gov.au](mailto:hans.daetwyler@ecodev.vic.gov.au)

**Introduction** It is now feasible to routinely measure many animals with several high-resolution omics technologies providing us with rich data resources to support breeders' selection decisions. Genomic prediction was the first large scale omics approach used in animal breeding. Similarly, mid-infrared (MIR) spectra have been used to predict cattle milk components for many years. More recently, MIR and other metabolomics (e.g. NMR) have been investigated to potentially predict other key dairy traits. Genome sequencing technologies have enabled several approaches to investigate regions of the genome that are associated with gene expression and regulation. Our aim was to combine information from several omics-derived datasets to establish predictor traits and to prioritize variants to increase the accuracy of genomic prediction.

**Materials and Methods** Several MIR datasets were accessed through testing centers in Australia and New Zealand on thousands of cows with other high-value phenotypes, such as subacute rumen acidosis, blood beta-hydroxybutyrate (BHB), hyperketonemia, blood urea, blood non-esterified fatty acids (NEFA), calcium, magnesium, milk phospholipids and oligosaccharides, dry matter intake and methane. MIR and other spectra were analyzed with partial least squares (PLS) for prediction and by band-wise genome-wide association analyses.

Seventeen million sequence variants identified in the 1000 Bull Genomes Project Run6 were imputed into 44,260 animals (about 75% Holstein, 20% Jersey and 5% Australian Red breeds) using Eagle2.1/Minimac3. Sequence variants associated with gene expression (eQTLs) and concentration of milk metabolites (mQTLs, phospholipids), and under histone modification marks in several tissues were discovered from multi-omics data of over 400 cattle. Variants were also identified from 1000 Bull Genomes database (N=2,330) beef-dairy selection signatures. These analyses defined 30 variant sets and for each set we estimated the genetic variance it explained across 34 complex traits in 11,923 bulls and 32,347 cows. Only sets that explained more variance than a random set were carried forward in the analysis. We defined a Functional-And-Evolutionary Trait Heritability (FAETH) score indicating the functionality and predicted heritability of each variant. Further linkage disequilibrium pruning and variant classification reduced the set to 40,000 variants that were included on a new Illumina XT SNP chip design. Finally, we tested whether this new variant set increased genomic prediction accuracy when compared to the standard Illumina 50k SNP chip in an independent cow dataset.

**Results** The  $R^2$  of MIR predictions of difficult-to-measure phenotypes were in the moderate range for most traits at 0.59, 0.58, 0.71, 0.35, 0.45, 0.50 and 0.30 for subacute rumen acidosis, BHB, hyperketonemia, urea, NEFA, dry matter intake and methane, respectively. More validations are needed, but with the potential routine collection of MIR on most cows they will become an attractive source of correlated traits for selection indices. Genomic mapping using MIR or metabolites has revealed known but also new genes involved in the major dairy traits. In several cases, using multiple omics for mapping (i.e. expression, metabolites, etc) has aided in better resolving variant-gene relationships.

In the variant prioritisation work, the per-variant trait heritability of variant sets across traits was highly consistent ( $r > 0.98$ ) between bulls and cows. Based on the per-variant heritability, the sets of mQTL, eQTL and variants associated with non-coding RNAs ranked the highest, followed by the young variants, those under histone modification marks and selection signatures. A XT SNP chip with 40,000 variants from the prioritisation (as well as 8,000 markers overlapping with the Low-Density Dairy SNP chip) is currently used for genotyping these variants directly (to avoid imputation errors). An early validation in cows not used in the prioritisation and using the imputed high-value variants has increased prediction accuracy on average by 3%. The increase in accuracy was more pronounced in crossbred, Jersey and Australian Red cattle, which is encouraging for these smaller breed groups.

**Conclusions** Prediction of important and difficult-to-measure dairy cattle traits, such as animal health, using MIR spectra is proving to be effective. This will increase the accuracy of selection decisions for dairy farmers going forward. Our strategy to prioritize variants from whole-genome sequence using functional genomic, annotation, and phenomic information combined with target trait phenotypes has increased genomic prediction accuracy in animals that are less related to the reference population. This results in genomic breeding values that are more widely applicable across breeds and more robust across generations.

## Why does the hen peck? – Employing omics-approaches to understand the motivation for an unwanted behaviour

J. Beier<sup>1</sup>, C. Falker-Gieske<sup>1</sup>, H. Iffland<sup>2</sup>, S. Preuß<sup>2</sup>, W. Bessei<sup>2</sup>, J. Bennewitz<sup>2</sup> and J. Tetens<sup>1,3</sup>

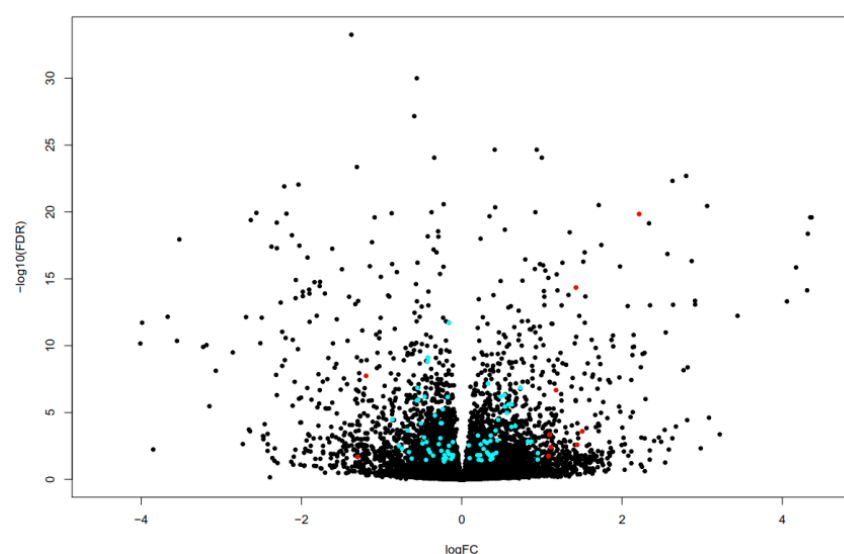
<sup>1</sup>Department of Animal Sciences, Georg-August-University, Göttingen, Germany; <sup>2</sup>Institute of Animal Science, University of Hohenheim, Stuttgart, German; <sup>3</sup>Center for Integrated Breeding Research, Georg-August-University, Göttingen, Germany

Email: jens.tetens@uni-goettingen.de

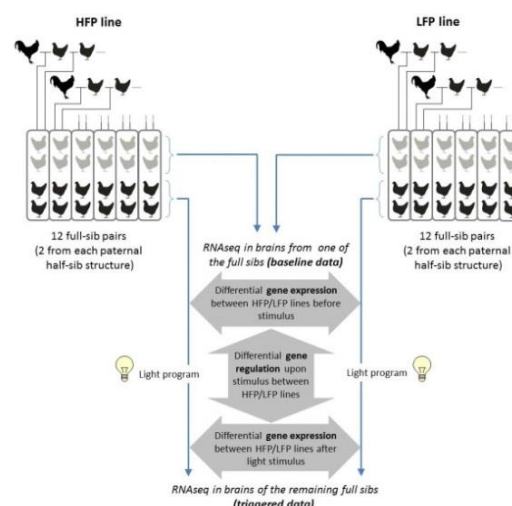
**Introduction** Feather pecking (FP) is a worldwide problem in the layer industry leading to serious impairments of animal welfare and causing considerable economic losses. The propensity to perform FP is a complex trait, which is influenced by numerous factors including a genetic component. Heritability estimates range from approximately 0.1 to 0.4 (Rodenburg et al., 2003; Bennewitz et al., 2014). Despite extensive research efforts, the motivation for this unwanted behavior is not completely understood, but a clear connection with monoamine-signaling has been established (e.g. Kops et al., 2014). Genomic studies conducted so far do partly support this finding, but also revealed conflicting results (for a review see Ellen et al., 2019) and underpinned the complex nature of this trait (e.g. Lutz et al., 2017). With the aim to unravel the genetic background of FP behavior, we set up a large experiment linking genomic sequence level to transcriptomic data in a system-oriented approach. In order to identify signaling pathways involved in FP, we analyzed the brain transcriptomes of White Leghorn layer strains divergently selected for FP behavior with two major research questions: 1) Are there genes and pathways differentially regulated between the strains possibly related to the general FP propensity? and 2) Which transcriptional changes are associated with the onset of the actual behavior upon light stimulation?

**Materials and Methods** A total of 48 hens comprising 12 full-sib pairs from each strain (high vs. low FP) were phenotyped according to established protocols at 27 weeks of age and subsequently kept under low light conditions to prevent the occurrence of FP. One bird from each full-sib pair was then sacrificed and brains were immediately collected for RNA isolation. The remaining birds were kept under increased light intensity ( $\geq 50$  lux) for several hours until they clearly showed FP and were then sacrificed as well (see Fig. 1 for the experimental design). Brain transcriptomic profiling was done by RNAseq (Illumina HiSeq4000, 2x75bp PE) aiming for 30 mio. reads per sample. Reads were mapped against latest chicken genome assembly using Star2pass. Differential gene expression analysis was done using DESeq2 and EdgeR; subsequent gene set enrichment analyses were performed with GAGE.

**Results and Discussion** Comparing the two strains under base line conditions (low light intensity, no FP shown) revealed 626 significantly (FDR<0.05) differentially expressed genes (DEG), while 834 DEG were detected under high light intensity. A considerable difference was found with respect to DEGs upon light stimulation when comparing the two strains (266 DEGs in the high FP strain vs. 688 in the low FP strain). Considering both factors (strain and light intensity) in one model resulted in 536 genes differentially expressed between strains (Fig. 2). A subsequent gene set enrichment analysis revealed three significantly enriched



**Figure 2** Volcano plot depicting the results of the differential gene expression analysis between high and low FP strains. Coloured dots indicate genes involved in significantly enriched pathways (red = sig. diff. expressed).



**Figure 1** Experimental design of the transcriptome study.

( $q < 0.05$ ) KEGG pathways: cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, and cell adhesion molecules. A major limitation of these analyses was the annotation status of the most recent chicken genome assembly leading to a high proportion of anonymous loci among DEGs, which reduces the power of enrichment and pathway analyses. Further analyses will be conducted to overcome this.

**Conclusions** Brain transcriptome profiling revealed differential gene expression between strains of layers divergently selected for FP. The DEGs are enriched for pathways involved in neural signal transduction. Currently, further analyses are conducted to better characterize these differences.

### References

Bennewitz et al. 2014, Poult Sci. 93:810; Ellen E D et al. 2019, Animals, in press; Kops M et al., 2017, Behav Brain Res 327:11; Lutz V et al. 2017, GSE 49:18; Rodenburg et al. 2003, Poult Sci. 82:861.

### Breeding for Resilience: New Opportunities in Modern Pig Breeding Programs

B. Harlizius, P. Mathur and E. F. Knol

TopigsNorsvin Research Center, Beuningen, The Netherlands

Email: [barbara.harlizius@topignorsvin.com](mailto:barbara.harlizius@topignorsvin.com)

**Introduction** Modern pig breeding schemes evaluate a large number of traits on specialized purebred breeding lines to improve the genetic makeup of crossbred animals used for pork production but also for the next generation of purebred breeding animals. A big leap has been achieved in breeding for production and reproduction traits through use of genomic information. However, the group of traits around resilience is not fully exploited and pure line production is mostly done under high health. Efforts are made to identify new phenotypes useful in breeding for resilience including phenotypes collected under more challenging commercial conditions as well as genes and mutations related to specific diseases. In this paper, resilience is defined as minimal changes in the overall performance of an animal in spite of diseases. We want to explore resilience and possibilities for selection by defining traits which describe resilience or parts thereof including state of the art genomic approaches.

**Materials and Methods** The backbone of breeding for resilient pigs is survival through life. Survival at birth and pre-weaning survival data are collected by an app (e.g. ToNo) on a mobile device in piglet production farms. Remarks for different diseases, injuries and other abnormalities were obtained from meat inspection data from 140,375 carcasses of finisher pigs inspected by trained meat inspectors through a close cooperation between Topigs Norsvin and slaughter houses in Germany (Mathur et al. 2018). Specific disease infection trials with PRRSV have been performed in collaboration with PigGenCanada and a natural disease challenge model that mimics commercial conditions with a mixture of field pathogens is currently explored. Performance data from commercial farms have been explored to improve resilience as describe in Herrero-Medrano et al., 2015, and Mulder, 2016. Genotype data from low density (50K) and high density (660K) SNP (single nucleotide polymorphisms) chips have been used to identify regions in the genome that contain deleterious alleles (Derks et al. 2019).

**Results** The definition of resilience implies to collect production data in the commercial environment. The underlying traits such as mortality in different stages of life, carcass remarks, mortality under specific and general disease infection but also variation in feed intake show considerable genetic variation. Carcass remarks clearly indicate that something was suboptimal during production. Heritability estimates of carcass remarks for pneumonia, pleuritis, pericarditis, liver lesions and joint disorders were 0.10, 0.09, 0.14, 0.24 and 0.17, respectively, on the liability scale suggesting existence of substantial genetic variation (Mathur et al. 2018). GWAS revealed a SNP on chromosome 6 (6:150824929) having significant effect on pericarditis (Mathur et al., 2018). Infection trials with PRRSV have shown that natural selection for disease resistance is possible. A new marker (WUR10000125) has favorable effects on viremia and weight gain even during co-infection porcine circovirus type 2b (PCV2b) (Dunkelberger et al., 2017). The results also show possibilities of using genetics to enhance vaccine response. First results of a natural disease challenge trial with a mixture of field pathogens show that variation in feed intake is correlated to mortality and number of veterinary treatments and can be used as novel phenotype for resilience (Putz et al. 2019). Reproduction data from multiplication farms can be used to improve resilience and genetic progress further enhanced by genomic selection. A Combined Crossbred and Purebred Selection (CCPS) program of Topigs Norsvin uses data from commercial farms, resulting in superior performance at the commercial level in spite of multiple diseases. From genotype data, several regions as well as mutations have been identified in 4 breeding lines being associated with embryonic and fetal death, stillbirths or pre-weaning mortality and phenotypes were proven from carrier by carrier matings (Derks et al. 2019). Until now, all deleterious variants have been shown to be line-specific and recessive and are therefore not expected to cause an increase in mortality in final crossbreds used for pork production. Currently, potentially deleterious variation from whole genome sequence data are investigated.

**Conclusions** Resilience traits have a sizable heritable component. The feedback of traits recorded on crossbred animals under challenging field conditions into the top of the purebred nucleus herds offers new opportunities for genetic improvement of overall resilience. Furthermore, genetic correlations between new disease traits from infection or challenge trials and production data such as feed intake can help to identify indicator traits for resilience that are recorded on large scale. Availability of genomic information at decreasing costs and new genetic selection tools increase the opportunities for breeding for resilience. Finally, genomic data deliver new possibilities to identify and control deleterious variation directly from DNA sequence. Nevertheless, for effective disease control and eradication measures, integrated approaches by geneticist, immunologists, virologists and other disciplines are necessary.

### References

- Derks MFL, et al. (2019): Loss of function mutations in essential genes cause embryonic lethality in pigs. *PLOS Genet.*
- Dunkelberger, J. et al. 2017. Effect of a major quantitative trait locus for porcine reproductive and respiratory syndrome (PRRS) resistance on response to coinfection with PRRS virus and porcine circovirus type 2b (PCV2b) in commercial pigs, with or without prior vaccination for PRRS. *Journal of Animal Science* 95: 584-598.
- Herrero-Medrano, J. et al. 2015. Estimation of genetic parameters and breeding values across challenged environments to select for robust pigs. *J. Anim. Sci.* 93: 1494-1502.
- Mathur, P. K., R. Vogelzang, H. A. Mulder, and E. F. Knol. 2018. Genetic selection to enhance animal welfare using meat inspection data from slaughter plants. *Animals* 8: 16.
- Putz AM et al. (2019). Novel Resilience Phenotypes Using Feed Intake Data From a Natural Disease Challenge Model in Wean-to-Finish Pigs. *Front Genet.* 2019 9:660

### From lab to farmyard: genome editing our livestock

C. Tait-Burkard

The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom

Email: [Christine.Burkard@roslin.ed.ac.uk](mailto:Christine.Burkard@roslin.ed.ac.uk)

With the world population predicted to reach almost 10 billion by 2050 there are a number of challenges in sustainable management of finite resources. The rising demand for food requires improved productivity of agricultural systems. One of the major burdens on the livestock industry is loss of animals and decrease of production efficiency due to disease. Furthermore, it is important to improve the health and welfare of animals by reducing and preferably preventing the effects of disease. For thousands of years humans have used selective breeding to improve desirable traits in both livestock and companion animals. Advances in sequencing technology and genome editing techniques provide the unique opportunity to generate animals with even further improved traits. One of the inherently difficult production traits to measure is resistance to a specific disease, as animals with less severe symptoms or pathology may simply have been exposed to less pathogen. Experimental infections guaranteeing equal pathogen exposures are expensive and require large numbers of animals for genetic association studies, making them ethically questionable. Genome editing offers new opportunities to livestock breeding for disease resistance, allowing the direct translation of laboratory research into disease resistant or resilient animals.

Recent examples of genome editing for disease resistance in pigs show that this technology can be successfully applied in generating disease resistant animals through the translation of *in vitro* research. For example, we showed that a small deletion of the CD163 gene can render pigs resistant to porcine reproductive and respiratory syndrome virus (PRRSV) infection. PRRSV is the etiological agent of PRRS, causing late-term abortions, stillbirths, and respiratory disease in pigs, incurring major economic losses to the worldwide pig industry. Current control strategies mainly involve biosecurity measures, depopulation, and vaccination. Thus far, they have failed in preventing the further spread and increased prevalence of the disease worldwide. We have edited the internal receptor of the virus, the CD163 protein, by removing one of the nine protein domains that arrange in a beads-on-a-string structure. This resulted in the animals being fully resistant to the virus infection. Removal of the PRRSV-interacting domain from the CD163 protein did not impact on any of the biological functions of the protein or overall pig health.

This and other examples show that genetic-control approaches through genome editing are promising approaches that could benefit animal welfare as well as the pork industry. They also highlight the potential risks, technological advances, as well as the political and societal changes that will be required to successfully integrate genome editing technology into livestock breeding.

### References

- Burkard, C., S. G. Lillico, E. Reid, B. Jackson, A. J. Mileham, T. Ait-Ali, C. B. Whitelaw, and A. L. Archibald. 2017. Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLoS pathogens* 13(2):e1006206. doi: 10.1371/journal.ppat.1006206
- Burkard, C., T. Opriessnig, A. J. Mileham, T. Stadejek, T. Ait-Ali, S. G. Lillico, C. B. A. Whitelaw, and A. L. Archibald. 2018. Pigs Lacking the Scavenger Receptor Cysteine-Rich Domain 5 of CD163 Are Resistant to Porcine Reproductive and Respiratory Syndrome Virus 1 Infection. *Journal of virology* 92(16):doi: 10.1128/JVI.00415-18
- Tait-Burkard, C., A. Doeschl-Wilson, M. J. McGrew, A. L. Archibald, H. M. Sang, R. D. Houston, C. B. Whitelaw, and M. Watson. 2018. Livestock 2.0 - genome editing for fitter, healthier, and more productive farmed animals. *Genome biology* 19(1):204. doi: 10.1186/s13059-018-1583-1

### Different genetic reactions of Simmental and Holstein dairy cattle concerning udder health along a continuous climate scale

B. Bapst<sup>1</sup>, M. Bohlouli<sup>2</sup>, S. König<sup>2</sup> and K. Brügemann<sup>2</sup>

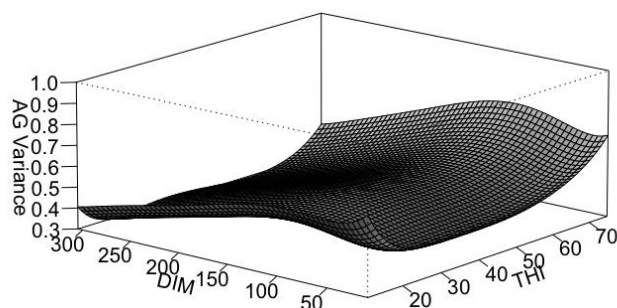
<sup>1</sup>Qualitas AG, Zug, Switzerland; <sup>2</sup>University of Giessen, Department of Animal Breeding and Pet Genetics, Giessen, Germany

Email: Beat.Bapst@qualitasag.ch

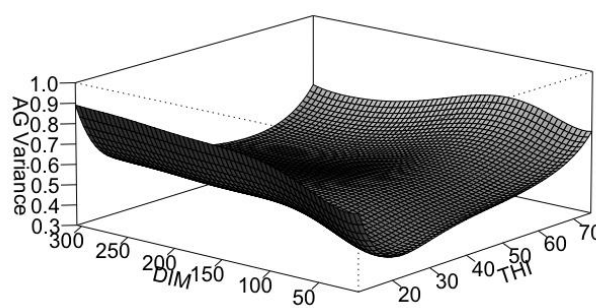
**Introduction** In the context of discussions on climate change the question arises whether dairy cows respond differently to changing weather conditions and therefore whether genotype x environment interactions (GxE) exist. Udder health (UH) is one of the most important functional traits in dairy cattle. UH is not only important from the point of view of quality requirements but also for economic reasons (Hogeveen et al., 2011) as well as animal welfare reasons. Somatic cell count (SCC) or somatic cell score (SCS) have been included in breeding strategies as auxiliary traits for UH because the genetic correlation between SCS and mastitis incidence is up to 0.67 (Coffey et al., 1986). The investigations presented here have been initiated with Swiss Holstein (HO) and Swiss Simmental (SI) by considering countrywide weather data in order to investigate the impact of the breeding strategy on the response to environmental changes. HO represents dairy breeds and SI is a classical dual purpose breed and our hypothesis was that dairy breeds react much more sensitive than dual purpose breeds on changing environmental conditions.

**Materials and Methods** We developed a reaction norm model, where we included weather data in a random regression test day model (RRTDM), which was derived from the official Swiss genetic evaluation system. The applied RRTDM was built based on previous work from Bohmanova et al. (2008). The trait of interest was SCS. Daily temperature-humidity indexes (THI, NRC (1971)) from 60 official federal weather stations were assigned to each HO and SI herd from swissherdbook (www.swissherdbook.ch) as a continuous environmental descriptor over a time period of 10 years. After data editing, 1,106,410 and 7,340,498 test day (TD) records remained from 61,064 SI and from 363,472 HO cows, respectively. For fitting the additive genetic and the permanent environment effects, the RRTDM was extended with the covariates days in milk (DIM) and THI, both with Legendre polynomials of order 3.

**Results** First results show that the additive genetic variance of SI and HO concerning SCS along the DIM and along the THI axis have not the same course (Figure 1 and 2). Changes in additive genetic variance along either gradient show sensitivity to this particular gradient. Therefore it can be observed that HO is more sensitive to DIM than to THI while SI is more sensitive to THI than to DIM. Further genetic parameters like heritability or genetic correlations differ between the two breeds along THI and along DIM as well. Genetic correlations of SI among specific THI combinations fall several times massively below the threshold of 0.8, which indicates that GxE exist (Robertson, 1959). HO usually stays above 0.8.



**Figure 1** Additive genetic (AG) variance of SCS of HO along DIM and THI



**Figure 2** Additive genetic (AG) variance of SCS of SI along DIM and THI

**Conclusions** The preliminary results point out that the dual-purpose breed SI reacts more sensitive to an environmental gradient than the dairy breed HO. This result is unexpected and requires further investigations. Additionally the results indicate that the impact of expanding the model with weather covariates on the breeding value estimation must be evaluated regarding a reranking of sires and dams.

#### References

- Bohmanova, J., F. Miglior, J. Jamrozik, I. Misztal, and P. G. Sullivan. 2008. Comparison of random regression models with Legendre polynomials and linear splines for production traits and somatic cell score of Canadian Holstein cows. *J. Dairy Sci.* 91:3627–3638.
- Coffey, E. M., W. E. Vinson, and R. E. Pearson. 1986. Potential of somatic cell concentration in milk as a sire selection criterion to reduce mastitis in dairy cattle. *J. Dairy Sci.* 69:2163–2172.
- Hogeveen, H., Huijps, K., and T.J.G.M. Lam. 2011. Economic aspects of mastitis: New developments. *New Zealand Veterinary Journal.* 59:1, 16-23.
- NRC. 1971. *A Guide to Environmental Research on Animals.* Natl. Acad. Sci., Washington, DC.
- Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. *Biometrics* 15:469–485.

### Exploiting massive genomic data to improve fertility and rearing success in Swiss dairy cattle

I. M. Häfliger<sup>1</sup>, F. R. Seefried<sup>2</sup> and C. Drögemüller<sup>1</sup>

<sup>1</sup>Institute of Genetics, University of Bern, Switzerland; <sup>2</sup>Qualitas AG, Zug, Switzerland

Email: irene.haefliger@vetsuisse.unibe.ch

Since genomic selection had been promoted and firstly introduced in 2008, thousands of cattle had been genotyped for ten thousands of single nucleotide variants (SNV). While genomic selection decreased the generation interval and increased the genetic gain for production traits immensely, many health traits were not yet taken into account. Rather than improving, these important traits worsened in the overall populations.

It is confirmed, that intensive selection for milk production affected fertility and immunity negatively. However, these days genomic breeding values for health traits are implemented and during the last years American Holstein breeders showed, that health and production traits can be improved simultaneously. Nevertheless, through the so-called hitchhiking effect, recessive lethal alleles affecting fertility negatively increased in frequency even though selection pressure was put to improve fertility traits. A reason for the accumulation of recessive embryonic lethal variants is the decreased population size and the thereby increased inbreeding.

Currently, we mine the available genotyping data for certain haplotypes, which impair fertility and rearing success. The general assumption applied is that haplotypes harboring a recessive lethal mutation do not occur in homozygous state within the genotyped normal breeding animals. Thus, we searched for haplotypes in the genome showing a significant depletion from the Hardy-Weinberg equilibrium and successfully identified numerous regions in four Swiss dairy breeds. In order to investigate the effects of such haplotypes, we estimated their impact on reproduction and production traits in a linear mixed model. Furthermore, we performed whole genome sequencing (WGS) of carrier animals to find deleterious variants responsible for embryonic lethality, abortion, stillbirth and other nonviable phenotypes. Up to date, we have more than 400 genomes available and use this data to screen for SNVs and larger structural variants in the genome regions corresponding to the mapped haplotypes.

In addition, we aim to apply the approach of reverse genetics. Thereby, the impact of protein-changing SNVs will be predicted and by genotyping those variants with a customized array, our aim is to demonstrate their effect on certain phenotypes. Through this approach, we intend to early detect reproduction and production diseases affecting calf survival. For example, the *APOB*-associated cholesterol deficiency in Holstein cattle, for which we have identified the causal mutation and which is phenotypically very unspecific, could have been revealed before thousands of calves had died and an economically significant damage had affected the dairy industry.

# Chromosomal imbalance in a boar causing cleft palate in the offspring and affecting the litter size in sows

A. Grahofner<sup>1,2</sup>, A. Letko<sup>3</sup>, I. M. Häfliger<sup>3</sup>, V. Jagannathan<sup>3</sup>, A. Ducos<sup>4</sup>, O. Richard<sup>5</sup>, V. Peter<sup>6</sup>, H. Nathues<sup>2</sup> and C. Drögemüller<sup>3</sup>

<sup>1</sup>Animal Physiology, Institute of Agricultural Sciences, ETH Zurich, Switzerland; <sup>2</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Switzerland; <sup>3</sup>Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland; <sup>4</sup>GenPhyse, INRA, INPT, ENVT, Université de Toulouse, France; <sup>5</sup>Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland; <sup>6</sup>Division of Clinical Radiology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

Email: alexander.grahofner@usys.ethz.ch

**Introduction** Unilateral and bilateral palatoschisis or cleft palate is a well-known congenital anomaly of craniofacial development in several animal species including pigs. Only limited information regarding the occurrence of palatoschisis or cleft palate in the pig population is available. Commonly, the affected piglets die within the first days of life due to the aspiration of colostrum or milk into the lungs and consequential associated complications. Little is known about its aetiology. Nevertheless, environmental and genetic factors have been described. The aim of this study was to apply recently improved genomic resources in pigs to evaluate a possible genetic cause for the occurrence of several piglets with palatoschisis in the progeny of a single boar. Furthermore, the effect on the litter size was analysed.

**Materials and Methods** Due to congenital anomalies in piglets on a satellite farrowing farm in Switzerland, further investigation were conducted to evaluate the possible cause for the palatoschisis (Figure 1) in the piglets. Six litters were analyzed in detailed and further samples were obtained from the farmer. In total twenty affected piglets were used in this study.

**Results** In these six litters a significant decrease (p-value: 0.0055) in total born piglets (14.7 (SD  $\pm$  0.9) to 9.7 (SD  $\pm$  2.7)) and an increase of stillborn piglets (0.8 (SD  $\pm$  0.6) to 1.7 (SD  $\pm$  1.2)) compared with the former litters was noticed in the affected litters (Table 1). A detailed description of the phenotype was derived from necropsy and by computed tomography revealing that all 20 cases also exhibited palatoschisis and renal cysts. Furthermore, a genetic origin was assumed due to dominant inheritance as all 20 recorded cases were confirmed offspring of a single boar. The boar, which sired the malformed piglets, carried a balanced translocation between Mb-sized segments of chromosome 8 and 14. This had not been previously observed during karyotyping. All affected offspring were shown to be carriers of a partial trisomy of chromosome 14 including the FGFR2 gene, which is associated with various dominant inherited craniofacial dysostosis syndromes in man, and partial monosomy of chromosome 8 containing MSX1 known to be associated with tooth agenesis and orofacial clefts in other species.

**Table 1** Reproductive parameters of six affected litters with malformed piglets in comparison with previous records of the sows.

Sow	Litter number	Total born piglets	Live born piglets	Dead born piglets	Palatoschisis
	(n)	(n/litter)	(n/litter)	(n/litter)	(n/litter)
		Status quo / before	Status quo / before	Status quo / before	Status quo / before
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean
1	2	6.0 / 14.0 $\pm$ 0	5.0 / 13.0 $\pm$ 0	1 / 1 $\pm$ 0	2 / 0
2	6	7.0 / 14.2 $\pm$ 0.8	4.0 / 13.0 $\pm$ 1.4	3 / 1.2 $\pm$ 1.3	1 / 0
3	6	9.0 / 15.6 $\pm$ 2.4	8.0 / 15.4 $\pm$ 2.5	1 / 0.2 $\pm$ 0.5	2 / 0
4	7	12.0 / 13.5 $\pm$ 1.0	9.0 / 13.3 $\pm$ 0.8	3 / 0.2 $\pm$ 0.4	6 / 0
5	10	12.0 / 14.9 $\pm$ 2.3	10.0 / 14.8 $\pm$ 2.4	2 / 0.4 $\pm$ 0.5	1 / 0
6	7	12.0 / 15.8 $\pm$ 1.7	12.0 / 14.2 $\pm$ 1.9	0 / 1.6 $\pm$ 1.9	4 / 0
Average	6.3	9.7 $\pm$ 2.7 / 14.7 $\pm$ 0.9	8.0 $\pm$ 3.0 / 13.9 $\pm$ 1.0	1.6 $\pm$ 1.2 / 0.8 $\pm$ 0.6	2.7 $\pm$ 2.0 / 0 $\pm$ 0



**Figure 1** Bilateral palatoschisis in a piglet

**Conclusions** In this report, a significant reduction in litter size of the affected litters was detected from the identified sire. Due to the extensive use of artificial insemination in the pig industry, an affected boar can cause tremendous economic losses in pig production. Therefore, the detection of a reciprocal translocation in a boar with recently established genomic resources at an early stage is essential.



### Entropion in Swiss White Alpine sheep is associated with the *CTNND1* gene region on chromosome 15

N. Hirter<sup>1</sup>, A. Letko<sup>1</sup>, I. Häfliger<sup>1</sup>, D. Greber<sup>2</sup> and C. Drögemüller<sup>1</sup>

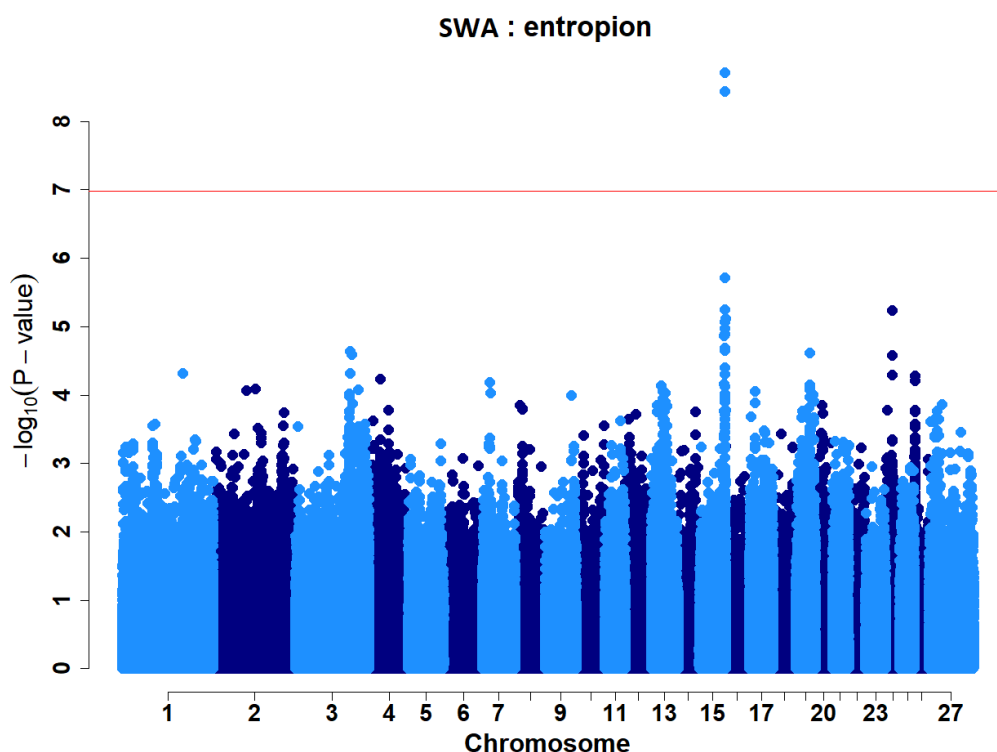
<sup>1</sup>Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland; <sup>2</sup>Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland

Email: [nathalie.hirter@vetsuisse.unibe.ch](mailto:nathalie.hirter@vetsuisse.unibe.ch)

**Introduction** Entropion is a known congenital disorder in different species including sheep. Lambs with entropion have an inward rolling of one or both lower eyelids since birth. The inward looking eyelashes and hairs lead to a constant irritation of the cornea and, in extreme cases, to blindness. In Switzerland, a recent survey showed that the most affected Swiss White Alpine sheep (SWA) breed showed a prevalence of entropion about 6%<sup>1</sup>. Entropion in sheep is supposed to be heritable; however, so far no causative genetic variant causing this disease phenotype has been identified. The aim of the present study was to discover the genetic cause of this disease.

**Materials and Methods** EDTA-Blood samples were collected in more than 400 sheep in the breeds SWA and Texel (TEX) and their crossbreeds. 150 (89 cases, 61 controls) of them were genotyped using the ovine high-density 600k SNP array, and a genome-wide association study (GWAS) was performed. The genomes of two pairs of affected and normal full sibs of SWA, a single affected SWA, and a case-control full sib pair of Texel sheep were sequenced and used for disease-associated variant filtering.

**Results** An associated genome region on chromosome 15 was identified with entropion. The best-associated marker (p-value: 1.95 E-09) was located close to the *CTNND1* gene encoding catenin delta-1. In human, recent studies have identified pathogenic variants in *CTNND1* in blepharocheilodontic syndrome 2, a rare autosomal dominant disorder characterized by eyelid anomalies. After filtering for disease-associated variants in the region around gene *CTNND1* using control genomes of other unrelated sheep breeds, we found two intronic single nucleotide variants and one missense variant in the *CTNND1* gene. Genotyping of more than 300 sheep with known phenotypes showed no association of these three variants with entropion in SWA sheep.



**Figure 1** Genome-wide association study, presented in Manhattan plot, with genome-wide significance threshold (red line = Bonferroni line)

**Conclusions** In conclusion, this study revealed a single locus associated with entropion in Swiss White Alpine sheep. Further sequence analysis is needed to identify the causative variant.

#### References

Greber D, Doherr M, Drögemüller C, Steiner A: Occurrence of congenital disorders in Swiss sheep. 2013



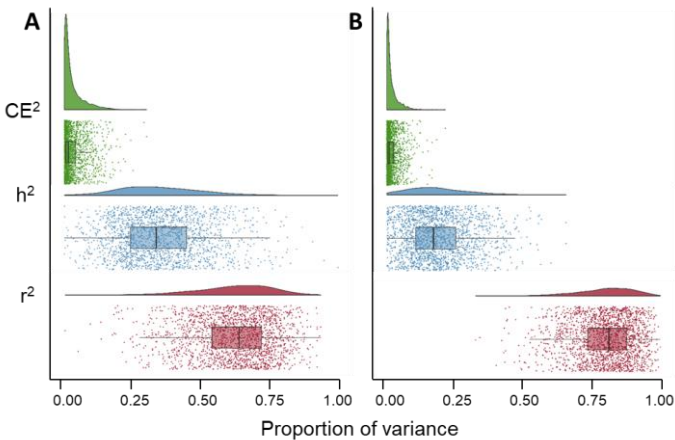
Heritability and phenotypic correlations of protein efficiency in a Swiss Large White pig population

C. Kasper, I. Ruiz-Ascacibar, P. Stoll and G. Bee  
Agroscope, La Tioleyre 4, 1725 Posieux, Switzerland  
Email : claudia.kasper-voelkl@agroscope.admin.ch

**Introduction** Pig production contributes importantly to environmental pollution through the emission of nitrogen waste. Many countries cannot meet the plant-protein demand of livestock with current feed cultivation practices, and an important part of high-quality protein feed, most importantly soybean meal, is imported from South America. The high global demand for protein results in an increased competition of agricultural land for human food and animal feed and ultimately to the large-scale loss of ecological habitats. It is therefore desirable to improve protein efficiency, i.e. the proportion of dietary protein that is fixed in the body, of pigs, an important livestock species, through selective breeding. However, little is known about the inheritance pattern and genetic architecture of protein efficiency as well as the genes underlying protein efficiency and their functions, which is essential for assessing the potential to breed pigs with higher protein efficiency.

**Materials and Methods** We used data of 294 offspring of 17 sires and 56 dams from previous experiments (Ruiz-Ascacibar et al., 2017). Nitrogen (~protein) efficiency of the empty body (*NEffEB*) and of the carcass (*NEffCarc*) were the proportion of fixed protein in the entire body and the carcass, respectively, from the total dietary protein intake. We estimated the genetic ( $h^2$ ) and environmental variance components ( $CE^2$ ) of *NEffEB* and *NEffCarc* using a mixed-effect Gaussian animal model (Henderson, 1984) following a Bayesian framework (Hadfield, 2010). We assessed phenotypic correlations of *NEffEB* with phosphorus efficiency, water, raw ash and fat content of the empty body of a subset (N=73) for which this information was available. The correlation of *NEffEB* with the number of days slaughtering was delayed because a pig did not reach the target body weight was calculated for the entire dataset.

**Results** The heritability of N efficiency of the empty body was higher than the one of the carcass ( $h^2_{NEffEB} = 0.32 [0.15, 0.59]$  and  $h^2_{NEffCarc} = 0.16 [0.08, 0.41]$ ) (Fig. 1). The common environment also contributed to the phenotypic variation in *NEffEB* ( $CE^2 = 0.13 [0.08, 0.27]$ ) and *NEffCarc* ( $CE^2 = 0.16 [0.09, 0.25]$ ). We found a low negative phenotypic correlation of *NEffEB* and the number of days an animal was delayed in reaching target weight (Table 1). *NEffEB* correlated moderately positively with phosphorus efficiency. Body composition traits (water/crude ash/crude fat content) were not correlated with *NEffEB*.



**Table 1** Correlations of *NEffEB* with traits of potential economic and ecological impact as well as with body composition.

trait	type	coefficient ± 95% CI
days delayed	Spearman	$\rho = -0.16 [-0.26, -0.07]$
P efficiency	Pearson	$r = 0.65 [0.48, 0.77]$
water content	Pearson	$r = -0.07 [-0.32, 0.18]$
crude ash content	Pearson	$r = -0.16 [-0.39, 0.09]$
crude fat content	Pearson	$r = -0.14 [-0.38, 0.11]$

**Figure 1** Heritability ( $h^2$ , blue), common environment effect ( $CE^2$ , green) and residual variance ( $r^2$ , red) of protein efficiency of the whole body (A) and protein efficiency of the carcass (B). Posterior distributions of the respective variance components (upper part), points representing single estimates are shown together with a box plot (with median, interquartile range and 5<sup>th</sup> to 95<sup>th</sup> percentile range).

**Conclusions** Our preliminary results indicate a potential for selective breeding towards increased protein efficiency but more research is needed to improve estimates of genetic parameters. Information on the association of genetic loci (SNPs) and protein efficiency will help understand the genes and their functions that underlie protein efficiency. We found no major trade-offs with other economically important traits. Breeding for increased protein efficiency could lead to a slower growth of the animals, but the decrease is expected to be small and might be offset by lower costs of protein-reduced feed. However, potential trade-offs with fertility, aggression and meat quality should be addressed by future studies. A reduction of proteins in pig feed can potentially facilitate both conventional and organic pig production in Switzerland.

**References**  
Hadfield, J.D., 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *J. Stat. Softw.* 33. <https://doi.org/10.18637/jss.v033.i02>  
Henderson C.R., 1984. Applications of Linear Models in Animal Breeding. University of Guelph, Guelph, CA, 462 pages  
Ruiz-Ascacibar, I., Stoll, P., Kreuzer, M., Boillat, V., Spring, P., Bee, G., 2017. Impact of amino acid and CP restriction from 20 to 140 kg BW on performance and dynamics in empty body protein and lipid deposition of entire male, castrated and female pigs. *animal* 11, 394–404. <https://doi.org/10.1017/S1751731116001634>

### The *APOB* loss-of-function mutation of Holstein dairy cattle reduces the capacities for cholesterol transport in circulation but does not imply a cholesterol deficiency

J. J. Gross<sup>1</sup>, A.-C. Schwinn<sup>1</sup>, F. Schmitz-Hsu<sup>3</sup>, A. Barenco<sup>4</sup>, T. F.-O. Neuenschwander<sup>5</sup>, C. Drögemüller<sup>2</sup> and R. M. Bruckmaier<sup>1</sup>

<sup>1</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland; <sup>2</sup>Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland; <sup>3</sup>Swissgenetics, Zollikofen, Switzerland; <sup>4</sup>swissherdbook, Zollikofen, Switzerland; <sup>5</sup>Holstein Switzerland, Posieux, Switzerland

Email: [rupert.bruckmaier@vetsuisse.unibe.ch](mailto:rupert.bruckmaier@vetsuisse.unibe.ch)

**Introduction** The loss-of-function mutation of the apolipoprotein (APO) B gene in Holstein cattle accounts for increased losses in calves that are homozygous for this mutation. Heterozygous carriers of the APOB mutation are supposed to be clinically healthy, but show decreased plasma concentrations of cholesterol and lipoproteins. So far the metabolic effects of the mutation had only been investigated in heterozygous calves, bulls, and non-lactating females. In high yielding dairy cows, a marked decrease in plasma cholesterol concentration during early lactation is part of the usual metabolic changes. Given the essential role of cholesterol in fatty acid and lipid metabolism, a specific impact of the APOB mutation on metabolism and performance in dairy cows is expected. Therefore, the aim of the present study was to investigate the effects of different APOB genotypes on metabolic parameters, hepatic metabolism, lactation and reproductive performance.

**Materials and Methods** Twenty pairs of full siblings with similar age, performance, and calving were investigated. Both animals of each pair were kept on the same farm and consisted of a heterozygous carrier (CDC) and a non-carrier (CDF) of the APOB mutation associated with cholesterol deficiency (CD). Blood samples were taken in early ( $25.5 \pm 4.7$  days in milk) and mid-lactation ( $158.2 \pm 11.1$  days in milk; mean  $\pm$  SD), and analyzed for non-esterified fatty acids,  $\beta$ -hydroxybutyrate, glucose, insulin-like growth factor-1, aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) activity, total cholesterol, free cholesterol, triglycerides (TAG), high density lipoprotein-cholesterol, and phospholipids (PL). The evaluation of milk production, milk gross composition, and lactation persistency was based on official DHIA recordings.

**Results** Milk production and milk composition (i.e., contents of milk fat, protein, lactose, and urea) did not differ between the cows with different APOB-genotypes. Furthermore, cumulative milk production at 100, 200, and 305 DIM, and average milk fat and protein content during these intervals did not differ between CDC and CDF cows. Lactation persistency of milk production and the interval between parturition until successful conception were similar across cows of both genotypes.

Cholesterol and lipoprotein concentrations were lower in CDC compared with CDF in early and mid-lactation ( $P < 0.05$ ). Metabolic parameters, and plasma TAG concentration did not differ between CDC and CDF.

**Conclusions** Cholesterol and lipoprotein concentrations were markedly lowered in Holstein cows heterozygous for the APOB mutation compared with non-carriers. The effect of the APOB mutation on the phenotypic expression of circulating cholesterol was persistent throughout the lactation in heterozygous carriers. However, animal health, lactational, and reproductive performance were not impaired compared with non-carriers. Parameters related to the intermediary energy metabolism were not affected by the mutation indicating that metabolic adaptations are successful and do not provoke a higher incidence of metabolic disorders. The low cholesterol concentrations associated with the APOB mutation are not due to a primary deficiency of cholesterol, as the term “cholesterol deficiency” suggests, but are rather a consequence of reduced capacities for transport in circulation. Our results indicate no need for eradication of APOB carriers from production. However, risk-matings of carriers should be avoided.

### Colostrum in swine: essential roles and factors of variation

C. Farmer

Agriculture and Agri-Food Canada, Sherbrooke R & D Centre, Sherbrooke, QC, Canada

Email: [Chantal.Farmer@canada.ca](mailto:Chantal.Farmer@canada.ca)

**Introduction** Colostrum is the elixir for life in newborn piglets. A minimum amount of 250 g of colostrum must be ingested by an average size neonatal piglet (1.4 kg) in order to acquire immune protection and sustain body growth (Quesnel et al., 2012). However, this does not occur in all litters. It was estimated that approximately one third of sows cannot produce enough colostrum to fully support their litters (Quesnel et al., 2012). It is therefore imperative to attempt to augment the amount of colostrum available to piglets. Unlike milk yield, the production of colostrum in sows is not determined by litter size and suckling intensity but is largely driven by sow-related factors. Colostrum yield and composition are highly variable among sows. Various nutritional and endocrine factors affect colostrum yield and composition in swine, yet mechanisms that regulate colostrogenesis are not fully known. Few studies have looked at factors affecting the onset or cessation of colostrogenesis, which would likely impact its duration. Such information would be most pertinent to assist in developing novel management strategies in periparturient sows to maximize colostrum availability to piglets.

**Importance of colostrum for piglets** Colostrum provides newborn piglets with the energy necessary for thermoregulation and body growth. It also provides passive immunity needed for protection of piglets against pathogens, and growth factors that stimulate growth and maturation of tissues and organs, especially the gastrointestinal tract. By transferring bioactive factors from the mother to the offspring, colostrum is crucial for the process of lactocrine signaling, which affects the offspring much beyond the neonatal period. Bioactive components of colostrum include such things as proteins, growth factors, peptides, oligosaccharides, fatty acid-derived molecules, steroids, and microRNAs.

**Factors of variation** The amount and composition of colostrum produced can be influenced by sow characteristics, such as endocrine status, nutrition, parity, immune status and level of stress, and by litter characteristics, especially vitality at birth. Environmental factors could also alter colostrum yield and composition through effects on the sow. Colostrum synthesis is under hormonal control, the prepartum peak of prolactin being essential for the initiation of lactation. Furthermore, colostrum yield is positively related to the prolactin/progesterone ratio in late pregnant sows. Neither farrowing induction using prostaglandins nor delaying farrowing with a progestagen affects colostrum yield. Other hormones also undergo drastic changes around parturition, namely estrogens and glucocorticoids whose concentrations increase before farrowing (Foisnet et al., 2010), yet, their role in the control of colostrogenesis has not yet been described in swine. Nutrition of the late-pregnant sow can alter the composition, but not amount, of colostrum produced. Energy intake and source have the greatest impact on colostrum composition, with fish oils affecting the type of colostrum fat via an increase in n-3 polyunsaturated fatty acids (Farmer and Quesnel, 2009).

**Duration of colostrogenesis** Hormonal manipulations can impact the onset and cessation of the process of colostrogenesis. As early as puberty, mammary cells can start to produce lacteal secretions when stimulated with prolactin. However, the impact of such a premature lactogenesis on future colostrum yield is not known. Increasing prolactin concentrations in late pregnancy also induces early lactogenesis and the effect on piglet growth seems to be related to the concentrations of prolactin achieved, growth rate being inhibited when prolactin concentrations are at pharmacological levels. Inducing farrowings with prostaglandins led to an earlier onset of colostrogenesis. Colostrogenesis generally lasts until approximately 24 h after farrowing, as indicated by changes in composition of colostrum to become transient milk, and this is linked with the closure of mammary tight junctions. The cessation of colostrogenesis can be delayed using oxytocin. Injecting a supraphysiological dose of oxytocin (75 IU) 16 h after birth of the last piglet alters the permeability of mammary tight junctions, as indicated by a greater Na/K ratio in milk, thereby delaying the occurrence of tightening of these tight junctions and leading to further passive transfer of immunoglobulins and bioactive components from the dam's circulation to the milk (Farmer et al., 2017). This prolongation of the colostrum phase could be advantageous for the survival of newborn piglets who are born immuno-deficient and with very poor energy reserves.

**Conclusions** Colostrum intake by piglets is a major determinant of their performance, but much remains to be learned on the control of this important process in order to develop novel management strategies that will maximize it. Recent findings show that by injecting a supra-physiological dose of oxytocin to sows 16 h after the end of farrowing, the colostrum quality of lacteal secretions is maintained for a longer period of time, thereby prolonging passive transfer of immunoglobulins, growth factors and hormones to the newborn piglets.

### References

- Farmer, C., M. Lessard, C. H. Knight, and H. Quesnel. 2017. Oxytocin injections in the postpartal period affect mammary tight junctions in sows. *J. Anim. Sci.* 124:3532-3539.
- Farmer, C., and H. Quesnel. 2009. Nutritional, hormonal and environmental effects on colostrum in sows. *J. Anim. Sci.* 109 (Suppl. 1): 56-65.
- Foisnet A, Farmer C, David C and Quesnel H 2010. Relationship between colostrum production by primiparous sows and sow physiology around parturition. *Journal of Animal Science* 110, 1672-1683.
- Quesnel, H., C. Farmer, and N. Devillers. 2012. Colostrum intake: influence on piglet performance and factors of variation. *Livest. Sci.* 145:105-114.

### Colostrum: Back to the Basics with Immunoglobulins

A. J. Geiger

Zinpro Corporation, Eden Prairie, MN, USA

Email: [AGeiger@Zinpro.com](mailto:AGeiger@Zinpro.com)

**Review Presentation:** Since maternal immunoglobulins (Ig) are unable to cross the placental barrier during the gestation period, young calves rely almost entirely on passive transfer of immunity for the first few weeks of life. This passive immunity is achieved via feeding of high-quality colostrum to neonatal calves immediately after birth. Indeed, it is well known that achievement of passive transfer (PT) of immunity by the calf is not only critical in the first few weeks of life until active immunity has been established, but also has long lasting impacts on cattle.

Recent research efforts have been heavily focused on assessing the impacts of various ‘biological’ factors in maternal colostrum (i.e., insulin-like growth factor-I and -II, lactoferrin, insulin, etc.) on developmental processes of the new-born calf (gut development, etc.). Although a majority of producers and industry representatives understand the importance of providing enough Ig to neonatal calves, it could be argued that the importance of these molecules and how they impact calf health and performance have taken a back seat to other aspects of colostrum in recent years. It is the author’s belief that revisiting the importance of various Igs (G<sub>1</sub>, G<sub>2</sub> M, A) is a worthwhile topic. Therefore, the purpose of this review is revisit the importance of feeding colostrum to calves immediately after birth with the goal of achieving PT via the consumption of high quantities of Ig. A focus will be given to differentiating the various Igs present in colostrum and also discussing differences between different Ig isotypes (G<sub>1</sub> and G<sub>2</sub> for example).

Quality and antibody profile of colostrum are impacted by a variety of on farm practices and conditions. The impact of these conditions and practices will be discussed in terms of their impact on colostrum quality and the quantity of colostrum that must be fed. Additional discussion will involve timeliness of colostrum collection and feeding along with bacterial contamination of colostrum and how it impacts the ability of a calf to achieve PT. Finally, Proper benchmarking protocols for both colostrum quality and rates of PT on farm will be discussed.

In times when the above criteria cannot be met or colostrum quality is unacceptable, alternatives exist. The primary alternatives are to feed stored colostrum on farm or a colostrum replacer. The feeding of stored colostrum on farm presents a significant challenge to producers as the quality of that colostrum is impacted by pooling, storage method, bacterial contamination, and time of pasteurization in relation to storage. Feeding a colostrum replacer also presents a significant financial commitment by dairy farms, and differences in colostrum replacer types will be discussed (i.e., serum-based vs. dried maternal vs. whey-based, etc.) to help producers make informed decisions.

Finally, although the importance of feeding high-quality colostrum to calves on day one of life cannot be denied, recent work suggests that feeding colostrum Igs post-gut closure (after day one of life) may have significant impacts on calf health and performance. The local impact of these Igs on the calf after 24 hours of life is worth of discussion as pressure continues to mount for the dairy industry to reduce antibiotic use on farm.

The goal of this review is not to discount the impact of ‘biological’ factors present in maternal colostrum on the calf, but to merely draw attention back to the pinnacle reason for colostrum feeding: Immunoglobulins. Through discussion of the above points, it is the author’s hope that new light can be shed on how colostrum is fed and managed on farm, resulting in healthier calves worldwide.

### Beyond immunoglobulins: The immunoregulatory role of colostrum

H.-J. Schuberth

Immunology Unit, University of Veterinary Medicine, Foundation, Hannover, Germany

Email: [Hans-Joachim.Schuberth@tiho-hannover.de](mailto:Hans-Joachim.Schuberth@tiho-hannover.de)

**Introduction** New born piglets and calves are equipped with a near complete immune system. The ability to respond to foreign antigens with a coordinated adaptive immune response is already present in late pregnancy. However, magnitude and speed of various immune mechanisms and cellular functions still need to develop in the neonate. Maternal colostrum is one the most decisive factor in this development. Of note, colostrum and its role has largely been narrowed to its protecting functions mediated by antigen- or pathogen-specific maternal antibodies. However, it becomes more and more apparent that colostrum and its ingredients have more immunoregulatory or –modulatory functions beyond the immunoglobulins and that the role of colostrum is not only to protect the newborn but to educate the immune system and to ensure balanced inflammatory responses in the gut and in the periphery.

The list of immunomodulatory colostral ingredients is remarking. Cytokines, chemokines, growth factors, cellular vesicles, micro RNA, viable cells and oligosaccharides are flooding the very immature gut of piglets and calves after uptake. They reach the yet underdeveloped gut epithelial cells and the circulation thereby affecting the maturation of the gut immune system as well as the development of secondary lymphoid organs in the periphery and hematopoiesis in the bone marrow.

Numerous publications addressing single aspects of these immunomodulatory components demonstrate their action and their potential role for the newborn calf and piglet. For instance, the uptake of viable maternal immune cells resulted in a different circulation behavior of immune cells in newborns and affected their response towards systemic vaccination (Langel et al. 2016). Interestingly, as proven for humans and mice, the transfer of maternal immune cells is not a transient phenomenon and can result in a life-long microchimerism in the offspring (Molès et al. 2018). Whether colostrum-induced microchimerism has a significant role in calf or piglet immune development, intestinal maturation and protection against infectious diseases remains to be investigated.

Apart from viable cells, cytokines and growth factors, one group of colostral ingredients, the colostral oligosaccharides (cOS), seem to be of special importance. cOS are variants of the lactose biosynthesis with, depending on the species, roughly 100+ variants. They promote and guide epithelial cell and villus growth, they are capable to inhibit the binding of putative pathogens to the epithelial surface, they favour balanced inflammatory responses and promote tissue repair mechanisms via their action on epithelial cells as well as on gut immigrating leukocyte subpopulations. This already remarkable array of cOS functions is complemented by their ability to favour as prebiotics the development of a diverse gut microbiome originating in part from colostrum-derived microbiota (Bode, 2012). Microbiota in turn produce immunomodulatory metabolites, and interact with developing epithelial and immune cells. From this point onward, it seems somehow difficult to dissect the relative role of colostral ingredients and the colostrum-favored expansion of certain families among the microbiota, since there is a massively growing body of data demonstrating the immune regulating, educating and guiding role of microbiota-generated metabolites for the immune system or various immune mechanisms (Le Doare et al. 2018).

**Conclusions** If it is accepted, that colostrum in calves and piglets is not just protection for a given time period, and that in absence of placental maternal transfer of maternal educating factors, this period of colostrum uptake is more than transferring antibodies, then it would be necessary to re-evaluate colostrum management strategies. This is especially important regarding the amount of transferred colostrum and its treatment (pasteurization, freezing/thawing). Especially the latter might keep antibodies and their concentrations largely unaltered but may alter the concentrations and bioactivities of other compounds (Fischer et al. 2017). The definition of ‘good quality’ colostrum needs refinement in light of the many roles colostrum has beyond passive protection by antibodies.

### References

- Bode L. Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology*. 2012, 22(9):1147-62.
- Gonzalez DD, Dus Santos MJ. Bovine colostral cells-the often forgotten component of colostrum. *J Am Vet Med Assoc*. 2017, 250(9):998-1005.
- Fischer AJ, Malmuthuge N, Guan LL, Steele MA. The effect of heat treatment of bovine colostrum on the concentration of oligosaccharides in colostrum and in the intestine of neonatal male Holstein calves. *J Dairy Sci*. 2018, 101(1):401-7.
- Langel SN, Wark WA, Garst SN, James RE, McGilliard ML, Petersson-Wolfe CS, et al. Effect of feeding whole compared with cell-free colostrum on calf immune status: Vaccination response. *J Dairy Sci*. 2016, 99(5):3979-94.
- Le Doare K, Holder B, Bassett A, Pannaraj PS. Mother’s Milk: A Purposeful Contribution to the Development of the Infant Microbiota and Immunity. *Front Immunol*. 2018, 9(361).
- Molès JP, Tuaillon E, Kankasa C, Bedin AS, Nagot N, Marchant A, McDermid JM, Van de Perre P. Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. *Pediatr Allergy Immunol*. 2018, 29(2):133-143.

### Update on Postpartum Dysgalactia Syndrome in Sows

N. Kemper

Institute of Animal Hygiene, Animal Welfare and Farm Animal Behavior, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Email: [Nicole.Kemper@tiho-hannover.de](mailto:Nicole.Kemper@tiho-hannover.de)

**Introduction** Postparturient disorders represent an economically important disease complex in sows worldwide, causing losses due to reduced productivity and high mortality rates. These disorders are commonly categorized under the terms mastitis-metritis-agalactia complex (MMA), postpartum dysgalactia syndrome (PDS) or periparturient hypogalactia syndrome. All these terms summarize the characteristic syndrome of greatly reduced milk production within 12 to 48 hours post partum. PDS affects therefore both sows' and piglets' health and welfare. The insufficient intake of colostrum by the piglets can lead to apathy, secondary infections, diarrhea or even death by starvation.

**Diagnosis and occurrence** The main clinical signs in sows are mastitis, dysgalactia and fever above 39.5°C in the sows. The most common practice for early diagnosis is to measure the rectal temperature post partum. The range of critical temperature thresholds varies between 39.3°C and 40.5°C. However, physiological hyperthermia is often observed in postparturient sows, especially gilts, leading to misinterpretations. Investigation of mammary glands, and especially of behavioral changes in sows and piglets, allow a more precise diagnosis. The use of inflammatory markers and hormonal and metabolic indicators for an early detection of PDS has been examined (for instance Kaiser et al. 2018 a, b), but no indicator has been put into practice, yet. In most cases, PDS is limited to few animals and may only be sporadic, but nearly epidemic cases in affected herds were described, too. Herds of different hygienic practices and standards may show the infection. It even occurs on excellently managed farms with optimized disinfection practices.

**Influencing factors** Mastitis is one of the central clinical signs, with mainly coliform bacteria of the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella* isolated from affected sows. Lipopolysaccharide (LPS) endotoxins, present in all gram-negative bacteria, play a major role in the etiology of PDS. However, the occurrence of bacteria alone does not lead to clinical signs. This is supported by findings in *Escherichia coli*, with no differences in the prevalence or in specific virulence gene profiles of isolates from either diseased or healthy sows detected (Gerjets et al., 2011). Any given strain can cause PDS, if further adverse factors are present. These adverse factors can be attributed to the environment or the host. Environmental factors such as husbandry and hygiene management as well as feeding can affect the clinical course of this disease. The host, including the genetic variation, individual factors, parity number and birth duration, is an important factor, too.

**Treatment and prevention** Treatment of PDS includes immediate administration of antibiotics if the sow's body temperature is above a defined threshold. This threshold is defined rather subjectively and the use of it might be regarded critically, since increases and decreases in temperature can appear physiological. To reduce the administration of antibiotics, it is therefore essential to perform diagnosis of PDS not only due to temperature increase, but also due to a combination of appropriate criteria. Prevention is the best way to cope with PDS in a population, but difficult to accomplish, due to the etiology. Even though various studies have been conducted to elucidate causative factors and the potential genetic background including individual resistance (Preissler et al. 2012), the reason for only some sows developing clinical signs of infection after contact with ubiquitous bacteria remains unknown still. However, providing a suitable environment and adapted feeding strategies for the pre-parturient sow accounts for a large proportion of successful prevention.

### References

- Gerjets I. & Kemper N. (2009) Coliform mastitis in sows: a review. *Journal of Swine Health and Production* 17, 97–105.
- Gerjets I., Traulsen, I., Reiners, K. & Kemper N. (2011) Comparison of virulence gene profiles of *Escherichia coli* isolates from sows with coliform mastitis and healthy sows. *Veterinary Microbiology* 152, 361-367.
- Kaiser M., Jacobson M., Andersen, P.H., Bækbo P., Ceron J.J., Dahl J., Escibano D., & Jacobsen S. (2018a) Hormonal and metabolic indicators before and after farrowing in sows affected with postpartum dysgalactia syndrome. *BMC Veterinary Research* 14:334
- Kaiser M., Jacobson M., Andersen, P.H., Bækbo P., Ceron J.J., Dahl J., Escibano D., & Jacobsen S. (2018b) Inflammatory markers before and after farrowing in healthy sows and in sows affected with postpartum dysgalactia syndrome. *BMC Veterinary Research* 14:83
- Preissler R., Tetens J., Reiners K., Looft H. & Kemper N (2013) A genome- wide association study to detect genetic variation for postpartum dysgalactia syndrome in five commercial pig breeding lines. *Animal Genetics* 44(5):502-8.

### Relationships Between Colostrum Consumption and Kid IgG Status

E. V. St. Clair, R. W. Russell and R. J. Van Saun

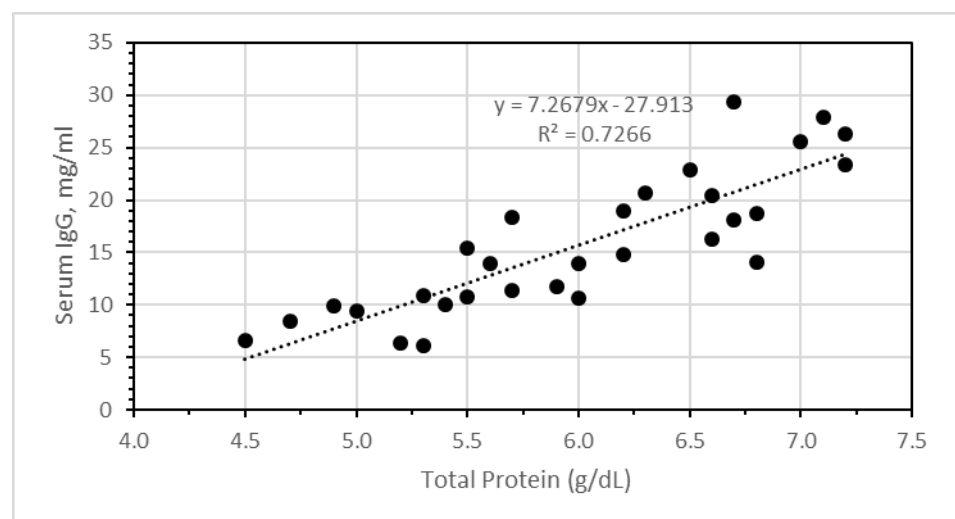
Department of Veterinary and Biomedical Sciences, College of Agricultural Sciences, Pennsylvania State University, University Park, Pennsylvania, USA

Email: [rjv10@psu.edu](mailto:rjv10@psu.edu)

**Introduction** Feeding of high-quality colostrum is the single most important event in the newborn kid's life to ensure viability and survivability. Quality is based on the concentration of immunoglobulin-G (IgG) in colostrum. Effectiveness of passive transfer then is based on delivering sufficient quantity of colostrum in a timely manner relative to birth to achieve adequate blood IgG concentration that would be protective from disease. In dairy calves achieving a serum IgG concentration of 10 mg/ml is desired, though this information is not available for the goat kid. The objective of this study was to document serum IgG concentrations in goat kids based on total IgG consumed and determine relationship between total protein and serum IgG concentration.

**Materials and Methods** All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. A commercial 900-doe goat dairy in Northern California was used for sample collection. Herd records provided information on amount and timing of colostrum feeding and method for kids. Blood samples were then collected between 1- and 4-days following birth from kids and serum harvested and frozen for analysis. Total protein (TP) determination (g/dL) was determined by digital refractometer (MISCO, Solon, OH). Total serum IgG (IgG<sub>1</sub>+IgG<sub>2</sub>) concentration was determined by goat IgG enzyme linked immunosorbent assay (ELISA, ZeptoMetrix, Buffalo, NY). Data were analyzed by ANOVA to determine significant effects of IgG consumed, timing and their interaction on serum IgG concentration. Correlation and regression modeling were used to determine a predictive relationship between serum IgG and total protein concentrations.

**Results** Hemolyzed samples resulted in extremely high TP and were removed from data analysis. Mean  $\pm$  standard deviation (median, range) serum TP (n=30) and total IgG concentration (n=57) were  $6.0 \pm 0.8$  g/dL (6.0, 4.5-7.2) and  $15.8 \pm 7.3$  mg/ml (15.3, 3.1-36.1), respectively. Calculated grams of IgG delivered at first feeding and total prior to bleeding was  $17.6 \pm 6.3$  g (range: 6.0-39) and  $35.0 \pm 11.2$  g (range: 11.9-64.3), respectively. Time to first feeding averaged  $106 \pm 136$  mins (median: 60 min). Serum TP measured between 1 and 4 days of life was highly associated (Figure 1;  $r^2=0.73$ ;  $P<0.0001$ ) with serum IgG concentration. Incorporation of age at bleeding ( $P=0.08$ ) only slightly improved the relationship between TP and serum IgG concentration ( $r^2=0.76$ ,  $P<0.0001$ ). Total IgG consumed showed the greatest influence ( $P=0.0020$ ) with no effect of method of feeding or time to feeding. Amount of IgG fed at 1<sup>st</sup> feeding ( $P=0.0069$ ), volume at first feeding ( $P=0.10$ ), time to second feeding ( $P=0.061$ ) and age ( $P=0.047$ ) accounted for more variation ( $r^2=0.45$ ,  $P=0.0093$ ) in serum IgG concentration than total IgG consumed ( $r^2=0.36$ ,  $P=0.013$ ). Mean total consumed IgG amount was  $35.0 \pm 11.2$  g (median 36.1; range 11.9-64.3). Assuming blood volume at 10% of birth weight, total blood IgG mass was determined from measured serum IgG concentration. Calculated efficiency of IgG absorption was  $16.8 \pm 8.8\%$  (median 16.4; range 3.6-52.9). Total IgG consumed was negatively associated ( $P=0.012$ ) with IgG absorption efficiency. Surprisingly time to second feeding ( $P=0.023$ ) and IgG amount at second feeding ( $P=0.015$ ) explained the greatest amount of variation ( $r^2 = 0.23$ ,  $P=0.0016$ ) in calculated efficiency of colostrum absorption. Further research is needed to explore these relationships.



**Figure 1** Relationship between serum IgG concentration and total protein concentration in goat kids ranging from 1-4 days of age.

**Conclusions** These data provide insights into goat kid passive transfer assessment. Although no assessment of health was performed these data suggest feeding 35 g IgG can achieve a serum IgG concentration of 15 mg/ml with an associated total protein concentration of 6.0 g/dL. Work with calves showed improved disease prevalence and severity with serum IgG concentrations  $\geq 15$  mg/ml (Furman-Fratczak et al., 2011). Based on this calf work it would seem desirable to attempt to achieve a serum IgG concentration of 15 mg/ml in goat kids. Further research to assess the relationship between health status and IgG transfer to better define passive transfer guidelines.

### References

Furman-Fratczak et al. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. J Dairy Sci 2011;94:5536-5543

### Evaluation of two different treatment procedures after calving to improve harvesting of high quantity and quality colostrum

F. Sutter<sup>1</sup>, S. Borchardt<sup>1</sup>, G. M. Schuenemann<sup>2</sup>, E. Rauch<sup>3</sup>, M. Erhard<sup>3</sup> and W. Heuwieser<sup>1</sup>

<sup>1</sup>Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany; <sup>2</sup>Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, Ohio, USA; <sup>3</sup>Department of Veterinary Sciences, Chair of Animal Welfare, Ethology, Animal Hygiene and Animal Husbandry, Faculty of Veterinary Medicine, LMU Munich, Germany

Email: [Franziska.Sutter@fu-berlin.de](mailto:Franziska.Sutter@fu-berlin.de)

**Introduction** Management and nutrition of the newborn calf during the first hours of life have the potential to permanently affect the lifetime performance of a dairy cow. It is known, that the timely delivery of colostrum, the colostrum quality and quantity, and the rate and amount of intestinal IgG absorption are essential components to guarantee a successful passive transfer in calves. In order to achieve these goals, it is important to harvest a sufficient quantity of high quality colostrum. Release of oxytocin is the prerequisite for milk ejection and complete colostrum harvest. A continuous ejection of colostrum is dependent on the presence of adequate circulating oxytocin concentration. Milk ejection is an innate neuroendocrine reflex, which involves the hypothalamus, the pituitary gland and sensory neurons in the teat. In neurosecretory terminals of the pituitary gland, oxytocin is stored and emptied into the bloodstream upon successful stimulation. Tactile stimulation of the teats results in the release of oxytocin and causes the contraction of myoepithelial cells around the mammary alveoli, whereby the alveolar milk fraction can be removed. The objective of this study was to evaluate two different treatment procedures at the first milking after calving to increase colostrum quantity and improve colostrum quality by applying exogenous oxytocin or to stimulate endogenous oxytocin secretion. We hypothesized that either exogenous treatment with oxytocin or the presence of the calf before first milking leads to higher colostrum quantity and higher IgG concentration.

**Materials and Methods** A total of 567 cows at the time of calving were enrolled, but for the final analyses only 521 animals were considered. The cows were randomly assigned on a daily basis into 1 of 3 groups: 1) control group (**CON**; n=177), 2) application of 20 IU oxytocin intramuscular (**OXY**; n=163), and 3) presence of the calf (**CALF**; n=181) before and during milking. Cows in the control and oxytocin group had no contact to their calves after calving and were milked in a separate milking parlor. Cows in the oxytocin group were injected with 20 IU oxytocin intramuscular 3 minutes before manual stimulation. For cows in the third group, the calf was placed into a calf cart and located in front of the cow 3 minutes before manipulation of the cow. Colostrum quantity was determined by a digital hanging scale. The colostrum quality was assessed with digital Brix refractometry and sandwich ELISA. To evaluate the effect of two different treatment procedures, a generalized linear mixed model was constructed using SPSS.

**Results** The mean colostrum quantity was  $4.17 \pm 0.30$  kg (min: 0.00 kg, max: 28.40 kg). The treatment procedure had no effect on colostrum quantity ( $P = 0.450$ ). Parity ( $P = 0.016$ ), calf birth weight ( $P = 0.001$ ), calving time ( $P = 0.093$ ), gestation length ( $P = 0.051$ ), and the square of gestation length ( $P = 0.050$ ) affected colostrum quantity. Parity 2 cows had the lowest quantity of colostrum ( $3.54 \pm 0.37$  kg) compared with cows in parity 1 ( $4.49 \pm 0.40$  kg) and cows in parity 3 or greater ( $4.49 \pm 0.35$  kg). Cows calving during the night shift ( $4.67 \pm 0.38$  kg) had the highest quantity of colostrum compared with cows calving in the morning ( $3.98 \pm 0.37$  kg) or afternoon shift ( $3.87 \pm 0.38$  kg). Gestation length and the square of gestation length affected the colostrum quantity positively.

The mean IgG concentration measured by sandwich ELISA was  $54.6 \pm 2.80$  mg IgG/mL (min: 14.5 mg IgG/mL, max: 146.3 mg IgG/mL). Colostrum quality was affected by the treatment procedure ( $P = 0.059$ ). In addition, colostrum quantity ( $P = 0.001$ ), parity ( $P = 0.001$ ), calving time ( $P = 0.002$ ), gestation length ( $P = 0.027$ ) and the day of the week ( $P = 0.049$ ) had an effect on the IgG concentration in colostrum. Both treatment procedures, i.e., OXY with mean IgG concentration results of 56.62 mg IgG/mL ( $P = 0.035$ ) and CALF with 56.08 mg IgG/mL ( $P = 0.047$ ), resulted in higher IgG concentrations in colostrum compared with CON (51.01 mg IgG/mL). With increasing colostrum quantity, the colostrum quality decreased in primiparous cows ( $r = -0.21$ ) and in multiparous cows ( $r = -0.13$ ). Gestation length affected the colostrum quality negatively. Concentration of IgG was higher for cows in parity 3 or greater ( $65.6 \pm 3.06$  mg IgG/mL) compared with cows in parity 1 ( $47.7 \pm 3.32$  mg IgG/mL) and cows in parity 2 ( $50.4 \pm 3.28$  mg IgG/mL). Cows calving during the night shift had greater IgG concentrations ( $60.5 \pm 3.32$  mg IgG/mL) compared to cows calving in the morning ( $49.9 \pm 3.31$  mg IgG/mL) or afternoon shift ( $53.3 \pm 3.32$  mg IgG/mL). Harvesting colostrum on Sundays resulted in the highest IgG concentration ( $62.4 \pm 4.04$  mg IgG/mL).

The mean Brix refractometry result was  $25.8 \pm 0.23\%$  Brix (min: 15.7% Brix, max: 39.7% Brix). Treatment procedure had no effect on colostrum quality ( $P = 0.515$ ). Colostrum quantity ( $P = 0.001$ ), parity ( $P = 0.001$ ), gestation length ( $P = 0.061$ ), and the square of gestation length ( $P = 0.061$ ) affected colostrum quality. There was a negative association between colostrum quantity and quality determined by Brix refractometry. The correlation coefficient was  $r = -0.09$  in primiparous cows and  $r = -0.17$  in multiparous cows. Brix readings were greater for cows in parity 3 or higher ( $27.5 \pm 0.29\%$  Brix) compared with cows in parity 1 ( $25.0 \pm 0.35\%$  Brix) and cows in parity 2 ( $24.8 \pm 0.33\%$  Brix). Similar to colostrum quantity, there was a negative association of colostrum quality and gestation length. The square of gestation length was positively associated with colostrum quality. The correlation coefficient between the analysis with sandwich ELISA and the assessment via Brix refractometry was  $r = 0.44$ .

**Conclusions** In conclusion, none of the treatment procedures improved colostrum quantity. However, the administration of parental oxytocin and presence of the calf increased IgG concentration in colostrum of cows measured by sandwich ELISA compared with the control group. The external validity in our study is limited; therefore, the results should be validated with a multicentric study design. Future studies should involve several farms to increase external validity.



### Practice report: Automatic calf feeding - scientific results successful implemented in practice

J. Plesse

Förster-Technik GmbH, Engen, Germany

Email: Juergen.Plesse@foerster-technik.de

**Introduction** Many studies in the younger past showed a huge impact of the calf nutrition to the vitality, health and performance of dairy cows. The University of Hohenheim (2012) and the Technical University of Munich (2015) in collaboration with Förster-Technik GmbH conducted two practical studies to verify the scientific findings under field conditions. To adopt these new findings, the software of automatic group feeders was improved to offer a so-called “controlled ad-lib” feeding scheme with limits on feed intake per visit but unlimited daily allowance. Another result was the development of a new automatic feeding system especially for calves in single pens after the colostrum phase that is able to feed age appropriate portion sizes up to 8 times a day thus encouraging high daily milk intake. The results with higher daily weight gain, lower morbidity and treatments at reduced labour were published in 2019.

**Materials and Methods** The first Study (Jurkewitz, 2012) compared the feed intake, growth and drinking behaviour between a restricted (G1) and ad libitum fed (G2) group. 54 female Holstein calves were split randomly into restricted (6l/d, 135 g/l skimmed milk powder) and ad libitum feeding groups until day 34. Afterwards they were weaned from the 35<sup>th</sup> to the 70<sup>th</sup> day from 6 to 2 respectively 12 to 2 l/d. All other factors were the same for both groups. The second study (Broghammer, 2015) followed the animals from 2012 as adult animals in first or second lactation and compared the fertility-, health- und performance data.

Further new sensor systems to monitor calf health and development embrace an automatic water intake measurement system, calf activity monitoring with LED finding light and Bluetooth rectal thermometers with connections to data clouds.

**Results** Concerning the drinking behaviour data showed a significant difference from G1=13 ( $\pm 5,6$ ) to G2= 1,5 ( $\pm 1,0$ ) in visits without entitlement during the pre-weaning period. In the same time G1 had 3 ( $\pm 0,5$ ) and G2 had 7 ( $\pm 1,7$ ) visits with entitlement and milk intake. The daily amount of milk of G2 was 62% higher than in G1. The daily gain of G2 was significant and three times higher (1037 g/d) from 13<sup>th</sup> to 27<sup>th</sup> day of life. After weaning the bodyweight of G2 was 10 kg (97,5 -  $\pm$  22,5) higher than G1 (85,2 -  $\pm$  20,9). The second study could not find significant differences in fertility data. The milk yield, however, was highly significant different. The 100 days milk yield of G1 was 2607 kg ( $\pm 453$ ) vs. G2 with 2774 kg ( $\pm 736$ ). Because of a higher persistence of the lactation the difference of milk yield at the 305<sup>th</sup> day was highly significant higher in G2 with 9090 kg ( $\pm 979$ ) vs. G1=7824 kg ( $\pm 1036$ ). That study also found significant less treatment days in G2 (6,8) vs. G1 (8,2).

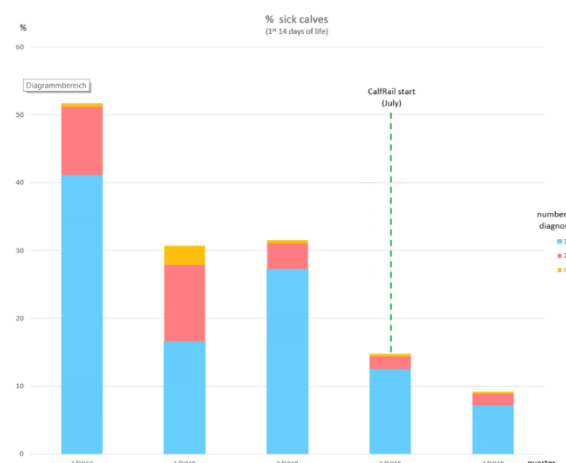
As a result of these studies Förster-Technik developed an automatic feeding system “CalfRail”. This is able to feed the new born calves up to 8 times a day completely automatically. It is established in about 100 farms worldwide as being described just recently in “top agrar”, the largest German agricultural journal. The featured farm has 1.500 milking cows with ~ 10.000 kg milk per 305-day lactation. The calves used to be housed in single hutches and fed twice a day with 8 l acidified milk. The growth rate was less than 600 g/day and the sickness risk was very high. A report from the local breeding organisation documented approx. 60% treated calves during the first two weeks before the installation of the CalfRail system. In July of 2018, the CalfRail was installed in a newly built barn with 80 calf boxes. The effect showed labour saving of ~5 h/day during the single pen period (14 days) in total ~2.200 h/year (Platen et. al, 2019). The health report showed a decrease of treatments during the first 2 weeks by more than 60% (Tab 1, Fig. 1). First results from a currently running study let expect increased growth rates probably from 700 ( $\pm 201$ ) g/d during the first two weeks of life.

**Table 1** Morbidity of calves (RBB, 2018)

Quarter	Number of diagnoses			
	0	1	2	>=3
4/2017	48,3	41,1	10,1	0,5
1/2018	69,3	16,6	11,3	2,8
2/2018	68,4	27,3	3,8	0,4
3/2018	85,1	12,5	1,9	0,4
4/2018	90,8	7,2	1,7	0,3

--- CalfRail started in July 2018

**Figure 1** Decreased disease rate after CalfRail implementation



**Conclusions** The higher nutrition plane for calves has a lifelong positive impact to the health and performance of the dairy cows because of a better development of the organ tissue and metabolic imprinting. With latest software improvements for group calves for “controlled ad-lib” supply it is possible to carry over this knowledge also to calves in single pens. With the CalfRail system it is possible to transfer the scientific knowledge of the last decades into practice of optimal calf rearing. It has a high potential to decrease the sickness incidence and fully exploit the growth potential during the important first weeks of life.

### Assessing the utility of leukocyte differential cell counts for predicting mortality risk in neonatal Holstein calves upon arrival and 72 hours post-arrival at calf rearing facilities

T. E. von Kongslow, D. L. Renaud, T. F. Duffield, C. B. Winder, V. Higginson and D. F. Kelton

University of Guelph, Guelph, ON, Canada

**Introduction** There is growing concern about the level of antimicrobial use and antimicrobial resistance in food producing animals. An area of opportunity to reduce antimicrobial use could be in the first week following arrival for young calves to calf rearing facilities. Group metaphylaxis is common due to the unknown age and history of calves that undergo several stressful events prior to arrival such as transportation, co-mingling and variable periods of fasting (Marcato et al., 2018). It may be possible to reduce antimicrobial use at this stage in the production cycle without sacrificing animal health and welfare if the calves at highest risk of morbidity and mortality could be identified and treated in a highly selectively manner. Recent studies have identified indicators of future risk for morbidity and mortality that can be measured at arrival such as biomarkers and physical exam factors (Renaud et al., 2018a and 2018b; Marcato et al., 2018). Bovine haematology, when used in conjunction with clinical examination findings, could be used to improve disease diagnosis (Roland et al., 2014). The objective of this study was to assess the utility of leukocyte differential cell counts taken at the time of arrival to a calf rearing facility and 72 hours post arrival for determining mortality risk during the production cycle.

**Materials and Methods** Calves were enrolled in this prospective cohort study at an independent grain-fed veal research facility in Ontario, Canada. All 240 calves that arrived from June to July 2018 into three rooms of 80 were eligible for enrolment. Calves received a risk assessment upon arrival to the facility using a standardized screening protocol (adapted from Renaud et al., 2018a) and blood samples were collected to evaluate serum total protein (TP) and machine leukocyte differential cell counts. Of the 240 calves eligible for enrolment, 7 were removed for missing mortality data or machine leukocyte differential cell count data. In total samples from 233 calves upon arrival and a subset of 158 calves 72 hours post arrival were evaluated by the QScout BLD test for leukocyte differential cell counts (Advanced Animal Diagnostic, Morrisville, NC). Calves were fed at the facility for 77 days and calf mortality was reported in a spreadsheet to researchers after calves were shipped from the facility. Cox proportional hazards models were performed using Stata 15 (StataCorp LP, College Station, TX).

**Results** Over the growing period 39 / 233 calves died, including 30 / 158 calves in the 72 hour post arrival subset. Preliminary univariable survival analysis using Cox proportional hazards models suggest that on the day of arrival every 1 g/dL increase in TP lowers the risk of mortality (Hazard Ratio (HR) = 0.38;  $P < 0.001$ ). At 72 hours post-arrival, it was found that for every  $10^9$  cells/L increase in neutrophils the risk of mortality increased (HR = 1.12;  $P = 0.007$ ).

**Conclusions** Preliminary results suggest that machine leukocyte differential cell counts taken on the day of arrival are not a good predictor of mortality but that they may predict an increased risk of mortality when taken 72 hours after transportation. Further studies are required to determine if machine leukocyte differential cell counts have a role in augmenting or refining the risk profile of calves arriving to veal and dairy beef facilities.

#### References

- Marcato, F., H. van den Brand, B. Kemp, and K. van Reenen. 2018. Evaluating Potential Biomarkers of Health and Performance in Veal Calves. *Frontiers Vet. Sci.* 5:133. doi:10.3389/fvets.2018.00133.
- Renaud, D.L., T.F. Duffield, S.J. LeBlanc, S. Ferguson, D.B. Haley, and D.F. Kelton. 2018a. Risk factors associated with mortality at a milk-fed veal calf facility: A prospective cohort study. *J. Dairy Sci.* doi:10.3168/jds.2017-13581.
- Renaud, D.L., T.F. Duffield, S.J. LeBlanc, D.B. Haley, and D.F. Kelton. 2018b. Clinical and metabolic indicators associated with early mortality at a milk-fed veal facility: A prospective case-control study. *J. Dairy Sci.* doi:10.3168/jds.2017-14042.
- Roland, L., M. Drillich, and M. Iwersen. 2014. Hematology as a diagnostic tool in bovine medicine. *J. Vet. Diagn. Invest.* 26:592–598. doi:10.1177/1040638714546490.

### Use of *Saccharomyces cerevisiae* fermentation products in comparison to halofuginone in *Cryptosporidium* infected newborn calves

J. Vélez<sup>1</sup>, M. K. Lange<sup>1</sup>, P. Zieger<sup>2</sup>, I. Yoon<sup>2</sup>, K. Failing<sup>3</sup> and C. Bauer<sup>1</sup>

<sup>1</sup>Institute of Parasitology, Justus Liebig University Giessen, Germany; <sup>2</sup>Diamond V, Cedar Rapids, USA; <sup>3</sup>Unit of Biomathematics and Data Processing, Faculty of Veterinary Medicine, Justus Liebig University Giessen, Germany

Email: christian.bauer@vetmed.uni-giessen.de

**Introduction** The protozoan parasite *Cryptosporidium parvum* is one of the four main enteropathogens causing diarrhea in newborn calves worldwide. Currently, halofuginone is the only anticryptosporidial drug approved by the authorities in Europe for use in newborn calves. However, its prophylactic effect against cryptosporidiosis is often equivocal, and additionally it has a low therapeutic index. Therefore, there is a demand for alternatives. Recently, it was reported that a 4-week feeding of *Cryptosporidium* infected calves with *Saccharomyces cerevisiae* fermentation products resulted in significantly less fragmented and atrophied villi of the lower small intestines in comparison to untreated controls, suggesting a preventive effect of these products against this parasite (Vázquez Flores et al., 2016). Therefore, a longitudinal field study was performed to determine the parasitological, clinical and economical effects of commercial non-GMO *S. cerevisiae* fermentation products against *Cryptosporidium* infections in calves in comparison with both a 'positive' (halofuginone treated) and 'negative' (untreated) control group.

**Materials and Methods** The study was performed on a *Cryptosporidium* endemic commercial dairy farm with approximately 1,600 cows in eastern Germany. A total of 123 newborn female calves, housed in individual hutches, were sequentially enrolled in 41 blocks of 3 animals based on their date of birth. Calves within each block were allocated randomly to one of 3 treatment groups: (i) remained untreated (CON); (ii) fed with *S. cerevisiae* fermentation products (Diamond V SmartCare® at 1 g/d in milk and NutriTek® at 5 g/d in starter grain) for the first 63 days (SCFP); (iii) treated daily with halofuginone (Halocur® at 2 ml/10 kg BW) for the first 7 days (HALO). Fecal samples collected daily during 4–21 days p.p. were examined semi-quantitatively for both *Cryptosporidium* oocysts and coproantigen as well as qualitatively for Rotavirus, Coronavirus and *E. coli* coproantigens. The presence and intensity of diarrhea were monitored by scoring daily for the first 4 weeks of life. Calves were weighed at 0, 21, 42 and 63 days of age. For each parameter arithmetic mean, standard deviation, minimum and maximum were calculated. Differences of the detection of pathogens between the 3 treatment groups were analyzed for significance using Chi<sup>2</sup>-test or Kruskal-Wallis test with Bonferroni corrected Dunn's post-test. To get a global basis for evaluation the intensity of the *Cryptosporidium* infection was estimated using the 'area under the curve' (AUC) of both the oocyst shedding and coproantigen scores over the time. Correlation analyses were done to analyze the relationship between the number of days with diarrhea and serum total protein concentrations, as well as AUC values of *Cryptosporidium* oocyst shedding or coproantigen scores.

**Results** The serum total protein concentration 4 days p.p. reached or exceeded the value of 55 g/l in almost all calves indicating a sufficient transfer of immunoglobulins by colostrum to calves. Almost all calves, regardless of the treatment group, were *Cryptosporidium* positive at least once during the study. Co-infections with Rotavirus, Coronavirus or *E. coli* were detected in a few calves and in few fecal samples only. The HALO treatment significantly reduced the number of *Cryptosporidium* positive fecal samples as compared to both the SCFP fed and CON calves. Based on coproantigen scores, both HALO treatment and SCFP feeding significantly reduced the intensity of *Cryptosporidium* infection as compared to the CON group. Diarrhea was recorded in almost all calves at least once. Overall, the intensity of *Cryptosporidium* infection (coproantigen scores) was positively correlated with the number of days with diarrhea. However, neither the proportion of diarrheic calves nor the intensity and duration of diarrhea differed between the 3 treatment groups significantly. The mean daily weight gain during the first 3 weeks of life was significantly lower in HALO treated calves than in the SCFP and CON groups; however, at the end of the 9-week study period the total weight gain did not significantly differ among the 3 treatment groups. Although the HALO treatment showed a partial anticryptosporidial activity, it did not significantly affect the onset, nor the frequency, duration and intensity of diarrhea in the present study. The same findings were recorded for the SCFP feeding that was, in other words, neither better nor worse than the HALO treatment.

**Conclusions** A pre-weaning supplementation with the two *S. cerevisiae* fermentation feed additives showed very similar clinical results and weight gains in *Cryptosporidium* infected newborn calves as a 7-day halofuginone treatment under the conditions on this farm. This suggests that feeding with these *S. cerevisiae* fermentation products may be a natural alternative measure, instead of halofuginone treatment, in bovine cryptosporidiosis.

#### References

Vázquez Flores, S., De Jesús Guerrero Carrillo, M., Scott, M.F., Hamann, J., Barrera Almanza S., Guizar Bravo, C., Baños Quintana A.P., Aranda Vargas, P.J. (2016). Effects of *Saccharomyces cerevisiae* fermentation products on intestinal villi integrity in neonatal calves naturally infected with *Cryptosporidium* spp. J. Anim. Sci. 94 (E-Suppl. 5), 714–715.

## Hypernatremia in diarrheic calves fed electrolytes mixed with milk replacer without access to water

J. Wilms, L. Leal and J. Martín-Tereso

Trouw Nutrition R&D, Amersfoort, the Netherlands

Email: juliette.wilms@trouwnutrition.com

**Introduction** Oral electrolyte solutions (OES) fed to diarrhoeic calves aim to mitigate dehydration and correct metabolic acidosis. However, the composition of OES products and the administration protocols present wide variations that can compromise the efficacy and safety of these treatments. Mixing OES with whole milk (WM) or milk replacer (MR) has become a common practice as it allows reducing labor associated with treatment of diarrhoeic calves. However, one should consider that WM and especially MR already contain high concentrations of lactose (140 to 230 mmol/L) and sodium (17 to 80 mmol/L; Byers et al., 2014). As a consequence, mixing sugar and electrolyte powder into WM or MR can exceed tolerance levels, especially in systems where water access is not available. In this light, the objective of the current study was to expose the risks associated with administration of OES into MR and water without access to fresh water.

**Materials and Methods** Sixty eight Holstein male calves ( $17 \pm 2$  d of age and  $45 \pm 2$  kg) were purchased from a collection center (Pali group, the Netherlands). Calves were enrolled in the study upon diarrhea incidence and the first day of which diarrhea was observed, was called d 1. Calves were blocked based on BW and within each block, calves were randomly assigned to one of two treatments. Treatments included a hypertonic OES (OES;  $n = 14$ ; 50 g/L in water and 25 g/L in MR), or a placebo consisting of whey powder (CON;  $n = 12$ ; 5 g/L of water and MR). Treatments were administered over a 4 d period into MR (2.5 L at 0800 h and 1730 h) and into water (3 L at 1300 h and 2200 h), without access to fresh water. Treatments were never drenched. Blood samples were taken from the jugular vein once daily at 0600 h for a length of 4 d, and fecal scores were assessed daily at 0900 h for a length of 15 d.

**Results** Intakes of treatments administered in water (4.6 L/d) and in MR (5.0 L/d) did not differ between treatment groups. Diarrhea incidence was higher in calves fed OES on d 3, d 4, and d 5 when compared to CON calves (Figure 1). Besides the digestive disturbances, the OES treatment resulted in a state of nutritional dehydration. Seventy one percent of calves fed OES developed hypernatremia (serum Na  $> 145$  mmol/L) within 48 h after the first OES administration (Table 1). Development of acute hypernatremia (serum Na  $> 160$  mmol/L) occurred in 21% of calves fed OES. Similarly to serum Na, serum osmolarity was higher in calves fed OES (313 mOsm/L) when compared to CON calves (295 mOsm/L;  $P < 0.001$ ). Serum SID did not differ across treatment groups as serum Cl increased with the OES treatment ( $P < 0.01$ ; Table 1). Additionally, there was a significant treatment by time interaction for serum urea, which was higher on d 3 and 4 in calves fed OES ( $P = 0.01$ ).

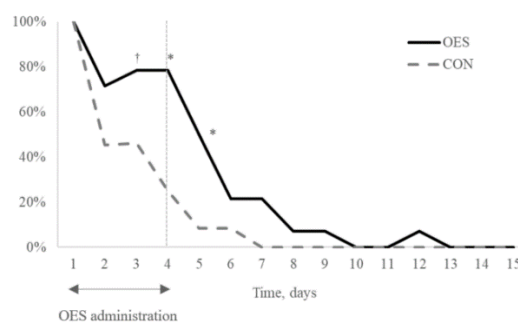
**Table 1** Effect of oral administration of OES in MR and water or a placebo solution without fresh water access on blood minerals, blood hematology, and blood chemistry in calves with naturally occurring diarrhea ( $n = 26$ ).

Blood parameter <sup>1</sup>	Treatments		Pooled SEM	P-values		
	CON	OES		Time	Treat	Treat*Time
Sodium	136.9	149.1	2.2	0.03	0.002	0.02
Chloride	101.6	113.8	2.6	0.03	0.003	0.13
SID <sup>2</sup> , mEq/L	40.5	40.0	1.3	0.16	0.74	0.55
Osmolarity <sup>3</sup> , mOsm/L	295.4	313.3	3.2	0.03	0.0007	0.02
Hematocrit, %	29.9	29.5	0.74	0.26	0.66	0.50
Urea	3.11	3.07	0.15	0.48	0.83	0.01
TPP	54.7	52.2	1.42	0.34	0.25	0.80
Glucose	4.98	4.76	0.13	0.80	0.23	0.31

<sup>1</sup>Expressed in mmol/L unless specified otherwise.

<sup>2</sup>Strong ion different (mEq/L) =  $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^-)$

<sup>3</sup>Serum osmolarity (mOsm/L) =  $2 \text{Na}^+ + 2\text{K}^+ + \text{Glucose} + \text{Urea}$



**Figure 1** Diarrhea incidence in diarrhoeic calves fed oral electrolyte solutions in MR and water or a placebo solution without fresh water access ( $n = 26$ ). Significant differences at each time point are indicated by \* ( $P \leq 0.05$ ) and † ( $P < 0.1$ ).

**Conclusions** Administration of OES in MR without access to water exacerbated digestive disturbances and prolonged recovery from diarrhea. Additionally, calves fed OES developed various degrees of hypernatremia. One should consider that not all diarrhoeic calves are able to drink water by themselves and water access for young calves is not always available in all dairy and veal farms. Providing OES into MR therefore represents a dangerous practice and proper guidelines for OES administration should be developed in order to emphasize the importance of water access.

## References

Byers, S.R., A.S. Lear, and D.C. Van Metre. 2014. Sodium balance and the dysnatremias. *Vet. Clin. North Am. - Food Anim. Pract.* 30:333–350.

### Immune modulating effect of a seaweed extract on specific IgG and total IgA titers in the colostrum and milk

F. Bussy, M. Le Goff and P. Collen

Olmix Group, Brehan, France

Email: FBussy@olmix.com

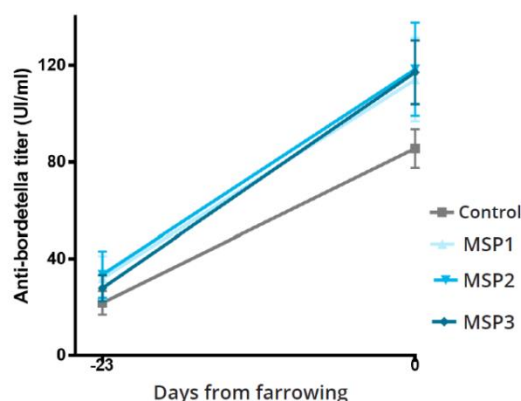
**Introduction** Investment in sow vaccination and feedback to improve their immune status and the immune status of their piglets has become more and more important. Therefore, the transfer of this protective immunity from the sow to its piglets is a key factor that could be optimized. A specific extract (MSP<sub>IMMUNITY</sub>) was prepared from the green algae *Ulva armoricana*. The ability of MSP<sub>IMMUNITY</sub> to stimulate the expression of a wide range of immune mediators was evidenced in vitro (Berri et al, 2016 and 2017). The aim of the present study was to test, in vivo, the capacity of MSP<sub>IMMUNITY</sub> to improve the transfer of immunity from gilts to piglets.

**Materials and Methods** Thirty-five gilts (Large White\*Landrace) were randomly distributed to 4 groups: 1 control group (n=10), and 3 test groups receiving MSP<sub>IMMUNITY</sub> at different doses 2g (n=8, MSP 1), 8g (n=8, MSP 2), 16g (n=9, MSP3) per gilt per day. Gilts were vaccinated against atrophic rhinitis (Porcilis AR-T DF) at 180, 200 and 322 days of age (ie 172, 152 and 30 days before farrowing). MSP<sub>IMMUNITY</sub> was distributed during the 3 days before the third vaccine booster and during 3 days one week before farrowing. The transfer of immunity from gilts to their piglets was evaluated by the measurement of IgG anti-Bordetella bronchiseptica (AB) level in serum and colostrum (using ELISA anti-FHA test, IBL International, Germany), and IgA level in milk (using ELISA polyclonal specific porcine IgA test, Bethyl, Montgomery, USA). Blood was collected 34 and 23 days before the theoretical day of farrowing. Colostrum samples were collected 2 hours after the beginning of farrowing and milk samples 7 and 21 days after farrowing.

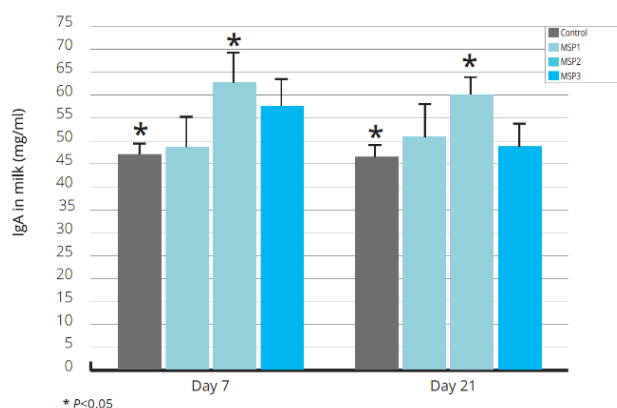
Data normality was validated by the Kolmogorov–Smirnov test, and immunoglobulin concentrations were compared between experimental groups by Student's t-tests, applying Bonferroni corrections for multiple comparisons. Data are shown as average  $\pm$  standard error of the mean (SEM) and differences were considered significant at  $p < 0.05$  in all tests. All statistical analyses were performed using GraphPad Prism 4.03 (GraphPad Software, San Diego, CA, USA).

**Results** The MSP<sub>IMMUNITY</sub> supplementation did not impact IgG AB levels in gilts' serum, but significantly increased their transfer to the colostrum, as shown by the increased level of IgG AB in the colostrum of the test groups compared to control (Figure 1, MSP1:  $P = 0.07$ , MSP2:  $P = 0.05$ , MSP3:  $P = 0.03$ ).

The MSP<sub>IMMUNITY</sub> supplementation had a dose-dependent effect on the IgA level in milk. The highest increase was obtained with MSP2, compared to the control group (Figure 2).



**Figure 1** IgG Anti-Bordetella in gilt's serum at D-23 and in the colostrum at D-0 from farrowing



**Figure 2** IgA level in milk at 7 and 21 days after farrowing

**Conclusions** I These results show that MSP<sub>IMMUNITY</sub> has the capacity to increase the amount of IgG AB in gilts' colostrum via an increased transudation from the serum, and to increase the level of IgA in gilts' milk. Therefore, this study highlights the capacity of MSP<sub>IMMUNITY</sub> to improve both systemic and mucosal immunity and makes it a good candidate to support immune status and health of sows and piglets in farms.

#### References

- Berri M., Slugocki C., Olivier M., Helloin E., Jacques I., Salmon H., Demais H., Le Goff M., Nyvall Collèn P. 2016. Marine-sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. *J Appl Phycol*, 28:1.
- Berri M., Olivier M., Holbert S., Dupont J., Demais H., Le Goff M., Nyvall Collèn P. 2017. Ulvan from *Ulva armoricana* (Chlorophyta) activates the PI3K/Akt signaling pathway via TLR4 to induce intestinal cytokine production. *Algal Research*, 28, 39-47.

### Effect of the maternal supply with essential fatty acids and conjugated linoleic acid on the immune and oxidative status in neonatal calves

K. L. Uken<sup>1</sup>, L. Vogel<sup>1</sup>, T. Gnott<sup>1</sup>, A. Tuchscherer<sup>1</sup>, A. Tröscher<sup>2</sup>, E. Trevisi<sup>3</sup>, T. Stefaniak<sup>4</sup>, H. Sauerwein<sup>5</sup> and H. M. Hammon<sup>1</sup>

<sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; <sup>2</sup>BASF SE, Lampertheim, Germany; <sup>3</sup>Università Cattolica del Sacro Cuore, Piacenza, Italy; <sup>4</sup>Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland; <sup>5</sup>University of Bonn, Germany

Email: hammon@fbn-dummerstorf.de

**Introduction** In modern dairy nutrition, the common utilization of corn silage based rations instead of pasture increases the intake of linoleic acid, whereas the supply with  $\alpha$ -linolenic acid (ALA) and conjugated linoleic acid (CLA) is reduced. During gestation and via the intake of colostrum and milk, an altered maternal fatty acid supply can be transferred to the neonatal calf (Uken *et al.*, 2018). This changed fatty acid status might be accompanied by immune modulatory and pro-oxidative effects of the fatty acids (Karcher *et al.*, 2014; Nair *et al.*, 1993). Therefore, the present study aims to investigate the effect of a divergent maternal fatty acid supply on parameters of the immune and oxidative status in neonatal calves.

**Materials and Methods** Thirty-eight Holstein-Friesian calves, subdivided into 5 blocks with 7-8 calves each, were investigated during their first 5 d of life. All calves were born from dams, which received a corn silage based ration and abomasal supplementations with either coconut oil (CNTR; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin® (CLA; n = 9), or a combination of EFA and CLA (EFA+CLA; n = 11) during the last 9 wk of gestation and the subsequent first 5 d of lactation (Vogel *et al.*, 2018). Each calf was fed with colostrum from its own dam according to its body weight. Colostrum was sampled from the first milking of each day for analysis of immunoglobulin G1 (IgG1), IgG2, and IgM. Concentrations of IgG, interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6,  $\beta$ -carotene, retinol, tocopherol, derivatives of reactive oxygen metabolites (dROM), ferric ion reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) were measured in calf plasma sampled daily before feeding from d 1 to 5. The apparent efficiency of absorption (AEA) was estimated for IgG according to Quigley and Drewry (1998) with an estimated plasma volume of 7% of birth weight. Data were analysed by repeated measures ANOVA of SAS using the mixed model. The model included the treatment (EFA, CLA, and their interaction), time, treatment  $\times$  time, block, and sex as fixed effects and the duration of supplementation and gestation length as covariates. For analysis of AEA, the factor time was excluded from the model and the interval between birth and first feeding was included as covariate.

**Results** Concentrations of IgG2 on d 2 were lower in colostrum from dams of the EFA group compared to CNTR ( $P = 0.017$ ), whereas IgG1 and IgM were not affected by fatty acid supplementation. Nevertheless, plasma concentrations of IgG and AEA in calves were similar between different groups. While IL-6 was not modulated by maternal fatty acid supplementation, plasma IL-1 $\beta$  concentration on d 2 was lower in calves of the group EFA+CLA than in CLA calves ( $P = 0.009$ ) as indicated by a significant interaction between EFA and CLA ( $P = 0.022$ ). Moreover, plasma IL-1 $\beta$  was lower at this time point if dams received EFA compared to calves, whose dams were not supplemented with these fatty acids ( $P = 0.017$ ). Maternal EFA supplementation increased concentrations of plasma retinol on d 5 ( $P = 0.048$ ) but  $\beta$ -carotene and tocopherol were not affected by maternal treatment. In calves, whose dams received EFA, dROM were increased ( $P = 0.049$ ). While plasma ORAC was not affected by maternal treatments, FRAP in calf plasma was lower if dams received EFA ( $P = 0.007$ ), and calves of the groups EFA, CLA, and EFA+CLA had a lower FRAP compared to CNTR on d 4.

**Conclusions** Maternal EFA supplementation might induce pro-oxidative effects in neonatal calves. Supplementing the dam with EFA induced minor effects on colostrum immunoglobulins, but plasma concentrations of cytokines with pro-inflammatory properties might be affected by maternal fatty acid supplementation in neonatal calves.

### References

- Karcher, E. L., Hill, T. M., Bateman II, H. G., Schlotterbeck, R. L., Vito, N., Sordillo, L. M., VandeHaar, M. J. (2014) *J. Dairy Sci.*, 97(4), 2329-2337.  
Nair, P. P., Judd, J. T., Berlin, E., Taylor, P. R., Shami, S., Sainz, E., Bhagavan, H. N. (1993) *Am. J. Clin. Nutr.*, 58(1), 98-102.  
Quigley III, J. D., Drewry, J. J. (1998) *J. Dairy Sci.*, 81(10), 2779-2790.  
Uken, K. L., Vogel, L., Gnott, M., Weitzel, J., Tuchscherer, A., Tröscher, A., Dannenberger, D., Hammon, H. M. (2018) *Proc. Soc. Nutr. Physiol.*, 27, 183.  
Vogel, L., Gnott, M., Kröger-Koch, C., Weitzel, J., Tröscher, A., Starke, A., Hammon, H. M. (2018) *Proc. Soc. Nutr. Physiol.*, 27, 181.

### Effect of different dietary selenium concentrations in dry cows on first colostrum IgG levels

J. Illek<sup>1</sup>, K. Mikulková<sup>1</sup>, R. Kadek<sup>1</sup>, V. Sujová<sup>1</sup> and D. Kumprechtová<sup>2</sup>

<sup>1</sup>University of Veterinary and Pharmaceutical Science Brno, Czech Republic; <sup>2</sup>Institute of Animal Science Prague, Czech Republic

Email: IllekJ@vfu.cz

**Introduction** Colostrum quality depends on multiple factors such as breed, age, health status and nutrition of late pregnancy cows. The quality of colostrum is also influenced by vitamins and trace elements in the diet. Colostrum obtained from the first milking after the calving shows the best quality. The first colostrum has a high protein concentration, with immunoglobulins making up the highest proportion. Furthermore, it has a high content of fat, minerals, vitamins and other biologically active substances. The concentration of immunoglobulins in colostrum is variable and thus the level of colostral immunity of calves is affected. The aim of the study was to determine the effect of different selenium concentrations in the diet on the concentration of immunoglobulins in colostrum.

**Materials and Methods** The study was performed in a Holstein dairy herd with low dietary selenium. The dry cow TMR diet (group A, n = 10) contained 0.18 ppm of selenium (Se) on dry matter (DM) basis. The TMR (total mixed ration) diet of the group B (n = 10) was supplemented with selenium in the form of selenium yeast, at 0.3 ppm Se on DM basis, with total dietary Se concentration of 0.48 ppm. All the cows included in the study were healthy, with optimum body condition. The dry period lasted 52 to 64 days. From each cow, a sample of first colostrum and blood sample from the coccygeal vein were taken immediately after the calving. Colostrum immunoglobulin G (IgG) was measured by immunodiffusion and Se concentration in whole blood was measured by the hydrid method AAS SOLAR 939. The data were statistically processed.

**Results** IgG concentration in colostrum from the cows with lower dietary selenium group A ( $55.8 \pm 5.5$  g/L) was significantly ( $P < 0.001$ ) lower than in the Se supplemented group B ( $81.7 \pm 9.4$  g/L). The difference between the group A and group B in whole blood Se levels was also significant at  $p < 0.001$  ( $77.6 \pm 9.2$  µg/L and  $110.7 \pm 17.1$  µg/L, respectively). The correlation between IgG in colostrum and whole blood Se levels was significant in all the cows ( $r = 0.8662$ ,  $P < 0.001$ ).

**Conclusions** Low Se concentrations in the dry cow diet resulted in significantly lower first colostrum IgG concentrations and blood Se concentrations. Dietary supplementation with Se at 0,3 ppm in the form of selenium yeast significantly increased IgG concentrations both in first colostrum and whole blood of the cows. A close correlation of  $r = 0.8662$  was found between whole blood and first colostrum selenium concentrations.

### The impact of warming of newborn Holstein calves on colostrum intake, blood parameters and vitality

L. Jürgensen<sup>1</sup>, J. Sohr<sup>2</sup> and M. Kaske<sup>3</sup>

<sup>1</sup>University of Veterinary Medicine, Hannover, Germany; <sup>2</sup>Veterinary Practice, Kropp, Germany; <sup>3</sup>Swiss Calf Health Service, Vetsuisse Faculty, Zurich, Switzerland

Email: mkaske@vetclinics.uzh.ch

**Introduction** In agricultural practice, most farmers stick to the recommendation to take the newborn calf as soon as possible out of the maternity pen and to transfer it to an individual hutch, where the newborn calves are housed under ambient temperature. Colostrum intake and vitality are crucial for the development of a newborn calf in a cold environment. The objective of this study was to evaluate whether a rapid drying of calves in winter within the first hours of life affects colostrum intake, metabolic and endocrinologic key blood parameters and vitality.

**Materials and Methods** A prospective field study on a dairy farm in Northern Germany was performed. A specific calf hutch equipped with fan heaters was constructed in order to keep the inside temperature between 28 and 34 °C. Newborn Holstein Friesian calves born between December and April were assigned to (a) a control group housed in individual calf hutches under ambient temperature (N=18) or (b) to a group housed in a heated calf hutch (28-34 °C) for the first 12 hrs after birth (N=20). Colostrum intake within 15 min from a nipple bottle at 2 and 12 hrs post natum (p. n.) was assessed and recorded. The vitality of the calves was assessed semiquantitatively by scoring the strength of the sucking reflex, the ability to stand and the interest in the environment. Blood samples from the jugular vein were taken from each calf 30 min, 2 hrs, 12 hrs and 96 hrs p. n.. The blood samples were analyzed for number of white blood cells, erythrocytes and platelets as well as metabolites (total protein, albumin, cholesterol, glucose, non-esterified fatty acids, urea) and hormones (cortisol, triiodothyronine, thyroxine). The calves were examined twice daily for the first 14 d of life to assess the incidence of diarrhea, undifferentiated fever and omphalitis using clinical score sheets.

**Results** Calves dried rapidly after delivery revealed a better vitality compared to the control group. The volume of colostrum ingested 2 hrs p. n. was 38 % larger for calves dried rapidly after delivery ( $p = 0.008$ ) compared to calves of the control group (3.6 vs. 2.6 l). A significant difference between calves dried rapidly and control group ( $p < 0.01$ ) was also found for the serum concentration of total protein 12 hrs p. n. (64.9 vs. 58.9 g/l). For all other parameters tested and points of time, no significant differences were found between the groups tested. The incidence of disease within the first two weeks of life did not differ between the groups investigated.

**Conclusions** It is concluded that irrespective of a highly developed thermoregulation capability of newborn calves there is a benefit in faster drying in winter and keeping them warm for the first 12 hrs p. n. as reflected by an improved vitality and a high colostrum intake. The recommendation for the routine management on dairy farms especially in cold climates is to ensure that the calves are kept warm until they are dry and ready to go into a calf hutch with ambient temperature. The benefits based on a better vitality and a higher colostrum intake in newborn calves should be taken advantage of in the everyday work on a dairy farm. There is a need for further investigations to characterize more in detail the metabolic and hormonal constellation in newborn calves during the first days of life as affected by the ambient temperature.



### Concentrations and reference intervals of trace elements, vitamin E, and other clinical-chemical parameters in serum of 1 to 6 day old calves

J. Muskens, S. van der Drift, R. Grotentraast and G. Counotte

GD Animal Health, Deventer, The Netherlands

Email: [J.muskens@gddiergezondheid.nl](mailto:J.muskens@gddiergezondheid.nl)

**Introduction** Reference intervals and cut-off values for clinical-chemical parameters in serum are usually based on results from adult cows. Specific reference values for young calves are, however, important to evaluate management interventions to improve calf vitality or health status and to advice on proper treatment and preventive measures. The aim of this study was to investigate concentrations of the trace elements, vitamin E and other clinical chemical parameters in serum of calves of 1 to 6 days old and to calculate reference intervals for these parameters for new-born calves. Results were compared with the reference and clinical decision values of adult cattle as used by GD Animal Health.

**Materials and Methods** Serum samples of 117 calves with the age of 1-6 days from 16 Dutch dairy herds were tested. The majority of the calves was 2-5 days old. Farms were chosen and sampled by their own veterinarian but not specifically selected for calf health problems. Samples were analysed in the routine laboratory of GD Animal Health for copper, zinc, selenium and iodine using inductively coupled plasma mass spectrometry and for Vitamin E using high-performance liquid chromatography. Concentrations of iron, urea, phosphorus, GLDH and gGT in serum were analyzed using commercial test kits on a multi-analyzer (UniCel Dx C 600 Synchron Clinical System, Beckman Coulter). In total, serum samples were tested for 39 parameters, of which only a selection is shown in this abstract. The number of tested samples per parameter varied from 89-117, which depended on the amount of serum material available for analysis. Of every parameter the average, SD, median and the ranges between 25<sup>th</sup> and 75<sup>th</sup> and were calculated with Statistix. Reference intervals for concentrations of clinical chemical parameters were calculated using the Reference Value Advisor (Geffre et al., 2011).

**Results** Descriptive statistics and reference intervals for concentrations of clinical chemical parameters in serum of young calves are shown in Table 1. The reference interval of Vitamin E is large (19 µmol/L), but the median value is low (2.9 µmol/L). The differences between the calves can be explained partially by the large differences in colostrum (IgG) uptake. Concentrations of gGT in serum of calves were high (interval 52-1777 U/L) when compared to values of adult cattle (0-34 U/L).

**Table 1** Concentrations of clinical chemical parameters in serum of calves of 1-6 days old, calculated reference intervals for calves, and reference values/clinical decision values for adult dairy cattle as used by GD Animal Health.

Parameter	Units	N	Average	SD	Median	25th and 75th percentiles	Reference interval <sup>1</sup>	Values of adult cows
Vitamin E	µmol/L	117	4.6	5.0	2.9	1.1-5.9	0.0-18.7	7.4-25*
Zinc	µmol/L	117	22.7	8.39	22.1	16.7-27.3	10-43	10-35*
Copper	µmol/L	117	8.6	2.21	8.3	7.0-10.2	5-14	10-23.5*
Selenium	µmol/L	117	0.5	0.11	0.5	0.4-0.6	0.2-0.7	0.5-3.8*
Iodine	µmol/L	117	2.4	1.43	2.0	1.4-2.8	0.8-6.9	0.4-5.5*
Iron	µmol/L	90	21.3	19.6	15.0	7.0-33.3	2.5-79.4	14-45**
Urea	mmol/L	89	4.7	2.1	4.0	3.1-6.1	2.0-9.7	3.3-6.6**
Phosphorus	mmol/L	90	2.9	0.7	2.7	2.5-3.2	2.1-5.4	1.1-2.4**
GLDH	U/L	90	29.6	41.9	14.1	9.0-27.2	6-156	0-22**
gGT	U/L	87	496	396	373	221-671	52-1777	0-34**

<sup>1</sup>Calculated with Reference Value Advisor (Geffre et al., 2011)

\*: clinical decision value (as used by GD Animal Health)

\*\*: reference values (as used by GD Animal Health)

**Conclusions** Reference values for several clinical-chemical parameters in serum of young calves differ from reference values for adult cows underlining the importance of knowledge on the age of tested animals when interpreting results of clinical-chemical blood tests. Vitamin E concentrations were low in young calves which indicate that vitamin E uptake from colostrum may not have been optimal. Further research should aim at the assessment of specific clinical decision values for clinical chemical parameters in young calves to evaluate management interventions to optimize calf health.

#### References

Geffre, D, Concordet, JP, Braun, C, Trumel. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol (2011) 40/1: 107-112

### Can Bovine Colostrum Microbiome be linked to Passive Transfer of Immunity?

I. Van Hese<sup>1,2</sup>, K. Goossens<sup>1</sup>, L. Vandaele<sup>1</sup> and G. Opsomer<sup>2</sup>

<sup>1</sup>Animal Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium; <sup>2</sup>Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Email: Ilke.Vanhese@ilvo.vlaanderen.be

**Introduction** Passive transfer of antibodies in calves is established through colostral ingestion during the first hours of life. This process is not always successful due to several factors, like timing of first feeding, method and volume of colostrum administration and immunoglobulin G (IgG) concentration in colostrum. When a calf could not absorb enough antibodies (also referred to as failure of passive transfer (FPT)), it is more vulnerable for diseases in its early life. Farmers are aware of the importance of adequate colostrum administration, still about 20 to 40% of newborns suffer from FPT (Raboisson et al., 2016). This triggers researchers to dig deeper into this mechanism of passive transfer of immunity and the contribution of other colostral components, besides IgGs, in this process. Colostrum contains a rich and diverse microbial community (Lima et al., 2017) and colostrum ingestion seems to be beneficial for bacterial colonization of the gut (Malmuthuge et al., 2015). The aim of the present study is to define the microbiome composition of bovine colostrum and to investigate potential associations between the colostral microbiome and the calf's health and immunity in early life.

**Materials and Methods** Samples of first colostrum were taken at the ILVO research barn from 76 dairy and 36 beef cows within 1h after parturition. Colostrum IgG concentration was measured with a digital brix refractometer. Calves received a total of 6 liter of good quality colostrum (>20% Brix) in the first 24 hours of life (3 feedings of 2L within 2, 6 and 24h after birth, respectively). Blood samples were taken from calves at the age of 3 days and serum IgG was measured using electrophoresis. Microbial DNA was extracted from colostrum with the Powerfood microbial kit (QIAGEN) and are currently sequenced (16S amplicon sequencing of the V3-V4 region) to determine colostrum microbiome composition. Sequencing results will be processed using the DADA2 pipeline in R. Next, correlations will be identified between the microbial composition of colostrum and serum IgG levels of the calf that ingested the colostrum in order to identify microbial groups that effect the passive immunity in positive or negative way.

**Results** Concentrations of IgG in first colostrum (mean  $\pm$  SD) averaged  $24.23 \pm 4.26$  % Brix. Concentrations of IgG in calves' serum (mean  $\pm$  SD) averaged  $20.13 \pm 8.42$  g/L. 10.3% of the sampled calves suffered from FPT (serum IgG <10g/L). A preliminary sequencing test showed that the five most abundant genera in colostrum were *Streptococcus*, *Pseudomonas*, *Sphingomonas*, *Staphylococcus* and *Acinetobacter*, which accounted for 29.81% of the total number of sequences obtained. Sequencing is currently performed for the 112 collected samples. Results of the microbiome composition in relation to the transfer of passive immunity will be available for presentation at the conference.

### References

Lima, S. F., A. G. V. Teixeira, F. S. Lima, E. K. Ganda, C. H. Higgins, G. Oikonomou, and R. C. Bicalho. 2017. The bovine colostrum microbiome and its association with clinical mastitis. *J. Dairy Sci.* 100(4):3031-3042.

Malmuthuge, N., Y. Chen, G. Liang, L. A. Goonewardene, and L. Guan le. 2015. Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. *J. Dairy Sci.* 98(11):8044-8053.

Raboisson, D., P. Trillat, and C. Cahuzac. 2016. Failure of Passive Immune Transfer in Calves: A Meta-Analysis on the Consequences and Assessment of the Economic Impact. *PLoS One* 11(3):e0150452.

### Use of Brix Refractometer in Assessing Goat Colostrum

E. V. St. Clair, R. W. Russell and R. J. Van Saun

Department of Veterinary and Biomedical Sciences, College of Agricultural Sciences, Pennsylvania State University, University Park, Pennsylvania, USA

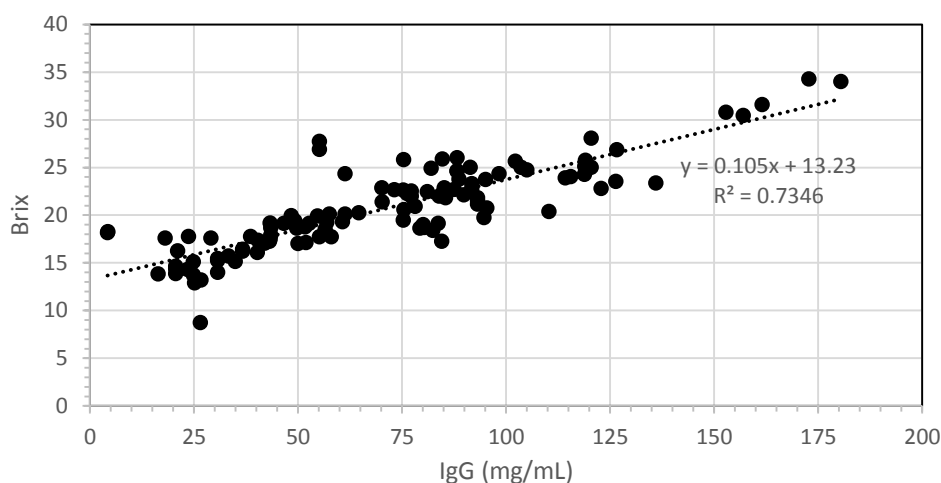
Email: [rjv10@psu.edu](mailto:rjv10@psu.edu)

**Introduction** Feeding high-quality colostrum is the single most important event in the newborn kid's life to ensure viability and survivability. Quality is based on the concentration of immunoglobulin-G (IgG) in colostrum. Use of the colostrometer used in cattle is not practical for goats. Dairy cattle colostrum quality assessment with a Brix refractometer has been validated (Bielmann et al., 2010). A practical application of using a Brix refractometer for testing goat colostrum would be useful if a valid method. The objective of this study was to determine if there exists a relationship between Brix reading and IgG concentration in goat colostrum similar to what has been documented in dairy cattle.

**Materials and Methods** All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. A commercial 900-doe goat dairy in Northern California was used for sample collection. First milk colostrum samples were collected from kidding does and Brix digital refractometer (MISCO, Solon, OH) used on the fresh sample. Individual doe and pooled colostrum samples were collected. Pooled colostrum included samples before and following heat treatment. All data relative to kidding, kid numbers, birth weights, time from kidding, lactation number (LN) were compiled. A subsample of colostrum was frozen and shipped to Penn State for a post-thaw Brix determination and measurement of total IgG concentration using radial immunodiffusion (RID, Triple-J Farms, Bellingham, WA). Data were analyzed by ANOVA to determine significant effects influencing the relationship between Brix and IgG concentration, correlation between measured parameters and use of regression modeling to determine a predictive relationship between Brix and RID measures.

**Results** A total 114 paired Brix and RID determinations were performed on 58 individual and 56 pooled doe colostrum samples. These samples were all post-thaw while 58 Brix determinations were made on fresh colostrum samples. Fresh colostrum samples were collected by  $6.4 \pm 7.3$  h post kidding on average (median: 3.25; range: 0-26.5 h). Colostrum quality declined exponentially with time from kidding ( $\text{IgG, mg/ml} = 97.9 \cdot e^{(-0.05t)}$ ,  $r^2=0.35$ ,  $P<0.0001$ ) but was highly variable. Mean  $\pm$  standard deviation (median, range) RID concentrations for all samples was  $71.0 \pm 36.8$  mg/mL (74.2, 4.2-180.5). Overall post-thaw Brix determinations were  $20.7 \pm 4.5$  (20.3, 8.7-34.3). Fresh sample mean Brix values were  $21.2 \pm 4.7$  (21.7, 9.1-34.0). All Brix and RID determinations were normally distributed. Brix measurements determined in fresh and thawed colostrum were highly correlated ( $P<0.0001$ ) for all ( $r=0.97$ ), individual ( $r=0.98$ ) or pooled ( $r=0.76$ ) samples. Similarly, post-thaw Brix and RID measures were highly correlated ( $P<0.0001$ ) in overall ( $r=0.85$ ), individual ( $r=0.89$ ) and pooled ( $r=0.77$ ) samples. Accounting for type of sample (individual vs pooled) influenced ( $P=0.045$ ) the association between Brix in fresh and post-thaw samples ( $r^2=0.94$ ,  $P<0.0001$ ). Brix determinations on individual samples tended to be influenced by LN ( $P=0.084$ ), but not dry period length or number of kids. Overall prediction model relating Brix to RID in goat colostrum (Figure 1) was  $\text{RID (mg/mL)} = 6.97(\text{Brix}) - 73.65$  ( $r^2=0.73$ ,  $P<0.0001$ ).

**Figure 1** Overall regression of Brix determination on colostrum IgG determination for 114 goat colostrum samples including individual (fresh and post-thaw) and pooled (fresh, post-thaw, and post-heated).



**Conclusions** These data suggest use of a digital Brix refractometer is useful in predicting colostrum IgG concentration for goats similar to what was validated for dairy cattle colostrum. In comparison to reported association in dairy cattle, colostrum containing 50 mg/ml IgG had a Brix value of 19 in goats compared to 22 in cattle (Bielmann et al., 2010). Brix measures were not different between fresh and post-thaw samples and could evaluate pooled colostrum samples, though not as accurately as individual samples. No differences in Brix or IgG concentration with heat treatment suggests no degradation in IgG or inadequate heat treatment. Further research to determine desired colostrum IgG concentration for successful passive transfer is needed.

### References

Bielmann V, et al., An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. J Dairy Sci 2010;93:3713-3721.

## Determination of the nutritional boundaries for lactose replacement with dextrose in milk replacers for rearing calves fed twice daily

J. Wilms, H. Berends, L. Leal, and J. Martín-Tereso

Trouw Nutrition R&D, Amersfoort, the Netherlands

Email: juliette.wilms@trouwnutrition.com

**Introduction** Lactose in milk replacer (MR) can be replaced by dextrose up to one third without affecting insulin sensitivity and growth performance in veal calves (Gilbert et al., 2014). However, the effect of replacing more than 20% of the lactose with dextrose on gastrointestinal health in young calves is unknown. Increasing dextrose concentration may affect absorption capacity, glucose signaling and intestinal osmotic pressure, thereby potentially affecting growth, development and health. In this light, the aim of this study was to determine the effect increasing levels of dextrose inclusion at the expense of lactose on glucose metabolism, digestion and growth performance in male Holstein calves.

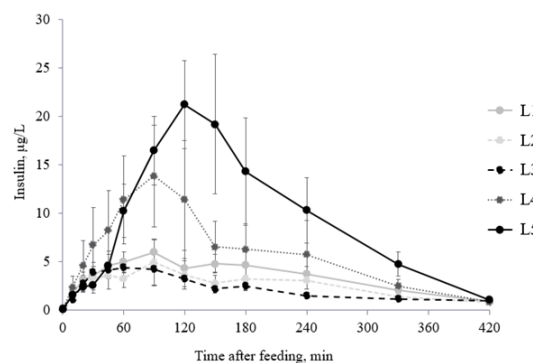
**Materials and Methods** In total, 110 Holstein male dairy calves ( $16 \pm 2.5$  d and  $50.3 \pm 0.2$  kg) were acquired from a collection center (Pali group, the Netherlands). After an adaptation period of 3 d, 100 calves were selected based on health parameters and divided over two departments. Based on body weight (BW) taken on d 4, calves were assigned to one of ten blocks. Then within each block, calves were randomly assigned to one of 5 treatments including 5 levels of dextrose inclusion (replacing lactose): 0% (L1, n = 20), 10% (L2, n = 20), 20% (L3, n = 20), 30% (L4, n = 20) and 40% (L5, n = 20). Carbohydrates were exchanged based on hexose equivalents and the rest of the formula (60%) remained unchanged. Across treatments, the estimated osmolality ranged from 490 (L1) up to 660 mOsm/kg (L5). Milk replacer was provided daily in two equally sized meals at 0700 and 1600 h, respectively. Meal size was 2.0 L during the adaptation period and gradually increased to 4.0 L on d 22. During weaning, meal size decreased to 2 L on d 36 and milk was withdrawn on d 48 after arrival. Calves were fed L1 during the adaptation period and were then exposed to their respective treatment until weaning. Straw and concentrates were offered *ad libitum* from d 26 onwards and calves had *ad libitum* access to water throughout the study. Measurements included feed intake, growth, and fecal parameters (pH, DM) in all calves and postprandial responses of insulin and glucose in 6 calves per treatment.

**Results** Growth was similar across treatment groups. However, mortality linearly increased with dextrose inclusion up to 25 % in L5 ( $P = 0.02$ ). Dextrose inclusion in MR did not affect fecal DM nor fecal pH suggesting an absence of impact on hindgut fermentation. At higher dextrose levels, calves needed greater serum insulin concentrations to control glycaemia as shown by a linear increase in the area under the curve (AUC; Table 1; Figure 1). Furthermore, calves needed more time to control glycaemia as indicated by a linear increase in  $C_{max}$  of insulin (Table 1). Consequently, there was a linear increase of AUC for glucose (Table 1).

**Table 1** Effect of dextrose inclusion in MR on postprandial curves for insulin and glucose (n = 30).

Item <sup>1</sup>	Treatments					Pooled SEM	P-values Linear response
	L1	L2	L3	L4	L5		
Glucose							
AUC, $\times 10^{-3}$	907	762	916	1281	1729	541	***
ng/ml $\times 420$ min							
Basal level, ng/ml	4431	4523	4167	2908	3673	1064	†
$C_{max}$ , ng/ml	1139	1124	1099	1112	1470	507	NS
$T_{max}$ , min	78	68	78	75	105	49.5	NS
Insulin							
AUC, $\mu\text{g/L} \times 420$ min	1390	1003	861	2418	3925	1198	***
ng/ml							
Basal level, ng/ml	0.16	0.23	0.15	0.13	0.13	0.11	NS
$C_{max}$ , $\mu\text{g/L}$	8.13	7.63	6.61	17.2	24.2	8.66	***
$T_{max}$ , min	130	98	60	150	120	54.8	NS

<sup>1</sup> Significant differences at each time point are indicated by \*\*\* ( $P \leq 0.001$ ), \*\* ( $P \leq 0.01$ ), \* ( $P \leq 0.05$ ), and † ( $P < 0.1$ ).



**Figure 1** Postprandial response of plasma insulin in calves fed MR with different dextrose inclusion levels (n = 30).

**Conclusions** In this study, we investigated extensive replacement of lactose with dextrose in MR. Our data suggest that, despite calves needed more time and higher insulin concentrations, they could control glycaemia up to 30% dextrose inclusion in MR. Increasing dextrose inclusion, at the expense of lactose in MR, did not affect growth but significantly increased mortality. Mortality was primarily associated with gastrointestinal disorders suggesting a potential loss of gut integrity due to increased osmotic pressure. The effect of dextrose inclusion on intestinal osmotic pressure, glucose signaling and absorptive capacity deserves further investigation, in order to explain the observed effect on mortality.

## References

Gilbert, M.S., A.J. Pantophlet, J.J.G.C. van den Borne, W.H. Hendriks, H.A. Schols and W.J.J. Gerrits. 2014. The effect of replacing lactose by glucose, fructose or glycerol in milk replacer on energy partitioning in Holstein-Friesian calves. In: Proceedings of the 30th Biennial Conference of the Australian Society of Animal Production. - Animal Production Science 30.

### Variation of colostrum composition and IgG content in goat and sheep breeds

E. C. Kessler, R. M. Bruckmaier and J. J. Gross

Veterinary Physiology, Vetsuisse Faculty University of Bern, Switzerland

Email: josef.gross@vetsuisse.unibe.ch

**Introduction** In small ruminants, the transfer of immunoglobulins to the fetus during gestation is prevented by the placenta. Thus, colostrum represents the sole source to acquire humoral immunity and is further an important energy source for newborn lambs and goats kids. Colostrum composition (i.e., the contents of IgG, fat, protein, and lactose) is affected by various factors such as parity, litter size and, potentially, by breed. According to published data, colostral IgG concentrations in Blackface, Suffolk and Rasa Aragonesa ewes ranged between 37 mg/mL, as found in Santa Inês ewes (Alves et al., 2015) and 79 mg/mL observed in Polypay ewes (al-Sabbagh et al., 1995). In dairy goats, IgG concentrations varied from 28.2 mg/mL in Murciano-Granadina (Romero et al., 2013) to 72 mg/mL in a Chinese strain of Saanen goats (Yang et al., 2009) while Majorera, Alpine goats and Weisse Deutsche Edelziege were intermediate. Considering the constituents of colostrum, average protein concentrations in sheep colostrum of Polish Merino, Chio, and Canarian sheep varied between 14 and 22% (Hadjipanayiotou, 1995; Ciuryk et al., 2004; Hernández-Castellano et al., 2016), and between 10 and 16% in Damascus, Majorera, and Murciano-Granadina goats (Hadjipanayiotou, 1995; Moreno-Indias et al., 2012; Romero et al., 2013). Fat and lactose contents in colostrum, however, showed less variation in the studies cited above. However, up to now, only single individual breeds have been investigated in terms of colostrum composition and direct breed comparisons are rarely performed. Due to the fact that small dairy ruminants are mainly concentrated in the Mediterranean and Black sea regions, most data on colostrum composition, as shown above, originate from those local breeds. Therefore, we aimed at investigating IgG, fat, protein, and lactose concentrations in colostrum of different goat and sheep breeds raised for milk and meat production in alpine areas in Switzerland and Germany.

**Materials and Methods** Colostrum samples (116 from goats and 100 from ewes) were collected from 10 goat (Anglo-Nubian, Appenzell, Boer, Bunte Deutsche Edelziege, Chamois-colored, Grisons Striped, Peacock, Saanen, Toggenburg, and Valais Blackneck) and 10 sheep breeds (Brown-Headed Meat, East Friesian Milk, German Blackheaded Mutton, Gray Horned Heath, Lacaune Dairy, Merino Land, Swiss Black- Brown Mountain, Swiss Charollais, Swiss White Alpine, and Valais Blacknose) by twenty-eight goat and sheep farmers in Switzerland and Germany. Farmers provided approximately 50 mL of goat or sheep colostrum (frozen at  $-20^{\circ}\text{C}$ ) per animal and completed a questionnaire containing information about the individual dams, time of parturition, and first milking of colostrum. Colostrum samples were taken before suckling, between 10 and 300 min after kidding in goats, and between 10 and 390 min after lambing in sheep. Two-thirds of the samples were collected within 2 h after parturition. Total IgG concentrations in sheep and goat colostrum were measured with a commercial ELISA kit and fat, protein and lactose concentrations were assessed by an infrared milk analyzer. Sheep colostrum samples were diluted 1:2 with distilled water before measurement because of their high viscosity.

**Results** Ovine and caprine colostral IgG concentrations showed a broad range and varied between 4.8 and 75.0 mg/mL in goats, and between 6.2 and 65.4 mg/mL in ewes. This high variation in colostral IgG concentrations was not due to different time intervals between parturition and milking ( $P > 0.05$ ) or different laboratory procedures for IgG determination. In goats, the highest IgG concentrations were found in Boer (meat-type;  $61.0 \pm 10.3$  mg/mL; mean  $\pm$  SD) whereas the lowest IgG concentrations were observed in Bunte Deutsche Edelziege (milk-type;  $26.5 \pm 12.5$  mg/mL). In sheep, East Friesian Milk and Lacaune Dairy showed the lowest colostral IgG concentrations ( $17.9 \pm 7.3$  and  $20.2 \pm 8.0$  mg/mL, respectively), while the highest values were observed in the Merino Land breed ( $44.2 \pm 15.7$  mg/mL). Ovine colostrum contained higher fat and protein concentrations than caprine colostrum. In both species, colostrum constituents varied widely, with fat, protein, and lactose concentrations between 1.3 and 16.5%, 4.9 and 25.1%, and 2.1 and 6.0% in goats, and between 1.1 and 24.8%, 7.3 and 30.5%, and 1.3 and 4.6% in sheep, respectively. The dairy sheep breeds, East Friesian Milk and Lacaune Dairy, had lower fat and protein with concomitantly higher lactose concentrations in colostrums compared with most of the meat-type ewes. In contrast, differences in colostrums composition between goat breeds were not striking.

**Conclusions** In this study of different goat and sheep breeds, colostral IgG, fat, protein, and lactose concentrations varied broadly between individual animals and breeds. Breed type (meat or dairy) of the dam affected IgG concentrations in colostrum, with meat-type goats showing the greatest and dairy sheep breeds the lowest IgG concentrations. This high variation in IgG concentration and colostral constituents has a great impact on the minimum colostrum quantity required by kids and lambs to meet their energy demands and to obtain adequate passive immunization.

### References

- al-Sabbagh, T.A., Swanson, L.V. and Thompson, J.M. (1995): The effect of ewe body condition at lambing on colostral immunoglobulin G concentration and lamb performance. *J. Anim. Sci.* 73:2860–2864.
- Alves, A.C., Alves, N.G., Ascari, I.J., Junqueira, F.B., Coutinho, A.S., Lima, R.R., Pérez, J.R.O., De Paula, S.O., Furusho-Garcia, I.F. and Abreu, L.R. (2015): Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. *J. Dairy Sci.* 98:3706–3716.
- Ciuryk, S., Molik, E., Kaczor, U. and Bonczar, G. (2004): Chemical composition of colostrum and milk of Polish Merino sheep lambing at different times. *Arch. Anim. Breed.* 47(Special Issue):129–134.
- Hadjipanayiotou, M. (1995): Composition of ewe, goat and cow milk and of colostrum and goats. *Small Rumin. Res.* 18:255–262.
- Hernández-Castellano, L.E., Almeida, A.M., Renaut, J., Argüello, A. and Castro, N. (2016): A proteomics study of colostrum and milk from the two major small ruminant dairy breeds from the Canary Islands: A bovine milk comparison perspective. *J. Dairy Res.* 83:366–374.
- Moreno-Indias, I., Sánchez-Macías, D., Castro, N., Morales-de-la-Nuez, A., Hernández-Castellano, L.E., Capote, J. and Argüello, A. (2012): Chemical composition and immune status of dairy goat colostrums fractions during the first 10 h after partum. *Small Rumin. Res.* 103:220–224.
- Romero, T., Beltrán, M.C., Rodríguez, M., Martí De Olives, A. and Molina, M.P. (2013): Short communication: Goat colostrum quality: Litter size and lactation number effects. *J. Dairy Sci.* 96:7526–7531.
- Yang, X.-Y., Chen, J.-P. and Zhang, F.-X. (2009): Research on the chemical composition of Saanen goat colostrum. *Int. J. Dairy Technol.* 62:500–504.

### Assessment of colostrum quality in dairy cows by color measurement, Brix refractometry, and specific gravity

J. J. Gross, E. C. Kessler and R. M. Bruckmaier

Veterinary Physiology, Vetsuisse Faculty University of Bern, Switzerland

Email: josef.gross@vetsuisse.unibe.ch

**Introduction** Since bovine calves are born agammaglobulinemic and therefore depend on passive immunization via colostrum-derived immunoglobulins (Ig), a timely supply with good quality colostrum is essential for calf health (Tyler et al., 1999). However, yield and quality of colostrum obtained after parturition in dairy cows vary markedly between herds and between animals. Instruments for on-farm determination of colostrum quality like refractometers and densimeters are increasingly used in dairy farms. Furthermore, the colostrum color is also supposed to reflect its quality. A pale or mature milk like color is associated with a lower colostrum value compared to a more yellowish and darker color. The objective of this study was to elucidate if Brix refractometry, specific gravity and color measurements (CIE L\*=from white to black, a\*=from red to green, b\*=from yellow to blue) are suitable to assess colostrum quality in cows and heifers.

**Material and Methods** Fourteen primiparous and twelve multiparous cows were milked for the first time exactly 4 h post-calving. Colostrum was analyzed for total IgG by ELISA and for fat, protein and lactose by a FTS Infrared Milk Analyzer (Bentley Instruments Inc., Chaska, MN, USA) (previously validated for use with colostrum). Fresh colostrum samples were analyzed at 20°C using a colostrum densimeter (DENS; Kruuse, Langeskov, Denmark). Specific gravity (SG) was determined by reading the scale (SG 1.023 to 1.077) above the submerged portion of the colostrometer. Additionally, all samples were measured with an optical Brix refractometer (BRIX; Manual Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany) with a scale ranging from 0 to 40% Brix. All measurements with the colostrometer (SG determination) and refractometer were performed in duplicate. Color [CIE 1976 (L\*, a\*, b\*) color space - CIELAB] was measured in thawed and homogenized samples in triplicate at 25°C using a calibrated Microflash 200d spectrophotometer (Datacolor International, Dietikon, Switzerland), with the coordinates L\* representing relative lightness (black to white), a\* giving the relative value between green and red, and b\* indicating the relative position between blue and yellow.

**Results** In multiparous cows, BRIX was closely correlated with DENS ( $r=0.72$ ,  $P<0.05$ ), protein ( $r=0.88$ ,  $P<0.01$ ) and lactose concentration ( $r=-0.85$ ,  $P<0.01$ ). Also DENS showed a clear relationship with milk protein ( $r=0.74$ ,  $P<0.01$ ) and lactose ( $r=-0.73$ ,  $P<0.05$ ) in cows. Both, BRIX and DENS did not correlate with IgG concentration in cows. In colostrum of primiparous cows, BRIX was less correlated with DENS ( $r=0.43$ ) and lactose ( $r=-0.50$ ) than in cows, but still high with protein concentration ( $r=0.75$ ,  $P<0.01$ ). Also DENS was less correlated with protein ( $r=0.44$ ) in primiparous than multiparous cows. For primiparous cows, BRIX and DENS did not correlate with IgG concentration. As for colour measurement, total IgG concentration was poorly correlated with L\*, a\*, and b\* ( $r=-0.13$ ,  $0.02$ , and  $0.12$ ;  $P>0.05$ ) in primiparous cows, while in multiparous cows correlations were higher ( $-0.40$ ,  $0.32$ , and  $0.06$ , resp.,  $P>0.05$ ). While DENS did not correlate with color measurements, BRIX was closely correlated with L\* ( $r=-0.68$ ,  $P<0.01$ ), and b\* ( $r=0.55$ ,  $P<0.0001$ ) in primiparous and for b\* in multiparous cows ( $r=0.52$ ,  $P<0.001$ ). Milk fat concentration was correlated with a\* ( $r=0.42$ ,  $P<0.001$ , and  $r=0.44$ ,  $P<0.001$ , for primi- and multiparous cows) and b\* ( $r=0.27$ ,  $P<0.05$ , and  $r=0.43$ ,  $P<0.01$ ), while milk protein concentration was more correlated to b\* ( $r=0.53$ ,  $P<0.0001$ , and  $r=0.30$ ,  $P<0.05$ ). Highest correlations were found between milk lactose percentage and b\* in primiparous ( $r=-0.59$ ,  $P<0.0001$ ) and multiparous cows ( $r=0.56$ ,  $P<0.0001$ ).



**Figure 1** Variation in colostrum color of dairy cows compared to mature milk (right)

**Conclusions** The refractometer and the densimeter were closest correlated with protein content in colostrum while the color measurements via spectrophotometer were also correlated with milk fat and lactose concentrations in colostrum. Total IgG concentration was not necessarily reflected by the three tested methods. The easier handling of the refractometer and the higher correlations of its results with colostrum constituents make it a more reliable instrument than DENS for on-farm assessment of colostrum quality. As for color measurement, an implementation of color measuring devices in automatic milking systems might be a potential instrument also to access colostrum quality besides detecting abnormal milk.

### References

Tyler, J.W., Hancock, D.D., Thorne, J.G., Gay, C.C. and Gay, J.M. (1999): Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. *J. Vet. Int. Med.* **13**: 335-33

### Effects of the novel concept "outdoor veal calf" on antimicrobial use, calf growth and mortality in Switzerland

J. Becker<sup>1</sup>, A. Steiner<sup>1</sup>, G. Schüpbach<sup>2</sup>, V. Perreten<sup>3</sup> and M. Meylan<sup>1</sup>

<sup>1</sup>Clinic for Ruminants Vetsuisse-Faculty, University of Bern, Switzerland; <sup>2</sup>Veterinary Public Health Institute, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>3</sup>Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Bern, Switzerland

Email: mireille.meylan@vetsuisse.unibe.ch

**Introduction** The aim of the intervention study 'outdoor veal calf' is to evaluate a novel concept for calf fattening in order to reduce antimicrobial use by 50% without compromising animal health. In Swiss calf fattening facilities both antimicrobial use and mortality are high, and the risk of selecting bacteria resistant to antimicrobials and economic losses are elevated accordingly. Reasons for this situation include related management practices such as commingling of calves from multiple birth farms and crowding, as well as suboptimal barn climate. For decades, pneumonia has been described as the most important reason for antimicrobial treatment, and the growing prevalence of resistant bacteria represents a threat to human and animal health by now.

**Materials and Methods** The "outdoor veal calf" concept, implemented in nineteen intervention farms (IF), is based on three main actions: first, purchased calves were transported directly among neighbors from the farm of birth to the fattening facility instead of commingling calves in livestock dealer trucks. Second, each calf was vaccinated against pneumonia immediately after arrival and completed a three-week quarantine in an individual hutch. Third, the calves spent the rest of the fattening period in outdoor hutches in groups not exceeding 10 calves. The group hutches and the covered and bedded paddock provided shelter from adverse weather conditions and direct sunshine, but constant access to fresh air was warranted. Nineteen conventional calf fattening operations of similar size producing according to the current regulations of the label IP-Suisse (with improved animal welfare and sustainability standards) served as controls. Every farm was visited once a month for a 12-month period, and data regarding animal health and welfare, treatments, and production parameters were collected.

**Results** Mean TI<sub>DDD</sub> was 5.3-fold lower in IF compared to control farms (CF): 5.9±6.5 and 31.6±27.4 days under treatment per animal year, respectively;  $p<0.001$ . Mortality was 2.1-fold lower in IF (3.1%±2.3 vs. 6.3%±4.9 in CF;  $p=0.032$ ). Animal health and welfare were assessed in live animals, at slaughter and by comparing management practices indicating an overall better health and welfare in IF. The prevalence of pneumonia at slaughter was 26% and 46% in IF and CF, respectively ( $p<0.001$ ), whereas the prevalence of abomasal ulceration did not differ between groups (IF: 65%, CF: 72%;  $p=0.216$ ). Average daily gain did not differ between groups (IF: 1.29±0.17 kg/day, CF: 1.35±0.16 kg/day;  $p=0.244$ ).

**Conclusions** A drastic reduction in antimicrobial use and mortality was achieved in the novel "outdoor veal calf" concept without compromising animal health and welfare. The principles of risk reduction used in designing the concept can be used to improve management and animal health, decrease the need for antimicrobial treatments, and increase profitability in veal operations.

### Customising dry period management: consequences for milk yield, body condition and disease incidence

A. T. M. van Knegsel<sup>1</sup>, R. J. van Hoeij<sup>1</sup>, B. Kemp<sup>1</sup> and A. Kok<sup>1,2</sup>

<sup>1</sup>Adaptation Physiology group, Wageningen University, the Netherlands; <sup>2</sup>Animal Production Systems group, Wageningen University, the Netherlands

Email: [ariette.vanknegsel@wur.nl](mailto:ariette.vanknegsel@wur.nl)

**Introduction** Advantages of shortening and omitting of the dry period are an improved energy balance and metabolic status in early lactation and potentially also an improved fertility (Van Knegsel et al., 2013). Disadvantages, however, are a reduction in milk yield in the subsequent lactation, loss of opportunity for selective dry cow therapy (DCT) and, in case of omitting of the dry period, reduction in concentration of antibodies in colostrum. Moreover, response of dairy cows to different dry period lengths was related to individual cow characteristics, like parity (Annen et al., 2004), milk yield level or SCC level (Van Hoeij et al., 2016). Therefore, it can be hypothesized that customising dry period management for individual cows could mitigate negative impacts of shortening and omitting the dry period on milk production and udder health, and at the same time retain benefits from both a dry period as well as benefits from a short or no dry period. In this study, we aimed to evaluate two decision trees to customize dry period length and selective DCT based on parity, milk production and SCC in late lactation.

**Materials and Methods** In total 183 Holstein-Friesian cows were selected, blocked for expected calving date, parity, milk yield level and SCC and within blocks randomly assigned to one of the three treatments. Treatments consisted of a control (C) treatment and one of two decision trees for customised dry period management (treatment T1 or T2). In C, all cows had a 60d dry period, with DCT if SCC >150,000 cells/mL prior to dry-off. In T1, heifers and older cows were assigned DCT if SCC ≥150,000 cells/mL and SCC ≥50,000 cells/mL, respectively, whereas in T2 the threshold for DCT was SCC ≥200,000 cells/mL for all animals. In T1 and T2, cows with DCT were assigned a 60d dry period, whereas cows without DCT were assigned a 30d or 0d dry period if their milk production remained >12kg/d. Decision trees were applied to individual cows using cow information from the last milk recording before 67 days before the expected calving date. Cows were monitored for milk production, milk composition, body weight, body condition, and disease incidence from - 60 till 100 DIM. Data were analysed using a repeated measurements model with fixed effects of treatment (C, T1 or T2), parity (2 or >2), week and their interactions and repeated cow effect included using SAS (PROC MIXED).

**Results** In the 8 weeks before calving, cows on average produced least milk in group C and most in group T2, with T1 being intermediate (0.1 vs 4.1 vs 7.3 kg/d in group C vs T1 vs T2;  $P<0.05$ ). In the first 14 weeks after calving, average milk yield of cows in both group T1 and T2 was lower than in group C (40.1 vs 37.1 vs 35.1 kg/d in group C vs T1 vs T2;  $P<0.05$ ). Lactose and fat content did not differ between the three groups, whereas protein content was greater for cows in groups T1 and T2 than for cows in group C. Precalving, body weight and BCS did not differ among treatments. Postcalving, cows in T2 gained more body weight in the first 100 DIM, compared with cows in T1 or C. Postcalving, SCC was lower for cows in T1, compared with other 2 groups (77 vs 61 vs 80 ×1000 cells/ml in group C vs T1 vs T2;  $P<0.05$ ). Postcalving, disease incidence tended to be lower for cows in T2, compared with other 2 groups (50/59 vs 34/63 vs 55/61 in group C vs T1 vs T2;  $P<0.10$ ).

**Conclusions** In conclusion, customising dry period management (dry period length and DCT) for cows based on parity, milk production and SCC in late lactation reduced milk losses due to short or no dry periods compared with applying these strategies for the complete herd. Attributing a large proportion of cows to a dry period with DCT (T1) reduced SCC in the first 100 DIM of the subsequent lactation. Attributing a large proportion of cows to a short or no dry period (T2) increased body weight gain and tended to reduce disease incidence in the first 100 DIM of the subsequent lactation.

#### References

Annen, E.L., Collier, R.J., McGuire, M.A., Vicini, J.L., Balam, J.M., Lormore, M.J. 2004. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. *J. Dairy Sci.* 87: 3746-3761

Van Hoeij, R.J., Lam, T.J.G.M., De Koning, D.B., Steeneveld, W., Kemp, B., Van Knegsel, A.T.M. 2016. Cow characteristics and their association with udder health after different dry period lengths. *J. Dairy Sci.* 99: 8330-8340.

Van Knegsel, A.T.M., Van der Drift, S.G.A., Cermáková, J., Kemp, B. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: a systematic review. *Vet. J.* 198: 707-713.



### Customising lactation length: impact of calving interval, parity, and lactation persistency on milk production of dairy cows

E. E. A. Burgers<sup>1,2</sup>, A. Kok<sup>1,3</sup>, R. M. A. Goselink<sup>2</sup> and A. T. M. van Knegsel<sup>1</sup>

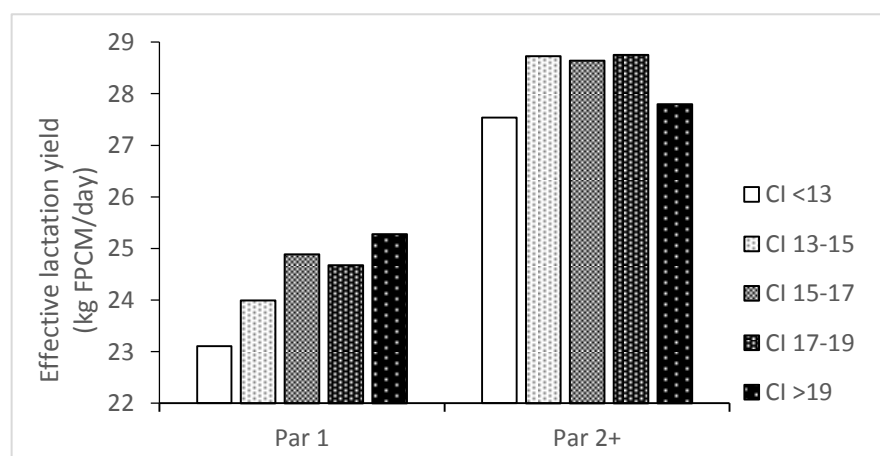
<sup>1</sup>Adaptation Physiology group, Wageningen University & Research, the Netherlands; <sup>2</sup>Wageningen Livestock Research, Wageningen University & Research, the Netherlands; <sup>3</sup>Animal Production Systems group, Wageningen University & Research, the Netherlands

Email: [eline.burgers@wur.nl](mailto:eline.burgers@wur.nl)

**Introduction** The transition period around calving is a critical phase for a dairy cow. Large changes in both physiology and management increase the risk for disease. In most modern dairy systems a cow faces this transition period every year, as a one-year calving interval (CI) is usually aimed for. Increasing CI reduces the relative amount of transition periods for a cow, thereby potentially reducing the risk for disease and improving fertility. Drawback of an increased CI is a possible reduction in milk yield although estimated effects of an increased CI on milk yield differ among studies. Some farmers in the Netherlands deliberately increase CI by increasing the voluntary waiting period for insemination (VWP), using various strategies to select cows that may benefit from an increased CI. This study aims to gain insight in cow characteristics that can be used to optimize CI for individual cows by assessing how CI, parity, and persistency impact milk production.

**Materials and Methods** We used milk production data (2014-2018) of Holstein Friesian cows from 10 commercial Dutch dairy farms which work with an extended VWP. The final dataset consisted of 50,065 six-weekly milk records of 3,656 cow lactations. Cow lactations were grouped by parity (1 or 2+), and CI class (<13, 13-15, 15-17, 17-19, >19 months). Milk yield was converted to fat and protein corrected milk (FPCM). Lactation curves were fitted with a mixed model in SAS (PROC MIXED), using a Wilmink curve extended with a linear negative effect of gestation on milk production. The model included fixed effects for parity, CI class, farm, and all significant interactions, on lactation curve parameters. From these curves, per farm, per parity, per CI class, we derived 305-day yield, effective lactation yield, peak yield, and persistency. The 305-day yield was expressed as mean FPCM yield in the first 305 days of lactation in kg/day (MY305). Effective lactation yield is the mean FPCM yield in kg per day of CI (MYeff); persistency was defined as the slope of the lactation curve from day 100 until day 212 in lactation (after peak yield and before the negative effect of gestation on milk yield) in kg per day.

**Results** Parity, CI class, and farm affected all lactation curve parameters. MY305 was lower for parity 1 than parity 2+ (28.9 vs 34.8 kg per day). Interestingly, MY305 was lowest for CI <13, intermediate for CI 13-15, and highest for CI 15-17, CI 17-19, and CI >19 (29.5 vs 31.6 vs 32.3, 32.9, and 32.9 kg per day). These differences were caused by a combination of peak yield and persistency, with cows in CI<13 having a lower peak yield and cows with longer CI having greater persistency. For parity 1, MYeff increased with increasing CI, whereas for parity 2+ highest MYeff was realised with intermediate CI (Figure 1). Lactations of parity 1 were characterised by greater persistency but lower peak yield compared with lactations of parity 2+. Persistency ranged between 0.052 and 0.004 kg milk reduction per day for parity 1, and between 0.102 and 0.043 for parity 2+; peak yield ranged between 20.4 and 38.5 kg for parity 1, and between 26.1 and 60.7 for parity 2+.



**Figure 2** Effect of CI class (legend) and parity (par) category on effective lactation yield. FPCM = fat-and-protein-corrected milk.

**Conclusions** Farmers realized higher 305-day yields with longer lactations. This is likely due to selection of cows with higher peak and greater persistency for a longer CI. Moreover, selection by farmers of parity 1 cows for an increased CI was always beneficial for effective lactation yield, while for parity 2+ there seems to be an optimal CI which maximizes effective lactation yield. Increasing CI has more effects than only on FPCM yield. Possible positive effects on health and fertility should be subject of further studies to conclude on the viability of increasing CI on farms.

### Dairy veterinarians' skill in Motivational Interviewing is linked to enhanced veterinary herd health management consultations

C. Svensson<sup>1</sup>, H. Wickström<sup>2</sup>, U. Emanuelson<sup>1</sup>, C. von Brömssen<sup>3</sup>, S. Betnér<sup>3</sup> and L. Forsberg<sup>4</sup>

<sup>1</sup>Dept. of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>2</sup>MeetMe Psykologkonsult AB, Gothenburg, Sweden; <sup>3</sup> Dept. of Energy and Technology, Unit of Applied Statistics and Mathematics, Swedish University of Agricultural Sciences, Uppsala, Sweden; <sup>4</sup>MICLab AB, Stockholm, Sweden

Email: catarina.svensson@slu.se

**Introduction** The importance of communication skills is increasingly being acknowledged in the veterinary profession. Veterinary herd health management (VHHM) services constitute an increasing proportion of professional work for cattle veterinarians and are often expected to lead to client behavior change. In these types of services, change-orientated communication skills may be of special importance to improve client engagement and resulting service efficacy. Cattle veterinarians have been shown to predominantly use a directive communication style when discussing herd health, directing the client in what to do and how to do it whilst leaving the client in a passive role (Bard et al. 2017). Such a communication style has been shown to evoke resistance to change in clients experiencing psychological ambivalence, a well-known aspect of farmers' herd health decisions. Hence, veterinary consultancies may run the risk of counteracting the purpose of encouraging clients to follow recommendations.

In the medical and psychological sciences, practitioners are increasingly adopting an evidence-based methodology called Motivational Interviewing (MI). MI is a client-centred communication methodology that aims to facilitate clients' internal motivation to change (Miller & Moyers 2017). There is strong research evidence supporting the efficacy of MI, both in its original application within drug and alcohol abuse counselling and in a diverse array of wider behavior change contexts (Miller and Moyers 2017).

In this methodology, *Change Talk* is a client's own statement expressing consideration of, motivation for or commitment to behavior change, whereas *Sustain Talk* is a corresponding statement related to maintaining the status quo. Client *Change Talk* is strongly correlated to clients later realizing behavior change (Apodaca & Longabaugh 2009), meaning the frequency and proportion of *Change Talk* expressed by a client can be considered predictive of future behavior change. The aim of the present study was to investigate the effect of dairy cattle veterinarians' MI skills on farmer *Change Talk* and *Sustain Talk* during VHHM consultations.

**Materials and Methods** VHHM consultancies on 169 Swedish dairy farms were audio recorded by participating cattle veterinarians (n=36). As part of a larger research study, veterinarians had randomly been designated to an MI-training group (n=18) where they participated in a six-month training program in MI and conducted consultancies after this training or a control group (n=18) where they conducted consultancies without such training. Before the consultancies took place, the veterinarians' MI skills were measured using the Motivational Interviewing Treatment Integrity code 4.2.1 (Moyers et al. 2014). Veterinarians' MI skills were categorised as untrained, poor, near moderate or moderate (none were better than "moderate") for the purpose of analysis. Client language was coded using the Client Language Easy Rating (CLEAR) coding system (Hagen & Moyers 2012) and the summary measurements *Change Talk*, *Sustain Talk* and *Proportion Change Talk* (*Change Talk* per sum total of *Change* and *Sustain Talk*) were calculated. The effects of differing MI skills on *Change Talk*, *Sustain Talk* and *Proportion Change Talk* was investigated using three multiple regression models (Poisson and logistic) with random intercepts for farm and veterinarian. Extra explanatory variables were the gender, VHHM experience and type of veterinarian, the age, education and role of the client, if both client and veterinarian felt that the time allocated for the consultancy was sufficient, if the gender of the client and veterinarian were the same, number of participants from the farm, visit type, and the client's satisfaction with the consultation. Predicted values and their confidence limits (CI) were calculated based on estimates from the models.

**Results** Veterinarian MI skill was significantly associated with the amount of client *Change Talk*; clients interacting with veterinarians who were of moderate MI competency expressed more *Change Talk* ( $P=0.016$ ). Predicted values ( $\pm$  95% CI) for the rate of *Change Talk* were 6.1 (2.2-16.5), 4.1 (1.5-11.4), 3.9 (1.4-11.0) and 4.0 (1.4-11.2) for veterinarians with moderate, near moderate, poor and untrained MI skills, respectively. No associations between MI skills and *Sustain Talk* or *Proportion Change Talk* were detected.

**Conclusions** Enhanced veterinarian MI skill increased client *Change Talk*. Improved veterinary-client communication in this way may result in higher rates of client behavior change in response to consultancies and greater efficacy of VHHM services.

#### References

- Apodaca T.R., and O.R. Longabaugh. 2009. Mechanism of change in motivational interviewing: a review and preliminary evaluation of the evidence. *Addiction* 104:705-715.
- Bard A.M., D.C.J. Main, A.M. Haase, H.R. Whay, E.J. Roe, and K.K. Reyher. 2017. The future of veterinary communication: Partnership or persuasion? A qualitative investigation of veterinary communication in the pursuit of client behavior change. *Plos One* 12(3):e0171380. <https://doi.org/10.1371/journal.pone.0171380>.
- Hagen L.G., and T.B. Moyers. 2012. Manual for the Client Language Easy Rating (CLEAR) Coding System: Formerly "Motivational Interviewing Skill Code (MISC) 1.1" Retrieved from <http://casaa.unm.edu/download/CLEAR.pdf>
- Miller W. R., and T.B. Moyers. 2017. Motivational interviewing and the clinical science of Carl Rogers, *Journal of Consulting and Clinical Psychology*, 85(8), pp. 757-766. doi: 10.1037/ccp0000179.
- Moyers, T. B., J.K. Manuel, and D.A. Ernst. 2014. Motivational Interviewing Treatment Integrity Coding Manual 4.2.1. Retrieved from [http://casaa.unm.edu/download/MITI4\\_2.pdf](http://casaa.unm.edu/download/MITI4_2.pdf)

### The potential of local administration of NSAID in mastitis treatment

O. Wellnitz and R. M. Bruckmaier

Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland

Email: [olga.wellnitz@vetsuisse.unibe.ch](mailto:olga.wellnitz@vetsuisse.unibe.ch)

**Introduction** The systemic use of nonsteroidal anti-inflammatory drugs (NSAID) as a supportive therapy for mastitis in cows is a common practice. NSAID alleviate pain, and they have been shown to improve the recovery from mastitis if administered in addition to antimicrobial treatments. However, the exact pathways of interaction between NSAID and the mammary immune system are unknown. Although the bovine mammary gland is most suitable for a local administration through the teat canal, which allows a precise and simple application of drugs at the site of inflammation, the potential of intramammary administration of NSAID to treat mastitis has to our knowledge not yet been investigated.

The aim of our studies was to investigate effects of NSAID on the immune response of mammary epithelial cells with and without simultaneous immune stimulation. In addition, effects on the mammary immune response, including effects on the blood-milk barrier after intramammary application of the commonly used NSAID ketoprofen and meloxicam in quarters without and with induced intramammary inflammation were investigated.

### Materials and Methods

#### *Effects on mammary epithelial cells in vitro*

Primary mammary epithelial cells from three cows were challenged with or without lipopolysaccharide (LPS, 0.2 µg/mL) from *Escherichia coli*, with or without addition of ketoprofen in two concentrations (1.25 or 2.5 mg/mL, respectively). Cells from four cows were challenged with LPS or with lipoteichoic acid (LTA; from *Staphylococcus aureus*, 20 µg/mL) with or without 1.5 mg/mL of meloxicam. At 3h, 6h, and 24h post treatment, the supernatants and the total RNA of the cells were harvested. A repeated measures mixed model analysis using the MIXED procedure in SAS was performed to analyze effects of ketoprofen and meloxicam on the mRNA abundance of factors related to the immune response by reverse transcription and quantitative real-time PCR. Prostaglandin (PG) E<sub>2</sub> concentrations in the supernatants were measured by ELISA.

#### *Effects on mammary immune response in vivo*

Lactating dairy cows received 50 mg ketoprofen or meloxicam/ quarter with or without additional challenge with 0.2 µg LPS from *Escherichia coli*/ quarter (n=6 per treatment). Another eight cows received ketoprofen and 20 µg LTA from *Staphylococcus aureus*/ quarter. Somatic cell count (SCC) in milk was measured with a DeLaval cell counter as a reference parameter of mammary immune response. Serum albumin (SA) in milk as a marker for blood-milk barrier opening and immunoglobulin (Ig)G concentration was measured by ELISA.

### Results

#### *Effects on mammary epithelial cells in vitro*

In vitro, an upregulation of immune relevant factors showed that LPS and LTA triggered an immune response in mammary epithelial cells. Meloxicam and ketoprofen without additional immune challenge did not change the mRNA abundance of almost all measured factors. However, both NSAID diminished ( $P<0.05$ ) the increase of mRNA abundance of several immune factors (Tumor necrosis factor  $\alpha$ , interleukin-8, serum amyloid A, prostaglandin synthase) after LPS or LTA challenge. Furthermore, concentrations of PGE<sub>2</sub> measured in the cell culture supernatant were lower if cells were additionally treated with ketoprofen or meloxicam compared to cells stimulated with LPS or LTA alone.

#### *Effects on mammary immune response in vivo*

In vivo, the intramammary administration of 50 mg ketoprofen or meloxicam into healthy quarters without additional immune challenge did not change the SCC or the blood-milk barrier integrity. The administration of LPS and LTA induced an increase of SCC from  $140\pm 73$  and  $126\pm 29 \times 10^3$  to  $1,576\pm 190$  and  $1,523\pm 579 \times 10^3$  cells/mL at 6 h after challenge, respectively. However, the addition of ketoprofen to the LPS challenge caused a reduced ( $P<0.05$ ) increase of SCC to  $824\pm 154 \times 10^3$  cells/mL at 6 h after challenge, whereas meloxicam did not diminish the increase of SCC. In LTA challenged quarters, ketoprofen did not influence the SCC within the experimental period of 6 h. In quarters only challenged with LPS, SA and IgG concentrations increased ( $P<0.05$ ) from  $0.10\pm 0.01$  to  $0.30\pm 0.09$  mg/mL, and from  $0.30\pm 0.06$  to  $0.49\pm 0.08$  mg/mL within 5.5 h after challenge, respectively, indicating an opening of the blood-milk barrier. With the addition of ketoprofen SA and IgG concentrations in milk did not increase after LPS challenge, whereas meloxicam did not prevent the opening of the blood-milk barrier. The administration of LTA with or without ketoprofen did not cause a significant change of both SA and IgG concentrations in milk.

**Conclusions** In conclusion, commonly used NSAID, like meloxicam or ketoprofen, have clear effects on the immune response of mammary epithelial cells to endotoxins of common mastitis pathogens in vitro by diminishing the increased expression of important immune factors. It is likely that these effects reduce the severity of inflammation after local administration of NSAID during mastitis. The intramammary administration of both NSAID do not appear to induce a response of the mammary immune system in the healthy gland. However, ketoprofen reduces the increase of SCC during inflammation, whereas this effect is not detected with meloxicam. In accordance with the effect on SCC the transfer of other blood components including immunoglobulins during inflammation is only reduced by ketoprofen but not by meloxicam. Data demonstrate that different NSAID exert different effects in the mammary gland. How the reduction of inflammation by ketoprofen and meloxicam, and the limitation of blood-milk barrier opening during mastitis after local administration of ketoprofen influences the elimination of pathogens from an infected mammary gland needs to be further investigated.

### A model for assessing management-driven alternatives for disease control: A case-study on *Streptococcus suis* disease in nursery pigs

E. Giang<sup>1</sup>, J. M. Sargeant<sup>1,2</sup>, Z. Poljak<sup>1,2</sup> and A. L. Greer<sup>1,2</sup>

<sup>1</sup>Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada; <sup>2</sup>Centre for Public Health and Zoonoses, Ontario Veterinary College, Guelph, ON, Canada

Email: [egiang@uoguelph.ca](mailto:egiang@uoguelph.ca)

**Introduction** *Streptococcus suis* is a significant production disease of swine that can cause severe clinical disease and mortality in nursery pigs. Outbreaks of this disease can involve up to 20% of the herd, but in some cases more than 50% in scenarios of poor hygiene and concurrent disease<sup>1,2</sup>. Treatment and prevention mainly rely on the use of antimicrobials; however, increasing frequency of antimicrobial-resistant *S. suis* strains isolated from pigs<sup>3,4</sup> and humans<sup>5</sup>, highlights the need for alternative approaches to prevent and control this disease. Changes in demographic parameters particularly in populations with high turnover of animals, can have a direct impact on the recruitment rate of susceptible animals to the population and thus on the overall infection dynamics. Recruitment rates in pigs are often determined by production-type and/or management-structure. In Canada, the conventional use of a continuous weekly farrowing system remains common; however narrow intervals that introduce a continuous surge of young and immunologically naïve piglets can change the contact-structure between pigs and facilitate disease spread. Changes in recruitment rates through modifications in farrowing-intervals (FI) are tangible approaches for reducing transmission and spread of disease; yet the effects have not been modelled for this disease. Therefore, the aim of this study is: 1) to develop a mathematical compartmental model that describes *S. suis* transmission in the nursery and 2) use the model to evaluate the effects of FI as a potential management-driven alternative for disease control.

**Materials and Methods** Prior to this study, production data was collected from a 6-month *S. suis* outbreak that occurred in a Canadian swine farm involving 20 weaning cohorts (N = 1975 pigs). A descriptive analysis of this dataset was performed to obtain realistic epidemiological parameters that were used to inform our model state transitions. Parameter values that could not be feasibly measured which included the transmission rate and latent period were determined using maximum-likelihood estimation or derived from literature. Using our parameters, we developed a compartmental susceptible-exposed-infectious (SEI) model with typical swineherd dynamics and used it to examine patterns of on-farm disease persistence and fade-out using R open-source software for computational techniques and simulations. Using our model, we simulated a range of commonly found FI's (1 – 5 weeks) used in swine production and evaluated their effectiveness as disease control approaches using a 1-week FI as the base case scenario. Estimates of the 'critical community size' will be calculated, which will estimate the likelihood of disease extinction on farm as a function of herd size and the basic reproduction number ( $R_0$ ).

**Results** We propose a simple, yet robust modelling framework that uses empirical outbreak data to capture the transmission dynamics of this disease, while enabling direct quantitative assessment of interventions strategies performed *in silico*. Results from our descriptive analysis show the average cumulative mortality rate for this outbreak was 0.30 with range [0.13, 0.71] per pig week, while periodicity of case occurrences were reported most frequently during week 3 of the nursery stage of production. On average, 100 new pigs entered into the nursery each week with a range of [66, 134] pigs, while the  $R_0$  for this outbreak was 1.09 [95% CI: 0.91, 1.27]. Using our model parameterized by herd-level outbreak data, preliminary analysis suggests increasing the interval between farrowing could be a potential way to reduce disease burden and persistence of *S. suis* among the herd. We are currently using our model to optimize the interpretation of our model outputs as a decision-support tool for herd health advisors and producers.

**Conclusions** Understanding and identifying factors that permit the persistence of *S. suis* within a swineherd can help to inform strategies to control this disease and decrease the risk of pig-to-pig transmission. Our model will help to enable producers to consider management-driven intervention as alternative forms of disease control while also minimizing the use of antimicrobials.

#### References

1. Wertheim HFL, Nghia HDT, Taylor W, Schultsz C. *Streptococcus suis*: An Emerging Human Pathogen. *Clin Infect Dis*. 2009;48(5):617–25.
2. Fulde M, Valentin-Weigand P. Epidemiology and pathogenicity of zoonotic streptococci. *Curr Top Microbiol Immunol*. 2013;(368):49–81.
3. Hernandez-Garcia, J., Wang, J., Restif, O., Holmes, M. A., Mather, A. E., Weinert, L. A., & Tucker, A. W. Patterns of antimicrobial resistance in *Streptococcus suis* isolates from pigs with or without streptococcal disease in England between 2009 and 2014. *Veterinary Microbiology*. 2017; 207:117–124.
4. Vela AI, Morena MA, Cebolla JA, Gonzalez S, Latre MV, Dominguez L and Fernandez-Garayzabal JF. Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. *Veterinary Microbiology*. 2005; 105:143-147.
5. Zhang C, Ning Y, Zhang Z, Song L, Qiu H and Gao H. In vitro antimicrobial susceptibility of *Streptococcus suis* strains isolated from clinically healthy sows in China. *Veterinary Microbiology*. 2008; 131: 386–392.

### Associations between pig health, welfare, performance and antibiotic use on farm and *post mortem* meat inspection outcomes

J. C. Pessoa<sup>1,2,3</sup>, M. R. Costa<sup>1,2</sup>, E. G. Manzanilla<sup>1,2</sup>, T. Norton<sup>3</sup>, O. Clear<sup>1</sup>, C. McAloon<sup>2</sup> and L. A. Boyle<sup>1</sup>

<sup>1</sup>Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; <sup>2</sup>Section of Herd Health and Animal Husbandry, School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland; <sup>3</sup>M3-BIORES-Measure, Model & Manage Bioresponses, KU Leuven, Leuven, Belgium

Email: Joana.Pessoa@teagasc.ie

**Introduction** The importance of meat inspection (MI) data as feedback information for producers and veterinarians to inform (and control) farm health and welfare management plans is well accepted. Health and welfare data collected on farm is not commonly used to inform MI but the interest on this practice is growing (Felin et al., 2019). The aim of this study was to assess if on farm data on health and welfare, performance and antibiotic (AB) use can be used to predict condemnations and pluck lesions.

**Materials and Methods** Twelve-week old pigs, individually identified and weighed, ( $n = 1573$ ,  $25 \pm 5.3\text{kg}$ ) were followed for 13 weeks until slaughter ( $114 \pm 15.4\text{kg}$ ) in a wean-to-finish commercial farm. All parenteral AB administrations were registered during that period, and there were no in-feed or water medication. At 18 weeks of age, pigs were individually scored for tail and ear lesions (scores 0 to 4), and hernias, lameness and bursitis were recorded as present or absent. At slaughter, all partial and total condemnations as per the decision of the acting veterinary inspector were registered. Data on lung lesions (pneumonia, dorso-caudal and cranial pleurisy) and pericarditis were also collected. Average daily gain (ADG) was calculated for the full time period, ear lesions were reclassified as 0 (normal) and 1 (partial or total loss of one or both ears); AB use was reclassified as 0 (no treatments) and 1 ( $\geq 1$  treatments/pig). Health and welfare variables recorded on farm, AB use and ADG were used as predictor variables, while slaughterhouse variables were used as dependent variables. Collinearity between independent variables was assessed using the chi-square test and ANOVA. Univariable analysis between dependent variables and predictor variables was performed using chi-square tests (categorical variables) and simple logistic regression (ADG). Pig was used as the experimental unit. All data were analysed in R v3.4.1 (R core team, 2018).

**Results** On farm, ear lesions were the most prevalent condition, affecting 33.3% of the pigs, followed by bursitis (20.0%), tail lesions (11.5%), lameness (4.5%) and hernias (3.4%). ABs were administered to a total of 244 pigs (15.5%). ADG was  $990 \pm 120.8$  g/day. Regarding slaughterhouse findings, pneumonia was the most prevalent lesion (21.4%), followed by pericarditis (17.8%), cranial (15.8%) and dorso-caudal pleurisy (12.1%). Pigs partially condemned amounted to 11.7%, with 12 carcasses being fully condemned (0.8%). There were no associations between the different health and welfare variables measured on farm. ADG was negatively associated with ear lesions ( $P < 0.001$ ) and AB use ( $P < 0.001$ ), and positively associated with bursitis ( $P = 0.009$ ).

The univariable analysis between on farm and slaughterhouse variables showed associations between: partial condemnations and AB treatment ( $P = 0.008$ ); full condemnations and hernias ( $P < 0.001$ ); pneumonia lesions and hernias ( $P = 0.039$ ); presence of dorso-caudal and cranial pleurisy and AB treatment ( $P = 0.025$  and  $P = 0.002$ , respectively); and pericarditis was associated with ear lesions ( $P < 0.001$ ). ADG was associated with dorso-caudal and cranial pleurisy (OR = 0.997,  $P < 0.001$  and OR = 0.998,  $P < 0.001$ ) such that the odds of having dorso-caudal or cranial pleurisy decreased by 0.3% and 0.2%, respectively, for an increase of 1 g/day in ADG. These findings indicate a detrimental association between ear lesions and performance and pericarditis. The classification of ear lesions as partial or total loss of one or more ears corresponds to severe lesions which may serve as an entry point for pathogens. This is a plausible explanation for the link between ear lesions and pericarditis. However, regarding ear lesions and performance the causal direction is less clear. The association between higher ADG and bursitis might be related to a greater mechanical stress on the joints in heavier pigs when resting or moving on concrete flooring. There were several associations between AB treatment and the slaughterhouse variables. As expected, AB use was negatively associated with ADG. This indicates that even when pigs are treated the burden of infectious disease influences performance. ADG was negatively associated with pleurisy, one of the most prevalent lesions found in finisher pigs at slaughter. This is in line with the results obtained in a cross-sectional study involving 56 Irish farrow-to-finish pig farms (Rodrigues da Costa et al., submitted). These results confirm the need for the implementation of prevention plans and management improvements to reduce AB use and improve performance.

**Conclusions** These data provide the first evidence of an association between severe ear lesions and pig performance and pericarditis. They also indicate the usefulness of on-farm measures of AB use, lesions related to pig welfare (i.e. ear lesions) and ADG in complementing the food chain information, safeguarding pig health, welfare, and public health.

### References

- Felin, E., Hälli, O., Heinonen, M., Jukola, E., & Fredriksson-Ahomaa, M. (2019). Assessment of the feasibility of serological monitoring and on-farm information about health status for the future meat inspection of fattening pigs. *Preventive Veterinary Medicine*, 162, 76–82. <https://doi.org/10.1016/j.prevetmed.2018.11.009>
- R core team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

### Management practices associated with lamb mortality on Irish sheep farms: a national survey

D. Shiels<sup>1,2</sup>, J. F. Mee<sup>3</sup>, C. Dwyer<sup>2</sup> and T. Keady<sup>1</sup>

<sup>1</sup>Teagasc, Athenry Research Centre, Ireland; <sup>2</sup>SRUC, Edinburgh, Scotland, <sup>3</sup>Animal and Bioscience Research Department, Moorepark Research Centre, Teagasc, Ireland

Email: [john.mee@teagasc.ie](mailto:john.mee@teagasc.ie)

**Introduction** Both the all-cause national lamb mortality rate (average lamb mortality in Irish sheep flocks is currently 8.4%; NFS) and the timing and cause-specific lamb mortality rates (51.6 and 21.5% of neonatal lamb mortality occurs at birth and within 24 hours of birth, respectively, and the main causes of lamb mortality during the neonatal period are infection and dystocia; Shiels et al. 2018) are known for Irish sheep flocks. However, the management factors associated with these losses have not been documented. The aim of this study was to establish farm management practices on sheep farms prior to and during lambing which may influence lamb mortality.

**Material and methods** A survey was undertaken with sheep farmers participating in the Teagasc National Farm Survey (NFS). The NFS is part of the Farm Accountancy Data Network (FADN) of the EU. The NFS uses a random representative sample of approximately 1,000 Irish farms which is selected annually in conjunction with Ireland's Central Statistics Office (CSO). The NFS sample represents a farming population of approx. 90,000 farms. A supplementary survey relating to the prevalence of on-farm practises that carry high and low risks for lamb mortality, and farmer opinions of what management factors carry high and low risk for lamb mortality was conducted as an additional section to the regular 2017 Teagasc NFS. A total of 183 NFS respondents who farmed sheep completed the additional survey. These farms represent a total of 18,284 Irish sheep farms. Only farmers with a sheep enterprise consisting of more than 20 breeding ewes were included in the final analysis. The surveys were completed on farm by a team of trained NFS recorders. The supplementary survey contained 12 multiple-part questions and was designed to create a profile of on-farm practices used by each individual farmer. The questions were predominantly closed-ended. Some open-ended questions were also included. On completion of data collection the dataset was cleaned and screened for anomalies. Descriptive statistics were compiled and preliminary analysis conducted using Microsoft Excel.

**Results** Of the 183 survey responses, 179 (98%) were usable. The mean (range) farm size, sheep grazing area, average number of ewes, stocking rate and number of lambs reared per ewe joined was, 67 (8-1,117) ha, 22 (1.07–330) ha, 135 (20-1,427) ewes, 7.09 (0.89-19.55) ewes/ha and 1.34 (0.23-2.05) lambs per ewe joined, respectively. Fifty six percent of respondents raddled rams prior to joining them with ewes. Sixty eight percent of farmers had their sheep scanned, of these 18% had >1.8 lambs per ewe joined and 3% had <1.2 lambs per ewe joined on scanning records. The majority of farmers (86%) vaccinated their ewes. Vaccinations against clostridial and/or pasteurella diseases were the most common vaccines used on ewes (68%). Twenty percent of farmers vaccinated their ewes against abortifacients (*Toxoplasma gondii* and/or EAE). The majority of farmers (76%) used individual lambing pens. Of those 51, 15 and 10% cleaned and disinfected, cleaned only, and disinfected only individual lambing pens after each ewe, respectively. Twenty three percent of farmers did not clean or disinfect individual lambing pens. The majority of farmers (80%) applied iodine to the navels of all lambs, 9% to some lambs and 11% did not apply iodine to the navels of any lambs. Nine and half percent of farmers administered antibiotics to all lambs in order to treat and/or prevent diseases (diarrhoea and/or joint ill), 46.5% treated some lambs with antibiotics, while 44% didn't administer antibiotics to any lambs. The majority of farmers (79%) vaccinated their lambs. Seventy eight percent of farmers assisted lambs to suck at birth, 68% used artificial colostrum, while 46% gave fresh colostrum from another ewe. Nine percent of all farmers used artificial colostrum as their only source of colostrum at lambing. Vaccinations against clostridial and/or pasteurella diseases were the most common vaccines used on lambs (79%). The majority of farmers (74%) did not vaccinate lambs against orf while 18% of farmers vaccinated all lambs against orf and 8% vaccinated some lambs. Thirty percent of farmers recorded lamb deaths. Diseases, predators and abnormal birthweight were ranked by farmers as the three main causes of live born lamb mortality in their flock. The majority of farmers (64%) tail-docked every lamb, 17% tail-docked some lambs and 19% did not tail-dock any lambs. Male lambs were reared entire on 64% of farms. Only 27% of farmers castrated all male lambs while 9% castrated some male lambs.

**Conclusions** While the majority of respondents implemented best practices, some deficiencies in management which may impact lamb mortality were detected. For example, pregnancy diagnosis rates, vaccination against abortifacients, lambing pen hygiene practices and recording of lamb mortality data could be improved on many farms.

**Acknowledgements** The authors acknowledge the Teagasc Walsh Fellowship scheme and the National Farm Survey staff for their assistance in the conduct of the survey.

### References

Shiels, D., Mee, J.F., Fagan, S., Dwyer, C.M., Grant, J., McGovern, F.M., Creighton, P., Keady, T.W.J., (2018). Causes and time of neonatal mortality in grass-based systems of lamb production, *Advances in Animal Biosciences* 9: 64.

### Demography and potential biosecurity threats associated with contract dairy heifer rearing

M. C. McCarthy<sup>1,2</sup>, J. Kennelly<sup>1</sup>, N. Byrne<sup>1</sup>, L. O'Grady<sup>2</sup> and J. F. Mee<sup>1</sup>

<sup>1</sup>Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Ireland; <sup>2</sup>School of Veterinary Medicine, UCD, Belfield, Dublin, Ireland

Email: [john.mee@teagasc.ie](mailto:john.mee@teagasc.ie)

**Introduction** With expansion of dairy herds in Europe after the abolition of the milk quota in 2015, farmers have been exploring ways of increasing milk output on a fixed land base. One way to achieve this goal is to move the non-lactating animals (calves and replacement heifers) off the milking platform to be reared by another farmer. Contract rearing (CR) involves moving dairy heifers from their source farm (SF) to another farm to be reared for a set duration and fee. This contract rearing model of dairy herd expansion has become more popular in recent years. However, this enterprise potentially presents unique biosecurity risks as animal movement is the most important route of disease transmission.

**Materials and methods** Recently (2018) an international (Teagasc, UCD, ICBF, AHI and DAFM, Univ. Gent, Belgium) research project was designed to investigate the biosecurity implications of CR (McCarthy *et al.*, 2018). This longitudinal study will follow the performance (health, fertility and productivity) of home-and contract-reared heifers from birth to first lactation. A total of 178 farms across 16 counties nationally were recruited; 66 farmers engaged in CR, 58 contract rearers and 54 farmers rearing heifers at home. Each farm was visited during spring and autumn, 2018 and spring 2019 and will be visited twice yearly for the duration of the four year project. Heifers (~6,800) were weighed and health-scored (Wisconsin scores) and blood, faecal and/or nasal samples were taken. Preliminary results to date are presented here.

**Results** On average, dairy farmers practicing CR reared more heifers (n=68; 11-200) than control farmers (n=43; 10-164). The majority (52%) of source farmers sent their heifers out for rearing between 2 and 4 months of age and the majority (55%) expected to bring them back between 18 and 21 months of age. The majority (75%) of source farmers sent heifers to a CR farm in the same county. The most common CR arrangement was 1source farmer: 1CR (62%), followed by >1source farmer: 1CR (36%).

**Conclusions** In conclusion, farmers who send their heifers for CR have larger than average herds (probably expanding herds) and most send their heifers between 2 and 4 months of age to a single CR within their county of origin. In over a third of CR arrangements more than one source herd of heifers is reared on the same contract rearing farm. This presents a biosecurity threat due to the risk of transmission of infectious agents between heifers from different source herds. When these heifers return to their home farms they will possibly carry new infections to which the home herd is naïve. Further data collection and analysis will allow a complete appraisal of the impact of CR on heifer lifetime performance and home herd biosecurity.

#### References

McCarthy, M., Mee, J.F. and O'Grady, L. (2018) Contract Rearing – a disaster waiting to happen or a fundamental component of herd expansion? *Veterinary Ireland Journal*, 8: (8) 474-475.

### The effect of peer group education on veterinary prescription practices

V.-B. Pucken<sup>1</sup>, M. Bodmer<sup>1</sup>, S. Schouwey<sup>1</sup>, F. Nägele<sup>1</sup> and G. Schüpbach-Regula<sup>2</sup>

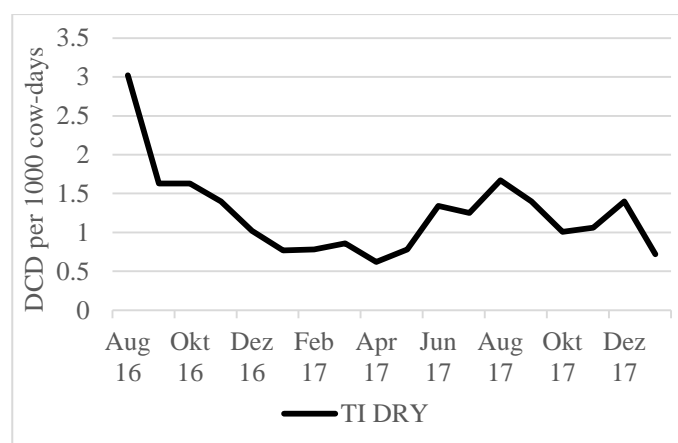
<sup>1</sup>Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland; <sup>2</sup>Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Switzerland

Email: [valerie.pucken@vetsuisse.unibe.ch](mailto:valerie.pucken@vetsuisse.unibe.ch)

**Introduction** Within the dairy industry, most antimicrobials are used for dry-cow therapy and mastitis treatment respectively. In previous studies participating in peer study groups supported knowledge transfer between farmers and helped them to maintain a good udder health with reduced use of antimicrobials (Tschopp et al., 2015; Vaarst et al., 2010). Therefore, the objective of our project was to evaluate the effect of peer-group education on antimicrobial prescription practices of veterinarians.

**Materials and Methods** 21 Swiss cattle practitioners were divided into three peer study groups. Each group met every 2 to 3 months, from August 2016 until January 2019, to discuss different topics. In addition, a moderator and a person with expertise in the respective topic were present at each meeting. For the same study period, the antimicrobial prescription data were extracted from the practice software for 5 dairy farm clients per participating veterinarian. Prescription data were divided into intramammary preparations used during lactation (IMM), intramammary preparations used for dry off (DRY) and systemic treatments (SYS). The monthly treatment incidence (TI) for the defined daily dose (DDD) per 1,000 cow-days (IMM and SYS) and the defined course dose (DCD) per 1,000 cow-days (DRY) were calculated on farm level for the intervention period.

**Results** Preliminary results show that the monthly TI IMM ranged between 5.04 and 11.51 DDD per 1,000 cow-days from August 2016 until January 2018, with a trend to lower TI IMM in the winter season and higher TI IMM in the summer season. The monthly TI SYS ranged between 0.81 and 2.03 DDD per 1,000 cow-days. The TI DRY ranged between 0.62 and 3.02 DCD per 1,000 cow days with a tendency to decrease over the study period.



**Figure 1** Mean of monthly Treatment Incidence (TI) for intramammary preparations used for dry off

**Conclusions** In the descriptive statistics, no reduction was recognizable for the intramammary antimicrobials used during lactation and the systemic antimicrobials. However, the observed reduction of intramammary antimicrobials used for dry off might indicate an implementation of selective dry cow therapy due to the participation in the peer study groups where this topic was discussed in depth. The data will further be analyzed with hierarchical multiple regression analysis.

### References

- Tschopp A, Reist M, Kaufmann T, Bodmer M, Kretzschmar L, Heiniger D, et al. A multiarm randomized field trial evaluating strategies for udder health improvement in Swiss dairy herds. *J Dairy Sci.* 2015 Feb;98(2):840–60. doi:<http://dx.doi.org/10.3168/jds.2014-8053>
- Vaarst M, Gratz E, Walkenhorst M, Ivemeyer S, Brinkmann J, March S, et al. Farmer groups for animal health and welfare planning in European organic dairy herds. In: 9th European IFSA Symposium. 2010. p. 683–91.



### Biosecurity on Finnish swine and cattle farms, with special emphasis on rodent control

L. Seppä-Lassila<sup>1</sup>, H. Rossow<sup>1</sup>, M. Simola<sup>2</sup>, J. Niemimaa<sup>3</sup>, O. Huitu<sup>3</sup> and P. Tuominen<sup>1</sup>

<sup>1</sup>Risk assessment unit, Finnish Food Authority, Helsinki, Finland; <sup>2</sup>Microbiology research unit, Finnish Food Authority, Helsinki, Finland; <sup>3</sup>Forest and Animal Ecology, Natural Resources Institute Finland, Helsinki, Finland

Email: [leena.seppa-lassila@ruokavirasto.fi](mailto:leena.seppa-lassila@ruokavirasto.fi)

**Introduction** Farm sizes are constantly increasing in modern livestock which leads to increased risk of endemic diseases. Farms have more contacts with each other and animals within the farms have more interactions predisposing them to infectious diseases. Importance of external and internal biosecurity on a farm level is rapidly increasing. In the current study farm biosecurity was explored on Finnish swine and beef farms, with special emphasis on rodent control.

**Material and methods** A convenience sample of 18 beef farms and 20 swine farms from Southern and Western Finland participated in the study. Farmers were interviewed on their premises using a structured questionnaire with multiple choice or open questions. A face-to-face interview was conducted in order to increase the trust of the farmer and thus the validity of the answers. A visit to the premises also gave the researchers an opportunity to verify some of the farmer's answers (e.g. organization of the farm, management of farm yard). Rodent trapping was carried out on each farm in fall 2017 and 2018. One hundred traps were placed on the premises of the farm for two nights. Traps were checked and emptied after each night.

**Results** The median size of swine and cattle farms was 950 (mean 1630, SD 1710) and 205 (mean 271, SD 184) animals per farm, respectively. The distribution of biosecurity measures on swine and cattle farms is presented in Table 1. Swine farms tended to have slightly stricter biosecurity than cattle farms, although the level was generally good in both. Very few farms kept track of the visitors; most farmers claimed not to have visitors other than the veterinarian, so track keeping was not considered necessary or relevant. Mean catches of rodents in 2017 and 2018 were 10.6 (SD 6.7) and 21.4 (SD 11.1) rodents per farm, respectively. In 2017 mean catches on swine and cattle farms were similar (mean 108, SD 7.3 and mean 10.3, SD 6.0, respectively). In 2018 the number of catches was higher on swine farms (mean 24.9, SD 13.2) than on cattle farms (mean 17.6, SD 6.6). The number of rodent catches on farms was not correlated between years ( $p = 0.36$ ).

A linear regression model was constructed to explain rodent catches in 2018. The initial model included several independent farm level variables regarding rodent control (number of animals, cattle/swine farm, open or closed feed storage, presence of cats on the farm, surface material of the farm premises; asphalt, gravel, or no constructed surface, only cart ways; maintenance of the vegetation on yard, feed leftovers on the yard, old/unused machines on the premises, having a rodent control plan on paper). The results indicate that having swines was positively associated with increased rodent numbers ( $p = 0.042$ ). A similar model for data from 2017 indicated that yards with only cart ways (no gravel or asphalt) and a high number of animals were positively associated with rodent numbers (coeff. 5.2,  $p = 0.037$  and coeff. 0.0016,  $p = 0.043$ , respectively).

**Table 1** Biosecurity measures used in Finnish swine (n=20) and cattle (n=18) farms.

	Swine farms, %	Cattle farms, %
Farmer uses protective clothes	90	78
Farmer uses clean boots	95	89
Farmer washes hands when finishing	95	100
Visitors use protective clothes	95	83
Visitors use clean boots	95	100
Visitors wash hands when entering	75	59
Visitors clean and disinfected tools they use	58	50
Farmer keeps track of the visitors	15	0
Farm has a hygiene lock*	60	6
Farm has a separate loading space	80	6
Doors of animal shelter are locked	75	6
Farmer has considered separate routes of feed, manure, carcasses etc. on farm premises	65	89
Farmer is satisfied with the biosecurity	85	72

\* A definite space where farmer changes into protective clothes and boots only used in animal premises

**Conclusions** The biosecurity measures on Finnish farms are at a reasonable level. Swine farms have more controlled premises than cattle farms. Swine farms seem to have more rodents, especially in years when the number of rodents is generally high (rodent peak years). Having asphalt or a gravel yard might be helpful in reducing number of rodents on the farm.

## Labour process during machine milking of ewes with regards to their biological needs and welfare

L. Mačuhová<sup>1</sup>, V. Tančin<sup>1,2</sup>, M. Uhrinčat<sup>1</sup> and J. Mačuhová<sup>3</sup>

<sup>1</sup>NPPC, Research Institute for Animal Production Nitra, Lužianky, Slovakia; <sup>2</sup>Slovak University of Agricultural in Nitra, FAFR, Department of Veterinary Science, Nitra, Slovakia; <sup>3</sup>Institute for Agricultural Engineering and Animal Husbandry, Freising, Germany  
Email: macuhova@vuzv.sk

**Introduction** Due to the management effort to save the labour force or a lack of people willing or having qualification for employment in this sector, the milking process is often performed inadequately what can result in disruption of the biological requirements of ewes during milking. Incorrect milking process may have a negative influence on the health of the mammary gland as well overall animal health or quality of ewe's milk. Overmilking (milking unit is on the udder in spite of that milk did not flow) is one of the consequences of incorrect work organization which does not respect biological needs of ewes during machine milking. The overmilking can arises teat damages and thereby susceptibility of animals to intramammary infection. The aim of this study was to find out how the biological needs of ewes are satisfied during machine milking on four selected Slovak dairy farms.

**Materials and Methods** The organization of work during machine milking of ewes was evaluated on four farms with different milking conditions. One day before evaluation of the organization of the work, the measurement of milkability using an electronic jar with a 2-wire compact magnetostrictive level transmitter continuously measuring the position of the float in the jar and recording it on a computer once per second was performed. Farm situation was as follow: Farm 1: breed – Tsigai (TS), 1x24 milking parlour and 8 ewes/milker, 0.238 L milk/milking/ewe; Farm 2: breed - TS, Improved Valachian (IV), and Lacaune (LC), crossbred – TSxLC and IVxLC, 1x24 milking parlour and 12 ewes/milker, 0.389 L milk/milking/ewe; Farm 3: breed - IV, 2x20 milking parlour and 20 ewes/milker, 0.200 L milk/milking/ewe; Farm 4: breed - TS, IV, LC, and crossbred - TSxLC and IVxLC, 1x24 milking parlour and 24 ewes/milker, 0.308 L milk/milking/ewe. The milking parlours were equipped with half number of milking units compared to the number of stands. Organization of work was evaluated during the whole milking. There were no interferences during milking process. The labour process was evaluated according to the recording of working time. The recorded parameters were: time of cluster attachment, time of beginning stripping, and time of milking end. A stopwatch was used to record these parameters. Moreover, the following traits were calculated from the recording of working time: waiting time (waiting time of dairy sheep on attaching of clusters on teats from the time of attaching of first sheep in batch), machine milking time (time from attaching clusters on udder to start of stripping), stripping time (time from start of stripping to end of stripping), total milking time (sum of machine milking time and machine stripping), and percentage of stripping time from total milking time. In parlours with more than one milker during the milking, the ewes of single milker were evaluated separately to be able to compare the effect individual milker on process of milking too. The study was supported by the Ministry of Education, Science, Research and Sports of the Slovak Republic/the Slovak Research and Development Agency (Project No. APVV-15-0072).

**Results and Discussion** Data of time needed to milk of ewes on evaluated farms are shown in Table 1. The significant effect of the farm was observed in all evaluated parameters. Machine milking time and also total milking time were significantly shorter on Farm 1 than on Farm 2, 3, and 4 ( $P < 0.0001$ ). The shortest milking time and total milking time were observed also on the Farm 1 and the longest on the Farm 4. The duration of milk flow on Farm 4, depending on the type of milk flow (4 types were evaluated: one peak, two peaks, plateau I, and plateau II; Mačuhová et al., 2008) was from  $45 \pm 4$  to  $77 \pm 5$ s by one peak milk flow and plateau II, respectively. Waiting time, machine milking time as well as total milking time were affected by number of ewes milked by one milker, and therefore these parameters have the shortest duration on Farm 1 (8 ewes per milker) and the longest on Farm 4 (24 ewes per milker). The shortest machine stripping time was recorded on Farm 4 and the longest on Farm 1. However, the time of machine stripping time seems to be influenced not only by the number of ewes per one milker, but also by the practice of the milking process on individual farms. Especially at the Farm 1, where ewes were multiple machine stripping during milking, the machine stripping time was notably longer.

**Table 1** Time needed to milk of the ewes on the evaluated farms.

Traits	Farms				F - test P
	Farm 1	Farm 2	Farm 3	Farm 4	
Waiting time, s	$51 \pm 1^a$	$63 \pm 2^b$	$83 \pm 1^c$	$117 \pm 1^d$	<0.0001
Machine milking time, s	$67 \pm 1^a$	$87 \pm 3^b$	$96 \pm 1^c$	$151 \pm 2^d$	<0.0001
Stripping time (ST), s	$23 \pm 1^a$	$12 \pm 1^b$	$7 \pm 1^c$	$9 \pm 1^d$	<0.0001
Total milking time (TMT), s	$90 \pm 1^a$	$99 \pm 3^b$	$102 \pm 1^b$	$160 \pm 2^c$	<0.0001
ST/TMT, %	$26.24 \pm 0.46^a$	$13.24 \pm 1.17^b$	$6.65 \pm 0.41^c$	$6.29 \pm 0.60^c$	<0.0001

<sup>a,b,c,d</sup> means in the same line without a common superscript letter were significantly different ( $P < 0.05$ )

**Conclusions** Waiting time, machine milking time as well as total milking time appear to be affected by number of ewes milked by one milker. Machine stripping time seems to be influenced not only by the number of ewes per one milker, but also by the practice of the milking process on individual farms.

## References

Mačuhová L., Uhrinčat M., Mačuhová J., Margetin, M. and Tančin, V., 2008. The first observation of milkability of the sheep breeds Tsigai, Improved Valachian and their crosses with Lacaune. Czech J. Anim. Sci. 53: 528-536

## Locomotion, metabolic and alimentary disorders in Holstein and Czech Fleckvieh dairy cows – comparison of occurrence in the Czech Republic

S. Šlosárková<sup>1</sup>, E. Kašná<sup>2</sup>, P. Fleischer<sup>1</sup>, A. Pechová<sup>1,3</sup> and L. Zavadilová<sup>2</sup>

<sup>1</sup>Veterinary Research Institute, Brno, Czech Republic; <sup>2</sup>Institute of Animal Science, Prague, Czech Republic; <sup>3</sup>University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Email: slosarkova@vri.cz

**Introduction** Large and national surveys of health event data were started in dairy cattle in progressive countries mainly for the introduction of direct health traits to genetic selection indexes (e.g. Hoof Health trait in the Nordic Total Merit index). The obtained data are also used for benchmarking including antimicrobials consumption control and farm management. The aim of the present study was to evaluate the occurrence of locomotion, metabolic and alimentary disorders in Czech Fleckvieh (C) and Holstein (H) dairy cows and to compare these two breeds, i.e. these two types of farms with (dominant) C or H breed.

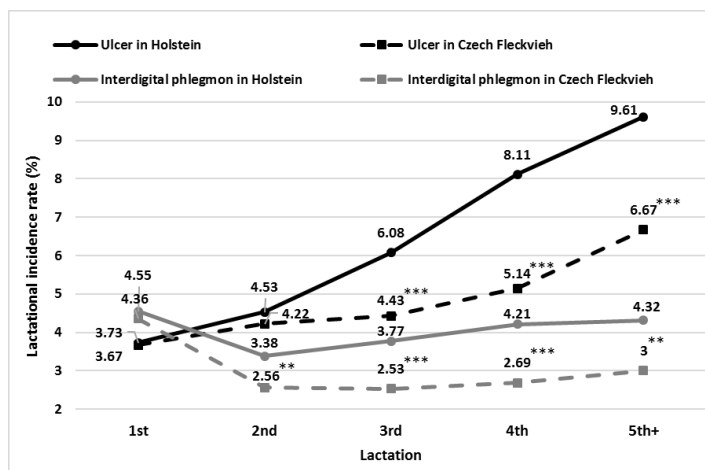
**Materials and Methods** The first national survey covered all diseases/health disorders occurring in Czech dairy cows. The data were gathered retrospectively from farmers via on-line survey from July 2016 through June 2017. The 46% of farmers who responded owned 78% of dairy cows in Czechia. We gathered 27 common health traits. The farmers reported 411,949 health events on 956 farms. Data editing: 1) Only lactations which started after 30 June 2016 and no later than 7 (for parturient paresis and the downer cow syndrome) and 60 (for all other disorders evaluated in this study) days before 30 June 2017 were evaluated to keep a minimum period to enable the disease to express (287,491 lactations). 2) Data were assigned according to the breed to C ( $\geq 75\%$  share of Czech Fleckvieh; 96,909 lactations, mean 279 per herd) and H ( $\geq 75\%$  share of Holstein; 165,922 lactations, mean 346 per herd). Cows of these breeds were kept on 916 farms (91.2% of these farms with  $>80\%$  lactations of one breed). This study evaluated the occurrence of digital dermatitis, interdigital phlegmon, interdigital hyperplasia (tyloma), ulcer (of the sole area), white line abscess, lameness, parturient paresis, downer cow syndrome, primary clinical ketosis, displaced abomasum, and all locomotion, metabolic and alimentary disorders. To identify the farms with incomplete data, each farm with more than 20 evaluated lactations (92.8% farms) had to report at least 1 record of the particular health disorder to be included in the evaluation of the disorder. Reported occurrences were expressed as lactational incidence rate LIR (number of affected lactations / number of lactations at risk)  $\times 100$  and at the first occurrence in lactation the parity was evaluated. For editing the database and calculations the SAS 9.4 software was used.

**Results** C and H lactations lasted  $317 \pm 86$  and  $340 \pm 94$  days, milk yield in lactations which lasted  $>240$  DIM reached  $7,627 \pm 1,961$  and  $10,100 \pm 2,025$  l milk and the mean parity was  $2.78 \pm 1.66$  and  $2.33 \pm 1.36$ , respectively. Many farms failed to report some diseases. The evaluation of locomotion disorders included 79% C and 82% H herds, but the evaluation of individual locomotion disorders only 18–52% C herds (tyloma-lameness) and 20–55% H herds (tyloma-ulcer). This study shows that H cows had a significantly higher LIR for four individual diseases (digital dermatitis, interdigital phlegmon, ulcer, primary clinical ketosis) and for all locomotion, metabolic and alimentary disorders, but lower LIR for white line abscess and parturient paresis (Table 1). Four disorders (tyloma, lameness, downer cow syndrome, displaced abomasum) did not differ significantly. Whereas the same level of LIR was found in interdigital phlegmon and ulcer in primiparous C and H cows, the pluriparous C cows had lower LIR than H cows (Figure 1) – except ulcer in the 2<sup>nd</sup> lactation. The LIR for interdigital phlegmon was higher in 1<sup>st</sup> than in 2<sup>nd</sup> lactation in both breeds ( $P < 0.001$ ).

**Table 1** Proportions of lactations affected by disease (LIR) in Czech Fleckvieh (C) and Holstein (H) dairy cows in Czechia

	Lactations (no.)		LIR%	
	C	H	C	H
Digital dermatitis	25,541	57,316	4.28	5.91***
Interdigital phlegmon	26,979	58,509	3.18	4.03***
Tyloma	13,282	27,463	2.23	2.06 <sup>ns</sup>
White line abscess	14,636	31,723	2.86	1.64 <sup>ns</sup>
Lameness	38,297	65,977	5.00	4.87 <sup>ns</sup>
Ulcer	33,490	71,726	4.57	5.23***
Locomotion disorders	58,228	102,887	10.27	12.75***
Parturient paresis	43,488	72,385	1.99	1.66***
Downer cow syndr.	17,587	19,774	1.02	1.06 <sup>ns</sup>
Primary ketosis	9,345	39,148	1.08	2.54***
Metabolic disorders	53,852	100,073	3.43	5.80***
Displaced abomasum	1,071	39,099	0.75	1.33 <sup>ns</sup>
Alimentary disorders	11,166	54,008	1.24	2.06***

\*\*\*  $P < 0.001$ ; <sup>ns</sup> not significant



**Figure 1** Comparison of proportions of lactations affected by ulcer and interdigital phlegmon between Holstein and Czech Fleckvieh dairy cows in the Czech Republic according to parity

**Conclusions** This first national comparison of health between Czech Fleckvieh and Holstein cows shows that H cows had more lactations affected by a locomotion, metabolic or alimentary disorder, but less by white line abscess (in all parities) and parturient paresis (in 1<sup>st</sup> lactation). The differences in the present study are less uniform (and smaller) than in the comparison of reproduction disorders (in a parallel study).

**Supported** by the Ministry of Agriculture of the Czech Republic, projects QK1910320, MZE-RO0518 and MZE-RO0718.

### Risk factor analysis for dairy cow production diseases by a system analysis - First results from the EIP-project “Die Entwicklung des KUH-mehr-WERT Navigators”

M. Schären<sup>1</sup>, B. Waurich<sup>1</sup>, F. Ebert<sup>2</sup>, A. Wöckel<sup>1,2</sup>, W. Wippermann<sup>1,2</sup>, A. Özcan<sup>1,2</sup>, G. Hermenau<sup>1</sup>, J. Wittich<sup>1</sup>, S. Dänicke<sup>3</sup>, H. Swalve<sup>4</sup>, P. Hufe<sup>1</sup>, D. May<sup>1</sup> and A. Starke<sup>2</sup>

<sup>1</sup>Lehr- und Versuchsanstalt für Tierzucht und Tierhaltung (LVAT) e.V., Groß Kreutz, Germany; <sup>2</sup>Clinic for Ruminants and Swine, Veterinary Faculty, University of Leipzig, Germany; <sup>3</sup>Institute for Animal Nutrition, Friedrich-Loeffler-Institute, Brunswick, Germany; <sup>4</sup>Institute of Agricultural and Nutritional Sciences, University of Halle, Germany

Email: melanie.schaeren@lvat-kmw.de

**Introduction** The EIP-project “Die Entwicklung des KUH-mehr-WERT Navigators” („The development of the COW-more-VALUE”) is a multicentric cooperative five-years project in which different partners from the dairy industry in Brandenburg (Germany) are working together to develop an economical risk analysis tool for dairy cow production systems. To properly assess risk factors and their consequences for animal health and production a sound and encompassing data collection regarding the housing, management, feeding, animal constitution and economical factors needs to be established and changes in the production system and their consequences monitored and documented. Therefore, a so called “system analysis” was elaborated.

**Materials and Methods** Twelve dairy farms were analysed throughout the year 2018. The farms are characterized by: an average size of 553 cows (235 - 1383), an average milk production of 10'561 kg per cow and lactation, 29.6 % culling rate, German Holstein breed, total mixed ration (TMR)-based total confinement system, using the herdmanagement software HerdeW or HERDEplus (dsp-Agrosoft GmbH, Ketzin, Germany). The analysis included aspects from housing and management (e.g. group size, stocking density, feed bunk space, water accessibility, freestall dimension and bedding, cleanliness of the environment, evaluation of the milking process in various aspects, synchronization of working processes and time budgeting), different animal scores adjusted to the production and age group (e.g. body condition score (BCS), lameness, hygiene, decubital lesions for cows and bovine respiratory disease (BRD) score and nesting score for calves), assessment of critical control points in the feeding routine, as well as the analysis of various key figures in animal production, health, feeding and economical aspects. The on-farm data collection lasted three days and was followed by an intense analysis within approximately one week, finalized by the composition of a presentation and a team meeting to consolidate on a list of recommendations. The results were then presented to the farm the following week. The participants in this one-day meeting included the farms management, commercial administration, the herdmanager, optionally additional employees, the farms external consultants (claw trimmer, veterinary and nutritionist) and the project team including an agricultural economist, veterinaries, a nutritionist and agricultural engineers. In the final part of the day collectively a plan of action for the following year was developed. To motivate and support the farms in implementing their personal action plan, as well as to monitor the changes on the farm closely, the team re-visits the farms on a six-weekly basis. Further, several workshops were and are organized to train the herdmanagers, accountants and different other personnel in data documentation as well as various practical aspects, and to increase the motivation for change by creating benchmarks for different analyses.

**Results** The system analysis revealed, even though the farms exhibit similar production levels (9'977 - 11'234 kg per cow and year) and housing systems, a wide range in key production figures: culling rate of 15.9 - 38.2 %, of which euthanized or died: 6.4 - 37.6 % (av. 18.9 %), life span production of 26'510 - 47'580 kg (av. 33'712 kg) and average lactation in herd until culling of 2.8 - 4.3 (av. 3.4), age at first calving of 23.9 - 29.2 months (av. 25.4 months) and stillbirth rates in cows and heifers of 2.5 - 11.1 % (av. 7.9 %) and 4.5 - 16.3 % (av. 9.7 %), respectively. The proportion of marketable milk of total milk production per cow and year ranged between 90.3 and 95.3 % (av. 93.2 %). The results of the scores also revealed large differences in animal constitution: proportion of animals with a BCS  $\leq 2$  (according to Edmonson et al. (1989)) of 6 - 29 % (av. 13 %), with a lameness score of  $\geq 3$  (mild to severe lameness; score adapted according to Offinger et al. (2013)) of 15 - 35 % (av. 22 %), decubital lesions at the hock with a score 3 (abrasion of hair with mild swelling to severe enlargement of the joint and lameness; according to Lombard et al. (2010)) of 4 - 97 % (av. 42 %) and a hygiene score of  $\geq 4$  (= dirty to severe dirtiness; score according to Reneau et al. (2005)) of 3 - 74 % (av. 34 %). Most frequent issues named on the plan of actions for the twelve farms were for the area of herdmanagement and housing: digital structuring of herd (7), stocking density (7), cleanliness of flooring and bedding (9), climate/ventilation (6), light intensity (8), harmonization/optimization of work processes (6) and biosecurity (12), for production and animal health: animal control protocols (e.g. fresh cow control, calf control (7)), BCS controlling (7), still birth rates (9), dry cow management (6), lameness (11) and udder health (6), for calf and youngstock: housing (7), daily gain in various growth stages (9) and pneumonia (8), for feeding: water availability (10), feeding routine precision (6), efficiency (6) and documentation (7); numbers indicate numbers of farms affected, only issues are named with six or more farms affected. The evaluations and presentations of the system analysis have been received positively and constructive by the farm members on all twelve farms. The continuous mentoring and control in implementing the plan of action has begun in all farms. Already now large differences in adaptation and motivation are observed.

**Conclusion/Outlook** The system analyses have confirmed the complex interrelations between management, housing, feeding, economical and animal health and production, and that only by a encompassing and profound analysis durable recommendations can be made. In the second year of the project new aspects will be included in the system analysis, such as the analysis of the efficiency of work processes. Next analysis steps are to find interrelations between different variables and their consequences for animal health, production and economical aspects. *This project was funded by the European Innovation Partnership (EIP)-AGRI.*

#### References

- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A Body Condition Scoring Chart for Holstein Dairy-Cows. *J Dairy Sci* 72(1):68-78. Lombard, J. E., C. B. Tucker, M. A. G. von Keyserlingk, C. A. Kopral, and D. M. Weary. 2010. Associations between cow hygiene, hock injuries, and free stall usage on US dairy farms. *J Dairy Sci* 93(10):4668-4676. Offinger, J., S. Herdtweck, A. Rizk, A. Starke, M. Heppelmann, H. Meyer, S. Janssen, M. Beyerbach, and J. Rehage. 2013. Postoperative analgesic efficacy of meloxicam in lame dairy cows undergoing resection of the distal interphalangeal joint. *J Dairy Sci* 96(2):866-876. Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Association between hygiene scores and somatic cell scores in dairy cattle. *Javma-J Am Vet Med A* 227(8):1297-1301.

## Metabolic and biochemical changes in sows around parturition in relation to farrowing progress, piglet vitality and housing condition

S. Blim<sup>1</sup>, D. Schupp<sup>1</sup>, T. Scheu<sup>2</sup>, C. Koch<sup>2</sup>, G. Thaller<sup>3</sup>, K. Failing<sup>4</sup> and H. Bostedt<sup>1</sup>

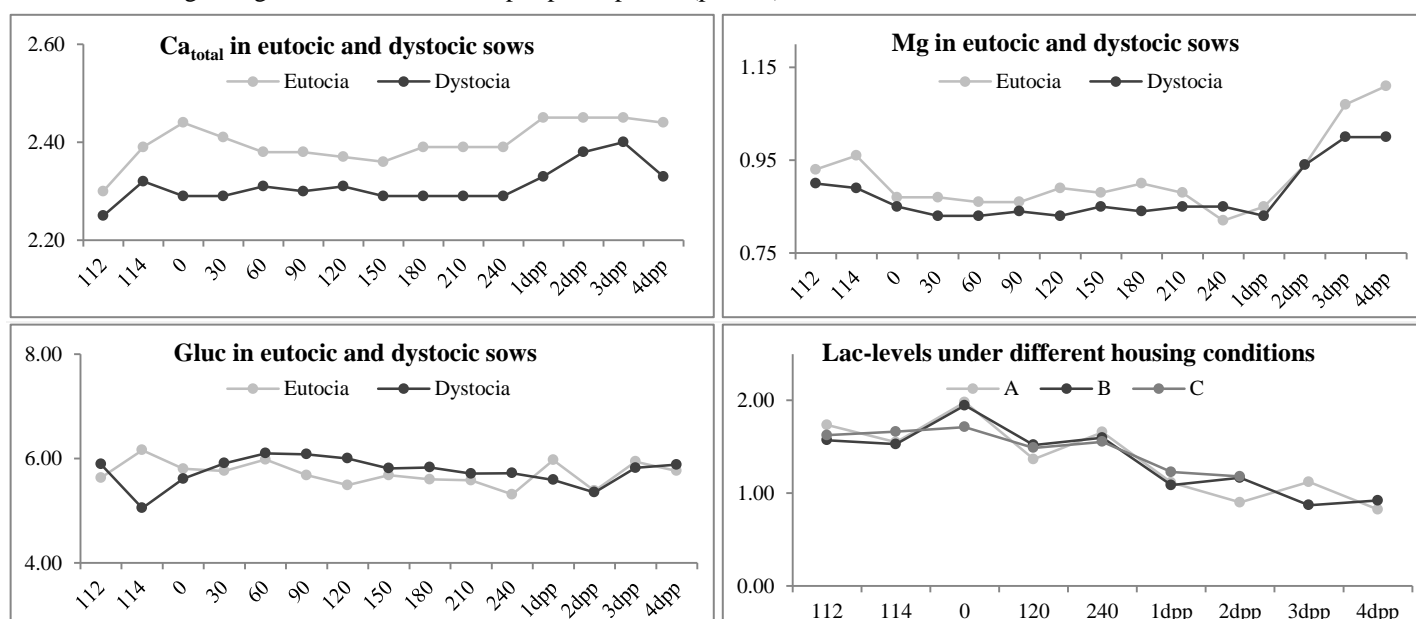
<sup>1</sup>Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals with Amulatory Service, Justus-Liebig-University Giessen, Germany; <sup>2</sup>Education and Research Centre for Animal Husbandry, Hofgut Neumühle, Münchweiler an der Alsenz, Germany; <sup>3</sup>Institute for animal husbandry and domestic animal genetics, Christian-Albrechts-University Kiel, Germany; <sup>4</sup>Institute for biomathematics and data processing, Justus-Liebig-University Giessen, Germany

Email: sarah.blim@gmx.de

**Introduction** The aim of the study was to examine the influence of three different housing conditions on clinical evidences, ethological signs and blood levels of multiple metabolic, biochemical and endocrine parameters around parturition in a modern pig genetic (BHZP Victoria). In this paper, due to ongoing investigation, only selected clinical and metabolic results will be presented.

**Materials and Methods** Surveys were carried out on 69 sows at the age of  $2.45 \pm 0.98$  years, which at least had reached second gestation. They were observed from day 112 of gestation until day 7 post partum. There was same feeding and nesting material for every sow in every housing condition. Mothers were either housed in farrowing crate (A, n=38), free farrowing pen (B, n=25) or group housing (C, n=8). In groups B and C sows were allowed to farrow without any confinement. No introduction of farrowing, neither drug treatment intra partum was performed in any housing condition. If there was an excess of 60 minutes (protraction) after the last piglet, a manual obstetric intervention took place. Farrowings without obstetric intervention were defined as eutocia, those with intervention as dystocia. Blood samples were taken on day 112 and 114 of gestation, in 30 minutes intervals intra partum, as well as once daily in the first week after parturition. Therefore on the first day of sampling central venous catheters were implanted through the ear veins under low-dose or without sedation (2 mg/kg azaperone IM).

**Results** Means of birth length were numerically different (eutocia 207, 190, 145; dystocia 333, 331, 259 min.), but statistical significance could not be demonstrated. Ratio of protractions per partus was  $1.49 \pm 1.83$  for A,  $0.96 \pm 0.98$  for B and  $0.63 \pm 0.74$  for C ( $p = 0.06$ ). 16.7 % of sows in A showed four or more protractions, while in B max. 3 (8%) or C max. 2 (12.5%) protractions occurred. Piglets were more vital in B (0.069) and C ( $p=0.035$ ) than in A. Piglet losses were 18.5, 27.7, 21.6 % for A, B and C. However largest portion of piglet losses was in the first 3 days of life. While there was no significant housing influence on metabolic parameters, some significant differences could be found in farrowing process. In dystocic partus concentrations of total Calcium ( $Ca_{total}$ ) were significant lower than in eutocic partus ( $p=0.001$ ). In the entire partus  $Ca_{total}$ -concentrations in B were higher than in A ( $p=0.04$ ). The same findings were made for ionic Calcium ( $Ca_{ionic}$ ) and Magnesium (Mg). In dystocia those levels were significant lower than in eutocia ( $p=0.04$  vs. 0.004). For Mg there was an effect of confinement on peripheral concentrations: C had significant ( $p=0.003$ ) higher Mg-levels than A. For A there were significant higher Mg-levels over time than in B ( $p=0.03$ ). The interdependence between protraction and time was significant ( $p=0.0008$ ). The levels of peripheral Glucose-concentrations (Gluc) underlie enormous intraparturial variability. In dystocic sows there was a more pronounced anteparturial decline, than it was found in eutocic sows. However, no significant differences could be proven. In the case of Lactat (Lac), clearly fluctuations were also found. In difference to Gluc the Lac-levels undergo a significant decline in the periparturial period ( $p=0.04$ ).



**Conclusions** The presented results provide some noteworthy indications that free ranging housing conditions have beneficial effects not only on farrowing sows, but also on vitality of neonates. The metabolic findings in our study show that sows with farrowing problems undergo lower Ca- and Mg-levels. Even though these findings are independent of housing conditions, substrate-deficiency seems to be more severe in confinement. Based on both – clinical evidence and blood status – possibility of free movement can be seen as a factor for a parturition progress without protraction. Consequently, free ranging sows give birth to more vigorous piglets. Considering the higher proportion of piglet losses, free housing conditions in the first days after parturition cannot be recommended.

Financed by Toennies-Research

### Effects on milk quality when replacing soybean meal with Spirulina in a hay-based diet for dairy cows

E. Manzocchi<sup>1</sup>, B. Guggenbühl<sup>2</sup>, M. Kreuzer<sup>1</sup> and K. Giller<sup>1</sup>

<sup>1</sup>ETH Zürich, Institute of Agricultural Sciences, Animal Nutrition, Zurich, Switzerland; <sup>2</sup>Federal Department of Economic Affairs, Education and Research, Agroscope, Food Microbial Systems, Bern, Switzerland

Email: [katrin.giller@usys.ethz.ch](mailto:katrin.giller@usys.ethz.ch)

**Introduction** There is a search for alternative protein sources produced on non-arable land to be able to replace the critically discussed but extensively used soybean meal in diets of livestock. The cyanobacterium *Spirulina* (*Arthrospira platensis*), often associated with the group of microalgae, is an interesting option due to its low growth requirements. By replacing soybean meal with *Spirulina*, more arable land would remain available for human food production. In addition to its high protein content, *Spirulina* contains high proportions of nutritionally favourable fatty acids and might thereby potentially improve the nutritional quality of milk for consumers. So far, only very few studies have evaluated the effects of feeding *Spirulina* to dairy cows and its effects on milk quality. Therefore, the present study aimed at investigating the effects of replacing soybean meal with *Spirulina* in dairy cows on compositional and sensory quality aspects as well as on coagulation properties of the milk.

**Materials and Methods** Twelve late-lactating dairy cows (6 Brown Swiss and 6 Holstein; 2±1 lactations, 329±69 days in milk) were housed in a single pen within a free-stall barn and randomly allocated (considering breed, parity and milk yield) to one of two experimental groups. Applying electronic access control, each animal had *ad libitum* access to only one individual feeding trough. One group (SOY; n=6) was fed with a total mixed ration consisting, on a dry matter (DM) basis, of 74% hay (18.7% crude protein (CP)/kg DM), 13% sugar beet pulp, 6% soybean meal (47.8% CP/kg DM), 4% wheat flakes and 3% molasses. For the second group (SPI; n=6), the soybean meal in that diet was replaced by 5% dried powdery *Spirulina* meal (67.6% CP/kg DM) and 1% wheat flakes. Ingredients were homogenously mixed in order to avoid feed selection. The diets were isoenergetic (5.3 MJ/kg DM) and isonitrogenous (20 g N/kg DM). In addition, the cows received NaCl (50 g/d) and a vitaminized mineral mix (120 g/d). The cows were allowed to adapt for 2 weeks to the diet. After this adaption period, 3 weeks of sampling followed, where feed intake and milk yield were recorded daily. At each milking, milk samples were collected and pooled per day according to milk yield. Analysis of milk composition was performed by mid infrared spectroscopy (MilkoScan FT1, Foss, Hilleroed, Denmark). Rennet coagulation properties were determined twice in the sampling period with a Lattodinamografo (Foss, Padua, Italy). The milk color was determined four times during the sampling period using a tristimulus colorimeter (Chroma-Meter CR-300, Minolta, Osaka, Japan). In addition, complete milkings from all cows were collected two times on two consecutive days. For standardization of the fat content, the milkings of two cows each were combined to result in three milk mixtures per experimental group. The resulting total 12 milk mixtures were then individually homogenized and pasteurized and subsequently evaluated in 15 triangle tests by a sensory panel trained on milk products. Data were analysed with the Mixed procedure of SAS considering diet, breed, and their interaction as fixed factors. Milk composition data from before the start of the experiment were included as covariate.

**Results** The complete replacement of soybean meal with *Spirulina* did not affect the DM intake (means ± SEM; SPI 17.6 vs. SOY 17.2 ± 0.9 kg DM/day; P=0.8), which points to a high palatability of *Spirulina* for dairy cows. Moreover, the milk yield (SPI 18.1 vs. SOY 16.7 ± 0.8 kg/day; P=0.2) and the milk composition as well as the rennet coagulation properties (rennet coagulation time, rate of firming and curd firmness) were also not affected (all P>0.1). However, the milk from *Spirulina*-fed cows had a higher (P=0.013) yellowness (b\*, SPI 14.9 vs. SOY 13.8 ± 0.2) at unchanged brightness (L\*, SPI 75.1 vs. SOY 75.1 ± 0.2; P=1.0) and redness (a\*, SPI -5.65 vs. SOY -5.65 ± 0.1; P=1.0). This was probably caused by the transfer of abundant pigments, especially carotenes and xanthophylls, from *Spirulina*. In case a higher amount of the vitamin A precursor β-carotene is indeed confirmed by the ongoing analyses, *Spirulina* might be additionally valuable for cows and consumers from a nutritional perspective. Furthermore, an increased carotenoid content in the lipid fraction of the milk from *Spirulina*-fed dairy cows might improve its oxidative stability and therefore its shelf life. With regard to the sensory evaluation, 12 of the 15 triangle tests with 13 to 17 panellists did not show any significant difference between the two milk types. This emphasizes that feeding *Spirulina* did not impair milk flavor.

**Conclusions** Our study shows that *Spirulina* can completely replace soybean meal in the diet of dairy cows without impairing feed intake as well as milk yield, composition and coagulation properties. Milk flavor also does not seem to be negatively affected. In order to provide a more in depth characterization of the milk nutritional properties, further analysis such as milk carotenoid and fatty acid profiling and determination of antioxidant properties are still ongoing.

**Acknowledgments** The staff of AgroVet-Strickhof is gratefully acknowledged for the help during the experiment. We thank the H. Wilhelm Schaumann Stiftung for funding of the project.

### Microbiological quality of raw sheep's bulk milk samples on selected Slovak farms

M. Vrškova<sup>1</sup>, V. Tančin<sup>1,2</sup>, L. Mačuhová<sup>1</sup> and M. Uhrinčat<sup>1</sup>

<sup>1</sup>NPPC, Research Institute for Animal Production Nitra, Lužianky, Slovakia; <sup>2</sup>Slovak University of Agricultural in Nitra, FAFR, Department of Veterinary Science, Nitra, Slovakia

Email: vrskova@vuzv.sk

**Introduction** The quality of raw milk is regularly checked, because milk is the ideal environment for developing microorganisms because of its high water and nutrient content. All unwanted bacteria may not be pathogenic to humans. There are species that cause technological problems by producing thermostable lipolytic and proteolytic extracellular enzymes that pass through pasteurization in the active form. In order to avoid risks, and to ensure hygiene-sanitary quality and raw cows', sheep's and goats' milk safety, in its Regulations (EC) Nos. 852/2004 and 853/2004 European legislation lays down general food hygiene rules and specific ones for food of animal origin. The aim of our work was to evaluate the occurrence of individual species of technologically significant microorganisms is influenced by the season and the hygiene of obtaining milk in the milking process.

**Materials and Methods** At the monitored 28 ewe's farms, we took bulk milk samples from evening or morning milking in March, April and May (spring), in June, July and August (summer) and September, October, November (autumn) during year 2018. Somatic cell count (SCC) were set on the Somacount 150 (Bentley Instruments, Chaska, MN, USA). We analyzed the total bacterial count (TBC, mandatory indicator according to EC Regulation No. 853/2004) according to STN ISO 4833 (1997, 2004). We established technologically important microorganisms (MO) of psychrotrophic MO according to STN ISO 6730 (2000) and coliform MO according to STN ISO 4832 (2000). The presence of thermoresistent MO was detected in the Plate-Count-Agar and the presence of spore-forming anaerobic MO by liquid paraffin irrigation. The study was supported by the Ministry of Education, Science, Research and Sports of the Slovak Republic/the Slovak Research and Development Agency (Project No. APVV-15-0072).

**Results** In Table 1, we presented the species of the most important technological types of bacteria. We found out that the total bacteria count (TBC) in raw sheep's milk complied with the requirements of EU Regulation No. 853/2004 with an average of  $132 \times 10^3$  CFU.ml<sup>-1</sup> per spring (min  $34 \times 10^3$  CFU.ml<sup>-1</sup> and max  $501 \times 10^3$  CFU.ml<sup>-1</sup>),  $300 \times 10^3$  CFU.ml<sup>-1</sup> in summer (min.  $31 \times 10^3$  CFU.ml<sup>-1</sup> and max  $640 \times 10^3$  CFU.ml<sup>-1</sup>) and in autumn with an average value of  $147 \times 10^3$  CFU.ml<sup>-1</sup> (min  $52 \times 10^3$  CFU.ml<sup>-1</sup> and max  $276 \times 10^3$  CFU.ml<sup>-1</sup>). The enormous occurrence of psychrotrophic bacteria was found in one farm in northern Slovakia during spring and summer, in the summer we increased our number to 3 farms, in the autumn of 2 farms. We did not, therefore, enter statistical evaluation. We explain this by contaminating the milk in insufficiently disinfected and cooled collecting containers. The remaining farms have a milking parlor and beside the dairy tank with cooling. At the other farms we evaluated the average value of  $12 \times 10^3$  CFU.ml<sup>-1</sup> per spring and  $33 \times 10^3$  CFU.ml<sup>-1</sup> in summer,  $13 \times 10^3$  CFU.ml<sup>-1</sup> in the autumn. The count of thermoresistent MO achieved 57 CFU.ml<sup>-1</sup> per spring, 15 CFU.ml<sup>-1</sup> in summer and 40 CFU.ml<sup>-1</sup> in the autumn. The presence of spore-forming anaerobic MO in raw ewe's milk was found during spring at six farms out of 15, but in the summer at just one in 9, in the autumn the count rose to two farms. The SCC is not yet a mandatory indicator as it is for dairy cows. In the spring, 7 farms of 15 had SCC above  $1000 \times 10^3$  cells in 1 ml. The remaining farms ranged from 131 to  $825 \times 10^3$  cells in 1 ml. In the summer, SCC was in 5 farms over  $1000 \times 10^3$  cells in 1 ml. The remaining four farms ranged from 60 to  $965 \times 10^3$  cells in 1 ml. In the autumn there were 2 farms out of 4. The remaining two farms reached SCC  $958 \times 10^3$  cells per 1 ml.

**Table 1** Hygienic quality of raw sheep's milk

Microbiological characteristics (x10 <sup>3</sup> CFU.ml <sup>-1</sup> )	spring (n=15)		summer (n=9)		autumn (n=4)	
	mean	St.deviation	mean	St.deviation	mean	St.deviation
TBC	132.13	87.71	300.00	217.01	147.00	124.78
Psychrotrophs MO	12.33	40.87	33.86	3.87	13.05	4.50
Coliforms MO	0.40	-	3.61	-	0.30	-
Thermoresistent MO 1 ml <sup>-1</sup>	57.69	29.31	13.33	11.15	40.50	33.27
SCC (x10 <sup>3</sup> .ml <sup>-1</sup> )	1229.93	818.40	1411.08	770.25	2468.25	1147.14

TBC - total bacteria count, SCC – somatic cell count.

**Conclusions** The count of microorganisms in milk gives us an overall picture of the level of hygiene in the primary production. The degree of contamination of raw ewes' milk with mesophilic and psychrotrophic microorganisms affects the dairy health and hygiene of dairy ewes, the hygiene of the milkers and the environment in which the ewes are bred and milked, the methods used for the preparation of the udder and the milking technique, the methods used for cleaning and sanitizing milking equipment and bulk tank milk. Depending on the species of microorganisms found in milk, we could identify the source of contamination and then use the correct methods to eliminate them. For small ruminants, milk hygiene is important for serious economic and sanitary consequences for farmers, the processing industry and consumers due to the interrelationship between loss of production, yield in cheese production, excreted milk (and its safe disposal) and consequently the safety of dairy foods for the consumer.

### Rethinking intensive animal agriculture and meeting public expectations for our future farms

M. A. G. von Keyserlingk<sup>1</sup>, M. J. Hötzel<sup>2</sup> and D. M. Weary<sup>1</sup>

<sup>1</sup>Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada; <sup>2</sup>Laboratório de Etologia Aplicada e Bem-Estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Brazil

Email: [nina@mail.ubc.ca](mailto:nina@mail.ubc.ca)

**Introduction** In 1964 Ruth Harrison published the book “Animal Machines” that challenged developments in production agriculture. Her descriptions of more intensive production systems, such as battery cage housing for hens, veal crates for calves, and gestation stalls for sows, shocked readers and resulted in a growing public movement questioning whether farming practices are detrimental to animal welfare. In the more than 50 years that have followed public opposition to these practices has hardened leading, for example, to bans on the use of battery housing for hens and gestation stalls for sows in some parts of Europe. However, in many parts of the world, the use of such systems is still common. Some stakeholders working within agriculture argue that these intensive systems are beneficial, in part because they perceive these to improve animal health and productivity, and that rather than changing the systems, those working in agriculture should instead seek to better educate regarding these benefits.

In this lecture we review work examining whether efforts to educate the public regarding agricultural practices improves willingness to accept these systems. We then critically review the literature examining if more intensive and less naturalistic practices do actually improve animal health and productivity. We then examine examples of practical ways to incorporate naturalness into current food animal production systems that result in improved welfare and potentially increase public acceptability of these systems. Finally, we discuss to what extent views of naturalness influence public support for emerging technologies, such as gene editing, and how the acceptability of these technologies may vary depending on whether the methods are thought to improve animal welfare.

**Educating the public** Research focused on exploring perceptions, knowledge, and values of animal welfare among citizens can help in identifying socially sustainable farm production systems. There is some evidence showing that although education can improve knowledge it does not appear to improve perceptions of welfare of intensively housed animals. Simply knowing more does little to change underlying values, and increased knowledge can lead to the development of new concerns. Thus, attempts to educate the public may do little to resolve societal concerns about animal welfare on livestock farms.

**Critically evaluating the purported benefits of intensive systems on health and productivity** A key justification for the more intensive systems is that these promote health and production, but the evidence in support of such claims is not always clear. For example, the common practice of early separation of dairy calves and cows is thought to benefit the health of cow and calf. We describe the results of a recent systematic review, showing that there is actually little evidence in support these claims. This example shows the value in critically assessing the evidence used within agriculture to defend or promote intensive rearing practices.

**Incorporating naturalness** Given that attempts to educate the public may be ineffective, and that the evidence used to justify intensive practices may be weak, we argue that new research and development efforts within animal agriculture should attempt to address public opposition by developing refined practices that allow for more naturalness while maintaining good levels of health and productivity. For instance, despite the public placing value on animals having access to pasture, many are still housed exclusively indoors. Pasture may not always be feasible on some farms, but alternative forms of outdoor access may provide some of the same benefits to animals and be more easily applicable for farmers. In new work we have examined cow preferences and usage of pasture and alternative forms of outdoor access. In addition, this work has considered the health and production implications of these new systems, sometimes showing unanticipated benefits. For example, we have found that providing dairy cows access to an outdoor bedded pack (as an alternative to pasture), resulted in increased expression of reproductive behaviour, improving heat detection. In other examples, however, more naturalistic rearing conditions can be detrimental to the animal and farmer. We review work showing that the public can take a nuanced perspective on these issues; in this example, we have found that respondents expect farmers to provide animals with protection for adverse environmental conditions including heat stress, and that a more natural environment are only preferred if they indeed provide welfare benefits.

**Concerns about naturalness as a barrier to the acceptability of new technologies** The research we have reviewed shows that concerns about the acceptability of rearing methods for animals often involve concerns about naturalness. Proponents of new technologies will likely need to understand these concerns if these technologies are to be implemented. Here again, the interplay with welfare is important. Some improvements in animal welfare might be achieved, for example, through gene editing (e.g. to create polled cattle that do not need to be dehorned, or more heat tolerant animals). Research indicates that the ability of these technologies to reduce suffering can increase support.

**Conclusions** Educating the public may not increase acceptance of current farm animal production systems if practices fail to resonate with public values. Work has begun on identifying ways of incorporating naturalness into modern animal agriculture practices. Support for the use of gene editing technologies in livestock production systems increases if the application reduces animal suffering.



### Refining the utility of precision behavior monitoring of dairy cattle

T. J. DeVries

Department of Animal Biosciences, University of Guelph, Ontario, Canada

Email: [tdevries@kemptvillec.uoguelph.ca](mailto:tdevries@kemptvillec.uoguelph.ca)

The dairy cattle industry has seen an increasing availability of equipment that is readily available for the automation of management tasks, including milking and feeding, as well as the precision monitoring of dairy cow behavior. Such automation not only has the ability to improve production and time efficiency on farm, but also increases our ability to monitor individual cows.

Much recent research has been focused on the use of individual behavioral monitoring to detect health disorders, both in occurrence and in advance of clinical symptoms. This, in turn, has the potential to allow producers to identify and implement prevention and treatment protocols at earlier time points. Many different individual cow behaviors can now be collected automatically on farm, including feeding, rumination, lying/standing, and milking behavior. Thus, much research has been done in recent times to investigate the association these behaviors have with various health issues, including metabolic and infectious transition disease, lameness, and mastitis (King and DeVries, 2018). Much progress has been made in this area, in particular through combination of use of multiple behavioral inputs, as well as other cow-level data (production, age, size) that may be collected in real-time, to improve our predictive ability of health events. The predictive abilities of using such information are currently being strengthened through investigation of more advanced data modelling techniques (e.g. machine learning). Deep learning models (e.g. neural network models) have, in particular, shown promise for developing disease probability indexes that are reliable and timely for disease identification.

Despite great potential for use of automated behavioral monitoring for early disease detection, the fact of the matter is that to make significant improvement in animal welfare as well as farm economic sustainability, our focus in disease control in dairy cattle must be more centred on disease prevention rather than detection. To that end, there is great opportunity to use behavioral monitoring to inform management decisions on farm, particularly those that are going to reduce the incidence of disease, and thus improve overall herd health, productivity, and efficiency.

Precision monitoring of feeding behavior, in particular its response to changes in nutrition and management, is one particular area where there is a direct opportunity to impact rumen health and metabolic activity, and thus production and efficiency. This information may, therefore, be used to optimize nutritional management. As example, we know that the feeding behavior patterns of dairy cows not only dictate the amount of feed consumed (Johnston and DeVries, 2018), but also have a direct impact on rumen health and digestion efficiency. Dairy cows that eat fewer and larger meals, more quickly, are at greater risk of incidence of ruminal acidosis (DeVries, 2019). Indeed, ruminal pH declines following meals, and the rate of pH decline increases as meal size increases and as dietary effective fiber concentration decreases. Further, as cattle spend less overall time feeding, and increase their rate of feed consumption, daily salivary secretion is reduced, decreasing the buffering capacity of the rumen and reducing rumen pH. Thus, by monitoring how eating patterns of dairy cows respond to nutritional and management changes, decisions can be made to minimize the impact of those changes.

There is also evidence that precision monitoring of post-ingestive eating behavior of dairy cows, rumination, may be important for maintenance of good rumen health. Rumination contributes to the breakdown of feed into smaller particles, which then increases feed surface area and decreases the amount of time it takes feed to be fermented in the rumen, thus increasing the rate of digestion of that feed. In addition, the saliva produced during chewing and bolus formation in rumination not only aids in re-swallowing of that bolus, but also acts to maintain rumen pH through its buffering abilities, thus helping avoid ruminal acidosis. Thus, through monitoring of rumination behavior, both at an individual and herd level, changes in response to nutritional and management alternations can be detected, and subsequent decisions can be made ensure continued good chewing behavior, and thus maintain cow health.

In conclusion, it is anticipated that through automated precision monitoring of dairy cattle behavior, dairy cattle producers will be able to make more timely decisions for altering and adjusting nutritional programs, housing, and management, both at a cow and herd level, in effort to optimize dairy cow health, welfare, production, and efficiency.

#### References:

- DeVries, T. J. 2019. Invited Review: Feeding behavior, feed space, and bunk design and management for adult dairy cattle. *Vet. Clin. Food Anim.* 35:61-76. <https://doi.org/10.1016/j.cvfa.2018.10.003>
- Johnston, C., and T. J. DeVries. 2018. Associations of feeding behavior and milk production in dairy cows. *J. Dairy Sci.* 101:3367-3373. <https://doi.org/10.3168/jds.2017-13743>
- King, M. T. M., and T. J. DeVries. 2018. Detecting health disorders using data from automatic milking systems and associated technologies. *J. Dairy Sci.* 101:8605-8614. <https://doi.org/10.3168/jds.2018-14521>

# Potential underlying factors of subacute rumen acidosis: Effects of frequency and duration of feed availability on performance and milk fatty acid profile in dairy cows

S. Abd Rahim<sup>1</sup>, H. van Laar<sup>2</sup>, J. Martín-Tereso<sup>2</sup>, W. H. Hendriks<sup>1</sup> and J. Dijkstra<sup>1</sup>

<sup>1</sup>Animal Nutrition Group, Wageningen University and Research, the Netherlands; <sup>2</sup>Trouw Nutrition R&D, Amersfoort, the Netherlands

Email: sholeha.bintiabdrahim@wur.nl

**Introduction** Subacute ruminal acidosis (SARA) is a digestive disorder that negatively affects both cow health and herd profitability. Several feeding management strategies have been proposed to mitigate the risk of SARA, one of them being to increase feeding frequency. Although the effects of feeding frequency have been extensively studied (González et al., 2012), findings for its effect on rumen fermentation and milk fat yield and fatty acid (FA) profile have not been consistent. Compared to milk fat content, which is frequently used as an indicator of SARA, specific milk FA have shown more promising results (Colman et al., 2010). Therefore this study aimed to determine the effect of feeding frequency and duration of access to feed on rumen fermentation and milk FA profile in lactating dairy cows.

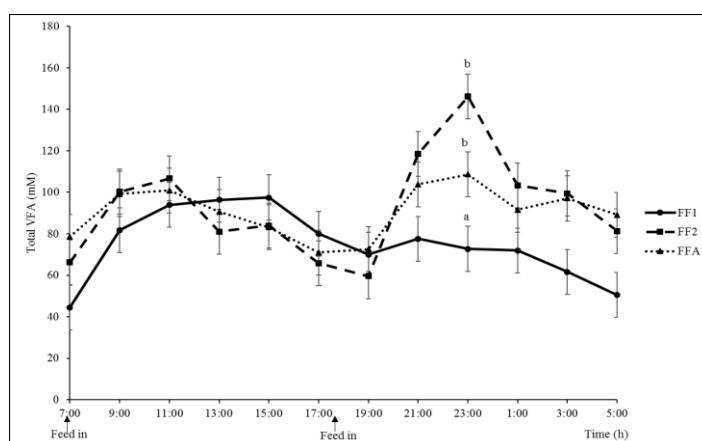
**Materials and Methods** Nine multiparous Holstein-Friesian dairy cows were randomly assigned to one of six treatment sequences in a 3 × 3 Latin square design, with 3 treatments and 3 periods. One of the blocks consisted of 3 ruminally fistulated cows. Data from one of the rumen fistulated cows was not available due to a metabolic disorder. The treatments consisted of daily frequency of access to feed and duration of feed availability. Treatments were: 1 × 4 h (FF1), 2 × 2 h (FF2), and 2 × free access to half of the daily allowance (FFA). Each period consisted of 4 days where feed was supplied 2 × 2 h, 10 days of adaptation to assigned treatments and 7 days of measurement. All cows were housed in a free-stall barn and fed the same mixture of 33.4% grass silage, 33.4% maize silage, 2.2% grass hay and 31.0% compound meal (on a DM basis), offered in 9 automated Roughage Intake Control (RIC) bins (Insentec, Marknesse, the Netherlands). Feed was restricted at 90% of the *ad libitum* intake observed during the adaptation period preceding the trial. Cows were milked twice daily. Approximately 100 ml of rumen fluid was collected from the rumen of fistulated cows on day-7 of each measurement period at every 2 h for 24 h for analysis of volatile fatty acids (VFA) and pH. Milk samples were taken at the milking parlour on Monday evening, Tuesday morning, Wednesday evening, and Thursday morning. Milk samples were pooled and analysed for FA composition by gas chromatography. All data were analyzed as repeated measurement with PROC MIXED of SAS 9.4. Differences were analyzed using the least square method with a Simulate adjustment for multiple comparisons. Significance was declared at  $P < 0.05$ , and a trend was considered at  $0.05 \leq P < 0.10$ .

**Results** Dry matter intake was lowest in FF1 (14.7, 17.0, and 16.4 kg/d for FF1, FF2 and FFA;  $P < 0.01$ ), resulting in the lowest total ruminal VFA concentration ( $P < 0.01$ ) compared with cows in FF2 and FFA (Figure 1). Molar proportion of propionic acid ( $P < 0.01$ ) was lower but molar proportion of acetic acid ( $P < 0.01$ ) was greater in FF1. Rumen pH (average = 6.75, 6.63, and 6.57 for FF1, FF2, and FFA) was not affected by treatment. Primary milk FA indicators of SARA, viz. C18:1 *trans*-9 ( $P = 0.003$ ), C18:1 *trans*-10 ( $P < 0.001$ ), and C18:1 *trans*-11 ( $P = 0.001$ ), were higher in FF1 compared with FF2 and FFA (Table 1).

**Table 1** Milk fatty acid (FA) composition of lactating dairy cows fed at different feeding frequency (FF) and duration

FA (g / 100 g of FA)	Treatment			SEM	P-value Treatment
	FF1	FF2	FFA		
C18:1 <i>trans</i> -9	0.21 <sup>a</sup>	0.18 <sup>b</sup>	0.18 <sup>b</sup>	0.007	0.003
C18:1 <i>trans</i> -10	0.33 <sup>a</sup>	0.25 <sup>b</sup>	0.23 <sup>b</sup>	0.024	0.036
C18:1 <i>trans</i> -11	1.16 <sup>a</sup>	0.90 <sup>ab</sup>	0.86 <sup>b</sup>	0.058	<0.001

<sup>a,b</sup> Means within a row with different superscripts differ ( $P < 0.05$ ).



**Figure 1** Effects of feeding frequency and duration on total rumen volatile fatty acid (VFA) concentration.

**Conclusions** Feed intake patterns influenced the concentration of fermentation acids in the rumen. Although ruminal pH was not severely affected, several milk FA parameters were shifted towards *trans* C18:1 formation in cows that were fed less frequently.

## References

González, L.A., X. Manteca, S. Calsamiglia, K.S. Schwartkopf-Genswein, and A. Ferret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (A review). *Anim. Feed Sci. Technol.* 172:66–79.

Colman, E., W.B. Fokkink, M. Craninx, J.R. Newbold, B. De Baets, and V. Fievez. 2010. Effect of induction of subacute ruminal acidosis on milk fat profile and rumen parameters. *J. Dairy Sci.* 93:4759–4773.

### Strong differences between early lactating primiparous and multiparous cows in feed sorting behavior, chewing activity, and rumen acidosis index

A. Stauder<sup>1</sup>, E. Humer<sup>1</sup>, V. Neubauer<sup>2</sup>, N. Reisinger<sup>3</sup>, A. Kaltenegger<sup>1</sup> and Q. Zebeli<sup>1</sup>

<sup>1</sup>Department for Farm Animals and Veterinary Public Health, Institute of Animal Nutrition and Functional Plant Compounds, Vetmeduni Vienna, Austria; <sup>2</sup>Institute of Animal Nutrition and Functional Plant Compounds, and Food Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria; Institute for Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria; <sup>3</sup>BIOMIN Research Center, BIOMIN Holding GmbH, Tulln, Austria

Email: Alexander.Stauder@vetmeduni.ac.at

**Introduction** During early lactation, cows are typically switched from the fiber-rich close up diet to a lactation diet, high in concentrates, to mitigate their negative energy balance. This dietary change often happens both in primiparous (PP) and multiparous (MP) cows. Yet, there are strong differences, particularly when taking into account that PP cows are still growing and have nutrient requirements that go beyond milk production. In addition, PP cows experience for the first time lactational, nutritional, and environmental stress, mostly being lower in hierarchy which can be expressed in different patterns of eating and feed sorting, and eventually rumen health indices than MP cows. However, this has not yet been carefully evaluated. The objective of this study was to determine effects of increasing amounts of concentrate in a total-mixed ration (TMR) on chewing activity, sorting behavior, and rumen acidosis index in PP and MP dairy cows.

**Materials and Methods** A total of 24 lactating Simmental cows (8 PP, 16 MP;  $50 \pm 22$  days in milk) were used for this study. Cows first received a medium concentrate (MC) diet with 40% concentrate on dry-matter (DM) basis for 2 weeks (MCwk1, MCwk2). Then cows were abruptly switched to a high-concentrate diet (60% concentrate on DM basis) for 4 weeks (HCwk1-wk4). During MCwk1, MCwk2, HCwk2, and HCwk4 chewing activity was measured using rumination halters (Rumiwatch System, ITIN + Hoch GmbH). Reticular pH was measured continuously by wireless pH-sensors (smaXtec, animal care GmbH) inserted at the end of MCwk1. Fresh TMR and remaining samples were collected every week for nutrient analysis and to determine feed sorting using a Penn State Particle Separator (model C24682N, Nasco, Fort Atkinson, WI). The samples were separated into 4 fractions: long ( $>19$ mm), medium (8-19mm), short (1.18-8mm), and fine ( $<1.18$ mm) particles. Feed sorting was calculated as a percentage of the actual DM-intake (DMI) of each particle fraction expressed as a percentage of its predicted intake. Statistical analysis was performed using the MIXED procedure of SAS, including the diets (MCwk1-2, HCwk1-4) and parity, as well as a possible interaction between them as fixed effects. SARA (subacute ruminal acidosis)-Index was calculated as the inverse area of the pH below 5.8 divided by the DMI.

**Results** As expected, the switch from MC to HC-diet caused an overall decrease in the time cows spent eating ( $P=0.03$ ) and ruminating ( $P<0.01$ ) per kg of DM. However, the PP cows spent more time eating ( $P=0.01$ ) and ruminating ( $P<0.01$ ) per kg of DM than MP cows and they had more ruminating chews/bolus ( $P=0.02$ ). During the HC-feeding, cows sorted for longer particles and less for fine particles. Yet, the PP cows sorted even stronger for long ( $P=0.05$ ) and medium particles ( $P=0.02$ ) than MP cows. Therefore, PP cows sorted more against fine ( $P<0.01$ ) and short particles ( $P=0.06$ ) than MP cows. Despite these changes on eating, ruminating and sorting as well as the lower DMI in the PP cows ( $P<0.01$ ), they had a higher SARA-Index than in MP cows ( $P=0.10$ ).

**Conclusions** In conclusion, this study demonstrated greater susceptibility of PP cows to SARA than MP cows. Although PP cows enhanced chewing and ruminating activity as well as modified sorting for longer and against finer feed particles, they still could not lower the risk of SARA. This indicates that PP cows need better shaped feeding strategies to mitigate rumen acidosis and fermentation disorders in early lactation.

### Feeding time, body condition and lameness during the dry period: associations with transition period disease in dairy cows

R. R. Daros, H. K. Eriksson, D. M. Weary and M. A. G. von Keyserlingk

Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada

Email: rrdaros@gmail.com

**Introduction** Metabolic and infectious disease during the transition period (around calving) have been associated with lower feeding time and body condition loss during the pre-calving period. Few studies have investigated lameness as a potential cause of reduced feeding time and body condition loss during the dry period. We hypothesized that lame cows during the dry period would have lower feeding times, greater body condition loss and be more likely to develop transition period diseases.

**Materials and Methods** A total of 461 predominantly of Holstein parous cows from 6 commercial freestall dairy farms were enrolled in this prospective longitudinal study. Average herd size (mean  $\pm$  SD) was  $361 \pm 137$  cows, and cow's average milk production was  $11866 \pm 179$  kg of milk per 305-d lactation. Cows were individually marked with alphanumeric symbols on their back, and followed weekly pre-calving – starting at wk 9 before calving – and twice a wk for 2 wk after calving. Pre-calving weekly gait scores based on 5-point scale ( $\leq 2$  = sound and  $\geq 3$  = lame) were summarized per animal to create groups of cows that were: always sound, chronically lame and other (cows that transitioned between being lame and sound or vice versa during the dry period). Body condition score (BCS; 5-point scale in 0.5 increments: 1.0 = thin to 5.0 = fat) was assessed at wk 8 before calving and then weekly from wk 4 before calving to the wk of calving. Change in BCS during the dry period was calculated as the difference between BCS at the wk of calving and BCS at wk 8 before calving. For each cow, metritis (0 = vaginal discharge not watery, not fetid, may contain pus; 1 = red/brown, fetid, watery vaginal discharge) and sub-clinical ketosis (measure by cow-side blood test, BHB  $\geq 1.2$  mmol/L) were assessed 4 times during the first 2 wk after calving. Data on retained placenta, parity and previous lactation milk production (305-d corrected) were retrieved from farm records. Feeding time per cow/d was measured by scan sampling every 10-min (cow's head over feed bunk = 1 and cow elsewhere = 0). Number of scans per cow/d was summarized as h/d for each cow. Feeding time was collected for 24 h on the day following gait scoring visits. Feeding time was available for a subset of 156 cows from 5 farms. Because we hypothesized that lameness would have an indirect association with the incidence of transition period diseases through feeding time and BCS loss, we initially built models to assess if feeding time and change in BCS were associated with the likelihood of cows having transition period diseases. Two multilevel ordinal logistic regression models, using farm as random intercept, were fitted to evaluate the association of 1) feeding time during the close-up period, and 2) BCS change during the dry period, with the likelihood of a cow developing 0, 1 or  $>1$  transition period disease. We then built two multilevel linear regression models with farm and cow within farm as random effects, to assess the association between 1) lameness and feeding time, and 2) lameness group and change in BCS. Finally, a multilevel ordinal logistic regression model evaluated the association between lameness group and the likelihood of cows developing 0, 1 or  $>1$  transition period diseases. In all models, parity and previous lactation milk production were included as possible confounding effects.

**Results** The proportion of cows that were always sound, chronically lame and other during the dry period was 33, 23 and 46%, respectively. The cumulative incidence of sub-clinical ketosis, metritis and retained placenta was 35, 28 and 10%, respectively. Cows that spent more time feeding during the close-up period had lower odds of developing transition period diseases. For each 1 h increase in daily feeding time during the close-up period there was a reduction in the odds (OR: 0.7; CI: 0.5 to 0.9;  $P = 0.02$ ) of having 1 or more transition period diseases, when controlling for the effects of parity, BCS at calving and previous lactation milk production. Similarly, cows that gained body condition during the dry period had lower odds of developing transition period diseases. For each point increase in BCS during the dry period we found reduced odds (OR: 0.4; CI: 0.2 to 0.7;  $P < 0.01$ ) of having 1 or more transition period diseases. Lame cows spent 20 min/d less time feeding (CI: -32 to -8 min/d;  $P < 0.01$ ) compared to sound cows, when controlling for the effects of parity, wk in relation to calving, period (close-up, far-off), and the interaction between wk in relation to calving and period. Overall, cows decreased feeding time throughout the dry period, though this decrease was markedly higher during the close-up period. There was no interaction between lameness and week in relation to calving, indicating that the rate of reducing feeding time throughout the dry period was not different between lame and sound cows. Lameness was not associated with changes in BCS during the dry period; chronically lame cows lost 0.07 BCS more points (CI: -0.2 to 0.01 BCS points;  $P = 0.17$ ) than cows that remained sound. Because lameness was not associated with BCS change, we included BCS change as covariate in the model that measured the association between lameness and incidence of transition period disease. Chronically lame cows had higher odds of having 1 or more transition period diseases (OR: 1.9; 95% CI: 1.1 to 3.3,  $P = 0.02$ ), compared to cows that remained sound during the dry period. Cows that changed their lameness status did not differ (OR: 1.3; CI: 0.8 to 2.1,  $P = 0.23$ ) from cows that were always sound. For each point of BCS gain during the dry period, the odds of having 1 or more transition period diseases decreased (OR: 0.4; CI: 0.2 to 0.7,  $P < 0.01$ ). Primiparous cows had lower odds (OR: 0.6; CI: 0.4 to 0.9,  $P = 0.02$ ) of having 1 or more transition period diseases, compared to multiparous cows.

**Conclusions** Lameness during the dry period was associated with the development of transition period disease. A potential causal mechanism for how lameness may contribute to disease development is through reduced feeding time during the dry period, but changes in body condition appears to be independently associated with the incidence of transition period diseases. This study is consistent with previous findings showing that reduced feeding time is associated with higher transition period disease incidence. Reducing lameness and managing cows so that they not lose body condition during the dry period may improve dairy cows' health.

### Analysis of behavioral changes for early detection of lameness in dairy cows

J.-B. Burla<sup>1</sup>, H. Weigle<sup>1</sup>, L. Gyax<sup>1</sup>, A. Steiner<sup>2</sup> and B. Wechsler<sup>1</sup>

<sup>1</sup>Federal Food Safety and Veterinary Office FSVO, Centre for Proper Housing of Ruminants and Pigs, Tänikon, Switzerland;

<sup>2</sup>University of Bern, Vetsuisse Faculty, Clinic for Ruminants, Bern, Switzerland

Email: joan-bryce.burla@agroscope.admin.ch

**Introduction** Lameness is one of the most prevalent diseases affecting the welfare of cows in modern dairy production. Diagnosis at an early stage results in a reduction of medications, faster recovery, and a shortened period of suffering for the cow. Previous research has reported clear differences in the behavior, i.e. locomotor activity and lying behavior, of severely lame cows in comparison to non-lame cows. However, effects of moderate lameness on cow behavior have rarely been investigated so far. The aim of this study was to identify behavioral changes in moderately lame cows that have the potential to be used for an automatic on-farm system for early lameness detection in the future.

**Materials and Methods** Ethical approval was obtained from the Veterinary Office of the Canton Zurich in Switzerland (Approval ZH061/15). The study was conducted on 17 Swiss dairy farms with cubicle housing systems that provided an indoor area ( $\geq 1$  cubicle per cow) and a permanently accessible outdoor area. Herd size ranged from 31 to 91 (mean  $\pm$  SD:  $55.9 \pm 17.4$ ) lactating cows. On each farm, cow behavior was recorded continuously during two periods (A, B) of 48 h each with an interval of 6 - 10 weeks in between. A sample of 5-11 non-lame (locomotion score 1 of 5) and 2-7 moderately lame (locomotion score 3 of 5) cows was selected based on visual lameness scoring (based on Sprecher et al., 1997). Overall, 219 cows were included in the study of which 142 cows non-lame and 66 were moderately lame in period A, and 128 cows were non-lame and 53 moderately lame in period B. In between the two periods, the cows underwent corrective claw trimming. Locomotor activity and lying behaviors (recorded by MSR145 accelerometers attached to left hind leg), feeding and rumination behaviors (by RumiWatch halter with noseband sensor), visits to the brush and the concentrate feeder, and the milking order position were compared between moderately lame and non-lame cows. Data was statistically analyzed in R (version 3.3.2) using linear-mixed effects models (lmer; package lme4). P-values were calculated using parametric bootstrap (PBmodcomp; package pbkrtest) by comparing the statistical models with and without the fixed effect locomotion score, whereby days in milk and parity were included in the models as nuisance factors.

**Results** In comparison with non-lame cows, moderately lame cows had a lower average locomotor activity per 24 h, a longer lying duration per 24 h and a longer average lying bout duration, but a similar number of lying bouts per 24 h. Eating time per 24 h and the number of eating chews per 24 h were reduced in moderately lame compared with non-lame cows, whereas no effect of moderate lameness was evident for ruminating time per 24 h and number of ruminating chews per 24 h. Moderately lame cows visited the concentrate feeder and the brush less frequently, and they were further back in the milking order compared with non-lame cows. The observed changes in activity, lying, and feeding behaviors are known to cause disadvantages with respect to the bodily constitution and energy supply, which may predispose moderately lame cows to other health problems and a shorter life. Moreover, the restriction in mobility is likely to further affect the cows' low resilience and social behaviors.

**Table 1** Recorded behaviors with mean values (minimum - maximum) of non-lame and moderately lame cows and the p-value.

Behavior	Non-lame cows	Moderately lame cows	p-value
Average locomotor activity [g/h]	315.2 (116.9 - 607.2)	288.5 (133.4 - 534.2)	p = 0.007
Lying duration [min/24 h]	655.8 (324.9 - 976.8)	696 (320.3 - 1089.6)	p = 0.027
Average lying bout duration [min]	85.6 (29.9 - 245.9)	101.4 (25.8 - 335.5)	p = 0.008
Lying bouts [n/24 h]	8.5 (2 - 20)	7.8 (3 - 27)	p = 0.11
Eating time [min/24 h]	460.4 (243.2 - 909.6)	414 (255.3 - 605.7)	p = 0.033
Eating chews [n/24 h]	31920 (11850 - 55030)	28200 (14650 - 43820)	p = 0.05
Ruminating time [min/24 h]	494.6 (84.62 - 677.4)	492.6 (285.5 - 663.5)	p = 0.53
Ruminating chews [n/24 h]	31520 (5889 - 50440)	29870 (12940 - 48080)	p = 0.86
Visits to concentrate feeder [n/24 h]	9 (0 - 26)	6.1 (0 - 17)	p = 0.014
Visits to brush [n/24 h]	2.3 (0 - 11)	1.8 (0 - 10)	p = 0.046
Milking order [relative position]	0.47 (0.01 - 0.99)	0.63 (0.02 - 0.99)	p $\leq$ 0.001

**Conclusions** The behavior of moderately lame cows differed in many of the investigated behaviors in a biologically relevant way from non-lame cows. The effects on the everyday life of moderately lame dairy cows in loose housing systems indicate that even an early stage of lameness already has a great potential effect on animal welfare.

The use of behavioral changes seems to be promising to elaborate an automatic on-farm system for early lameness detection. However, further analyses are necessary to assess their reliability for lameness detection and to create algorithms considering several parameters in combination or track changes within cows over time to achieve sufficient sensitivity and specificity.

### References

Sprecher, D. J., D. E. Hostetler, and J. B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology* 47, 1179–1187. [https://doi.org/10.1016/S0093-691X\(97\)00098-8](https://doi.org/10.1016/S0093-691X(97)00098-8)

### Effects of social deprivation in pigs on their behavioural, neuroendocrine and immunological responses to acute endotoxemia

R. Brückmann<sup>1</sup>, M. Tuchscherer<sup>1</sup>, A. Tuchscherer<sup>2</sup>, U. Gimsa<sup>1</sup> and E. Kanitz<sup>1</sup>

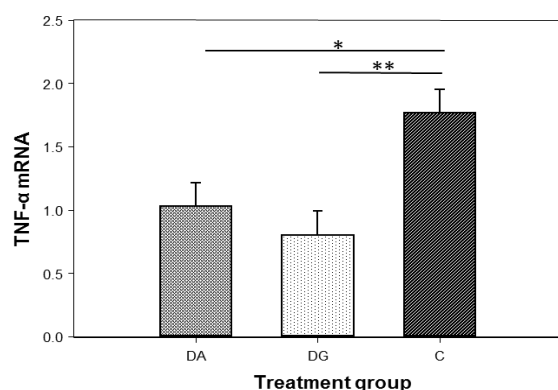
Leibniz-Institute for Farm Animal Biology (FBN), <sup>1</sup>Institute of Behavioural Physiology; <sup>2</sup>Institute of Genetics and Biometry, Dummerstorf, Germany

Email: brueckmann@fbn-dummerstorf.de

**Introduction** Social stress during early postnatal life induces persistent alterations in behavioural and physiological mechanisms of adaptation that may increase an organism's susceptibility to disease in later life. In farm animals, physiological consequences of disrupting mother-offspring interactions are relatively unknown in terms of animal health and welfare. Therefore, the present study aimed to investigate the effects of maternal and littermate deprivation without and with social support on physiological and behavioural responses to acute endotoxemia in piglets.

**Materials and Methods** On days 2-15 of age, piglets from eight multiparous Landrace sows were deprived from mother and littermates either alone (DA) or as a group (DG, i.e. deprivation in the presence of familiar conspecifics). Non-socially deprived piglets from another eight sows served as controls (C). At 42 days of age, two randomly assigned piglets per sow and deprivation treatment were intraperitoneally injected with 50 µg/kg body weight LPS (*Escherichia coli* O111:B4). Blood samples were taken by vena cava puncture for analyses of cortisol and cytokines (TNF-α, IL-6, IL-10) before LPS and 1, 3, 6 and 24 h thereafter. Signs of sickness behaviour (panting, somnolence, vomiting) was observed using scan sampling every 5 min over a period of 6 h after LPS. For mRNA expression analysis, hypothalami were collected 24 h after LPS. Data were analysed by the GLIMMIX procedure of SAS/STAT software.

**Results** Peripheral LPS administration provoked increases in plasma cortisol, TNF-α, IL-6 and IL-10 concentrations in a time-dependent manner and caused serious sickness behaviour in socially deprived and control piglets. The GLIMMIX procedure indicated a main effect of the interaction deprivation treatment × sex × time on the plasma TNF-α level ( $P < 0.01$ ). One hour after the LPS challenge, male DA and DG piglets responded with a smaller TNF-α increase compared to C piglets ( $P < 0.001$ ). Additionally, male DG piglets reacted to the challenge with a lower release of IL-10 than controls ( $P < 0.05$ ). TNF-α/IL-10 ratios in male DA piglets were significantly lower than in male DG and C piglets ( $P < 0.05$  and  $P < 0.001$ , respectively). The cortisol and IL-6 levels after LPS were not significantly affected by deprivation treatment. Statistical analysis revealed an effect of social deprivation on LPS-induced TNF-α mRNA expression in the hypothalamus ( $P < 0.01$ ). The mRNA expression of TNF-α was significantly decreased in DA ( $P < 0.05$ ) and DG ( $P < 0.01$ ) piglets compared to controls. This effect was more pronounced in male piglets. LPS-induced IL-6 and IL-10 mRNA expressions were not affected by previous deprivation treatments. However, the prior social deprivation treatment enhanced signs of sickness after LPS. During the 2<sup>nd</sup> hour post challenge, DA and DG piglets showed higher frequencies of panting (DA:  $P < 0.01$ ; DG:  $P < 0.05$ ) and somnolence (DA:  $P = 0.06$ ; DG:  $P < 0.05$ ) than control piglets. Furthermore, the latency to the onset of panting was shorter in DA and DG piglets compared to controls ( $P < 0.05$ ).



**Figure 1** LPS-induced mRNA expression of TNF-α in the hypothalamus of socially deprived piglets. DA: deprivation alone, DG: deprivation in a group of familiar conspecifics, C: control without deprivation. Significant differences are indicated by asterisks (\* $P < 0.05$ ; \*\* $P < 0.01$ )

**Conclusions** The present data indicate that repeated social deprivation in pigs causes long-lasting effects on defence reactions to an acute peripheral LPS challenge; consequently social deprivation could increase disease susceptibility. Furthermore, the deprivation with familiar conspecifics alters the cytokine balance of the male piglets compared to controls and may have a positive effect on coping with diseases.

### Management of parturition in the hyper prolific sow

O. A. T. Peltoniemi, S. Björkman and C. Oliviero

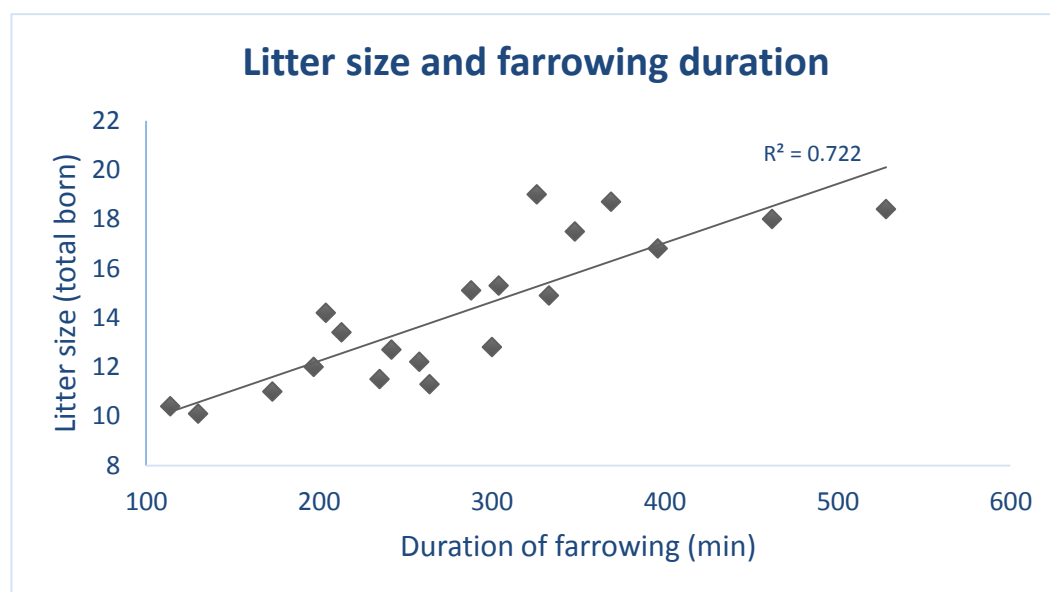
University of Helsinki, Saarentaus, Finland

Email: [olli.peltoniemi@helsinki.fi](mailto:olli.peltoniemi@helsinki.fi)

**Background** Over the past three decades, efficient breeding and management has almost doubled the litter size of the sow. In the same period of time, duration of farrowing has extended remarkably (Oliviero et al., 2019). In many cases (breeds, circumstances), the expulsion phase of parturition is now 4-5 times longer than it used to be in the early 1990's (Figure 1). In the same period, we have seen a constant downward trend in the birth weight of the piglet – along with a similar trend in colostrum intake of the piglet, which has been shown as one of the most important risk factors for piglet mortality. Along with these trends, an increase in complications of farrowing such as PDS and retention of placenta has been reported.

**Successful farrowing** It is essential to focus on the success factors of the process of parturition. Successful parturition in the pig includes the possibility to express adequate maternal behaviour, rapid expulsion of the piglets, complete expulsion of the placenta, neonatal activity and colostrum intake. New insights, such as adding more fibre to sow diets during pregnancy, and especially during the period prior to farrowing, may prevent constipation, increase water intake of the sow around parturition and increase milk intake and performance of piglets. Maternal characteristics, including maternal behaviour, ease of parturition, colostrum production, and piglet quality parameters, may be utilized to improve success rate of reproductive management during farrowing and early lactation.

**Management** This paper aims to address the changes in production environment and management tools to alleviate problems seen with the present hyper prolific sow model. In short, the sow needs to be given space and enrichment materials for adequate expression of nest building behavior. New diagnostic imaging technology provides tools with improved monitoring of physiology and pathology of the female genital track around parturition. These tools have shown their power in peripartum management of prolonged, complicated farrowing of the present hyper prolific sow lines. In feeding, adequate percentage of appropriate quality of fiber appears as a best practice in the battle with constipation. Appropriate management of the sow body condition during the last trimester of pregnancy appears to be essential for mammary development and colostrum production. Adequate breed selected for ease of management of the litter during early lactation is becoming an issue of growing significance. Management of parturition have an impact on the immune system and microbiota of the newborn piglets. It will also be questioned how far the industry can go to push the limits of physiology of pig reproduction without compromising sustainability of production.



**Figure 1** The relationship between litter size and the duration of farrowing in 20 studies from 1992 to 2018 (Oliviero et al., 2019).

**Conclusions** The pig industry needs to address the growing concern over prolonging parturition in the pig. The decreasing birth weight along with the vulnerable immunity is subject for vigorous research in search for necessary management tools. The industry needs to rethink the strategies regarding over size litters. Robust breeds with somewhat lower size of the litter but increased birth weight may be needed for cross breeding in the near future.

#### References

Maded and Leon 1992; Oliviero et al., 2013; Björkman et al., 2018; Yun et al., 2018 Oliviero et al., 2019

### Diagnosis of endometritis and cystitis in sows: use of biomarkers

A. Grahofner<sup>1,2,3</sup>, S. Sipos<sup>4</sup> and W. Sipos<sup>1</sup>

<sup>1</sup>Clinic for Swine, VetmedUni Vienna, Austria; <sup>2</sup>Animal Physiology, Institute of Agricultural Sciences, ETH Zurich, Switzerland;

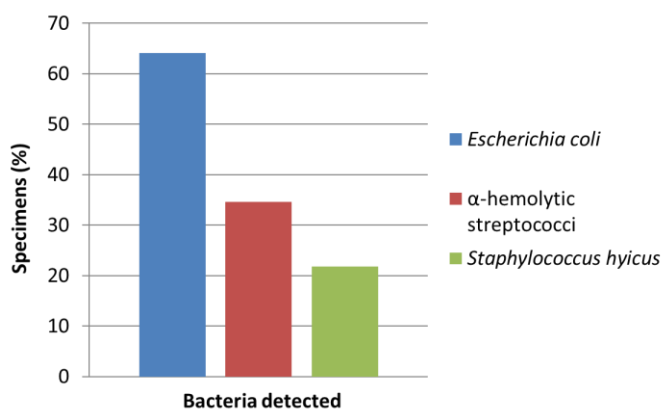
<sup>3</sup>Clinic for Swine, Vetsuisse Faculty, University of Bern, Switzerland; <sup>4</sup>Veterinary Practice Entenfellner, Stössing, Austria

Email: alexander.grahofner@usys.ethz.ch

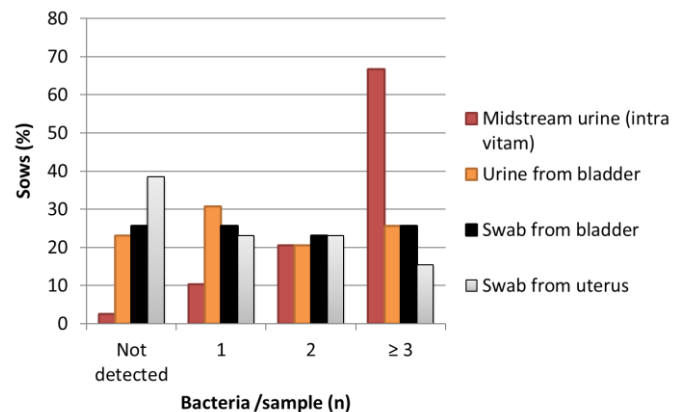
**Introduction** Reduced fertility in sows, often caused by urinary tract infection (UTI), plays a major role in sow mortality and their replacement, thus causing economic losses to piglet producers. The aim of this study was to analyse the prevalence of UTI in sows with reproductive failure and to compare different sample sites and methods to diagnose UTI. Moreover the bacteriological aetiology of cystitis in sows located in Lower Austria and the correlation with urine analysis data was examined.

**Materials and Methods** Thirty-nine sows with reproductive disorders from 9 farms in Lower Austria were sampled. Midstream urine was examined macroscopically and physically-chemically one day before slaughter. Another urine sample was taken under sterile conditions directly out of the urinary bladder immediately after slaughter. Furthermore, swabs were taken of bladder and uterus mucosa. A bacterial investigation of all four matrices was conducted.

**Results** More than three fourths (84.6%) of the sows showed a UTI (Fig.1). Nearly identical culture results were obtained from urine collected immediately after slaughter and from swabs of bladder mucosa. Samples obtained after slaughter most often yielded pure cultures of one bacterium, whereas midstream urine samples often led to isolation of two or more different bacterial species (Fig.2). The most frequently detected bacteria were *Escherichia coli* in 64.1% of the samples,  $\alpha$ -hemolytic streptococci in 34.6% and *Staphylococcus hyicus* in 21.8%. No statistically significant correlation between the physical and chemical parameters and the bacteriological results were found.



**Figure 1** Overview of most frequently detected bacteria in the collected samples.



**Figure 2** Differences between the number of bacteria per sample and the used sample.

**Conclusions** In this study also results with low quantity of bacterial agents (+ growth compared to ++ and +++ growth) were considered. So it was difficult to interpret culture results for midstream urine samples, likely due to environmental contamination. Therefore, any utilization of a similar strategy of interpretation in practice may lead to an overestimation of UTIs. Apart from this, pathogens not specific for UTI were found and might have led to reproductive disorders in the sow herds. It was concluded that sterile urine or swabs from the bladder of culled sows should be tested, if there are problems with the genitourinary system, i.e. presumption of UTIs as a herd problem.



### Metabolism and host-pathogen interactions shape the development of uterine disease in dairy cows

I. M. Sheldon

Swansea University Medical School, Singleton Park, Swansea, United Kingdom

Email: [i.m.sheldon@swansea.ac.uk](mailto:i.m.sheldon@swansea.ac.uk)

Multiple pathogenic bacteria are present in the uterus of cattle after parturition. Controlling these bacteria to maintain uterine health depends on a robust immune response to resist the pathogens, and the ability to tolerate the damage that pathogens cause (Sheldon et al, 2019). Unfortunately, metabolic stress impairs resistance and tolerance, and 20 to 40% of modern dairy cattle develop some form of postpartum uterine disease.

Resistance to pathogens in the uterus is highly dependent on innate immunity, which relies on receptors on host cells binding to pathogen-associated molecular patterns, such as bacterial lipopeptides and lipopolysaccharide. Activation of innate immunity leads to the secretion of inflammatory mediators, including chemokines and cytokines. These inflammatory mediators attract and regulate the neutrophils that clear pathogens and damaged cells from the endometrium. Of course, neutrophils and damaged cells form the pus that is the cardinal sign of postpartum uterine disease.

The innate immune response scales to match the severity of the pathogen challenge and tissue damage. However, innate immunity is metabolically demanding, and glucose and glutamine are important to fuel the inflammatory response in the endometrium. Conversely, metabolic stress, caused by limiting glucose or glutamine, impairs the ability to mount robust inflammatory responses. The close integration of immunity and metabolism is highlighted by the regulation of endometrial inflammatory responses by AMP-activated protein kinase (AMPK), which is the most fundamental sensor of cellular energy homeostasis (Turner et al, 2016).

Tolerance is the ability to cope with the presence of pathogens by limiting the tissue damage they cause. Tissue damage in the endometrium is caused by peripartum trauma and by pore-forming toxins secreted by a wide range of pathogenic bacteria. For example, *Trueperella pyogenes* secretes pyolysin, which is a cholesterol-dependent cytolysin that forms pores in cholesterol-rich areas of the plasma membrane of cells. The impact of pore-forming toxins is dependent on the type of cell, with endometrial epithelial cells more tolerant to pyolysin than stromal cells. However, cell metabolism also modulates tolerance, with glucose or glutamine deprivation impairing the ability of cells to tolerate cholesterol-dependent cytolysins. Conversely, reducing cellular cholesterol increases cell tolerance to pyolysin, as does inhibiting enzymes in the cholesterol synthesis pathway (Griffin et al, 2018).

In conclusion, postpartum uterine disease is a consequence of compromised resistance and/or failure to tolerate the pathogens in the endometrium. The severity of disease is shaped by the inflammatory response to pathogenic bacteria and the damage pathogens cause. Metabolic stress increases the risk of uterine disease by impairing innate immunity and tolerance. Enhancing the ability of animals to tolerate pathogens and controlling metabolic stress may help counter the impact of uterine disease on animal health and production.

#### References

- Griffin S, Healey GD, Sheldon IM (2018) Isoprenoids increase bovine endometrial stromal cell tolerance to the cholesterol-dependent cytolysin from *Trueperella pyogenes*. *Biology of Reproduction*, 99(4), 749–760
- Sheldon IM, Cronin JG, Bromfield JJ (2019) Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. *Annual Review of Animal Biosciences*, 7, 361–384
- Turner ML, Cronin J.G, Noleto PG, Sheldon IM (2016) Glucose availability and AMP-activated protein kinase link energy metabolism and innate immunity in the bovine endometrium. *PLOS ONE*, 11(3), e0151416

### **Repeat breeding: The cause and the possible treatment to restore fertility**

S. Katagiri, Y. Yanagawa and M. Nagano

Laboratory of Theriogenology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

Email: [katagiri@vetmed.hokudai.ac.jp](mailto:katagiri@vetmed.hokudai.ac.jp)

**Introduction** Poor reproductive efficiency is a worldwide problem that has affected the dairy industry during the last several decades. In an attempt to explain the changes in reproductive physiology caused by high milk production, a model of elevated steroid metabolism in lactating dairy cows has been proposed. A slow increase in levels and low peak levels of estradiol ( $E_2$ ) and progesterone ( $P_4$ ) characterize endocrine changes in high producing cows. Similar changes have been reported in repeat breeder cows. The abnormal changes in  $E_2$  and  $P_4$  concentrations of these cows may cause an improper uterine environment due to disturbed expression of growth factors and cytokines in the endometrium. This presentation focuses on the alteration in epidermal growth factor (EGF) profile in the uterine endometrium during the estrous cycle.

**Seeking hidden causes of infertility** The reasons for the decline in fertility of modern dairy cows are multifactorial; thus, it is most important to exclude animals with any known causes of infertility when seeking hidden causes of infertility in repeat breeder cows. The study should exclude cows with any signs of abnormality in the reproductive organs and function. Repeat breeder cows should be examined for inflammation of the uterus by using ultrasonography and endometrial cytology and for oviductal patency. Cows showing extended estrous periods, delayed ovulation and those that fail to show clear signs of estrus should also be excluded from the study. In addition, cows conceived by either 1 or 2 artificial inseminations (AI) immediately after the study might be used as the normal (fertile) control cows. The strict selection policy for recruitment of animals for a study may be essential to enabling the detection of a hidden difference between the repeat breeder and normal cows.

**Dairy cows failed to conceive after 3 AIs** In our study with about 2,500 cows in 18 farms, 397 cows (16%) failed to conceive after 3 AIs, but we found potential causes of infertility in about half of them (8.0%) after detailed examinations as above. The potential causes are an extended estrous period (9.1%, 36 cows), an irregular estrous cycles (11.6%, 46 cows), delayed ovulation (6.3%, 25 cows), periparturient disorders (4.5%, 18 cows), mastitis and foot diseases (6.3%, 25 cows), urovagina (4.5%, 18 cows), inflammation of the uterus (6.3%, 25 cows), abnormal oviductal patency (1.5%, 6 cows). When cows were treated to the identified causes of infertility, 40 to 66% of them, depending on the causes, conceived by 2 AIs. While we could not find any causes in the other half (198 cows) of cows. They were diagnosed as repeat breeders. We use the same strict selection of repeat breeder cows in all studies.

**Endometrial EGF and fertility** Growth factors and cytokines in the endometrium form a regulatory network of uterine function. The network is primarily regulated by  $E_2$  and  $P_4$ . EGF is one of components of the network and has been shown to replace  $E_2$  action in the uterus. In cows, preimplantation embryos express EGF receptors, but not EGF ligand. This may indicate that EGF acts as a maternal signal to an embryo to support its development or survival. The normal cow has two peaks of endometrial EGF concentrations on Days 2-4 and 13-14. In fertile animals, peak levels of EGF concentrations were higher in heifers than cows and the concentrations decreased as the number of parities increased in Holstein cows. Low concentrations of EGF on these days distinguished both high-producing and repeat breeder cows from normal cows. Alteration of the EGF profile could be found in 70 and 25% of repeat breeder and high-producing Holstein cows between 50 and 90 days postpartum, respectively. In an embryo transfer experiment with 439 recipient cows, those with low endometrial EGF concentrations (5 mg/g tissue weight) on Day 3 showed a lower conception rate (33%) than controls (77%). Size of embryos on Day 16 of pregnancy was smaller in cows with low EGF concentrations on Day 3 than controls with the normal EGF profile. Further, an abnormal EGF profile persisted over the 3 estrous cycles, if cows were not treated. This abnormality was found less frequently in Japanese Black (JB) repeat breeder cows (about 20%) than Holstein cows. However, many of JB repeat breeder cows with low EGF peaks were with high BCS.

**Hormonal treatment to normalize EGF profile and fertility** Treatment with a high dose (5 mg) of estradiol benzoate and an intravaginal progesterone-releasing device restored the normal EGF profile and restored fertility. In a controlled study, the treatment normalized the EGF profile in about 70% and restored fertility in about 60% of treated cows. However, efficacy of this treatment protocol differed between farms (25-70%) and decreased in the presence of some factors (high levels of lactation, obesity, heat stress).

**Treatment with seminal proteins** Recently, we have shown that seminal plasma (SP) contains an activity to normalize the endometrial EGF profile and restore fertility in repeat breeder cows. When SP was infused into the vagina, the concentrations of EGF in the uterus increased in about 50-70% of repeat breeder cows. The activity of SP on the EGF profile differed between bulls, but the activity was consistent between ejaculates from the same bulls. The effect of SP treatment on fertility was consistent between farms. However, repeat breeder Holstein cows under heat stress and repeat breeder JB cows with high BCS (> 4.0) did not respond to SP treatment as well as hormonal treatment. Interestingly, repeat breeder JB cows became responders to treatment after reducing BCS by 0.75 or greater.

**Identification of seminal proteins** SP was separated by gel chromatography and SDS-PAGE. The activity was found in protein fractions with molecular weight of 16-29kDa with pI5.8-7.0. This protein fraction contains 14 protein spots on 2D-PAGE. Protein spots were subjected to TOF-MS and 11 protein spots have been identified. One of the 14 protein spots showed the activity to increase the EGF concentrations on Day 3 in a dose-dependent manner in repeat breeder and high-producing cows.

**Conclusions** We could identify a common abnormality in strictly-selected repeat breeder cows. This abnormality of the EGF profile in the uterine endometrium could be corrected by hormonal treatment and seminal proteins. We have identified seminal proteins in the protein fraction with the activity to normalize the EGF profile. Currently, we are preparing a field study to confirm the activity of the identified seminal proteins. This study will provide a new strategy to deal with repeat breeding in dairy cows.

### Factors affecting reproductive wastage in beef cattle herds

D. A. Kenny<sup>1,3</sup>, M. G. Diskin<sup>2,3</sup> and P. Lonergan<sup>3</sup>

<sup>1</sup>Teagasc Animal and Bioscience Research Department, Grange Research Centre, Dunsany, Co. Meath, Ireland; <sup>2</sup>Mellowes Research Centre, Athenry, Co. Galway, Ireland; <sup>3</sup>Agriculture and Food Science Centre, University College Dublin, Belfield, Dublin, Ireland  
Email: david.kenny@teagasc.ie

Worldwide, a reproductively efficient beef cow herd is fundamental to meeting the protein and specifically, red meat demand of an ever increasing global human population. However, achieving a high level of reproductive efficiency is underpinned by producers recognizing and attaining many key targets throughout the production cycle and requires significant technical competency (Diskin and Kenny, 2014). While not receiving the same degree of attention as for their dairy contemporaries, the impact of poor fertility in beef cattle is arguably of greater economic significance, given that the production of a weaned calf is the primary output from beef cow based enterprises. Following insemination the greatest increment of cow reproductive wastage occurs in the form of early embryo mortality with approximately 80% of this occurring within 14-16 days (Diskin et al., 2016). Coincidentally, during this period, embryo development is occurring at an exponential rate and is heavily reliant on its maternal uterine environment (Sánchez et al., 2018). Although the extent of late embryo loss is numerically lower than early embryo mortality, it causes serious economic losses in all production systems, but particularly in seasonal calving herds because it is often detected after the end of the season breeding period resulting in increased culling rates.

The lifetime productivity of the beef bred female commences from the onset of puberty and is dictated by subsequent critical events including age at first calving, duration of the postpartum interval for each successive calving, conception and pregnancy rate and ultimately manifested as length of intercalving intervals and number of calves weaned over her lifetime. Puberty in heifers is a consequence of the interactive effects of genetics and both pre and post-weaning nutrition. Early onset of puberty is essential to achieving the first main reproductive target for beef cow herds; first calving at two years of age. Our work and others has now clearly shown that nutrition and in particular during calfhood, critically influence the rate of sexual development and in particular the ontogeny of the hypothalamic-pituitary-ovarian axis (Kenny et al., 2018). In calved heifers and mature cows, the onset of ovarian activity, postpartum is a key event dictating the calving interval. Again, this will be the product mainly of prepartum nutrition, manifested through body condition and the strength of the maternal bond between cow and calf, though there is increasing evidence of genetic influence, while modest, on this trait. Following the initiation of postpartum ovarian cyclicity, conception and subsequent pregnancy rate is generally a function of bull fertility in natural service herds and heat detection and timing of insemination in herds bred through AI. Where natural service is practiced producers must be cognisant of the potential for infertility and perhaps more worryingly, subfertility in their stock sires. Breeding females should be maintained on a steady plane of nutrition during the breeding season, but the contribution of significant excesses or deficiencies of nutrients including protein and trace elements is likely to be minor where adequate pasture is available. Many bacterial, viral, protozoan and fungal infections have been associated with early embryo, late embryo, fetal and abortion losses, as well as with perinatal losses in cattle. While the overall impact of pathogens on the extent of reproductive wastage in beef cattle is likely to be small, where such losses do occur, they are often incurred from mid to late gestation; therefore representing a potentially significant economic loss to the producer and highlighting the importance of control measures to prevent infection. Systemic concentrations of progesterone during both the cycle preceding and following insemination affect embryo survival, with evidence of either excessive or insufficient concentrations being negatively associated with survival rate. Genomic technology will not only provide scientists with an improved understanding of the underlying biological processes involved in fertilisation and the establishment of pregnancy, but also, in the future, could identify genes responsible for improved embryo survival. Indeed, data from our own laboratory have revealed remarkable differences in uterine endometrial transcriptomes of high compared to low fertility heifers during both the mid and late luteal phases of the oestrous cycle. Such information could be incorporated into breeding objectives in order to increase the rate of genetic progress for embryo survival. While, increased efforts are being made internationally to genetically identify and select for more reproductively efficient beef cows, this is a more long-term strategy and will not replace the necessity for a high level of technical efficiency and management practice at farm level. The correction of minor deficits in several areas can have a substantial cumulative positive effect on herd reproductive performance.

### References

- Diskin M.G. and Kenny D.A. 2014. Optimising reproductive performance of beef cows and replacement heifers. *Animal*. Suppl. 1:27-39.  
Diskin M.G., Waters S.M., Parr M.H. and Kenny D.A. 2016. Pregnancy losses in cattle: potential for improvement. *Reproduction, Fertility and Development*. 28: 83-93.  
Kenny D.A., Heslin J. and Byrne C.J. 2018. Early onset of puberty in cattle: implications for gamete quality and embryo survival. *Reproduction, Fertility and Development*. 30: 101-117.  
Sánchez J.M., Mathew D.J., Passaro C., Fair T., Lonergan P. (2018). Embryonic maternal interaction in cattle and its relationship with fertility. *Reprod Domest Anim*. Suppl 2:20-27.

### Sensing sperm by maternal immune crosstalk: A potential mechanism for interfering with fertility in the cow

A. Miyamoto<sup>1</sup>, M. A. Ezz<sup>1,2</sup>, I. Akthar<sup>1</sup>, M. S. Yousef<sup>1,3</sup>, K. Imakawa<sup>1,4</sup>, M. Shimada<sup>1,5</sup> and M. A. Marey<sup>1,6</sup>

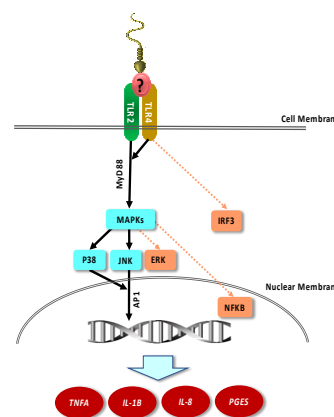
<sup>1</sup>Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; <sup>2</sup>Mansoura University, Mansoura, Egypt; <sup>3</sup>Assiut University, Assiut, Egypt; <sup>4</sup>Tokai University, Kumamoto, Japan; <sup>5</sup>Hiroshima University, Higashi-Hiroshima, Japan; <sup>6</sup>Damanhur University, Behera, Egypt

Email: akiomiya@obihiro.ac.jp

The female genital tract, one of the initial barriers for foreign antigens, orchestrates pivotal functions in reproduction such as sperm transport, fertilization, conceptus implantation, placentation, and maintenance of pregnancy until parturition. Concurrently with these functions, the uterus and oviduct require a defence system against invading pathogens as well as tolerating allogenic sperm and semi-allogenic embryo. Therefore, the uterine and oviduct milieu should be equipped with a well-developed and strictly controlled immune system that can respond effectively to various antigens to which it is exposed.

At artificial insemination (AI), millions of sperm are deposited into the bovine uterus, but most of these sperm are eliminated and only a few thousands reach the oviduct where fertilization takes place. Recently, we demonstrated that live, but not dead, sperm attach to bovine endometrial epithelial cells (BEECs) *in vitro* which subsequently produce an acute pro-inflammatory response [1]. Using a newly developed *ex-vivo* uterine explant culture model, it was shown that sperm immediately penetrate into endometrial glands, and activate a local innate immune response, suggesting that endometrial glands are working as a sperm-sensing system which triggers the whole cascade for uterine pro-inflammatory response to sperm after AI. Furthermore, it became evident that the recognition of sperm-inflammatory response in BEECs was mediated by TLR2/4 signalling pathway *in vitro* [2]. Indeed, a time-lapse *in vivo* investigation of the local appearance of sperm and PMNs in uterine horn illustrated that a number of sperm pass through the uterine horn within 1 h post-AI, and completely disappear from uterine horn 6 h after AI. On the other hand, PMNs appear after 3 h, reach maximum 6 h and disappear 10 h after AI. This rapid removal of sperm could help in preventing acquired immune responses against paternal antigens and at the same time, eliminating the ascending infection that may be impregnated with sperm during AI.

Once sperm reach the oviduct, they attach to epithelia of the isthmus and form a so-called sperm reservoir, but some move to the ampulla where fertilization occurs. In contrast to the uterine milieu, it was shown that sperm attachment to bovine oviduct epithelial cells (BOECs) induces gradual anti-inflammatory response with up-regulation of IL10 and TGFβ *in vitro* [3]. Moreover, LH-stimulated BOEC conditioned media suppressed sperm phagocytosis by PMNs that identified in the oviduct fluid in order to support sperm survival until fertilization [4]. Importantly, these anti-inflammatory responses of BOECs to sperm were disrupted by the semi-physiological concentrations of urea or mycotoxin, which derived from concentrate feeding and deteriorated silage to dairy cows, respectively [5,6]. The findings suggest that nutritional and environmental factors from forage disrupt the oviduct immunity toward sperm, thus the similar negative impact could be induced in the uterine immunity. In particular, it is essential to investigate such an interaction with TLRs that sense sperm as well as pathogens in the uterus.



**Figure 1.** A working hypothesis of the mechanism by which sperm activate TLR2/4 signaling pathway in BEECs [2].

**Conclusions** It became evident that the sperm sensing system to generate necessary maternal immune crosstalk is strictly regulated based on the local balance of Th1/Th2 in the uterus and the oviduct. This immune balance could be easily disrupted by various risk factors including those derived from undesirable nutrition and environment, thus may negatively affect fertility of dairy cows.

#### References

- 1) Elweza AE, Ezz MA, Acosta TJ, Talkder AK, Shimizu T, Hayakawa H, Shimada M, Imakawa K, Zaghloul AH, Miyamoto A. A proinflammatory response of bovine endometrial epithelial cells to active sperm *in vitro*. *Mol Reprod Dev* **85**: 215-226. (2018)
- 2) Ezz MA, Marey M, Elweza A, Kawai T, Heppelmann M, Pfarrer C, Balboula A, Montaser A, Imakawa K, Zaabel S, Shimada M, Miyamoto A. TLR2/4 signaling pathway mediates sperm-induced inflammation in bovine endometrial epithelial cells *in vitro*. *PLoS ONE* **14**: In press. (2019)
- 3) Yousef MS, Marey MA, Hambruch N, Hayakawa H, Shimizu T, Hussien HA, Abdel-Razek ARK, Pfarrer C, Miyamoto A. Sperm binding to oviduct epithelial cells enhances TGFβ1 and IL10 expressions in epithelial cells as well as neutrophils *in vitro*: Prostaglandin E2 as a main regulator of anti-inflammatory response in the bovine oviduct. *PLoS ONE* **11**: e0162309. (2016)
- 4) Marey MA, Yousef MS, Kowsar R, Hambruch N, Shimizu T, Pfarrer C, Miyamoto A. Local immune system in oviduct physiology and pathophysiology: Attack or tolerance? *Dom Anim Endocrinol* **56**: S204-S211. (2016)
- 5) Kowsar R, Marey MA, Shimizu T, Miyamoto A. Urea induces T helper 2 (Th2) type environment at transcriptional level and prostaglandin E2 secretion in bovine oviduct epithelial cells in culture. *J Dairy Sci* **99**: 5844-5850. (2016)
- 6) Yousef MS, Takagi M, Talukder A, Marey M, Rasoul K, Abdel-Razek ARK, Shimizu T, Fink-Gremmels J, Miyamoto A. Zearalenone (ZEN) disrupts the anti-inflammatory response of bovine oviductal epithelial cells to sperm *in vitro*. *Reprod Toxicol* **74**: 158-163. (2017)

### Relationship between resumption of ovarian activity, days open, energy balance and metabolic status in dairy cows with different dry period lengths in early lactation

J. Ma<sup>1</sup>, R. J. van Hoeij<sup>1</sup>, T. J. G. M. Lam<sup>2</sup>, B. Kemp<sup>1</sup> and A. T. M. van Kneegsel<sup>1</sup>

<sup>1</sup>Adaptation Physiology Group, Wageningen University & Research, The Netherlands; <sup>2</sup>Department of Farm Animal Health, Utrecht University, The Netherlands

Email: [junnan.ma@wur.nl](mailto:junnan.ma@wur.nl)

**Introduction** Negative energy balance (NEB) in dairy cows results from a fast increase in milk production post calving while feed intake is limited in this period. NEB is accompanied with an altered metabolic status, which triggers metabolic disorders. Metabolic status in early lactation is related with reproductive performance, e.g. reduced concentrations of insulin and IGF-I, which contributes to reduced follicular responsiveness to gonadotrophic stimulation, and thus prevents the dominant follicle to ovulate, resulting in a delay in the resumption of cyclicity. Omitting or shortening the dry period (DP), adjusting dietary energy level or feeding different dietary energy sources is of interest because that could minimize the risk of NEB, postpartum metabolic diseases and suboptimal fertility like delayed resumption of postpartum ovarian cyclicity. The aim of this study is to investigate the effect of DP length, dietary energy level, dietary energy source and interactions among these factors on fertility (resumption of ovarian cyclicity and days open) of dairy cows postpartum. Additionally, the relation between energy balance and metabolic status of dairy cows during early lactation and resumption of ovarian cyclicity and days open will be evaluated.

**Materials and Methods** Holstein-Friesian dairy cows (N=128) were blocked for expected calving date, milk yield in the previous lactation and parity. Within each block of 6 cows, 4 cows were randomly assigned to no dry period (0DP) and 2 cows to a short dry period of 30 days (30DP). Cows with 0DP were randomly assigned to either low dietary energy level (LOW) or standard dietary energy level (STD). Standard dietary energy level was based on the energy requirement for expected milk yield in 30DP cows. All 30DP cows were fed STD. Low dietary energy level was based on the energy requirement for expected milk yield in 0DP cows. All cows were fed either glucogenic (GLU) or lipogenic (LIPO) ration in week 8-44. All cows were allocated in one of 6 treatment groups: 0DP-LOW-GLU (n=22), 0DP-LOW-LIPO (n=20), 0DP-STD-GLU (n=23), 0DP-STD-LIPO (n=20), 30DP-STD-GLU (n=21), 30DP-STD-LIPO (n=22).

Milk samples were collected thrice a week from the day of parturition until 100 DIM. Milk progesterone (P4) concentration was measured by enzyme immunoassay to analyse postpartum onset of luteal activity. Days open was defined as the number of days from parturition to conception. Plasma glucose, NEFA, BHBA, IGF-1, insulin concentration and EB were measured weekly from 4 weeks prepartum until 7 weeks postpartum. Blood was collected weekly from 3 weeks prepartum until 7 weeks postpartum. Statistical analyses were performed using repeated measures analysis in a mixed linear model (PROC MIXED) in SAS, using cow as the repeated subject.

**Table1** Metabolic status and energy balance of dairy cows during early lactation after 0d and 30d dry period categorised according to days open (LSM  $\pm$ SEM).

	Days open			SEM	P-value				
	<80d	80-130d	>130d	Not pregnant	Days open	DPL	Parity	Days open $\times$ parity	Days open $\times$ DPL
Cows (n)	36	35	37	20					
Glucose (mmol/l)	3.86	3.81	3.81	3.71	0.62	0.89	<0.01	0.04	0.73
NEFA (mmol/l)	0.17 <sup>b</sup>	0.21 <sup>ab</sup>	0.25 <sup>a</sup>	0.26 <sup>a</sup>	0.03	0.04	0.04	0.08	0.01
BHBA (mmol/l)	0.72	0.75	0.85	0.71	8.53	0.23	0.01	<0.01	0.26
IGF-1 (ng/ml)	122.26	116.49	111.48	105.99	0.80	0.72	0.73	0.37	0.42
Insulin ( $\mu$ IU/ml)	14.88 <sup>a</sup>	14.07 <sup>a</sup>	13.75 <sup>ab</sup>	12.12 <sup>b</sup>	0.98	<0.01	<0.01	0.42	<0.01
EB (kJ/kg <sup>0.75</sup> $\times$ day)	-48.34 <sup>a</sup>	-93.09 <sup>b</sup>	-92.98 <sup>b</sup>	-118.71 <sup>b</sup>	31.64	<0.01	0.09	0.90	<0.01

**Results** Cows in 0D(LOW) group had less days postpartum to onset of luteal activity compared with cows in 30D(STD) (19.43 vs 27.33 days) ( $P<0.01$ ). Cows in 0D(LOW) group had less days open compared with cows in 0D(STD) group (96 vs 129) ( $P<0.01$ ). Cows with less days postpartum (<21d) to onset of luteal activity had higher concentration of insulin in plasma (15.85 vs 11.47) ( $P<0.01$ ) and a lower NEB (-17.84 vs -170.01) ( $P<0.01$ ) compared with cows with more days postpartum (>30d) to onset of luteal activity. Cows with less days open (<80d) had lower concentration of NEFA in plasma and greater concentration of insulin and also lower NEB compared with cows with a long days open period (>80d) (Table1).

**Conclusions** Omitting the DP (0D) reduced the interval from calving to onset of luteal activity compared with a 30D DP in dairy cows. Within 0D groups, cows with low energy level (0D-LOW) had less days open compared with cows with standard energy level (0D-STD) group. A low number of days open (<80d) was associated with less severe NEB and better metabolic status, as reflected by increased insulin and decreased NEFA concentrations in plasma during weeks 1 to 7 weeks postcalving.

### Effect of lecorelin acetate intravaginal administration on rabbit reproductive parameters and welfare

C. Munari<sup>1</sup>, P. Ponzio<sup>1</sup>, A. R. Alkhawagah<sup>2</sup>, A. Schiavone<sup>1</sup> and C. Mugnai<sup>1</sup>

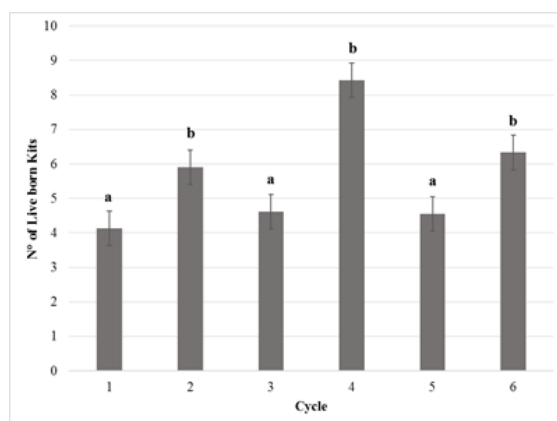
<sup>1</sup>Department of Veterinary Sciences, University of Turin, Grugliasco (TO), Italy; <sup>2</sup>Theriogenology Department, Faculty of Veterinary Medicine, Benha University, Egypt

Email: chiara.munari@unito.it

**Introduction** The most frequently used method for ovulation induction in commercial rabbit does is the intramuscular administration of gonadotropin-releasing hormone (GnRH) or its synthetic analogues. Unfortunately, these exogenous substances may negatively affect rabbit welfare and cause fertility disorders in does, such as oocytes maturation alteration (Zapletal and Pavlik, 2008). Moreover, hormonal treatments, particularly when repeatedly used, are generally followed by a decreased fertility due to the appearance of plasmatic anti-GnRH antibodies (Theau-Clement et al. 2008). In 2005, The European Food Safety Authority (EFSA) recommended that pharmacological treatments should be used in a limited manner, as infrequently as possible, and that they have to be replaced, if possible, with alternative methods having no impact on animal welfare. Several authors recently demonstrated ovulation induction by including GnRH analogues directly into the seminal dose, and administering them through vaginal absorption (Dal Bosco et al. 2014). The aim of the present study was to investigate if lecorelin acetate, a Gonadotropin Releasing Hormone analogue, could be used for does insemination by its inclusion in the seminal dose.

**Materials and Methods** Twenty does of 9 months age were individually housed and divided into two experimental groups: control group which received 0.2 ml of intramuscular (C group) lecorelin (Dalmarelin, Fatro®) and intravaginal group (IV group) inseminated by adding 0.3 ml of the same GnRH in the seminal dose. The experiment was performed for six consecutive reproductive cycles with an intensive rhythm (artificial insemination 11 days *post partum*). Sperm motility and morphological characteristics were evaluated on each of the heterospermic pooled semen before each insemination. Descriptive statistics were used to prove the significant differences in seminal parameters including sperm motility, livability and acrosomal status (expressed as mean  $\pm$  SE) and reproductive parameters including receptivity, fertility and live-born kits (expressed as mean  $\pm$  SD) between the 2 experimental groups (C and IV group) during the different insemination cycles. Multiple comparisons of the means were done with Duncan test and P value was set at  $< 0.05$ .

**Results** Does inseminated by intravaginal addition of lecorelin acetate showed a higher or equal sexual receptivity compared to the control group ( $P < 0.001$ ), which resulted in a higher fertility rate as the cycle increased. Regarding the number of live-born kits, only the cycle resulted significant ( $P < 0.01$ ). The volume of the seminal dose was very low and this could explain the best results of the intravaginal group which had a similar amount of hormone (0.3 ml/doe) to the control (0.2 ml/doe). Furthermore, the negative performance of the control group may be due to the formation of anti-GnRH antibodies and to a more stressful method for ovulation induction.



**Figure 1** Effect of insemination cycle on live-born kits number.

Values are expressed as mean  $\pm$  SD;

a, b: significant difference between different letters ( $P < 0.01$ ).

**Conclusions** In conclusion, our present findings indicated that GnRH can be used for ovulation induction in rabbit does, by its incorporation in the seminal dose, reaching levels of sexual receptivity, fertility and number of live born kits equal or higher than conventional administration.

### References

- Dal Bosco A, Cardinali R, Brecchia G, Rebollar PG, Fatnassi M, Millan P, Mattioli S, Castellini C. Induction of ovulation in rabbits by adding Lecirelin to the seminal dose: in vitro and in vivo effects of different excipients. *Anim Reprod Sci* 2014;150:44-9.
- Theau-Clement M, Lebas F, Beckers JF, Drion PV. Evolution of anti-eCG antibodies in response to eCG doses and number of injections. Relationship with productivity of rabbit does. *Animal* 2008;2:746-51.
- Zapletal D, Pavlik A. The effect of lecorelin (GnRH) dosage on the reproductive performance of nulliparous and lactating rabbit does. *Anim Reprod Sci* 2008;104:306-15.

### Extended lactation in high-yielding dairy cows: effects on reproductive performance and milk production

G. Niozas<sup>1</sup>, G. Tsousis<sup>2</sup>, I. Steinhöfel<sup>3</sup>, A. Römer<sup>4</sup>, S. Wiedemann<sup>5</sup>, H. Bollwein<sup>6</sup> and M. Kaske<sup>6</sup>

<sup>1</sup>University of Veterinary Medicine, Hannover, Germany; <sup>2</sup>Aristotle University, Thessaloniki, Greece; <sup>3</sup>Saxon State Office for Environment, Dresden, Germany; <sup>4</sup>State Research Institute of Agriculture and Fishery Mecklenburg-Western Pomerania, Dummerstorf, Germany; <sup>5</sup>Rhine-Waal University of Applied Sciences, Kleve, Germany; <sup>6</sup>Department for Farm Animals, Vetsuisse Faculty, Zurich, Switzerland

Email: mkaske@vetclinics.uzh.ch

**Introduction** Parity is the prerequisite for milk yield. A 12-month calving interval has been proposed traditionally as the economically most beneficial breeding pattern, i. e., cows should conceive around 85 d p.p. However, the remarkable increase in milk yield in dairy cattle over the past decades has been accompanied by a decrease in reproductive performance which makes it more and more difficult to achieve a 12 month calving interval. The objective of this prospective field study was to evaluate the effects of extending the lactation period on various reproductive parameters and milk production of high yielding Holstein cows.

**Materials and Methods** The study took place at a commercial dairy farm in Saxony, Germany. Based on data from 2012, the farm had a total of 1,092 lactating Holstein-Friesian cows and a mean milk production of 11,488 kg/305 d (4.09 % fat, 3.27 % protein). Animals were housed indoors in a free stall barn with concrete slatted floor and cubicles with mattresses. Body condition scoring (BCS) and backfat thickness (BFT) were estimated at the day of calving, at the 15th, 30th, 45th, 60th, 90th, 120th, 180th day of lactation and thereafter every two months until the end of the lactation period. Lactating cows were milked with an automatic milking system (AMS). The AMS provided individual daily milk records throughout the lactation and data concerning milk conductivity and temperature. Milk production including the milk fat, protein, urea and lactose concentration, as well as SCC were measured monthly by the State Control Association (Landeskontrollverband). On d 40 postpartum, cows were examined gynecologically (transrectal palpation, sonography, vaginoscopy). Cows without signs of clinical endometritis were blocked by parity and were randomly allocated to one of three experimental groups with a voluntary waiting period (VWP) of 40, 120 and 180 days, respectively (G40, n=135; G120, n=141; G180, n=139). Cows of G120 and G180 were reexamined at the end of the VWP. If natural estrus was detected within 46 d after the end of the VWP, an AI was performed. If no estrus was detected, the respective cows were synchronized by applying the classical OvSynch protocol. A pregnancy check was conducted by sonography between d 42 and 49 post insemination (p.i.). A second pregnancy check by rectal palpation was performed within 90-100 days after successful AI. For cows diagnosed as pregnant 40 d p.i. and empty 100 d p.i., embryonic or early fetal mortality was stated.

**Results** There was no difference in the proportion of cows that expressed estrus between 40 to 86 DIM or in the days to first estrus between the three groups. The proportion of cows that expressed estrus within 46 d after the VWP was greater in G120 (88.9 %) and G180 (90.8 %) compared to G40 (70.4 %). These effects were more apparent in cows with high ECM. The rate of estrus expression and of becoming pregnant in this period was greater for G120 (hazard ratio 2.2 and 1.6, respectively) and for G180 (hazard ratio 2.4 and 1.8) compared to G40. Cows in both groups with extended lactation had greater overall first service conception rates (G120: 48.9 %; G180: 49.6 %) and a lower number of services per pregnant cow (G120:  $1.56 \pm 0.1$ ; G180:  $1.51 \pm 0.1$ ) compared to G40 (36.6 %;  $1.77 \pm 0.1$ ). There was no difference in pregnancy loss or in the proportion of cows culled up to 305 d of lactation between the three groups. Regarding daily milk production (kg/milking day) throughout the study period, G40 performed better than G180 (LSmeans 37.1 vs. 35.0 kg, respectively), with the values of G120 being intermediate (36.2 kg). However, regarding daily Energy Corrected Milk (ECM) production, there was no difference between the 3 groups (LSmeans 35.6 vs. 35.2 vs. 34.7 kg for G40, G120 and G180, respectively). In late lactation (201 to 320 days in milk) G40 had lower average productivity (LSmeans 30.4 kg) compared to G120 (32.6 kg) and to G180 (32.4 kg). In the extended lactation groups, primiparous cows showed greater production compared to multiparous (29.0 vs. 27.3) from day 320 to day 400 of lactation. There was no difference between the three groups regarding the evaluated udder health parameters (development of somatic cell count, incidence of mastitis and days off milk due to mastitis). More cows in G180 (7.9 %) were culled due to low productivity compared to G40 (0.7 %) and as a tendency compared to G120 (2.8 %). Moreover, cows of G180 showed higher median BCS at the time of dry-off compared to cows of both G40 and G120 (3.50 for G180 vs. 3.25 for both G40 and G120). At the time of dry-off, cows of the G180 had also greater backfat thickness (25.0 mm) compared to both cows of the G40 (22.2 mm) and of the G120 (21.6 mm).

**Conclusions** Extending the lactation of dairy cows can improve reproductive parameters in high yielding cows. These positive effects are more prominent in higher yielders (in our study the 50 % of cows with the higher milk production) than low yielders. The extension of the voluntary waiting period of high yielding cows up to 120 days has no adverse effects regarding milk production efficiency, udder health or BCS gain. In future, it may be more useful based on the economical framework, to relate the expenditures for fertility to the amount of milk produced. We did that using the means of the three groups investigated. Accordingly, 30-35% less inseminations were needed in G180 and G120 per 10,000 kg ECM compared to G40 (G40: 1.50; G120: 1.02; G180: 0.97 inseminations/10'000 kg ECM). The number of Ovsynch protocols per 1,000,000 kg ECM was reduced by 75 % in G180 and by 74 % in G120 compared to G40 (5.9 vs. 7.1 vs. 25.1).

### References

Niozas G, Tsousis G, Steinhöfel I, Brozos C, Römer A, Wiedemann S, Bollwein H, Kaske M (2019): Extended lactation in high-yielding dairy cows. I. Effects on reproductive measurements. *J. Dairy Sci.* 102, 799-810.

Niozas G, Tsousis G, Malesios C, Steinhöfel I, Boscos C, Bollwein H, Kaske M (2019): Extended lactation in high-yielding dairy cows. II. Effects on milk production, udder health, and body measurements. *J. Dairy Sci.* 102, 811-823.



### Association between uterine involution in sows and reproductive performance in their next gestation

A. Meile<sup>1</sup>, H. Nathues<sup>1</sup> and A. Grahofner<sup>1,2</sup>

<sup>1</sup>Department of Clinical Veterinary Medicine, Clinic for Swine, University of Bern, Switzerland; <sup>2</sup>ETH Zurich, Animal Physiology, Institute of Agricultural Sciences, Zurich, Switzerland

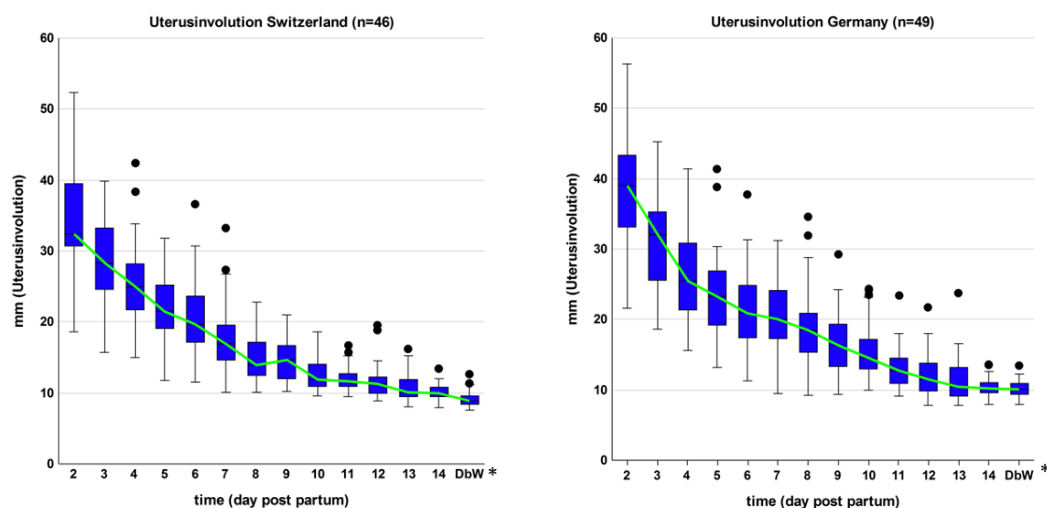
Email: [anja.meile@vetsuisse.unibe.ch](mailto:anja.meile@vetsuisse.unibe.ch)

**Introduction** A physiological uterine involution during the puerperium is essential for the reproductive health of sows. Until today, the uterine involution in sows has mainly been described by macroscopic and histological examination after slaughter. Therefore, the aim of this study was to describe the continuous regression of the uterine diameter from day 2 until day 14 after parturition and on the day before weaning by means of ultrasound in sows housed in a free farrowing system and in farrowing crates, respectively, and to compare these findings with the reproductive performance of the subsequent gestation. Furthermore, it was hypothesized that the reproductive performance of the next litter can be predicted by measuring the diameter of the uterus on single days.

**Materials and Methods** The diameter of three uterus cross-sections was measured in 24-hours intervals in 46 sows housed in a free farrowing system in Switzerland and in 49 sows housed in farrowing crates in Germany. After weaning, the sows were artificially inseminated by one person with a standardized protocol using only semen from one boar in each country. For the evaluation of the reproductive performance the following parameters were collected: the weaning to oestrus interval in days, number of total-born and live-born piglets, as well as the gestation length. Statistical processing of all data was done in NCSS 12 Data. Continuous variables were first tested for normality and homogeneity of variance using a Shapiro-Wilk normality test. Furthermore, the uterus involution was visualized with box plot graphics from day 2 to 14 postpartum and the day before weaning. The association between the uterine involution and the reproductive performance was evaluated with the spearman rank test. Furthermore the odds ratio was calculated. In all tests the level of statistical significance was considered being  $P < 0.05$ .

**Results** Overall, a continuous regression of the uterine diameter was observed during the lactation period in both groups (Figure 1). The median diameter of the uterus decreased from 32.4 mm (min: 18.6 mm, max: 52.3 mm) on day 2 to 9.0 mm (min: 7.6 mm, max: 12.7 mm) on the day before weaning in sows in free farrowing systems. The median diameter of the uterus of sows in farrowing crates decreased from 38.5 mm on day 2 (min: 21.6 mm, max: 56.3 mm) to 10.1 mm (min: 8.8 mm, max: 13.6 mm) the day before weaning. In both groups no relation between the uterine diameter and the weaning to oestrus interval as well as the gestation length could be detected. Only on day 11 a negative correlation between uterine diameter and total-born piglets ( $p = 0.022$ ) as well as live-born piglet ( $p = 0.002$ ) in the next litter was detected.

On day 11 postpartum, sows in the free farrowing system with a uterus diameter smaller than 12 mm showed a 2.2 times higher probability to get more than 16 total-born piglets in the next litter compared to sows with a uterine diameter larger than 12 mm. In addition, the probability of having more than 15 live-born piglets in the next litter increased by 1.4 times in sows with a uterine diameter smaller than 12 mm compared to sows with diameters higher than 12 mm.



**Figure 1** Regression of the uterine diameter (median; min, max) from day 2 to 14 after parturition and one day before weaning of sows in Switzerland and Germany. \*day before weaning

**Conclusions** The ultra-sonographic examination is a suitable method to evaluate the diameter of the uterus during puerperium and to describe intra vitam the physiological uterus involution as shown in this study. However, with the current data, no clear effect of the uterine involution on the reproductive performance in the subsequent gestation could be confirmed.



### Timing of artificial insemination using fresh or frozen semen after automated activity monitoring of estrus in lactating dairy cows

C. M. Tippenhauer<sup>1</sup>, J. L. Plenio<sup>2</sup>, A. M. Madueira<sup>3</sup>, R. L. Cerri<sup>3</sup>, W. Heuwieser<sup>1</sup> and S. Borchardt<sup>1</sup>

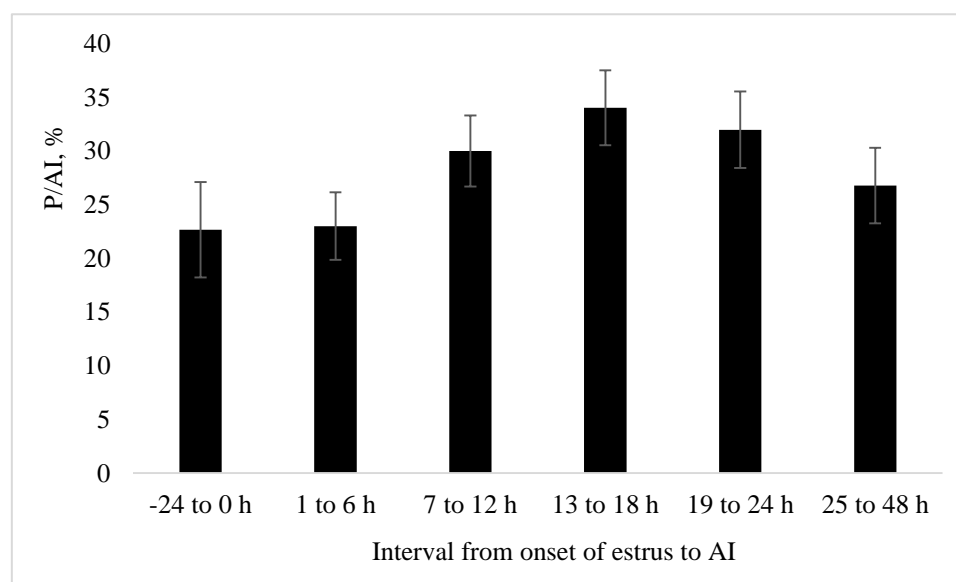
<sup>1</sup>Clinic of Animal Reproduction, Freie Universitaet Berlin, Germany; <sup>2</sup>Institute for Veterinary Epidemiology and Biostatistics, Freie Universitaet Berlin, Germany; <sup>3</sup>Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada

Email: [Stefan.Borchardt@fu-berlin.de](mailto:Stefan.Borchardt@fu-berlin.de)

**Introduction** Timing of artificial insemination (AI) relative to ovulation is of particular importance for pregnancy outcomes. It represents a compromise between fertilization rate and embryo quality. The major challenge is to identify the time of ovulation. Typically, this was based on visual observation of cows eligible to be bred. Poor estrus detection, however, is a major contributor to impaired reproductive performance in high yielding dairy cows in confinement systems. To overcome this challenge automated activity monitoring systems (AAM) have been implemented to improve estrus detection. However, there is only little research on the optimal timing of insemination relative to changes in activity using commercial automatic monitoring systems. The objective of this observational experiment was to determine the association between the interval from reaching an activity threshold (AT) using an AAM and the time of artificial insemination on P/AI for lactating Holstein cows inseminated with either fresh or frozen semen.

**Materials and Methods** Lactating Holstein cows ( $n = 3,420$ ) from 7 commercial dairy farms were inseminated based on visual heat detection and/or based on the alert of an accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel). Pregnancy diagnosis was performed by transrectal palpation  $35 \pm 7$  d after AI. Data were analyzed using the GENLIMIXED procedure in SPSS. Onset of estrus was defined as the time when the AT (index value  $\geq 35$ ) was reached. Estrus intensity was categorized based on peak activity change into low (index value 35 to 80), medium (81 to 99), and high intensity estrus (100).

**Results** The mean ( $\pm$  standard deviation) interval from onset of estrus to AI was  $15.0 \pm 8.9$  hours. Pregnancy per AI was affected by parity ( $P = 0.001$ ), season ( $P = 0.001$ ), estrus intensity ( $P = 0.001$ ), and the interval from reaching AT to AI ( $P = 0.001$ ). Type of semen ( $P = 0.231$ ) and the interaction of type of semen with interval from reaching AT to AI ( $P = 0.981$ ) had no effect on P/AI. Primiparous cows (31.1 %) had higher P/AI compared with multiparous cows (24.9 %). Cows inseminated in the hot season (July until September; 23.7 %) had lower P/AI compared with the cold season (October until January; 32.4 %). Cows with a high intensity estrus (32.7 %) were more fertile compared with medium (29.1 %) or low intensity estrus (22.4 %). There was a quadratic effect of the interval from reaching AT and AI on probabilities of pregnancy. Pregnancy per AI at 35 d after AI was highest for cows inseminated between 13 to 24 hours after onset of estrus ( $-24 - 0$  hours = 22.7 %,  $1 - 6$  hours = 23.0 %,  $7 - 12$  hours = 30.0 %,  $13 - 18$  hours = 34.0 %,  $19 - 24$  hours = 32.0 %,  $25 - 48$  hours = 26.8 %).



**Figure 1** Effect of the interval from onset of estrus to AI on pregnancy per AI (P/AI) using predicted probabilities ( $\pm$  SEM) from the generalized linear mixed model ( $n = 3,420$ ).

**Conclusions** In conclusion, inseminating cows 13 to 24 hours after passing the AT yielded the highest P/AI irrespective of type of semen. Inseminating cows before or 6 h after reaching AT was detrimental on P/AI. A high intensity estrus was favorable for P/AI.

### Intrauterine antibiotic treatment and bacterial growth in dairy cows with mild endometritis at the time of insemination

K. Wagener<sup>1,\*</sup>, P. Panagiotis<sup>2</sup>, U. Reinländer<sup>1</sup>, R. Schlegl<sup>1</sup>, M. Ehling-Schulz<sup>2</sup> and M. Drillich<sup>1</sup>

<sup>1</sup>Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, Vetmeduni Vienna, Austria; <sup>2</sup>Functional Microbiology, Institute for Microbiology, Vetmeduni Vienna, Austria; \*current address: Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Switzerland

Email: karen.wagener@uzh.ch

**Introduction** Clinical endometritis (CE) is one of the most common postpartum diseases in dairy cows (LeBlanc et al. 2002; Sheldon et al. 2006). CE has a significant impact on reproductive performance, e.g. on calving to conception interval and involuntary culling (Borsberry and Dobson 1989). Untreated or severe cases of CE may persist beyond the voluntary waiting period and can be detected at the time of artificial insemination (AI). Insemination of cows with CE is often postponed to the next estrus, although affected cows have a chance to become pregnant but with a lower odds (Lambertz et al. 2014). Another strategy for cows with endometritis at the time of intended breeding is the intrauterine treatment shortly after AI. Decades ago, the so-called Aström method was described as intrauterine infusion of an iodine-potassium solution a few days after AI (Aström 1935). Nowadays, the use of antibiotics instead of iodine solution is common and the treatment is performed a few hours after AI, although scientific evidence of this treatment strategy is poor. Therefore, the objective of this study was to evaluate the effect of this modified Aström method on pregnancy rates (proportion of cows pregnant after AI) and to characterize the intrauterine bacterial flora at the time of insemination.

**Materials and Methods** The study was conducted on a large dairy farm in Slovakia. A total of 323 Holstein Friesian cows were included in the study at the day of AI. Before enrolment, vaginal discharge was evaluated with the Metricheck device. Animals with clear discharge were assigned to a healthy comparison group (HE=112) and animals with cloudy discharge or fleck of pus in the mucus were divided into a treatment and a control group. The treatment group (MET; n=108) received an intrauterine treatment with 500 mg cephapirin (Metricure®, Intervet Deutschland GmbH) 6±1 hours after AI. The control group (CO; n=103) remained untreated. For the characterization of intrauterine bacteria, uterine samples were taken from a subset of cows with (E0; n=58) and without uterine discharge (E1; n=64) by the cytobrush technique. Bacteria were cultivated aerobically and identified by Fourier-transform infrared spectroscopy.

**Results** Pregnancy rate after first AI was similar in all groups (HE 37%; MET 32%; CO 31%). Non-pregnant animals were re-inseminated at the next estrus. For cows with a second AI, pregnancy rate was significantly higher in the treated (MET 59%) compared with the untreated group (CO 44%). Interestingly, the lowest pregnancy rate was observed in HE (32%; P<0.05). Cumulated results of the first and second AI showed a similar trend with the highest pregnancy rate in MET (73%) followed by CO (63%) and HE (58%), but it has to be mentioned that differences were not significant. The bacterial growth density was similar in the groups E1 and E0. Furthermore, no striking differences regarding the bacterial composition were detected. Most frequently detected bacteria in group E1 were representatives of the genera *Staphylococcus* (16%), *Bacillus* (12%), *Corynebacterium* (10%) and *Lysinibacillus* (10%). Most frequently detected isolates in group E0 were members of the genera *Bacillus* (25%), *Corynebacterium* (16%), *Micrococcus* (13%) and *Staphylococcus* (10%). Pathogenic bacteria, such as *T. pyogenes* and *E.coli*, were isolated rarely.

**Conclusions** In summary, post-insemination treatment with cephapirin had a significantly positive effect on pregnancy rates for the second AI but not for the AI before treatment. One reason for this delayed effect could be that the time for recovery of the inflamed endometrium after treatment was too short before the embryo entered the uterus. This finding and the fact that typical uterine pathogenic bacteria were rarely detected questions the indication of an intrauterine antibiotic treatment in cows with mild CE at breeding.

#### References

- Aström, B. 1935. Eine Abänderung der Methode Albrechtsens bei der Behandlung steriler Kühe. *Münchener Tierärztliche Wochenschrift* 8: 88–89.
- Borsberry, S, and H Dobson. 1989. Periparturient Diseases and Their Effect on Reproductive Performance in Five Dairy Herds. *The Veterinary Record* 124 (9): 217–19.
- Lambertz, C, D Völker, U Janowitz, and M Gaulty. 2014. Evaluation of Vaginal Discharge with the Metricheck Device and the Relationship to Reproductive Performance in Postpartum Dairy Cows. *Animal Science Journal* 85 (9): 848–52.
- LeBlanc, S J, T F Duffield, K E Leslie, K G Bateman, G P Keefe, J S Walton, and W H Johnson. 2002. Defining and Diagnosing Postpartum Clinical Endometritis and Its Impact on Reproductive Performance in Dairy Cows. *Journal of Dairy Science* 85 (9): 2223–36.
- Sheldon, I M, G S Lewis, S LeBlanc, and R O Gilbert. 2006. Defining Postpartum Uterine Disease in Cattle. *Theriogenology* 65 (8): 1516–30.

## Mastitis and reproduction disorders in Holstein and Czech Fleckvieh dairy cows – comparison of occurrence in Czechia

P. Fleischer<sup>1</sup>, E. Kašná<sup>2</sup>, S. Šlosárková<sup>1</sup>, A. Pechová<sup>1,3</sup> and L. Zavadilová<sup>2</sup>

<sup>1</sup>Veterinary Research Institute, Brno, Czech Republic; <sup>2</sup>Institute of Animal Science, Prague, Czech Republic; <sup>3</sup>University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Email: [Fleischer@vri.cz](mailto:Fleischer@vri.cz)

**Introduction** Large and national surveys of health event data were conducted or started in dairy cattle in progressive countries mainly for the introduction of direct health traits to selection indexes. The obtained data are also used for benchmarking including antimicrobials consumption control and farm management. The aim of the present study was to evaluate the occurrence of most important clinical diseases/health disorders in Czech Fleckvieh (C) and Holstein (H) dairy cows and to compare these two breeds, i.e. these two types of farms with (dominant) C or H breed.

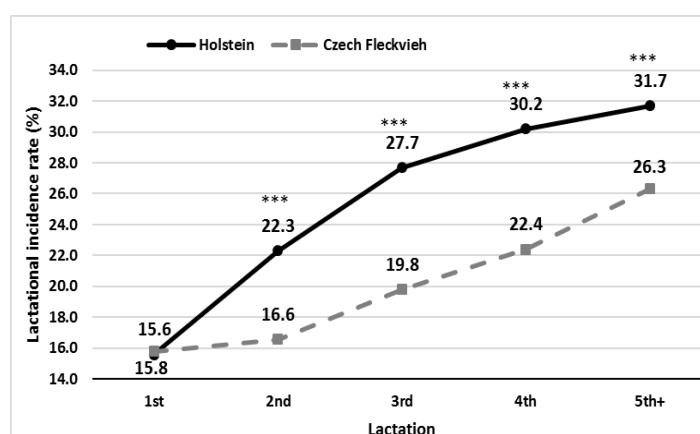
**Materials and Methods** The first national survey covered all diseases/health disorders occurring in Czech dairy cows. The data were gathered retrospectively from farmers via on-line survey from July 2016 through June 2017. The 46% of farmers who responded owned 78% of dairy cows in Czechia. We gathered 27 common health traits. The farmers reported 411,949 health events on 956 farms. Data editing: 1) Only lactations which started after 30 June 2016 and no later than 60 (e.g. for mastitis), 20 (metritis), 7 (e.g. for retained placenta) days before 30 June 2017 were evaluated to keep a minimum period to enable the disease to express (287,491 lactations). 2) Data were assigned according to the breed to C ( $\geq 75\%$  share of Czech Fleckvieh; 96,909 lactations, mean 279 per herd) and H ( $\geq 75\%$  share of Holstein; 165,922 lactations, mean 346 per herd). Cows of these breeds were kept on 916 farms (91.2% of these farms with  $>80\%$  lactations of one breed). This study evaluated the occurrence of clinical mastitis, dystocia, retained placenta, metritis (uterus inflammation up to 20 days in milk - DIM), endometritis (after 20 DIM), cystic ovary disease, and all health events summarized in two groups (udder diseases and reproduction disorders). To identify the farms with incomplete data, each farm with more than 20 evaluated lactations (92.8% farms) had to report at least 1 record of a particular health disorder to be included in the evaluation of the disorder. Reported occurrences were expressed as lactational incidence rate LIR (number of affected lactations / number of lactations at risk)  $\times 100$  and at the first occurrence in lactation the parity and DIM (in 30 d periods till DIM  $>300$ ) were evaluated. For editing the database and calculations the SAS 9.4 software was used.

**Results** C and H lactations lasted for  $317 \pm 86$  and  $340 \pm 94$  days, milk yield in lactations which lasted  $>240$  DIM reached  $7,627 \pm 1,961$  and  $10,100 \pm 2,025$  l, and the mean parity was  $2.78 \pm 1.66$  and  $2.33 \pm 1.36$ , respectively. Our data show that H cows had a significantly higher LIR for most of the evaluated disorders (Table 1). Median DIM at individual diseases was different in clinical mastitis (C 47; H 63), cystic ovary disease (C 71; H 83) and similar in metritis (C 7; H 8) and endometritis (C 37; H 40). Whereas the same level of LIR was found in clinical mastitis in primiparous C and H cows, the pluriparous C cows had lower LIR (Figure 1). The same parity pattern in both C and H cows was found for dystocia, metritis and retained placenta. The highest LIR in 1<sup>st</sup> lactation was found in dystocia (C 4.1%; H 3.9%), metritis (C 10.4%; H 16.8%), and a continuous increase of LIR was in retained placenta (C 3.0-7.6%; H 4.4-7.8%). First cases of clinical mastitis occurred up to 30 DIM in C cows in 7.8% but in H cows only in 7.5% lactations ( $P < 0.01$ ). Lower occurrence of first cases of clinical mastitis up to 30 DIM in H cows (despite higher milk yield) could be caused by better conditions/management starting from drying off to transition period.

**Table 1** Comparison of percentages of lactations affected by disease/health disorder between Czech Fleckvieh (C) and Holstein (H) dairy cows in the Czech Republic

Disease/disorder	Lactations (no.)		Lactational incidence rate (%)	
	C	H	C	H
Clinical mastitis	65,234	111,322	19.1	22.2***
Udder diseases	66,095	112,509	20.7	24.1***
Dystocia	26,308	48,437	2.5	2.6 <sup>ns</sup>
Retained placenta	61,566	94,752	5.1	5.9***
Metritis ( $\leq 20$ d)	55,557	97,780	8.7	14.4***
Endometritis	43,477	76,001	5.0	5.1 <sup>ns</sup>
Cystic ovary disease	36,932	53,606	11.1	12.6***
Reproduction disorders	70,997	120,710	23.8	29.4***

\*\*\*  $P < 0.001$ ; <sup>ns</sup> not significant



**Figure 1** Comparison of proportions of lactations affected by clinical mastitis between Holstein and Czech Fleckvieh dairy cows in the Czech Republic according to parity

**Conclusions** This first national comparison of health between Czech Fleckvieh and Holstein cows shows that Holstein cows as expected suffered from most of the evaluated health disorders more frequently than Czech Fleckvieh cows but, surprisingly, not from clinical mastitis in the first lactation and also not in the first 30 DIM (all parities together).

*Supported by the Ministry of Agriculture of the Czech Republic, projects QK1910320, MZE-RO0518 and MZE-RO0718.*

### Pre-farrowing supplement added to drinking water reduces stillbirth in sows

P. Langendijk, J. Bergman and M. Fleuren

Swine Research Centre, Trouw Nutrition R&D, Amersfoort, The Netherlands

Email: [pieter.langendijk@trouwnutrition.com](mailto:pieter.langendijk@trouwnutrition.com)

**Introduction** The majority of stillborn piglets are potentially viable, but die due to asphyxia during birth (Randall, 1971). Data from our research centre have shown that oxygenation of piglets deteriorates and stillbirth rate increases with duration of parturition (Langendijk et al., 2018). As a consequence, stillbirth rate is exaggerated in piglets that are born in the latter part of the farrowing process, especially in sows with long parturition. Our data also show that liveborn piglets with signs of asphyxia at birth (elevated blood lactate), have lower performance in terms of colostrum intake, and daily gain up to at least ten weeks of life.

**Materials and Methods** In this study, multiparous sows (Hendrix Genetics, Boxmeer, The Netherlands, n=73) were allocated to receive either normal water, or to receive a supplement dosed at 1% through their drinking water from 5 days before the average due date, until they had finished farrowing. The supplement (formulation cannot be disclosed due to pending patent) was designed to increase oxygen levels in the piglets at birth. Assistance during farrowing was restricted to a minimum (7% of sows). Sows were monitored continuously during farrowing, and piglets were weighed immediately at birth, and 24 h later to estimate colostrum intake, according to the algorithm developed by Theil et al. (2014). Average total born was  $15.4 \pm 0.3$ . Data were analysed using SAS. Number of born alive was analysed with GLM using total born as a covariate, to provide comparison between treatments as if total born was equal:  $BA = \mu + TRT + tb + e$ , with BA number born alive, TRT treatment, and tb number total born (covariate).

**Results** Water intake before farrowing (10 to 15 L/d) was not affected by the supplement. Number of piglets born alive was increased from  $13.9 \pm 0.2$  to  $14.5 \pm 0.2$  ( $P < 0.05$ ). Intake of colostrum by piglets from sows that received the supplement tended to be increased ( $P < 0.10$ ). Numbers of piglets surviving after equalising the litters was increased by 0.2 for sows that received the supplement (not significant).

**Table 1** Reproductive performance around farrowing for control sows and sows treated with a pre-farrowing water additive.

	Control	Water supplement
N	36	37
Total born, n	$15.0 \pm 0.6$	$15.3 \pm 0.6$
Born alive <sup>1</sup> , n	$13.9 \pm 0.2^a$	$14.5 \pm 0.2^b$
Colostrum intake per litter <sup>1</sup> , g	$5548 \pm 154^x$	$5753 \pm 151^y$
Pre-wean mortalities, n	$2.3 \pm 0.4$	$2.1 \pm 0.3$

<sup>1</sup> Corrected for litter size; <sup>a,b</sup>  $P < 0.05$ ; <sup>x,y</sup>  $P < 0.10$

**Conclusions** In conclusion, the pre-farrowing water supplement increased the number of piglets born alive by 0.6 piglet. The extra piglets were viable piglets, since pre-wean mortality was not affected and even numerically reduced, and intake of colostrum tended to be increased.

### References

- Langendijk P, Fleuren M, van Hees H, van Kempen T. The Course of Parturition Affects Piglet Condition at Birth and Survival and Growth through the Nursery Phase. *Animals* 2018, 8, 60-67.
- Randall, GCB. The relationship of arterial blood pH and pCO<sub>2</sub> to the viability of the newborn piglet. *Can J Comp Med* 1971, 35, 141–146.
- Theil PK, Flummer C, Hurley WL, Kristensen NB, Labouriau RL, Sørensen MT. Mechanistic model to predict colostrum intake based on deuterium oxide dilution technique data and impact of gestation and pre-farrowing diets on piglet intake and sow yield of colostrum. *J Anim Sci* 2014, 92, 5507-5519.

### Relations between negative energy balance and embryo harvest in donors of Latvian native cow breeds

I. Sematovica<sup>1</sup>, G. Ringa-Karahona<sup>1</sup>, V. Antane<sup>1</sup> and I. Kanska<sup>2</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Latvia; <sup>2</sup>Animal Breeders Association of Latvia, Latvia

Email: isem@inbox.lv

**Introduction** Latvian native cow breeds (Latvian Blue (LZ) and Latvian Brown (LB)) are actually located on the line of extinction because of small number of animals (LZ 300 animals and LB 150 animals). Thanks to ERAF project No. 1.1.1.1/16/A/025, *BioReproLV* it is possible to promote the saving of these breeds using multiple ovulation and embryo transfer (MOET). Thus MOET in Latvia has been restarted after interruption for more than 35 years. Unfortunately, many of genetically valuable LZ and LB animals are in age, they live in small private farms without calculated feeding ration, and many of these small private farm owners do not have appropriate education relevant to the cow physiology beside their own experience. This is the reason why condition of these cows could not be suspected as acceptable for donor cow's role. Owners are not informed enough about MOET and very limited count of animals are available to be intend for MOET. The aim of the present study was to investigate the relations between parameters of negative energy balance and embryo harvest and quality in Latvian native cows intended to MOET up to now.

**Materials and Methods** Thirty cows were intended for donor cow role in 2017-2019. They were in  $3.8 \pm 2.77$  lactation. The oldest cows were 15 and 12 years old. Six cows had inaccuracies regarding to reproductive health history, poor body condition score (BCS), elevated  $\beta$ -hydroxybutyric acid (BHB) in blood, ovarian cyst or no response to MO inducing medicine. PMSG (13 cows) or FSH (17 cows) were used to induce MO. Cow productivity (kg in a current day, milk fat (MF), milk protein (MP), somatic cell count (SCC), body condition score (BCS), hematological (red blood cells (RBC), white blood cells (WBC), hemoglobin (Hgb), hematocrit (HTC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), leucocyte formula (Sg, St, Eo, Mo), and biochemical parameters (alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), gamma glutamyl transferase (GGT), urea, total protein (TP), albumins, cholesterol (CHOL), triglycerides (TG), glucose (Glc) and BHB were established. Statistical parameters of data such as mean values, standard deviation, Two Independent Samples t-test, One-Way-Anova test and Bivariate correlations (two-tailed) were provided using *SPSS-21*.

**Results** Productivity of intended cows was  $16.0 \pm 7.02$  kg on current day with MF  $4.68 \pm 0.890\%$ , MP  $3.91 \pm 0.813\%$ , MF/MP ratio  $1.22 \pm 0.24$ . They were in very different days in milk. No significant disturbances were found regarding to hematological and biochemical parameters excepting Glc, TG and BHB in blood and SCC in milk of some individuals. No one of cows had clinical mastitis. All the cows received additional treatment (vitamins, micro- and macro- additives, propylene glycol) and recommendations for feeding some weeks before MO inducing. BCS was in range from 1.75 up to 3.75 (5-point scale). Success of MO was influenced by level of Glc ((means  $\pm$  SD)  $3.1 \pm 0.58$  (successful MO) and  $2.3 \pm 0.26$  mmol/L (unsuccessful MO) respectively) and BHB ( $0.6 \pm 0.28$  mmol/L and  $1.7 \pm 0.21$  mmol/L respectively); ( $P=0.001$ ). In cows with unsuccessful MO also were higher MF and MF/MP ratio ( $5.47 \pm 1.130\%$ ;  $1.44 \pm 0.313$  and  $4.46 \pm 0.695\%$ ;  $1.16 \pm 0.187$  respectively); ( $P<0.05$ ). Totally 87 embryos were received from all donor cows together but just 37 embryos were classified as transferable. An average total embryo harvest was  $7.35 \pm 6.78$  embryos from cow (min. 0 and max. 18 embryos). Total count of embryos from one cow depended on level of TG ( $P=0.018$ ). No significant influence on MO and embryo quality was established regarding to RBC, WBC, HTC, MCV, MCH, MCHC and leucocyte formula ( $P>0.05$ ) except monocytes ( $P=0.037$ ). Transferable embryo count depended on SCC in milk ( $236.3 \pm 192.29$  ( $\geq 3$  transferable embryos) and  $1139.1 \pm 855.33$  (0-2 transferable embryos) thousand/mL respectively;  $P<0.05$ ), and TG ( $0.17 \pm 0.05$  and  $0.26 \pm 0.032$  mmol/L respectively;  $P<0.05$ ). Should be mentioned that the total embryo harvest and embryo quality depended on medicine used to induce MO ( $r=0.59$ ;  $P=0.011$ ). The negative correlation was found between count of transferable embryos and SCC ( $r=-0.44$ ;  $P<0.05$ ). All the seven pregnancies acquired up to now are from cows with SCC below 100 thousand/mL.

**Conclusions** In conclusion, there must be given more time to elevate level of Glc and decrease BHB in blood in donor cows. Elevated SCC in donor cow milk is not permissible despite of appropriate WBC count in blood. Because of limited count of available healthy cows it could be better choice to intend heifers for embryo donor's role.

### The role of mammary gland control of calcium homeostasis during the periparturient period

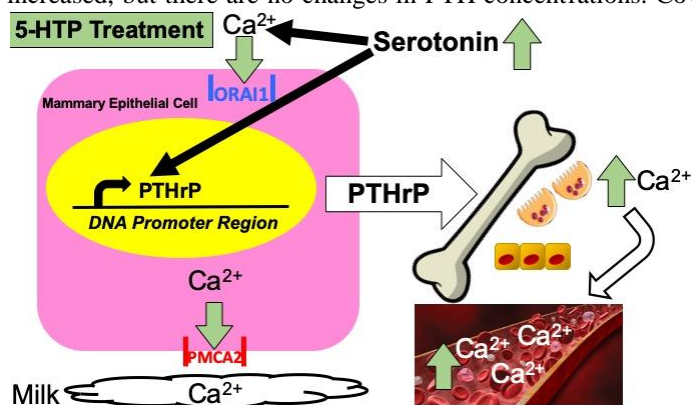
L. L. Hernandez

Department of Dairy Science, University of Wisconsin-Madison, WI, USA

Email: llhernandez@wisc.edu

**Introduction** During late pregnancy and lactation, the mammary gland differentiates immensely in order to support the copious production of milk. Calcium is the major mineral in milk, placing a substantial nutritional and physiological demand on the periparturient cow to support milk production, of which calcium is excreted into in large amounts, while simultaneously maintaining maternal health and physiology. During the periparturient period, the mammary gland produces and secretes biologically active compounds, which impact not only milk synthesis and secretion, but interact with maternal tissues to coordinate maternal metabolism and physiology, which holds true for the mineral calcium. Calcium is transported into the mammary gland for milk synthesis as well as for intracellular calcium signaling. Research in mice and humans has demonstrated that the mammary gland produces parathyroid hormone related-protein (PTHrP), which is responsible for regulating bone turnover to support maternal calcium homeostasis during lactation. We have demonstrated that serotonin produced by the mammary gland stimulates PTHrP synthesis as well as calcium transport in the mammary gland in a rodent model (Hernandez et al., 2012). Production and secretion of PTHrP from the mammary gland is critical for the bone mobilization necessary to sustain maternal calcium homeostasis during lactation. It is critical for animal health and welfare to optimize the ability of the dairy cow to mobilize calcium early in lactation to prevent hypocalcemia. Further understanding of the mammary gland's ability to control calcium homeostasis in early lactation will be critical for maintaining the health of the early lactation dairy cows.

**Research Summary** Our research is currently focused on determining if serotonin mediates calcium transport in the mammary gland to drive a negative feedback response in the dairy cow to induce bone calcium mobilization. Our early work providing late lactation dairy cows with 5-hydroxy-L-tryptophan (5-HTP), the immediate precursor to serotonin, resulted in an immediate reduction in circulating calcium concentrations (Laporta et al., 2015). Furthermore, we demonstrated that 5-HTP treatment, immediately decreased total calcium concentrations and resulted in increased milk calcium concentrations and reduced urinary calcium concentrations. These data suggest that serotonin appears to be directing calcium movement into the mammary gland for milk synthesis. In several studies, when we treated prepartum dairy cows were treated with 5-HTP, postpartum total calcium concentrations were increased compared to control cows (Weaver et al., 2016; Hernandez-Castellano et al, 2017; Slater et al., 2018). These data suggest that 5-HTP treatment influences the transport of calcium into the milk through, potentially through the plasma membrane calcium ATPase 2 pump (PMCA2) located on the apical membrane of the mammary epithelium. PMCA2 expression is increased in mammary tissue collected from dairy cows treated with 5-HTP, which corroborates our findings in mouse models (Slater et al., 2018). Additionally, we have determined that 5-HTP treatment influences methylation of promoter regions that result in increased PTHrP expression in the mouse mammary gland. These findings lead us to believe that serotonin influences calcium homeostasis both directly through methylation of promoter regions influencing mammary PTHrP production, and indirectly through modulating flux of calcium into the milk causing bone resorption through negative feedback mechanisms. We have further determined that serotonin and calcium homeostasis during this time period appear to regulate calcium independent of the classical PTH-Vitamin D pathway known to regulate calcium homeostasis. Our research has demonstrated that there are no significant changes in serotonin concentrations in response to Vitamin D supplementation during the periparturient period (Rodney et al., 2018). Furthermore, we have demonstrated that when administering 5-HTP prepartum, not only are postpartum calcium concentrations increased, but there are no changes in PTH concentrations. Cows that had lower postpartum calcium concentrations and did not receive prepartum 5-HTP treatment in the same experiment had increased PTH concentrations (Hernandez-Castellano et al., 2017). These data support the hypothesis that serotonin appears to regulate calcium through a different feedback loop than the classical PTH-Vitamin D pathway. Research in other mammalian species have consistently supported the notion that calcium homeostasis during lactation is largely regulated by PTHrP rather than PTH, however this has not been fully elucidated in the dairy cow. We are currently working on further determining the pathway(s) by which serotonin regulates calcium homeostasis during the periparturient period. We are interested in understanding the homeostatic feedback loop by which serotonin and calcium interact during the periparturient period in order to improve postpartum calcium metabolism in dairy cows.



**Figure 1** Model of proposed mechanism by which serotonin influences calcium homeostasis.

#### References

- Hernandez LL, KA Gregerson, and ND Horseman. (2012). Serotonin is an upstream regulator in the breast-to-bone axis. *Am. J. Physiol. Endocrinol. Metab.* 302:E1009-E1015. // Laporta, J, Moore SAE, Weaver SR, Cronick CM, Olsen M, Prichard AP, Schnell BP, Crenshaw TD, Peñagaricano F, Bruckmaier RM, Hernandez LL. (2015). Increasing serotonin alters calcium and glucose metabolism in late-lactation dairy cows. *J. Endocrinol.* 226(1):43-55. // Weaver SR, Prichard AP, Endres EL, Newhouse SA, Peters TL, Crump PM, Akins MS, Crenshaw TD, Bruckmaier, Hernandez LL. (2016). Elevation of circulating serotonin improves calcium dynamics in the dairy cow transition period. *J. Endocrinol.* 230(1):105-123. // Hernandez-Castellano LE, Hernandez LL, Weaver SR, Bruckmaier RM. (2017). Increased serum serotonin improves parturient calcium homeostasis in dairy cows. *J. Dairy Sci.* 100(2):1580-1587. // Slater CJ, EL Endres, SR Weaver, AA Cheng, MR Lauber, SF Endres, E Olstad, A DeBruin, PM Crump, E Block, and LL Hernandez. (2018). Interaction of 5-hydroxy-L-tryptophan and negative dietary cation-anion difference on calcium homeostasis in multiparous peripartum dairy cows. *J. Dairy Sci.* 101(6):5486-5501. // Rodney RM, N Martinez, E Block, LL Hernandez, P Celi, CD Nelson, JEP Santos, IJ Lean. (2018). Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Vitamin D, mineral, and bone metabolism. *J. Dairy Sci.* Mar;101(3):2519-2543.

### Mineral metabolism in the periparturient period: implications to health and productivity

J. E. P. Santos and A. Vieira-Neto

Department of Animal Sciences, University of Florida, Gainesville, USA

Email: [Jepsantos@ufl.edu](mailto:Jepsantos@ufl.edu)

The onset of lactation often results in disturbances in mineral metabolism, particularly a transient hypocalcemia that can be accompanied by hypophosphatemia and, in some cases, hypomagnesemia. Clear evidence exists that acute loss of calcium (Ca) from the plasma pool starts hours before calving and most of the loss is caused by sequestration of Ca in the mammary gland for synthesis of colostrum and milk. Removal of the mammary gland in dairy cows abolished the decline in blood Ca concentrations in spite of calving. Nevertheless, evidence exists that inflammatory diseases can perturb blood concentrations of Ca and further compromise homeostasis, likely because of reduced gastrointestinal absorption and losses from increased vascular permeability. Synthesis and secretion of colostrum results in irreversible losses of Ca, phosphorus (P) and magnesium (Mg) of approximately 20, 10, and 3 g, respectively. Total loss in the first day postpartum for cows milked twice can be 1.5 times the amounts in colostrum, which represent up to 10, 6, and 6-fold the total plasma pools of the respective minerals. Approximately 3 to 5% of the dairy cows succumb to clinical hypocalcemia, but a much larger proportion of cows develop subclinical hypocalcemia (total Ca,  $tCa < 2.0$  mM) in the first 3 d of lactation. Cows with hypocalcemia have reduced blood concentration of  $tCa$ , which also reflect reductions in concentrations of cytosolic ionized Ca ( $iCa$ ). Perturbations of cytosolic concentrations of  $iCa$  compromise cellular functions, among them muscle contractility and activation of immune cells, thereby compromising measures of innate immune response that predispose cows to increased risk of diseases such as retained placenta, metritis, and mastitis. Subclinical hypocalcemia induces anorexia and stasis of the gastrointestinal tract, which likely predisposes cows to displaced abomasum and ketosis. The reduced concentrations of blood  $iCa$  affect pancreatic beta cells, thereby blunting insulin release and further increasing lipomobilization, therefore, affecting intermediary metabolism. Hypocalcemia predisposes cows to hypophosphatemia, which compromises cellular ATP synthesis and muscle tone. On the other hand, prepartum hyperphosphatemia can increase the risk of hypocalcemia through alterations of vitamin D metabolism affected by fibroblast growth factor 23. Prevention of hypocalcemia through dietary intervention involve two methods. The first is reduced gastrointestinal Ca absorption either by underfeeding Ca or preventing its absorption with sequestering agents, both of which with the goal of inducing a negative Ca balance prepartum. This strategy prevents clinical hypocalcemia with less documented effects on subclinical hypocalcemia or overall health and productivity of cows. Diets high in sodium (Na) and potassium (K) content result in metabolic alkalosis and increased risk of hypocalcemia. Thus, the second strategy for prevention of hypocalcemia involves altering the acid-base balance of the cow by inducing a compensated metabolic acidosis prepartum. Acidosis frees up  $iCa$  from albumin, increases parathyroid hormone (PTH) secretion, enhances PTH-mediated control of Ca concentrations, improves gastrointestinal Ca absorption supposedly mediated by vitamin D, increases bone remodelling resulting in release of Ca from hydroxyapatite, and increases urinary losses of Ca caused by renal tubular acidosis. Collectively, these mechanisms that underlie the use of acidogenic diets enhance Ca homeostasis in the first days postpartum, which prevents hypocalcemia, but also reduce the risk of uterine diseases and improve lactation performance. Prevention of hypocalcemia is suggested to be improved by adequate Mg nutrition in late gestation and early lactation. A commonality between hypocalcemia and hypomagnesemia is intake of K. The latter depolarizes the apical membrane of the rumen epithelium and compromises active transport of Mg. Therefore, diets low in K prepartum and adequate in Mg pre- and postpartum are suggested as part of the preventative methods for mineral-related disorders in dairy cows.

### **Skeleton health: Nutritional and management interventions**

D. M. Karcher<sup>1</sup>, P. Regmi<sup>2</sup>, C. I. Robison<sup>3</sup> and W. K. Kim<sup>4</sup>

<sup>1</sup>Department of Animal Sciences, Purdue University, West Lafayette, IN; <sup>2</sup>Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC; <sup>3</sup>Department of Animal Science, Michigan State University, East Lansing, MI; <sup>4</sup>Department of Poultry Science, University of Georgia, Athens, GA, USA

Email: [dkarcher@purdue.edu](mailto:dkarcher@purdue.edu)

**Introduction** Keel bone problems were first reported in extensive housing systems in the 1930s<sup>1,2</sup>. However, the adoption of conventional cages for laying hens in the 1940s resulted in keel abnormalities being associated with calcium, phosphorus or Vitamin D deficiencies. Seventy years later, keel bone deformities and breakage in extensive housing systems starts to be reported<sup>3,4</sup>. Perch design and row spacing in extensive housing has been explored as ways to mitigate keel damage in addition to a few studies on nutritional interventions. A more robust approach to nutritional and management interventions needs to be explored to understand the contribution each plays in reducing keel damage. The materials to be covered will include a review of the nutritional strategies and management approaches to mitigate keel bone fracture prevalence.

**Materials and Methods** Several different experiments and discussion of literature will be covered. The species investigated is the laying hen in extensive, mostly cage-free, housing systems across the US and Europe. Nutritional strategies have included n-3 polyunsaturated fatty acids and 25-(OH)D3 supplemented diets. These diets have been fed at various stages of life as early as 12 weeks of age through 70 weeks. Different methods of keel assessment have been used including computed tomography on live hens, palpation, and dissection to determine keel deformation or breakage. The management approaches have not been as well investigated and different methods that have been explored will be discussed.

**Results** Results from other published studies will be discussed in context with current on-going research related to nutritional and management interventions on keel bone damage.

**Conclusions** Keel bone damage is a multifactorial problem that will require more than just nutrition and management interventions. No single solution will solve the problem but a systems approach to keel bone damage will provide a way forward to reduce the incidence observed in extensive housing systems world-wide.

### **References**

- Heerkens JLT, Delezie E, Rodenberg TB, Kempen I, Zoons J, Ampe B and Tuytens FAM. 2016. Risk factors associated with keel bone and foot pad disorders in laying hens housed in aviary systems. *Poult. Sci.* 95(3):482-488
- Warren DE. 1937. Physiologic and genetic studies of crooked keels in chickens. *Kansas Agricultural Experimental Station Technical Bulletin.* 44.
- Wilkins LJ, Brown SN, Zimmerman PH, Leeb C and Nicol CJ. 2004. Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. *Vet. Rec.* 155(18):547-549.
- Winter AR and Funk EM. 1941. *Poultry science and practice.* Chicago: J.B. Lippincott Co. p 388



### Lessons learned from the hypovitaminosis D kyphotic pig model

L. A. Amundson<sup>1,2</sup> and T. D. Crenshaw<sup>2</sup>

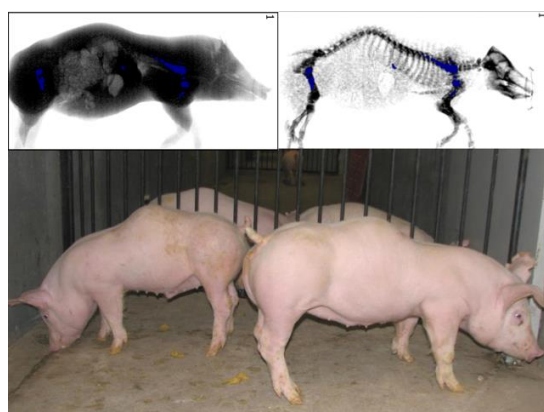
<sup>1</sup>Zinpro Corporation, Eden Prairie, MN, USA; <sup>2</sup>University of Wisconsin-Madison Animal Sciences Department, Madison, WI, USA

Email: LAMundson@Zinpro.com

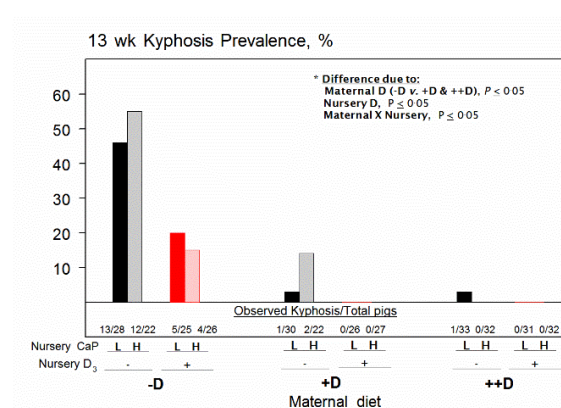
**Introduction and Background** Skeletal unsoundness, ultimately leading to lameness in production livestock, and human diseases associated with loss of bone integrity are problems that continue to plague agriculture and human medicine. An overarching issue which hinders discovery of technologies for prevention and treatment of such issues is the lack of controlled models to study the factors that initiate cartilage and bone abnormalities. Most studies have focused on characteristics of the final lesion, at which time most of the damage cannot be reversed. Consequently, the contribution of various nutrients on the initiation of bone lesions is not completely understood, which hampers early detection, prevention, and treatment. In recent veterinary and human health practices, the approach has often been to recommend excessive amounts of supplemental vitamin D (D), given the classical and accepted role of D in bone mineralization. This approach may appear to temporarily alleviate immediate issues, but long-term effects in these recommendations remain unknown. The implications of over-fortifying diets are often ignored.

**Experimental Approaches and Results** Results reported herein are a brief summary of multiple studies that demonstrate our ability to produce and reproduce the hypovitaminosis D kyphotic pig model through perturbations of sow and growing pig diets. Sow diets included 0 (-D), 325 (+D), or 1750 (++)D IU D<sub>3</sub>/kg throughout gestation and lactation. At weaning (3 wk) pigs were fed diets with 0 (-D) or 280 (+D) IU D<sub>3</sub>/kg and relatively minor modifications to dietary Ca and P to exacerbate responses to dietary D through the nursery phase.

Pigs produced by -D sows had an 11% reduction in growth ( $P < 0.05$ ) and 25% reduction in bone mineral content ( $P < 0.05$ ), regardless of nursery diet. Significant interactions between maternal and nursery dietary treatments were detected in pig responses. Pigs produced by -D sows had the greatest prevalence at of displaying kyphosis. Importantly, bone and soft tissue responses to maternal and nursery dietary D were not explained by serum 25-OH-D<sub>3</sub> concentrations. Significant maternal and nursery diet effects on mRNA expression of genes involved in D homeostasis (1 $\alpha$ -hydroxylase and 24-hydroxylase) and bone metabolism (matrix metalloproteinases and fibroblast growth factor 23) were also evident.



**Figure 1** Hypovitaminosis D Kyphotic Pig Model.



**Figure 2** Prevalence of kyphosis at 13 wk of age.

**Conclusions** Maternal dietary D carryover effects on soft tissue growth, skeletal mineralization, and expression of genes related to D metabolism and endochondral ossification were evident in young pigs. However, changes in serum 25-OH-D<sub>3</sub> concentrations were not consistent with cartilage and bone abnormalities. Therefore, serum concentrations of the circulating metabolite of vitamin D may not be the best indicator of cartilage and bone health and integrity. In future experiments we hope to characterize potential epigenetic effects of maternal dietary D through measurement of methylation patterns in response to maternal supplemental D and to ultimately identify a biomarker for early detection of cartilage and bone abnormalities prior to final lesion manifestation.

### References

1. Rortvedt, L. A. and T. D. Crenshaw. 2012. J. Anim. Sci. 90:4905-4915.
2. Crenshaw, T. D., L. A. Rortvedt-Amundson, J. A. Cuaron, J. R. Bergstrom, and G. Litta. 2014. J. Anim. Sci. 92:883-886.
3. Amundson, L. A., L. L. Hernandez, J. Laporta, and T. D. Crenshaw. 2016. British J. Nutr. 116:771-787.
4. Amundson, L. A., L. L. Hernandez, and T. D. Crenshaw. 2017. British J. Nutr. 118:30-40.

### Effects of diets high or low in dietary cation-anion difference combined with adequate or restricted calcium supply on renal calcium homeostasis in sheep

J. Freitag<sup>1,2</sup>, M. R. Wilkens<sup>3</sup>, A. S. Muscher-Banse<sup>3</sup>, N. Schnepel<sup>3</sup> and A. Liesegang<sup>1,2</sup>

<sup>1</sup>Institute of Animal Nutrition, Vetsuisse-Faculty, University of Zurich, Switzerland; <sup>2</sup>Centre for Clinical Studies, Vetsuisse-Faculty, University of Zurich, Switzerland, <sup>3</sup>Department of Physiology, University of Veterinary Medicine, Foundation Hannover, Germany  
Email: jfreitag@nutrivet.uzh.ch

**Introduction** Adapting to the enormous increased calcium (Ca) demand and maintaining required Ca plasma concentrations in late gestation and at the initiation of lactation is challenging for most ruminants. This frequently results in subclinical or even clinical hypocalcaemia (milk fever, parturient paresis). Goff et al. (2014) demonstrated a lower sensitivity of the parathyroid hormone receptor (PTHr) in cows fed diets high in dietary cation-anion difference (DCAD), inducing a pseudohypoparathyroidism. Therefore, the administration of anion-rich diets is thought to improve the responsiveness of the target tissues, but the details of the mechanism of action as well as the most suitable Ca content for the formulation of the ration are still unclear. It was the purpose of our experiment to study basic effects of different DCADs while considering the Ca supply in a sheep model. We hypothesized that changes in the expression of receptors crucial for Ca homeostatic mechanisms would occur in sheep kept on lowDCAD with sufficient Ca supply.

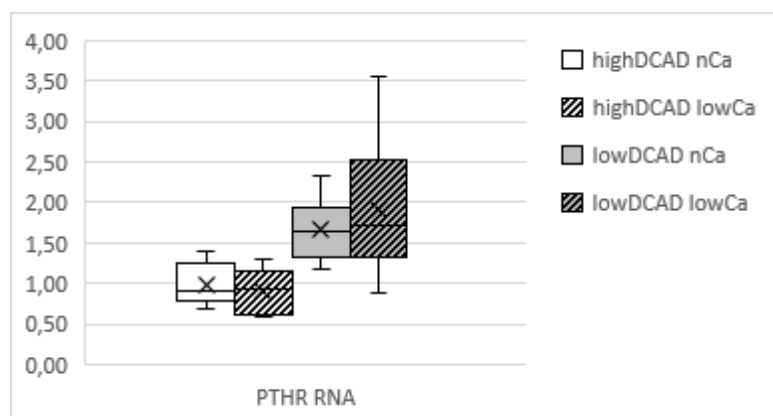
**Materials and Methods** Twenty-three male castrated East Friesian dairy sheep, aged 11-12 months, were randomly assigned to four different feeding groups. The concentrate of each group was modified for DCAD and Ca content. Diets high or low in DCAD (high/lowDCAD) were combined with normal or low Ca supply (nCa/lowCa). On day -14, serum and urine were collected from all sheep. Furthermore, a peripheral quantitative computed tomography (pQCT) of the left metatarsus was performed. After a 14-day adaptation period to the new diet, the experiment started (day 0). Urine and serum were collected on days 0, 4, 7, 14, and 22, and pQCT was performed on days 0, and 22. On day 22, the animals were sacrificed. Immediately post mortem, renal cortex was collected, frozen in liquid nitrogen, and stored until further analyses. In the collected samples, analyses of relevant Ca associated parameters were performed, including analyses of RNA expression in the renal cortex.

All statistical analyses were conducted with Excel and the statistical software R version 3.4.3 using a generalized linear mixed model for all analyses with repeated measurements and a general linear model for non-repeated measurements.

**Results** LowDCAD was significantly associated with lower urine pH ( $P < 0.001$ ), greater urinary Ca excretion ( $P < 0.001$ ), greater iCa in blood ( $P = 0.045$ ), and greater serum Ca concentrations ( $P = 0.030$ ). LowCa was also associated with greater urinary Ca excretion, albeit to a smaller extent than DCAD ( $P < 0.001$ ). LowDCAD and lowCa in interaction weakened these associated increases on urinary Ca excretion ( $P < 0.001$ ).

Blood pH, as well as bone parameters analyzed by pQCT did not differ significantly between groups at any point in time.

Furthermore, lowDCAD was associated with a greater renal RNA expression of PTHr ( $P < 0.001$ ). PTHr RNA expression was positively correlated with serum Ca concentration ( $r = 0.55$ ,  $P = 0.010$ ) and ionized Ca ( $r = 0.53$ ,  $P = 0.011$ ).



**Figure 1** RNA expression of parathyroid hormone receptor (PTHr) in renal cortex tissue of the different sheep feeding groups.

**Conclusions** Sheep were responsive to the anionic diets as shown by decreased urine pH in combination with an unaffected blood pH, indicating a compensated metabolic acidosis independent of Ca supply. In acidotic states, blood Ca concentration can be easily maintained when Ca homeostasis is challenged, as demonstrated by greater concentrations of circulating Ca and iCa in sheep fed lowDCAD. Serum Ca concentration decreased below the reference range in sheep kept on highDCAD with lowCa, but in none of the sheep given the lowDCAD diets, although urinary Ca excretion increased greatly with lowDCAD.

PTHr RNA expression in renal cortex tissue was greater in groups fed anion-rich diets, independent of Ca supply. This might contribute to the greater tissue responsiveness to PTH, already demonstrated by Goff et al. (2014) in cows fed anionic diets. However, it is unclear where the surplus calcium that is excreted with urine originates. As no differences in pQCT could be found in the bone, the contribution of the intestine should be focused on. In conclusion, lowDCAD significantly altered Ca homeostasis independent of Ca supply. PTHr expression likely plays an important role in this process.

#### References

Goff, J.P.; Liesegang, A.; Horst, R.L., 2014: Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. *Journal of Dairy Science* 97, 1520–1528.

## Alteration in response of negative feedback to subclinical hypocalcemia induction in different lactational stages of Holstein dairy cows

M. K. Connelly and L. L. Hernandez

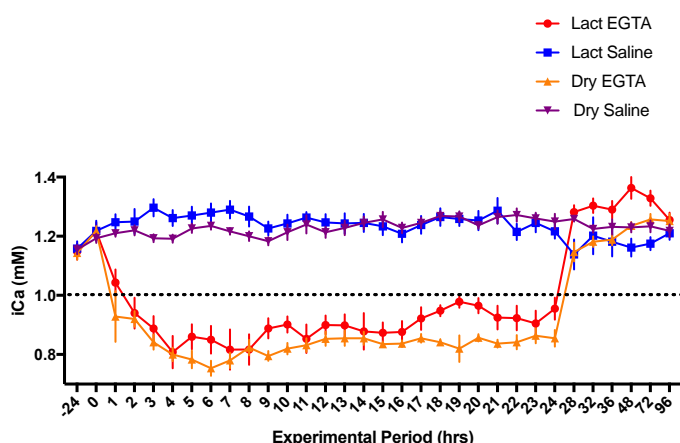
Department of Dairy Science, University of Wisconsin - Madison, Madison, WI, USA

Email: mkconnelly@wisc.edu

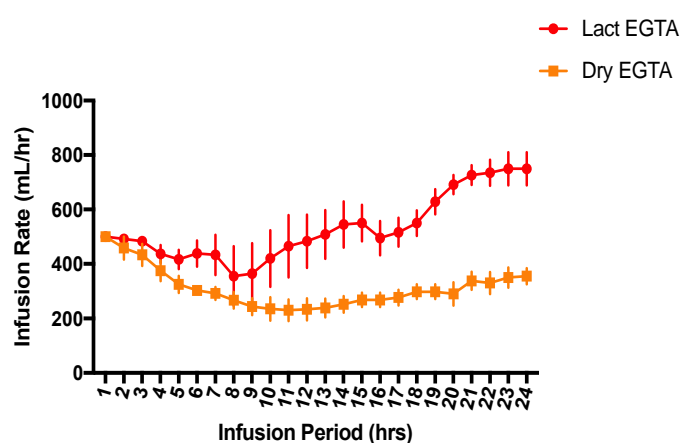
**Introduction** The peri-parturient dairy cow undergoes a dynamic change in metabolism at the onset of lactation. During this time if poor maintenance and regulation of circulating calcium concentrations occurs, detrimental impacts on cow health and productivity often ensue. When the mechanisms to support and maintain calcium concentrations in the blood fail, hypocalcemia ensues. The deleterious effects of this disease can often follow a cow throughout lactation and impede on other normal physiologic responses that are required to maintain homeostasis during this period. In order to aid the cow in the adaptation to the initiation of lactation, a more thorough understanding of the mechanisms that govern calcium homeostasis in the transition cow need to be elucidated. Furthermore, a greater understanding of how the mammary gland itself may help manipulate calcium metabolism during this dynamic timeframe is also needed. The objective of this study was to characterize the physiologic responses of a subclinical hypocalcemic challenge in lactating and non-lactating dairy cows.

**Materials and Methods** In a randomized complete block design, 12 dry, non-pregnant multiparous Holstein cows and 12 early lactation (5-20 days in milk) multiparous Holstein cows were blocked by parity and day of infusion. Cows were randomly assigned to receive either *i*) a continuous 24hr intravenous solution of 0.9% saline (n=6 lactating, n=6 non-lactating) or *ii*) 5% ethylene glycol tetraacetic acid (EGTA) in 0.9% saline (n=6 lactating, n=6 non-lactating) with the aim of maintaining blood ionized calcium (iCa) at less than 1.0 mM for the duration of the 24h period. Mammary gland biopsies were performed 24h prior to start of infusion, immediately after and 48h after termination of infusion. Blood samples were collected immediately prior to infusion, hourly during infusion, and 4, 8, 12, 24, 48 and 72h after termination of infusion. Groups were compared using a mixed model ANOVA with time included as a repeated measure (SAS 9.4).

**Results** Infusion of EGTA effectively decreased circulating iCa concentrations in both lactating and dry, non-pregnant cows compared to saline infusion ( $0.90 \pm 0.01$  mM,  $0.84 \pm 0.01$  mM vs  $1.25 \pm 0.01$  mM,  $1.23 \pm 0.01$  mM;  $P < 0.0001$ ). Lactating-EGTA infused cows had increased iCa during the infusion period compared to dry, non-pregnant EGTA cows ( $P = 0.003$ ). Lactating-EGTA cows had increased iCa concentrations compared to all other treatments 24h post-infusion ( $1.36 \pm 0.026$  mM;  $P < 0.05$ ). Additionally, dry, non-pregnant, EGTA cows required less EGTA to maintain subclinical hypocalcemia compared to lactating EGTA cows ( $P < 0.0001$ ). During the infusion period lactating cows had decreased ( $P < 0.0001$ ) glucose concentrations compared to dry, non-pregnant cows regardless of EGTA or saline infusion. Interestingly, EGTA infusion resulted in increased ( $79.94 \pm 2.31$  mg/dl vs  $76.16$  mg/dl;  $P = 0.27$ ) glucose concentrations in dry, non-pregnant compared to saline infusion in dry, non-pregnant cows. However, EGTA infusion in lactating cows resulted in lower circulating glucose concentrations compared to saline infusion in ( $58 \pm 2.31$  mg/dl vs  $61.59 \pm 2.31$  mg/dl;  $P = 0.29$ ).



**Figure 1** Concentrations of ionized calcium in whole blood of cows during the experimental period. Error bars represent SEM.



**Figure 2** Infusion rate of EGTA infused cows during infusion period. Error bars represent SEM.

**Conclusions** Homeostatic responses to calcium perturbations across different lactational stages in the Holstein dairy cow elicit different mineral and energetic responses, corresponding to the proposed theory that bone, calcium, and energy metabolism are highly integrated and aid in the dairy cow's adaption to lactation. This suggests that calcium regulation and control may be influenced by the ability of the mammary gland to help coordinate availability of calcium to help maintain blood calcium concentrations in times of substantial need. The early lactation dairy cow is capable of adapting to alterations in calcium availability much more quickly than a dry, non-pregnant cow. In addition, this rapid response to calcium depletion in the blood elicits an improved feedback mechanism for the dairy cow during lactation with higher calcium concentrations post-infusion in response to a short-term hypocalcemia challenge. Together this demonstrates that early lactation cows have a unique, improved feedback system that allows a more robust and sudden reaction to calcium challenges when compared to dry, non-pregnant cows.

## Effect of phosphorus intake during the transition period on plasma phosphorus content and hypocalcemia in dairy cows

P. Keanthao<sup>1</sup>, R. M. A. Goselink<sup>2</sup>, J. T. Schonewille<sup>1</sup>, J. Dijkstra<sup>3</sup> and W. H. Hendriks<sup>3</sup>

<sup>1</sup>Utrecht University, the Netherlands; <sup>2</sup>Wageningen Livestock Research, the Netherlands; <sup>3</sup>Wageningen University, the Netherlands

Email: p.keanthao@uu.nl

**Introduction** In early lactation, high producing cows frequently suffer from negative energy and mineral balances including a negative phosphorus balance (Grünberg et al., 2014; Goff, 2004). Farmers and veterinarians in the Netherlands worry that due to legislative pressure to reduce dietary phosphorus (P), P intake in early lactation may be insufficient and will increase the risk of milk fever-like health problems. The objective of this study is to observe the effect of reduced P intake in dairy cows in the first 8 weeks postpartum on P and calcium (Ca) content in blood plasma and clinical hypocalcemia. Interaction effects of feeding a dry cow diet at or above P requirements on performance after calving are also investigated.

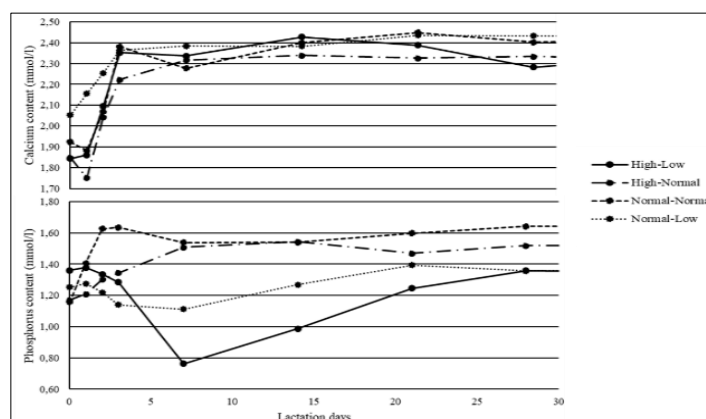
**Materials and Methods** Sixty multiparous dairy cows (HF) were, approximately 6 weeks before calving, randomly allocated to either a dry cow ration with a P content commonly encountered in practice in the Netherlands (i.e. Dry Period Normal (DP-N), 3.7 g P/kg DM) or a reduced P content, matching their calculated P requirements (CVB, 2005; i.e. Dry Period Required (DP-R), 2.0 g P/kg DM). After calving cows from each group were subjected to a ration containing either a normal dietary P content (i.e. Lactation Normal (LAC-N), 3.7 g P/kg DM) or a ration below calculated P requirements (i.e. Lactation Low (LAC-L), 2.6 g P/kg DM until 8 weeks postpartum, in a 2 × 2 factorial design.

The cows received a roughage mixture *ad libitum* and concentrates were fed individually by means of transponder-controlled concentrate feeders. Cows were milked twice daily and throughout the experiment animal health was monitored daily. Blood samples were taken 1, 2, 3, 7, 14, 21, 28 and 56 days after calving. Data were statistically analyzed according to the following model:  $Y_{ij} = \mu + DP-P_i + LAC-P_j + (DP-P \times LAC-P)_{ij} + e_{ij}$ ; where  $Y_{ij}$  = repeated measure of the response variable,  $\mu$  = overall mean,  $DP-P_i$  = Dietary P during the dry period (DP) ( $i = N$  or  $R$ ),  $LAC-P_j$  = Dietary P during lactation ( $j = N$  or  $R$ ),  $(DP-P \times LAC-P)_{ij}$  = interaction term between DP-P and LAC-P and  $e_{ij}$  = residual error. A cow with 2 out of 3 clinical signs (i.e. sternal or lateral recumbency, cold extremities, no rumen contractions) was considered a case of clinical milk fever; subclinical milk fever was defined as a blood calcium concentration lower than 2.0 mmol/l (Reinhardt et al., 2010).

**Results** The incidence of clinical and subclinical hypocalcaemia is shown in Table 1. Phosphorus and Ca content in blood plasma were significantly affected by dietary P content of the lactation diet: LAC-L group showed lower P content in blood plasma than LAC-N group ( $P < 0.05$ ). Phosphorus content in blood is lowest at day 7 postpartum in cows fed LAC-L (Figure 1). Calcium content in blood plasma varied less but was significantly higher in group LAC-L ( $P < 0.05$ ). There was no interaction between dry cow diet and lactation diet; however, cows fed a reduced P diet during the dry period (DP-R) had higher plasma P and Ca concentrations postpartum compared with DP-N ( $P < 0.05$ ).

**Table 1** Number of cows with (sub-) clinical hypocalcemia

Dietary P during dry period	Normal		Required	
Dietary P during lactation	Normal	Low	Normal	Low
No of cases / no of total cows				
clinical hypocalcemia	6/15	6/15	4/15	4/15
Plasma Ca < 2.0 mM first week postpartum	14/15	13/15	12/15	7/15
Plasma Ca < 2.0 mM weeks 2-8	3/15	2/15	0/15	1/15



**Figure 1** Means phosphorus and calcium content in blood plasma from calving to 56 days in lactation of cows receiving High or required levels of P during the dry period and normal or reduced levels of P during lactation

**Conclusions** Feeding below P requirements during the first 8 weeks of lactation does not seem to increase the incidence of clinical milk fever; it reduces plasma P content but increases plasma Ca, likely by increased bone mobilization. Dietary P content of the dry cow ration also affects plasma content postpartum. When feeding at ~185% of calculated dry cow P requirements, postpartum plasma P and Ca content are reduced compared to a diet at P requirements.

## References

- Goff P. J., 2004. Macromineral disorders of transition cow. *Vet Clin Food Anim.* 20:471-494  
 Grünberg W., 2014. Treatment of Phosphorus Balance Disorders, *Veterinary Clinics of North America: Food Animal Practice*, 30 (2) : 383-408,  
 Reinhardt T.A., J.D. Lippolis, B.J. McCluskey, J.P. Goff, and R.L. Horst. 2010. Prevalence of subclinical hypercalcemia in dairy herds. *The Veterinary journal* 188: 122-124

### Dietary phosphorus restriction induces bone mobilization, affects acid-base balance and downregulates synthesis of fibroblast growth factor 23 in bone

W. Grünberg<sup>1</sup>, A. S. Muscher-Banse<sup>2</sup>, O. Köhler<sup>3</sup>, A. Rajaeerad<sup>4</sup>, N. Schnepel<sup>2</sup>, K. Hansen<sup>2</sup> and M. R. Wilkens<sup>2</sup>

<sup>1</sup>Clinic for Cattle, University of Veterinary Medicine Hannover, Germany; <sup>2</sup>Institute of Physiology and Cell Biology, University of Veterinary Medicine Hannover, Germany; <sup>3</sup>Department of Ruminant Nutrition, Faculty of Agricultural Sciences, Georg-August-University, Göttingen, Germany; <sup>4</sup>Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Iran

Email: [Walter.Gruenberg@tiho-hannover.de](mailto:Walter.Gruenberg@tiho-hannover.de)

**Introduction** Recently, we demonstrated that restricted dietary phosphorus (P) supply and hypophosphatemia in late gestating dairy cows, resulted in bone mobilization and increased 1,25-dihydroxyvitamin D (1,25-(OH)<sub>2</sub>D) concentration at parturition and markedly lower peak in serum parathyroid hormone compared to cows on adequate P supply (Cohrs et al., 2018). We hypothesized that a decline of the serum phosphate (P<sub>i</sub>) concentration ([Pi]) stimulated osteoclastic activity and resulted in a downregulation of fibroblast growth factor 23 (FGF23), a phosphatonin synthesized by osteocytes that was shown to modulate vitamin D metabolism.

**Materials and Methods** To determine if results obtained in cattle can be reproduced in sheep and to test the hypothesis above 11 female, non-pregnant, non-lactating sheep, four to nine years of age were either fed a diet with adequate (AP; 0.25% P, 0.93% Ca) or low P content (LP; 0.11% P, 0.93% Ca) over a period of 6 weeks. Blood samples were obtained once a week to determine the serum concentration of P<sub>i</sub>, Ca and the bone resorption marker CrossLaps® (CL). After six weeks, animals were sacrificed one hour after feeding concentrate. Bone tissue samples were obtained for analysis of RNA expression of Receptor Activator of NF-κB Ligand (RANKL), a crucial factor for osteoclast activation, and osteoprotegerin (OPG), a glycoprotein inhibiting bone mobilization by binding RANKL, and FGF23.

**Results** While neither treatment- nor time effects were determined for the serum [Pi] and [Ca], a significant increase in serum CL concentrations was observed in animals assigned to LP. The difference in CL concentration after 5 weeks on experimental diets between treatments did not reach significance level ( $0.55 \pm 0.13$  for LP vs.  $0.28 \pm 0.15$  ng/ml AP). At the time of sacrifice, [Pi] in saliva ( $12.8 \pm 2.64$  vs.  $17.4 \pm 3.49$  mM) and rumen fluid ( $12.8 \pm 3.23$  vs.  $21.4 \pm 2.59$  mM) were significantly lower in sheep on LP than sheep on AP diet. While rumen pH was not altered, LP sheep showed significantly lower urinary pH in comparison to AP animals ( $7.57 \pm 0.36$  vs.  $8.05 \pm 0.14$ ).

The ratio of RANKL to OPG expression in bone was negatively associated with serum [Pi] ( $P < 0.01$ ,  $R^2: 0.61$ ). RNA expression of FGF23 was significantly down-regulated in LP sheep ( $P < 0.05$ ).

**Conclusions** The results presented here show that dietary P restriction without concomitant Ca deficiency induces bone mobilization in sheep as this was described to occur in dairy cattle. The negative relationship between [Pi] and the ratio of the two key factors involved in osteoclast activation indicate that this process is associated to the serum mineral status although it occurs in the absence of overt hypophosphatemia. The down-regulation of FGF23 -a compound shown to increase renal Pi excretion while inhibiting the activation of vitamin D in monogastric species- that was observed in the sheep on LP diet is presumably a direct effect dietary P restriction. It remains to be determined to what extent the vitamin D metabolism is affected in these non-pregnant, non-lactating sheep used as a model. A reduction of the inhibitory effect of vitamin activation with lower serum FGF23 concentrations occurring in late gestation potentially also facilitates the adaptation of the Ca homeostasis to a sudden increase in Ca demand, occurs at the onset of lactation.

#### References

Cohrs I, Wilkens MR, Grünberg W. (2018): Short communication: Effect of dietary phosphorus deprivation in late gestation and early lactation on the calcium homeostasis of periparturient dairy cows. *J Dairy Sci.* 101:9591.

## Effects of green LED light during incubation and dietary mineral variety during rearing on tibia characteristics of broiler chickens at slaughter age

B. C. Güz<sup>1</sup>, R. Molenaar<sup>1</sup>, I. C. de Jong<sup>2</sup>, M. van Krimpen<sup>2</sup> and H. van den Brand<sup>1</sup>

<sup>1</sup>Adaptation Physiology Group, Wageningen University and Research, Wageningen, The Netherlands; <sup>2</sup>Wageningen Livestock Research, Wageningen University and Research, Wageningen, The Netherlands

Email: [bahadir.guz@wur.nl](mailto:bahadir.guz@wur.nl)

**Introduction** Suboptimal leg health, which can be observed in fast growing broiler chickens, is known to result in pain, stress and mortality, and consequently affects welfare and natural locomotion-related behaviours negatively. Incubation conditions, such as lighting, may stimulate bone development of the embryo and leg health in later life. Moreover, mineral variety and consequently mineral availability for the chicken could affect post-natal bone development, and might reduce locomotion-related problems and risk on injuries during depopulation, transportation and slaughtering. This study was designed to evaluate effects of green LED light during incubation and dietary mineral variety for both macro (Ca and P) and trace (Fe, Cu, Mn, Zn, Se) minerals during the rearing period on tibia morphological, biophysical and mechanical characteristics in fast-growing broiler chickens at slaughter age.

**Materials and Methods** The experiment was setup as a 2 x 2 x 2 factorial arrangement in two phases; incubation and grow-out. A total of 2400 eggs (Ross 308) were obtained from a commercial hatchery. Half of the eggs (n=1200) were incubated under monochromatic green LED light, whereas the other half (n=1200) were incubated in darkness. After hatch, a total of 864 male broiler chickens were reared until slaughter age (d42) and provided one of four types of diet: 1. inorganic macro + inorganic trace; 2. inorganic macro + organic trace; 3. organic macro + inorganic trace; 4. organic macro + organic trace minerals during the rearing period in a complete randomized block design with 8 treatments and 9 replicates per treatment, and each replicate contained 12 chickens. At slaughter age (d42), two male chickens per replicate were randomly selected and tibia bones were obtained. Tibia weight (TW, g), length (TL, cm), thickness (TT, cm), osseous volume (TOV, cm<sup>3</sup>), pore volume (TPV, cm<sup>3</sup>), total volume (TTV, cm<sup>3</sup>), mineral content (TMC, g), mineral density (TMD, g/cm<sup>2</sup>), ultimate strength (TUS, N), and stiffness (TSF, N/mm) were determined.

**Results** Interactions between macro and trace mineral variety were found for several variables (Table 1). The organic macro + organic trace minerals and organic macro + inorganic trace minerals treatments had longer TL ( $P=0.05$ ), higher TOV ( $P=0.03$ ), higher TMC ( $P=0.002$ ), higher TMD ( $P=0.02$ ), higher TUS ( $P=0.004$ ), and higher TSF ( $P=0.003$ ) compared to inorganic macro + inorganic trace minerals group, whereas the inorganic macro + organic trace minerals treatment was in between. Groups of organic macro + organic trace minerals and organic macro + inorganic trace minerals had higher TTV ( $P=0.04$ ) compared to groups of inorganic macro + inorganic trace minerals and inorganic macro + organic trace minerals. Dietary organic macro minerals resulted in higher TW ( $P=0.04$ ), TT ( $P=0.03$ ), and TPV ( $P=0.05$ ) than inorganic macro minerals. Green LED light during incubation or darkness did not affect any of the tibia characteristics.

**Table 1** Green LED light during incubation and dietary mineral variety during rearing on tibia morphological, biophysical and mechanical characteristics of fast-growing broiler chickens at slaughter age

	TW	TL	TT	TOV	TPV	TTV	TMC	TMD	TUS	TSF
<b>INCUBATION</b>										
DARKNESS	16.29	12.51	1.21	24.48	4.72	29.42	14.42	0.24	251.4	245.1
GREEN	16.49	12.72	1.32	24.79	4.80	29.59	14.62	0.25	256.7	249.5
<b>MACRO MINERALS</b>										
INORGANIC	16.20 <sup>b</sup>	12.60	1.23 <sup>b</sup>	24.77	4.60 <sup>b</sup>	29.23	14.11	0.24	254.5	238.0
ORGANIC	16.71 <sup>a</sup>	12.97	1.41 <sup>a</sup>	25.28	4.79 <sup>a</sup>	29.90	14.89	0.26	270.3	250.6
<b>TRACE MINERALS</b>										
INORGANIC	16.19	12.80	1.24	24.72	4.73	29.14	14.32	0.24	255.1	232.1
ORGANIC	16.31	12.93	1.30	24.92	4.72	29.30	14.64	0.26	268.5	251.5
<b>MACRO MINERALS * TRACE MINERALS</b>										
INORGANIC MACRO + INORGANIC TRACE	16.39	12.12 <sup>b</sup>	1.21	24.52 <sup>b</sup>	4.58	29.23 <sup>b</sup>	14.44 <sup>b</sup>	0.24 <sup>b</sup>	255.7 <sup>b</sup>	241.1 <sup>b</sup>
INORGANIC MACRO + ORGANIC TRACE	16.42	13.10 <sup>a</sup>	1.28	24.81 <sup>a</sup>	4.73	29.53 <sup>a</sup>	14.78 <sup>a</sup>	0.28 <sup>a</sup>	270.9 <sup>a</sup>	254.7 <sup>a</sup>
ORGANIC MACRO + INORGANIC TRACE	16.51	12.81 <sup>a</sup>	1.29	24.82 <sup>a</sup>	4.82	29.64 <sup>a</sup>	14.98 <sup>a</sup>	0.29 <sup>a</sup>	274.5 <sup>a</sup>	264.4 <sup>a</sup>
ORGANIC MACRO + ORGANIC TRACE	16.38	12.44 <sup>ab</sup>	1.31	24.62 <sup>ab</sup>	4.58	29.37 <sup>b</sup>	14.50 <sup>ab</sup>	0.26 <sup>ab</sup>	261.9 <sup>ab</sup>	251.8 <sup>ab</sup>
<b>P VALUES</b>										
INCUBATION	0.12	0.14	0.11	0.09	0.08	0.10	0.35	0.25	0.08	0.14
MACRO MINERALS	0.04	0.03	0.03	0.02	0.05	0.006	0.002	0.004	0.003	0.004
TRACE MINERALS	0.62	0.09	0.12	0.11	0.46	0.13	0.17	0.24	0.01	<0.001
MACRO MINERALS * TRACE MINERALS	0.23	0.05	0.81	0.03	0.12	0.04	0.02	0.02	0.004	0.003

a-b Values within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

**Conclusions** It can be concluded that green LED lighting during incubation did not affect tibia characteristics. Dietary organic macro minerals (Ca and P) positively affected most of the tibia morphological, biophysical and mechanical characteristics. Organic trace minerals (Fe, Cu, Mn, Zn, Se) have influenced just a few tibia mechanical characteristics. It can be suggested that organic sourced Ca and P in the fast growing broiler chickens diet might improve bone development and consequently has positive effects on locomotion, welfare, and might decrease the risk on injuries during depopulation, transport and slaughtering.

### Calcium and nitrogen metabolism in growing sheep are affected by a menthol-based feed additive

A. K. Patra<sup>1,2</sup>, S. Geiger<sup>1</sup>, A. Cieslak<sup>3</sup>, M. Szumacher-Strabel<sup>3</sup>, H.-S. Braun<sup>4</sup>, K. T. Schrapers<sup>4</sup> and J. R. Aschenbach<sup>1</sup>

<sup>1</sup>Institute of Veterinary Physiology, Freie Universität Berlin, Germany, <sup>2</sup>Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata, India, <sup>3</sup>Department of Animal Nutrition, Poznan University of Life Sciences, Poznan, Poland, <sup>4</sup>PerformaNat GmbH, Berlin, Germany

Email: [schrapers@performanat.de](mailto:schrapers@performanat.de)

**Introduction** Specific plant bioactive lipid compounds (PBLC) have been shown to modulate the activity of several TRP channels, a family of non-selective cation channels. Ruminal epithelia express TRPV3, which is known to conduct cations such as  $\text{Ca}^{2+}$ . Menthol elevated  $\text{Ca}^{2+}$  transport via the ruminal epithelium significantly (Rosendahl et al., 2016) and a HEK cell line overexpressing the bovine TRPV3 increased its  $\text{Ca}^{2+}$  conductance upon menthol addition (Rosendahl et al., 2016; Schrapers et al., 2018). The physiological relevance of menthol-stimulated  $\text{Ca}^{2+}$  absorption was finally confirmed in a feeding study with lactating dairy cows where serum calcium levels increased upon dietary menthol supplementation (Braun et al., 2018).

Since ruminal TRPV3 does not only conduct  $\text{Ca}^{2+}$  but also  $\text{NH}_4^+$  (Schrapers et al., 2018) which, in turn, can influence metabolism of amino acids and urea, the present study tested whether menthol impacts on the serum concentrations of amino acid and urea in addition to those of calcium. The effects of menthol were tested at two practically relevant doses in growing sheep.

**Materials and Methods** 24 growing Suffolk sheep were allotted into three groups following a randomized block design. They were fed hay ad libitum and 600 g of concentrate feeds daily for a period of 28 d. The animals were either supplemented with no PBLC (control), a low dose (PBLC-low, 80 mg/d) or high dose (PBLC-high, 160 mg/d) of a PBLC with menthol as lead compound (OAX17, PerformaNat GmbH, Germany). Daily feed intake was monitored for the last 21 d to determine protein intake. Before the start and at the end of the trial, blood samples were taken to measure serum concentrations of calcium, magnesium and urea. Furthermore, serum concentrations of amino acids were analyzed using UPLC. Data were analyzed using the mixed model procedure of SAS including treatment, block, experimental run and sex into the model. Pre-treatment values were included as covariate for the serum biochemical analysis. Linear and quadratic effects were tested as well as control versus both PBLC groups.

**Results** After 28 d, PBLC supplementation induced minor changes in mineral homeostasis. Serum calcium tended to increase linearly with increased PBLC concentration ( $p = 0.089$ ), whereas magnesium was not influenced by PBLC feeding. The serum concentrations of urea and total free amino acids were not influenced by PBLC feeding ( $p > 0.1$ ); however, the amino acid profile changed. PBLC supplementation increased the sum of aspartate (Asp) and asparagine (Asn) ( $p = 0.005$ ) as well as the sum of glutamate (Gln) and glutamine (Glu) ( $p = 0.041$ ) linearly. Lysine concentrations tended to increase ( $p = 0.054$ ). The changes remained after including crude protein intake in the model as a covariable. Other amino acids were not significantly influenced by PBLC feeding.

**Discussion** The present study supports the hypothesis that menthol supplementation positively influences calcium absorption in ruminants. Furthermore, it provides first evidence for a potential impact of menthol on nitrogen metabolism. The observed changes in amino acid profile are most plausibly explained by an increase in ruminal  $\text{NH}_4^+$  absorption following the activation of a non-selective cation channel by menthol. The ruminal TRPV3 would be the most likely candidate for that channel given its proven activation by menthol and its ability to conduct  $\text{NH}_4^+$ . Increased ruminal absorption of  $\text{NH}_4^+$  would stimulate hepatic glutamine synthesis, which could explain the elevated serum level of Glu and Gln in the present study. Glu and Gln can act as precursors for Asp and Asn synthesis from oxaloacetate (Murray et al., 2003), which were increased by PBLC feeding, too. Surprisingly, blood urea levels were not affected. In a recent study with lactating dairy cows, urea levels were even decreased by menthol-containing PBLC (Braun et al., 2018). A potentially higher uptake of  $\text{NH}_4^+$  without an increase in nitrogen excretion in form of urea indicates that menthol supplementation could potentially lead to a higher nitrogen retention.

**Conclusion** Menthol-containing PBLC influence the calcium homeostasis of ruminants such as growing sheep, most likely, via increased calcium absorption. The latter is likely caused by an interaction of menthol with ruminal TRPV3 channels. Simultaneously increased  $\text{NH}_4^+$  absorption via ruminal TRPV3 channels could explain the positive effect of menthol supplementation on the blood levels of ureagenic amino acids. Further research shall evaluate whether the latter may promote nitrogen retention, as indicated by the missing increase in urea itself.

### References

- Braun, H.-S., Schrapers, K.T., Mahlkow-Nerge, K., Stumpff, F., and Rosendahl, J. (2018). Dietary supplementation of essential oils in dairy cows: evidence for stimulatory effects on nutrient absorption. *Animal* 1–6.
- Murray, R.K., Granner, D.K., Mayes, P.A., and Rodwell, V.W. (2003). A LANGE medical book. Harper's Illustrated Biochemistry. 26th Ed. New York: McGraw-Hill Companies, Inc.
- Rosendahl, J., Braun, H.S., Schrapers, K.T., Martens, H., and Stumpff, F. (2016). Evidence for the functional involvement of members of the TRP channel family in the uptake of  $\text{Na}^+$  and  $\text{NH}_4^+$  by the ruminal epithelium. *Pflügers Archiv-European Journal of Physiology* 468, 1333–1352.
- Schrapers, K.T., Sponder, G., Liebe, F., Liebe, H., and Stumpff, F. (2018). The bovine TRPV3 as a pathway for the uptake of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{NH}_4^+$ . *PLOS ONE* 13, e0193519.



### Concentrations of trace elements in serum of dry cows and corresponding reference intervals for the dry period

S. van der Drift, R. Grotentraast, S. Carp – Van Dijken, J. Muskens and G. Counotte

GD Animal Health, Deventer, The Netherlands

Email: s.v.d.drift@gddiergezondheid.nl

**Introduction** Trace elements are required by all animals in minute amounts and play essential roles in the body, usually as part of functional organic molecules such as enzymes. For example, zinc, copper and selenium are a functional part of enzymes that participate in the anti-oxidant system of the body. Iodine is an important constituent of thyroid hormones, which play a central role in metabolism and growth. Serum concentrations of trace elements in cows are used in the field to monitor (short term) uptake of these elements from the ration. Reference intervals and cut-off values are, however, usually based on serum results from dairy cows and specific dry cow reference values are often lacking. The objective of this research was to investigate concentrations of the trace elements copper, zinc, selenium and iodine in serum of dry cows under field conditions in the Netherlands and to calculate reference intervals for these trace elements for the dry period.

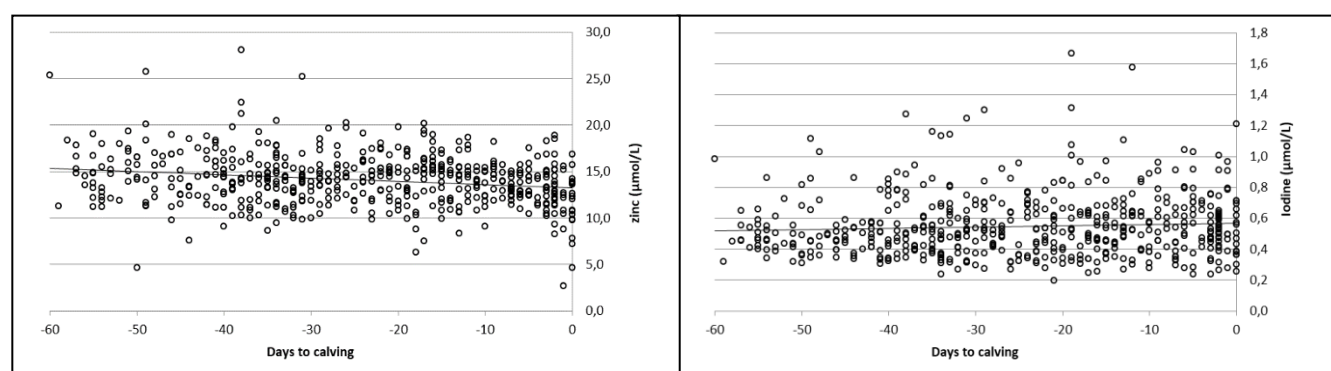
**Materials and Methods** From September 2014 to April 2015, serum samples of 625 dry cows from 40 Dutch dairy herds were collected during two farm visits (interval between visits was approximately three months). At each farm visit, all dry cows present were sampled. The number of cows sampled per farm ranged from 2 to 32. Collected serum samples were stored below – 20 °C until analysis. Only samples of cows with complete data records including calving date were analysed (n=541). Samples were analysed in the routine laboratory of GD Animal Health for copper, zinc, selenium and iodine using inductively coupled plasma mass spectrometry. Four samples did not contain enough sample material to complete the analysis. Two cows with were excluded from the dataset because they were sampled outside the interval of 60 to 0 days before parturition. The final dataset contained observations from 535 cows. Descriptive statistics were performed on serum results using Statistix. Linear regression was used to indicate time trends in trace element concentrations towards parturition. Reference intervals for concentrations of trace elements for dry cows were calculated using the Reference Value Advisor (Geffre et al., 2011).

**Results** Descriptive statistics for concentrations of trace elements in serum of dry cows are shown in Table 1. As an example, zinc and iodine concentrations are also plotted against day relative to calving in Figure 1. Serum zinc concentrations were significantly lower in cows with fewer days towards partition ( $P<0.05$ ); no such trend was present for the other elements ( $P>0.05$ ). Iodine concentrations were low in dry cows (22% of cows had a serum concentration below the common cut-off value of  $<0.4 \mu\text{mol/L}$ ).

**Table 1** Descriptive statistics for concentrations of zinc, copper, selenium and iodine in serum of 535 dry cows from 60 to 0 days to parturition from 40 Dutch dairy herds and corresponding reference intervals for the dry period.

Trace mineral ( $\mu\text{mol/L}$ )	Mean ( $\pm$ SD)	Median	Min - Max	25 <sup>th</sup> – 75 <sup>th</sup> perc.	Reference interval <sup>1</sup>
Zinc	14 ( $\pm$ 2.8)	14	3 – 28	12 – 16	9 - 20
Copper	11 ( $\pm$ 2.4)	11	3 – 21	10 – 13	8 - 17
Selenium	0.7 ( $\pm$ 0.18)	0.7	0.2 – 1.4	0.6 – 0.8	0.3 – 1.0
Iodine	0.5 ( $\pm$ 0.20)	0.5	0.2 – 1.7	0.4 – 0.7	0.3 – 1.1

<sup>1</sup>Calculated with Reference Value Advisor (Geffre et al., 2011)



**Figure 1** Concentrations of zinc (left) and iodine (right) in serum ( $\mu\text{mol/L}$ ) of 535 dry cows from 60 to 0 days to parturition from 40 Dutch dairy herds (including linear trend lines).

**Conclusions** This research shows that variation exists in serum concentrations of trace elements in dry cows. The iodine status of dry cows may need extra attention to optimize cow health and calf vitality. Reference intervals for dry cows were calculated.

#### References

Geffre, A., Concordet, D., Braun, J.P., Trumel, C. 2011. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet. Clin. Pathol. 40(1): 107–112.



# Application of alternative lime amendment to soil increases forage molybdenum inducing copper deficiency

R. J. Van Saun<sup>1</sup>, M. Hall<sup>2</sup> and J. T. Spargo<sup>3</sup>

<sup>1</sup>Department of Veterinary and Biomedical Sciences; <sup>2</sup>Department of Plant Sciences, <sup>3</sup>Agricultural Analytical Services Laboratory, College of Agricultural Sciences, Pennsylvania State University, PA, USA

Email: rjv10@psu.edu

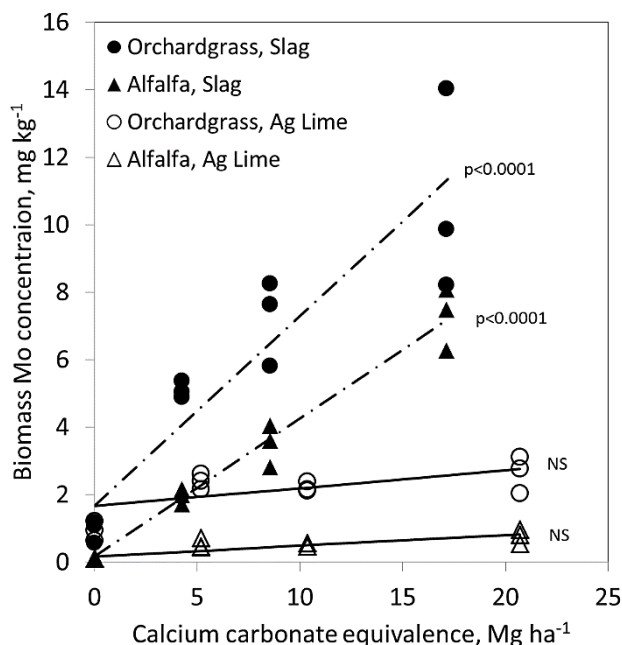
**Introduction** A clinical situation occurred where a sheep flock experienced high death rate (24 of 25) in only their two year-old ewes and high stillborn and neonatal deaths (27%). All diagnostic information on lamb and ewe necropsies showed no infectious agent and low liver Cu concentration. The interaction of molybdenum (Mo), sulfur, and copper (Cu) in ruminant animals has been well described (Suttle, 2010). The Cu deficiency was believed to be induced by application of an alternative lime (slag) product being applied to the farm's land for the previous two years resulting in high forage Mo content. This slag was found to contain between 50 and 80 mg/kg Mo where cautionary labels are required for fertilizers containing >10 mg/kg Mo. Over a 4-year period following the initial application of the slag product, home raised forages fed to the sheep had 196% higher Mo, no difference in Cu and a 46% reduction in Cu:Mo ratio compared to previous forage data. The objective of this study was to determine if the slag lime product compared to traditional agricultural lime would increase plant tissue Mo content and reduce Cu:Mo ratio in the plant.

**Materials and Methods** To test the hypothesis of the slag lime directly increasing forage Mo content a controlled greenhouse study was performed with a factorial split plot experimental design. Two common agronomic forage plants, Orchardgrass (*Dactylis glomerata*) and alfalfa (*Medicago sativa*), were used to mimic common pasture plants for the region. Seeds (10 each, 20 per pot) were planted into soil (5 mm depth) that was treated with dolomitic lime or slag at 4 different rates of application (0, 0.5, 1.0 and 2.0x) based on recommended calcium carbonate equivalents (CCE) required to amend soil pH to a final value of 7.0 (8.96 Mg CCE/ha). Soil used in pots was matched in composition and pH to that of the region of the farm. Soil mineral content was determined prior to the study to ensure no biases. Pots were fertilized with phosphorus and potash and provided 16 hr daylight. Upon emergence, plants in each pot were reduced to 5 of each species. Plants were harvested at 150 mm height when alfalfa had bloomed, and plant material separated into species and dried for compositional analysis. Nitrogen fertilization was applied following the first harvest based on browning of the orchardgrass suggesting soil nitrogen depletion. A total of three harvest cuttings were collected and analysed separately and cumulatively as a weighted average. Analysis of variance (ANOVA) and regression modelling were applied to the data. Independent variables in the ANOVA model performed by plant species with model effects of treatment (lime vs slag) and dose (4 levels) and their interactions on plant tissue Mo and Cu content and calculated Cu:Mo ratio.

**Results** Both lime and slag effectively increased soil pH ( $P<0.0001$ ), though the slag product was more effective ( $P=0.009$ ) at an equivalent CCE suggesting readily available calcium. Addition of the slag product to soil increased ( $P<0.0001$ ) plant tissue Mo concentration in both orchardgrass and alfalfa for all three harvests in a dose-dependent response from a single soil application to amend pH. There was no effect of either lime or slag on plant Cu content. Rate of application ( $P=0.041$ ) and slag by rate interaction ( $P<0.0001$ ) reduced plant tissue Cu:Mo ratio for both plant species.

Measured changes in Mo and Cu:Mo ratio in this study reflect the observed changes in forage on the affected sheep farm. Weighted average cumulative data showed a similar slag effect across all harvests on increasing ( $P<0.0001$ ) plant Mo content (Figure 1). Contrary to what was expected, orchardgrass had higher Mo content compared to alfalfa when it is often assumed legumes accumulate Mo better than grasses. Weighted average biomass Cu:Mo ratio was fitted to a three-parameter decay model. Regression analysis to determine Mo uptake coefficient ( $UC = \text{mg Mo kg}^{-1} \text{ forage/ kg Mo ha}^{-1}$ ) showed much higher UC (5.58 orchardgrass; 4.32 alfalfa) than what has been previously estimated (2.25) for Mo (O'Connor et al., 2001).

**Conclusions** Molybdenum in an alternative lime product seems readily available to growing plants as evidenced by increased plant tissue content in a dose-dependent manner. Plant Mo content remained high in all three harvests without any further addition of lime product. The elevation in plant Mo was consistent with observed Mo content in the case study and suggests the slag product was responsible for the sheep flock's health issues related to Cu deficiency.



**Figure 3. Weighted average biomass molybdenum (Mo) content in orchardgrass and alfalfa grown in soil amended with either slag or agricultural lime at 4 doses based on adjusting soil pH to 7.0.**

## References

Suttle N.F. 2010. The mineral nutrition of livestock, 4<sup>th</sup> ed. O'Connor GA, et al., A modified risk assessment to establish molybdenum standards for land application of biosolids. J Environ Qual 2001;30(5):1490-1507.

### Maternal-fetal hepatic mineral interactions

R. J. Van Saun

Department of Veterinary and Biomedical Sciences, College of Agricultural Sciences, Pennsylvania State University, PA, USA

**Introduction** Transfer of minerals from maternal blood to the gravid uterus is recognized as a critical physiologic process for development and survival of the fetus and neonate (Hostetler et al., 2003). The fetal liver can concentrate minerals to equal or exceed maternal hepatic concentration when corrected to a dry weight basis. Trace mineral content of milk is generally low, especially relative to iron, copper and selenium thus, requiring the nursing animal to mobilize hepatic stores of minerals. Most studies have reported comparisons of maternal and fetal liver mineral concentrations and attempted to develop regression models to predict the association. The study objective was to further characterize relationships between paired maternal and fetal hepatic mineral concentrations calculated as a ratio to better understand the dynamics in mineral homeostasis during pregnancy.

**Materials and Methods** Paired maternal and fetal livers (n=185), including 11 twin fetuses, were collected during five visits to an abattoir. Cattle breeds were identified as dairy (Holstein mostly) or beef (Angus and Hereford mostly). Fetal crown-to-rump length was measured and used to estimate gestational age (GA). Maternal and fetal liver mineral concentrations were determined by inductively coupled plasma spectroscopy (ICP/MS) methods. Mineral concentrations ( $\mu\text{g/g}$ ) were determined on a wet weight basis and converted to dry weight basis using determined liver dry matter content. Measured minerals included calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). Paired maternal-fetal liver mineral concentrations were used to calculate the fetal-maternal mineral concentration ratio (FMR) or maternal-fetal mineral concentration ratio (MFR). Mineral data were assessed for normality and transformed as necessary. Analysis of variance (ANOVA) models evaluated main effects of breed (Dairy or Beef), GA, gender, twin, sampling period on FMR or MFR. Correlation coefficients and linear and nonlinear regression modeling was used to determine relationships between fetal or maternal liver mineral concentrations or GA and FMR or MFR.

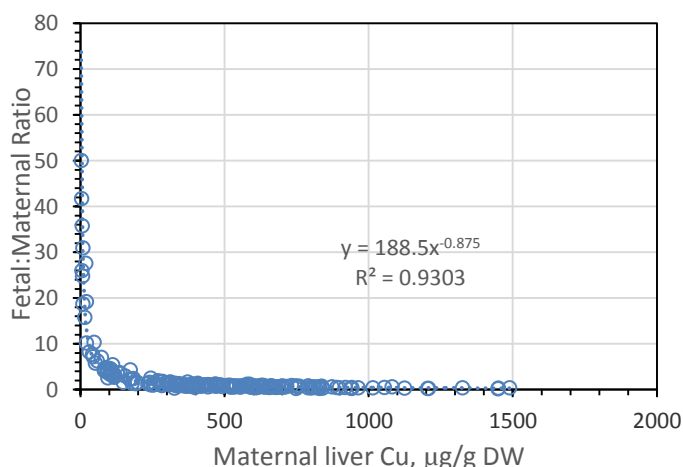
**Results** Mean ( $\pm$  SD) fetal age was  $6.4 \pm 1.5$  mo (range: 3.7-9.4 mo). Mean ( $\pm$  SD) FMR values were  $>1$  indicating fetal concentrating ability for Ca ( $1.69 \pm 0.64$ ), Cu ( $3.01 \pm 6.91$ ), Fe ( $4.93 \pm 4.50$ ), Mg ( $1.33 \pm 0.23$ ), Se ( $1.71 \pm 1.25$ ) and Zn ( $4.50 \pm 2.72$ ), whereas FMR was  $< 1$  for Mn ( $0.76 \pm 0.30$ ), Mo ( $0.21 \pm 0.12$ ) and Co ( $0.38 \pm 0.33$ ). Mean MFR were reverse values from FMR relative being  $>$  or  $< 1$ , except for Cu ( $1.19 \pm 0.87$ ).

Association between maternal and fetal liver mineral concentrations were only found with Cu ( $r=0.29$ ;  $P<0.0001$ ) and Se ( $r=0.64$ ;  $P<0.0001$ ). Fetal-maternal ratio and MFR were influenced by GA for Mg ( $P<0.0001$ ), Ca ( $P<0.004$ ), Mn ( $P<0.0001$ ), Fe ( $P<0.0002$ ) and Mo ( $P<0.0001$ ) when adjusting for breed and sampling period. In general FMR declined and MFR increased with GA, though these were not always linear relationships. Both Mn and Mo showed increasing FMR and declining MFR with GA. Both FMR and MFR for all minerals were modeled relative to respective fetal and maternal liver mineral concentration. Both Cu (Figure 1) and Co showed a high association between FMR and maternal Co concentration; best fitted by a power function. These models indicate at low maternal liver Cu or Co concentration a high FMR whereas above a minimal maternal concentration FMR was quite reduced. This relationship was seen in a strong linear association between MFR and maternal Cu ( $r^2=0.76$ ,  $P<0.0001$ ) or Co ( $r^2=0.89$ ,  $P<0.0001$ ) liver concentration. A similar power function was found between FMR and maternal mineral concentrations for Se ( $r^2=0.42$ ,  $P<0.0001$ ), Fe ( $r^2=0.24$ ,  $P<0.0001$ ), Zn ( $r^2=0.57$ ,  $P<0.0001$ ) and Ca ( $r^2=0.53$ ,  $P<0.0001$ ). There was a quadratic relationship between maternal ( $r^2=0.39$ ,  $P<0.0001$ ) and fetal ( $r^2=0.56$ ,  $P<0.0001$ ) liver Mg concentrations and Mg-FMR. Maternal-fetal Mg ratio was linearly associated with maternal ( $r^2=0.37$ ,  $P<0.0001$ ) and quadratically with fetal ( $r^2=0.60$ ,  $P<0.0001$ ) liver Mg concentration. Both Mn and Mo showed similar stronger relationships between fetal liver mineral concentration and FMR and MFR. Fetal Mo ( $r^2=0.83$ ,  $P<0.0001$ ;  $r^2=0.77$ ,  $P<0.0001$ ) and Mn ( $r^2=0.51$ ,  $P<0.0001$ ;  $r^2=0.69$ ,  $P<0.0001$ ) concentrations were associated linearly with FMR and nonlinearly with MFR, respectively. Additionally, Fe ( $r^2=0.74$ ,  $P<0.0001$ ), Zn ( $r^2=0.60$ ,  $P<0.0001$ ) and Ca ( $r^2=0.53$ ,  $P<0.0001$ ) showed a lower fetal liver mineral concentration with higher MFR, but this relationship was not observed with Cu, Co or Se.

**Conclusions** These data show a complex relationship between maternal and fetal liver mineral concentrations over the last two-thirds of pregnancy. Elevated FMR were most often associated with low maternal liver mineral concentration suggesting an efficient or facilitated transfer mechanism. A lower FMR at higher maternal liver mineral concentration suggests some underlying protective mechanism to minimize excessive fetal mineral accumulation. Further work is necessary to define underlying mechanisms.

### References

Hostetler CE, et al. The role of essential trace elements in embryonic and fetal development of livestock. Vet J 2003;166:125-139.



**Figure 4. Plot of fetal-maternal ratio for liver copper (Cu) to maternal liver Cu concentration.**

### Effect of dietary phytase and lactic acid treatment of cereals on gene expression related to Ca and P homeostasis along the intestinal tract, bones, and kidney in pigs

J. C. Vötterl<sup>1</sup>, J. Klinsoda<sup>1</sup>, Q. Zebeli<sup>1</sup>, I. Hennig-Pauka<sup>2</sup> and B. U. Metzler-Zebeli<sup>1</sup>

<sup>1</sup>Institute of Animal Nutrition and Functional Plant Compound, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria; <sup>2</sup>Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Bakum, Germany

Email: Julia.Voetterl@vetmeduni.ac.at

**Introduction** The positive effect of dietary phytase on phosphorus (P) digestibility, performance and bone quality in pigs is well established. Soaking of cereal grains in lactic acid (LA) recently showed to reduce the phytate-P content in wheat and corn. However, it has not been evaluated *in vivo* whether this P release is similar to the action of phytase or may potentiate the effect of phytase and therefore have the same regulatory effect in the intestine and bones. In the present study, we investigated the effect of LA-treatment of cereals and dietary phytase, alone or coupled with each other, on the expression of genes related to intestinal calcium (Ca) and P absorption, bone and kidney metabolism in pigs.

**Materials and Methods** Thirty-two castrated male crossbred pigs ( $13.1 \pm 2.3$  kg) were randomly assigned to one of the four diets with  $2 \times 2$  arrangement of treatments in a completely randomized design in four 18-day replicate batches, resulting in a total of eight observations per diet. The diets were: Con) diet with untreated wheat and corn and no phytase; Con-Phy) diet with untreated wheat and corn and phytase supplementation; LA) diet with LA-treated wheat and corn and no phytase; LA-Phy) diet with LA-treated wheat and corn and phytase supplementation. The LA treatment consisted of soaking wheat and corn for 48 hours in a 2.5% LA solution. Phytase was supplemented at 500 FTU/kg diet. The pigs were individually fed three times daily. Metacarpal bones and gut tissues from duodenum, jejunum, ileum, caecum, and colon were collected on day 18. Relative expression of genes related to barrier function and mineral uptake in the intestine and mineral homeostasis in metacarpal bones were determined using quantitative PCR. Data were subjected to ANOVA using the Mixed procedure of SAS with the fixed effect of treatment and the random effect of replicate batch and differences at  $P < 0.05$  were considered as significant.

**Results** The phytase supplementation had a stronger effect on the modulation of the gene expression related to Ca and P metabolism along the small and large intestines, and kidneys compared to the LA-treatment of cereals. Results further indicated changes in the intestinal response that can be related to the continuously advancing digestion and absorption of Ca and P as well as fermentation along the small and large intestines. Phytase influenced the vitamin D-dependent Ca uptake by decreasing the expression levels of vitamin-D deactivating enzyme *CYP24A1* on average by 80% in the duodenum and jejunum as well as of the vitamin-D receptor *VDR* expression in the ileum ( $P < 0.05$ ). This was accompanied by the down-regulated expression of the Ca transporter *TRPV6* by on average 67% in the jejunum, ileum, and colon ( $P < 0.05$ ) and that of the Ca transporter *TRPV5* by 49% in the cecum in pigs fed Phy diets ( $P < 0.05$ ). In the small and large intestines, the LA treatment of cereals only decreased the expression of the Ca transporter *TRPV6* by 44% in the jejunum ( $P < 0.05$ ). Interestingly, both dietary treatments did not modify the intestinal expression levels of sodium dependent P transporter, indicating postabsorptive modulation of P metabolism. In the kidney, the phytase supplementation increased the expression level of vitamin-D receptor *VDR*, the calcium-transporting protein *CALB1* and the Ca transporter *TRPV5* ( $P < 0.001$ ), whereas the LA treatment of cereals downregulated the renal expression of *CYP24A1* ( $P < 0.001$ ). In contrast to the intestines and kidney, the LA treatment of cereals influenced more strongly the gene expression levels in the bones than phytase addition. As such, the phytase supplementation only reduced the expression of vitamin-D deactivating enzyme *CYP24A1* by 70.6% in the metacarpal bone ( $P = 0.015$ ). The LA treatment of cereals, in turn, decreased the expression of the vitamin D-activating enzyme *CYP27B1*, and osteoclastogenesis regulators *OPG* and *RANKL* in the bone ( $P < 0.05$ ). Moreover, the LA treatment of cereals decreased the phosphaturic hormone *FGF23* by 40% ( $P = 0.009$ ), whereas the bone modeling marker *OCN* was decreased by on average 72% by both dietary treatments ( $P < 0.05$ ).

**Conclusions** The present results showed that dietary phytase supplementation and LA treatment of cereals differently modulated the gene expression related to intestinal absorption and renal and bone metabolism of Ca and P. This may be linked to their diverging effect to improve the intestinal Ca and P availability, leading to different systemic Ca and P levels. Since pigs were in the resorptive phase of digestion, alterations in intestinal gene expression levels may reflect adaptations to luminal Ca and P concentrations in the small and large intestines. Improved systemic utilization and the corresponding regulation of the metabolism of Ca and P may be indicated by the upregulation of gene expression levels related to reabsorption of Ca and P in the kidney especially with the phytase and by the decreased gene expression levels of genes for osteoclastogenesis in bones with LA treatment of cereals.

## Effect of voluntary calcium supplementation at calving on survival to the next lactation, milk yield and milk composition of subsequent lactation

J. B. Daniel, J. Wilms and J. Martín-Tereso

Trouw Nutrition R&D, Amersfoort, the Netherlands

Email: [juliette.wilms@trouwnutrition.com](mailto:juliette.wilms@trouwnutrition.com)

**Introduction** Hypocalcaemia is a common metabolic condition during the transition period characterized by a transient loss of calcium (Ca) homeostasis with serum total Ca concentrations below 2 mmol/L (Martín-Tereso and Martens, 2014). Administration of Ca supplementation products to all cows or all multiparous cows after calving is common in calving protocols. Oral Ca supplements provide a source of available Ca and have proven to positively affect blood Ca concentrations (Fowers, 2014). In this light, the objective of this study was to evaluate the effect of prophylactic administration of an oral Ca supplement (from CaCO<sub>3</sub>) at parturition on survival to the next lactation, milk yield and milk composition.

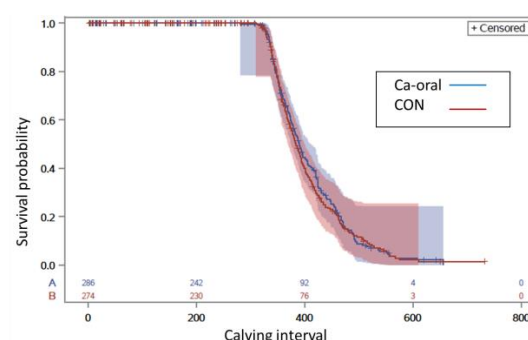
**Materials and Methods** This prospective, randomized and blind study was conducted in 10 commercial dairy farms in the Netherlands. A total of 613 Holstein dairy cows were enrolled in the study upon calving. Cows were blocked within farm by calving sequence and parity (primiparous or multiparous). Within each block of 2 animals, cows were randomly assigned to one of two treatments, including an oral Ca supplement (**Ca-Oral**, n = 312) or a placebo (**CON**, n = 301), offered for voluntary consumption. The CON treatment consisted of 100 g of highly purified food grade cellulose pulp (Jelucel®) and 20 g of dextrose mixed in 20 L of water. Cows in the Ca-Oral group were offered a 20 L commercial Ca suspension (48 g of Ca) containing Ca carbonate, Ca formate, Ca propionate and other minerals and vitamins (Farm-O-San Reviva®, Trouw Nutrition, Amersfoort, the Netherlands). Both treatments were initiated within 3 h after calving and the liquid supplements were offered to cows in a 25 L bucket for 15 min. Measurements included intakes of treatments (fully consumed or not), survival to the next lactation, calving interval, and milk yield and composition of the subsequent lactation. Analyses of survival to the next lactation and calving interval were performed using the GENMOD and LIFETEST procedure of SAS respectively (SAS Institute Inc., 2013). Milk yield and composition were analyzed in two ways: 1. Using milk yield and composition measured monthly by the standard periodic milk control done by the breeding cooperative (CRV, Arnhem, the Netherlands) and 2. Using residual performance obtained by subtracting the measured performance from the predicted performance estimated by the breeding model of CRV. Briefly, these predictions were made with a herd test day model using breeding values and multiple fixed effects, estimated from a much larger reference population than the animals included in the study. Both performance analysis were done using the MIXED procedure of SAS (SAS Institute Inc., 2013), including time, treatment and their interaction as fixed effects and block as a random effect. The cow was considered the experimental unit and time was included as a repeated statement.

**Results** Eighty-two percent of the Ca-Oral cows fully consumed the treatment while only 51% of CON cows did ( $P < 0.01$ ). The treatment had no significant effect ( $P = 0.48$ ) on the probability of reaching the next lactation (i.e. 70.6% of Ca-Oral cows had a next calving against 67.9% in CON). There were also no differences in calving intervals ( $P = 0.62$ ) with medians of 390 and 384 days for Ca-Oral and CON treatments respectively (Figure 1). When comparing the observed performance, no significant difference could be detected for milk yield, nor for composition (with the exception of a time to treatment interaction for milk fat yield, Table 1). In contrast, after subtracting the predicted to the measured performance, significant increase in milk protein yield (+23 g/d) and tendencies ( $P < 0.097$ ) for increased milk yield and milk fat yield (+0.52 kg/d and +19 g/d, respectively) were observed for cows that received the Ca-Oral treatment.

**Table 1** Effect of oral administration of calcium on milk yield and milk composition in dairy cows at calving (n = 613).

Item <sup>1</sup>	Treatments		Pooled SEM	P-values		
	Ca-Oral	CON		Time	Treat	Treat*Time
Analysis on measured performance						
MY kg/d	32.4	32.2	0.4	<0.001	0.772	.
MFY, g/day	1412	1388	16	<0.001	0.173	0.023
MPY, g/d	1486	1477	18	<0.001	0.494	0.826
Analysis on residuals (measured – predicted by CRV genetic model)						
Res MY, kg/d	0.78	0.26	0.22	0.001	0.065	.
Res MFY, g/d	37	18	9	0.203	0.097	0.291
Res MPY, g/d	34	11	7	0.008	0.010	0.917

<sup>1</sup>MY=Milk yield, MFY=Milk fat yield, MPY=Milk protein yield.



**Figure 1** Survival analysis for calving interval ( $P=0.62$ ).

**Conclusions** The supplementation of the tested oral Ca supplement at calving did not increase significantly the probability or time to reach a next lactation. However, the oral Ca supplement positively influenced milk yield and components.

## References

Fowers, R., A. Navarro-Villa, and J. Martín-Tereso. 2015. Effects on general health, energy status and calcium metabolism of a suspension of glucogenic precursors, vitamins and minerals supplemented to dairy cows immediately after calving. 2015. 8th international congress on farm animal endocrinology. Abstract no. 47. • Martín-Tereso, J., and H. Martens. 2014. Calcium and Magnesium Physiology and Nutrition in Relation to the Prevention of Milk Fever and Tetany (Dietary Management of Macrominerals in Preventing Disease). Vet. Clin. North Am. - Food Anim. Pract. 30:643–670.

## Effect of supplementing Zn and Se in different ration around parturition on mammary health and natural antibodies in dairy cows

N. Mayasari<sup>1</sup>, E. Y. Setyowati<sup>1</sup>, A. Arfiana<sup>2</sup> and L. B. Salman<sup>1</sup>

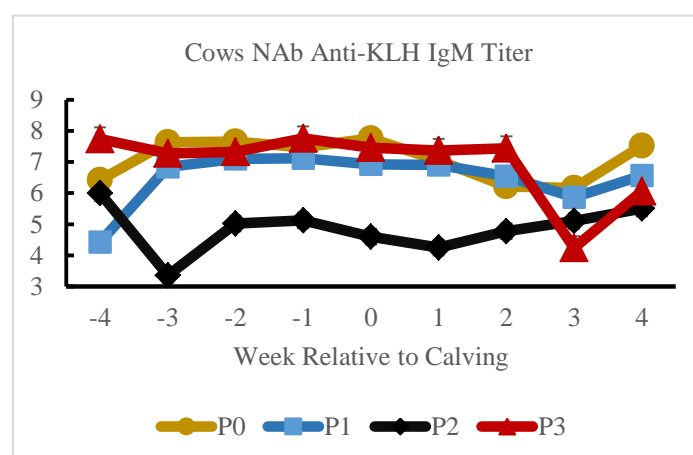
<sup>1</sup>Faculty of Animal Husbandry, Universitas Padjadjaran, Jatinangor, Indonesia; <sup>2</sup>Dairy Cows Breeding and Development Institution, Food and Livestock Security Services, Province of West Java, Indonesia

Email: novim001@gmail.com

**Introduction** Severe negative energy balance in early lactation may impair the function of immune system and consequently increase the risk of high somatic cell count (SCC) and mastitis. Natural antibodies (NAb) play an important role in the activation of innate immune responses against infections like mastitis. Trace minerals like Zinc (Zn) and Selenium (Se) have important roles in immune function and may affect health in transition dairy cows. Zinc (Zn) has antioxidant effects and is involved in the immune system. Selenium (Se) is involved in the antioxidant system via its role in the enzyme glutathione peroxidase. Inadequate dietary Zn or Se decreases immune function around parturition. It was hypothesized that Zn and Se supplementation in different ration around parturition may increase plasma natural antibodies, reduce SCC, and increase incidence of mastitis. The aim of the present study was to investigate effect of supplementing Zn and Se in different ration around parturition on mammary health and natural antibodies in dairy cows.

**Materials and Methods** Twenty-two healthy Holstein-Friesian dairy cows (13 primiparous and 9 multiparous) were randomly assigned to four dietary treatments. Treatments are a control group (P0, n=7), control diet with 15% *Indigofera zollingeriana* substitution of concentrate (P2, n=5) a control diet supplemented with ZnO and Se (P3, n=5), and control diet with 15% *Indigofera zollingeriana* substitution of concentrate supplemented with ZnO and Se (P4; n=5). The doses of Zn and Se supplementation were 40 ppm and 0.3 ppm, respectively (according to Nutrient Requirements of Dairy Cattle (NRC), 2001). Zn and Se content in the control diet (forage and concentrate) and *Indigofera zollingeriana* were analyzed with Neutron Activation Analysis (NAA). The study was conducted for a period of 70 days. The treatments were given at 4 weeks before estimated calving moment. Both NAb titers in plasma and SCC in milk were measured. Plasma samples for NAb determination were sampled weekly from week 1 until 4 postpartum (pp). Milk samples for SCC measurement were collected 2 times per week from week 1 until 4 pp. The data collected were statistically analyzed using analysis of covariance and logistic regression. Analysis of covariance was performed with dietary treatments, parity and 2-way interactions as fixed effect for all cows.

**Results** Supplementation of mineral Zn and Se in the diet without substitution *I. zollingeriana* (P2) reduced titers of NAb IgM binding keyhole limpet hemocyanin (KLH) compared with other dietary treatments (4.9 vs. 7.1 vs. 6.5 vs. 6.9 for P2 vs. P0 vs. P1 vs. P3, respectively;  $P=0.01$ , Figure 1). We found a significant interaction between treatments and parity ( $P=0.01$ ). Moreover, there is no effect of dietary treatments on SCC. Numerically, supplementation of Zn and Se in different ration reduced SCC in milk, (199,246 vs. 221,481 for P2 vs. T0, respectively) and (382,972 vs. 251,999 cell/ml in milk, for P1 vs. P3, respectively). Older cows were having significantly higher NABs binding KLH IgG ( $P<0.05$ ) compared with younger cows. Titers of NAb IgM was lower was partly explained by milk production and infections of some animal in group. When the milk yield was included in the model as correction factor, the effect of treatment was not significant to both IgG and IgM NAb. In this study, IgM was not related to SCC in milk.



**Figure 1** Titers IgM binding Keyhole limpet hemocyanin for cows with different dietary treatments a control group (P0, n=7), control diet with 15% *Indigofera zollingeriana* substitution of concentrate (P2, n=5) a control diet supplemented with ZnO and Se (P3, n=5), and control diet with 15% *Indigofera zollingeriana* substitution of concentrate supplemented with ZnO and Se (P4; n=5). Values represent means and error bars.

**Conclusions** In conclusion, supplementation of mineral Zn and Se in the diet around parturition reduced titers of NAb IgM and IgG concentrations in plasma. Lower IgM titers are related with infections of some animals. Low titers of IgM were not related to SCC but could be partly influenced by milk production at the first week after calving.



### Diselementosis as a risk factor of preeclampsia in dairy cows

V. Safonov<sup>1</sup> and A. Chernitskiy<sup>2</sup>

<sup>1</sup>Vernadsky Institute of Geochemistry and Analytical Chemistry of the Russian Academy of Sciences, Moscow, Russia; <sup>2</sup>All-Russian Veterinary Research Institute of Pathology, Pharmacology and Therapy, Voronezh, Russia

Email: safonovbio@gmail.com

**Introduction** A disruption of the bioelemental composition in an animal's body (excess, deficiency, imbalance), denoted as "diselementosis", is always accompanied by the development of various hidden or clinically obvious pathological conditions. The soil of the Republic of Belarus is generally poor in iodine, selenium, zinc, manganese, cobalt and copper. This fact refers its territory to a biogeochemical province. Preeclampsia (PE) is a pathological condition of pregnant cows, manifested by a syndrome of multiple organ failure, endogenous intoxication, systemic endotheliosis, disorders of hormonal status, uteroplacental and fetoplacental circulation. In highly productive herds, PE is clinically manifested after 32-36 weeks of gestation and diagnosed in 42.0-69.4% of cows and heifers. Presumably, diselementosis may play an important role in the pathogenesis of PE in dairy cows. The purpose of this study is to conduct a comparative assessment of the bioelemental status of dairy cows with a physiological pregnancy and PE in biogeochemical province of the Republic of Belarus (Mogilev region, Glusky district).

**Materials and Methods** 20 Holstein cows (10 with PE and 10 with a physiological pregnancy) with single gestation at 248-255 days of gestation were examined. In samples of hair that were taken from the tips of the tails of cows, mass spectrometry with inductively coupled plasma (Nexion 300D, Perkin Elmer, USA) was used to determine the content of aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), iodine (I), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se), silicon (Si), tin (Sn), strontium (Sr), vanadium (V) and zinc (Zn). All data were expressed as mean  $\pm$  standard deviation (SD) and median. The significance of differences between a group of cows with PE (PE group) and a group of cows with a physiological pregnancy (control group, CG) was determined by comparing the medians by using the Wilcoxon nonparametric test, correlation analysis was performed using the non-parametric tau-Kendall test in IBM SPSS Statistics 20.0 (IBM Corp., USA). The null hypothesis was rejected at  $P < 0.05$ .

**Results** The reliability of differences between groups of cows was established by 10 out of the 25 studied chemical elements (data are presented in the Table 1). Cows with PE showed a decrease in Ca concentration in their hair by 40.8% ( $P = 0.009$ ), P by 32.7% ( $P = 0.009$ ), Co by 60.0% ( $P = 0.015$ ), Mn by 71.6 % ( $P = 0.023$ ), B by 63.7% ( $P = 0.023$ ), Si by 29.8% ( $P = 0.023$ ), Ni by 30.0% ( $P = 0.035$ ), Sr by 34.5% ( $P = 0.023$ ) and Hg by 25.0% ( $P = 0.023$ ) as compared to the CG. The concentration of arsenic in the hair of cows with PE, by contrast, was increased by 25.0% ( $P = 0.035$ ) as compared to the CG. The Al, Cd, Cr, Cu, Fe, I, K, Li, Mg, Na, Pb, Se, Sn, V and Zn content in the cows' hair did not differ significantly between the groups. An inverse relationship was found between the presence of PE in the hair of cows and the concentration of Hg ( $r = -0.626$ ,  $P = 0.002$ ), Ca ( $r = -0.507$ ,  $P = 0.005$ ), P ( $r = -0.507$ ,  $P = 0.005$ ), Co ( $r = -0.526$ ,  $P = 0.006$ ), B ( $r = -0.447$ ,  $P = 0.011$ ), Mn ( $r = -0.447$ ,  $P = 0.011$ ), Si ( $r = -0.447$ ,  $P = 0.011$ ), Sr ( $r = -0.447$ ,  $P = 0.011$ ) and Ni ( $r = -0.422$ ,  $P = 0.017$ ). A direct relationship was found between the concentration of As in the hair and the presence of PE in cows ( $r = 0.487$ ,  $P = 0.012$ ). Earlier, we showed the role of Se deficiency in the pathogenesis of PE in dairy cows (Safonov, 2018). However, the results of this study did not reveal any connection between the concentration of Se in the hair of cows and the presence of PE in them. This can probably be explained by the low content of Se in the bodies of cows of both groups, due to the deficiency of this element in the region's soil.

**Table 1** The concentration of chemical elements in the hair of cows,  $\mu\text{g} / \text{g}$

Element	PE group (n = 10)		Control group (n = 10)		<i>P</i>
	Mean $\pm$ SD	Median	Mean $\pm$ SD	Median	
As	0.044 $\pm$ 0.008	0.050	0.036 $\pm$ 0.005	0.040	0.035
B	2.76 $\pm$ 1.38	1.77	4.97 $\pm$ 0.83	4.87	0.023
Ca	818.0 $\pm$ 328.0	711.0	1211.4 $\pm$ 151.4	1200.0	0.009
Co	0.008 $\pm$ 0.001	0.008	0.017 $\pm$ 0.005	0.020	0.015
Hg	0.030 $\pm$ 0.001	0.030	0.038 $\pm$ 0.008	0.040	0.023
Mn	7.02 $\pm$ 4.73	3.11	12.35 $\pm$ 6.11	10.94	0.023
Ni	0.078 $\pm$ 0.024	0.070	0.116 $\pm$ 0.034	0.100	0.035
P	165.6 $\pm$ 34.2	148.0	216.2 $\pm$ 43.2	220.0	0.009
Si	22.0 $\pm$ 5.2	21.8	29.5 $\pm$ 7.5	31.1	0.023
Sr	2.06 $\pm$ 0.97	1.94	2.87 $\pm$ 0.34	2.96	0.023

**Conclusions** The results of the study showed significant differences in the bioelemental status of Holstein cows with physiological pregnancy and PE. The diselementosis, manifested by a deficiency of Ca, P, Co, Mn, B, Si, Sr, Ni, Hg and As excess in the bodies of dairy cows in the biogeochemical province of the Republic of Belarus, may be a risk factor for PE. Conditions of deficiency of the elements I, Se, Zn, Mn, Co and Cu in the soil, lead to low levels of Mn and Co, but not I, Se, Zn and Cu, in the bodies of pregnant cows and are associated with PE.

### References

Safonov V.A. (2018) Biological role of selenium and correction effects of its content in the organism of animals. *Geochemistry International* 56: 1046-1050.

**Change in plasma pH in cows induced hypocalcemia with Na<sub>2</sub>EDTA**Y. Ro<sup>1</sup>, J. Park<sup>1</sup>, W. Choi<sup>2</sup>, E. Choe<sup>2</sup> and D. Kim<sup>1,2</sup><sup>1</sup>Department of Farm Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul, Korea; <sup>2</sup>Farm Animal Clinical Training and Research Center, Institutes of Green-Bio Science and Technology, Seoul National University, Pyeongchang, Korea

Email: danilkim@snu.ac.kr

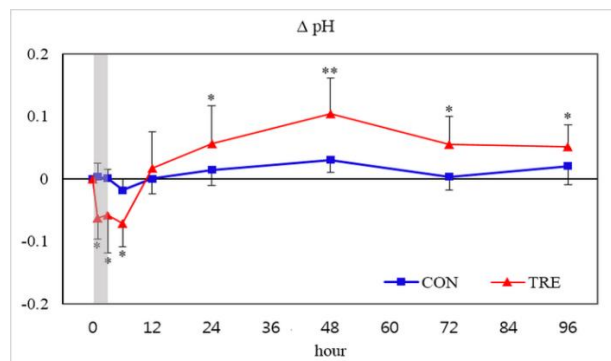
**Introduction** Like in human and other animals, the blood levels of minerals such as calcium (Ca), phosphate (P) and magnesium (Mg) are regulated relevantly in cows, and various factors regulate the balance of these minerals. Abrupt changes in the minerals in the blood can affect the physiological condition. And if the balance is broken, it can lead to serious diseases. When the level of mineral in the blood is high, various factors act to increase the excretion through the feces, urine and saliva. Conversely, when the mineral levels are low, the physiological responses try to increase the absorption from intestine and bone, to decrease the excretion and to limit the use of minerals throughout the body in order to restore normal levels of blood minerals. In cows initiating lactation, the blood mineral level changes more rapidly than in other animals, and these changes affect productivity. In addition, blood pH may affect the proportion of Ca ion (Ca<sup>++</sup>) among total Ca in blood, but it has not evaluated in cows induced hypocalcemia. Therefore, the purpose of this study was to investigate the changes in parameters of complete blood cell count (CBC), blood biochemistry and blood gas analysis as well as urinary mineral excretion in hypocalcemia-induced cows.

**Materials and Methods** The study was conducted with 4 non-pregnant and non-lactating Holstein Friesian cows (622.5 ± 63.49 kg, 2.5 ± 0.6 parities) using 2 × 2 cross-over design. In two cows, Na<sub>2</sub>EDTA (120 mg/kg of body weight) dissolved in 540 mL of distilled water was infused intravenously for 3 hours to induce hypocalcemia (TRE group), and other cows were infused with 540 mL of 0.9 % saline (CON group). The second administration was conducted by changing the groups following a washing period of 3 days. During the experimental period, feed materials were supplied at the same time and amounts, and water was prepared to drink *ad libitum*. Blood and urine were collected from catheter indwelled in a jugular vein and through induced urination or urinary catheter at each sampling time, respectively. CBC was performed immediately after sampling using whole blood collected with EDTA tube (HEMAVET® 950FS, Erba® Diagnostics, Germany). Heparinized plasma was separated with centrifugation in 2000 × g for 15 min and stored at -70 °C until the analyses. Plasma biochemistry (BS-400, Mindray, Chiana) and blood gas analysis (i-STAT, Abbott point of care Inc., USA) were performed using heparinized plasma. The urine was also centrifuged and the supernatant was stored in the same condition. Urinary creatinine (CREA), Ca, P and Mg were measured to determine the excreting amount of minerals in the urine which was calculated as the ratio of minerals to CREA (Ca/CREA, Mg/CREA and P/CREA, respectively). All statistical analyses were performed with two-way repeated measures ANOVA.

**Results** Total Ca and Ca<sup>++</sup> following Na<sub>2</sub>EDTA infusion was decreased with high correlation ( $r = 0.966$ ,  $P < 0.001$ ); total Ca and Ca<sup>++</sup> concentrations gradually decreased until 3 h (1.25 ± 0.22 mmol/L and 0.64 ± 0.12 mmol/L, respectively). A significant difference in total Ca between groups and time points in TRE was not detected from 12 h after infusion. No significant differences in CBC and plasma biochemical analysis were between groups as well as time points. However, there was a significant decrease in alkaline phosphatase (ALP) activity during the induction of hypocalcemia in TRE ( $P < 0.05$ ). This means that the bone remodeling is temporarily suppressed to restore low plasma levels of Ca, P and Mg in hypocalcemia. In addition, Ca/CREA, P/CREA and Mg/CREA was also decreased in TRE group at the same time point (Table 1). Plasma pH was significantly decreased immediately after infusion of Na<sub>2</sub>EDTA and maintained low level until 6 h after hypocalcemia induction. Then, Δ pH was increased with a significant increase from 24 to 96 h compared to 0 h (Figure 1). When blood Ca concentration decreases, parathyroid hormone (PTH) is secreted primarily. It is known that more PTH is secreted and PTH binds to its receptor efficiently when the blood pH is slightly acidic than normal. Therefore, it seems that the blood pH in hypocalcemic cows is lowered simultaneously with the induction of hypocalcemia to maximize the effect of PTH.

**Table 1** Results of Two-way repeated measures ANOVA

Parameter		ANOVA <i>P</i> -value		
		TIME	GROUP	TIME × GROUP
Blood	total Ca	< 0.001	0.009	< 0.001
	P	0.026	0.687	0.053
	Mg	< 0.001	0.010	< 0.001
	ALP	< 0.001	0.116	< 0.001
	Δ pH	< 0.001	0.881	< 0.001
	Ca <sup>++</sup>	< 0.001	0.012	< 0.001
Urine	Ca/CREA	< 0.001	0.963	0.399
	P/CREA	0.064	0.336	0.112
	Mg/CREA	< 0.001	0.100	< 0.001

**Figure 1** Change in plasma Δ pH. Gray zone means Na<sub>2</sub>EDTA infusion time. A significant difference compared to 0 h in TRE was denoted (\*,  $P < 0.05$ ; \*\*,  $P < 0.001$ ).

**Conclusions** In hypocalcemic cows, blood pH was decreased immediately after the initiation of infusion of Na<sub>2</sub>EDTA and maintained low level until recovering normocalcemic state fully. This decrease in blood pH suggested that another physiological mechanism related to maximization of PTH effect against hypocalcemia may exist to maintain Ca homeostasis in cows. Further study is needed to elucidate the mechanism to alter the blood pH in hypocalcemic state.

### Diagnosis and Control of Viral Diseases of Reproductive Importance in Cattle and Pigs

M. D. Givens

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL, USA

Email: [givensmd@auburn.edu](mailto:givensmd@auburn.edu)

**Introduction** The effective diagnosis and control of viral diseases of reproductive importance is critical to optimize reproductive efficiency. Multiple viruses can be rapidly and effectively transmitted via semen from boars and bulls if adequate control measures are not consistently maintained. Appropriate control measures can ensure the freedom of shipped semen from viruses that cause significant disease in cattle and pigs. However, if appropriate control measures are overlooked, shipment of semen and artificial insemination can accelerate the dissemination of disease to various farms and individual animals.

The potential for dissemination of pathogens in semen depends on the duration, distribution, and concentration of virus in the male reproductive tract. During uncomplicated acute infections, the concentration of virus shed in semen may rise and fall over time such that virus sufficient to transmit disease to an inseminated female can be detected continuously from an initiating time point to a concluding time point. For example, classical swine fever virus (hog cholera virus) can be detected continuously in the semen of some boars from 7 to 53 d after initial infection. In contrast, for latent infections in boars with Aujeszky's disease virus (pseudorabies virus) or bulls with bovine herpes virus 1 (infectious bovine rhinotracheitis virus), virus can be shed sporadically in semen over time such that the detectable concentration of virus in semen is variable or only intermittently detectable. Furthermore, some infections such as classical persistent infections with bovine viral diarrhea virus (BVDV) will result in shedding of virus in semen consistently throughout the entire post-pubertal life of the bull. In contrast, other infections such as persistent testicular infections with BVDV will result in constant shedding of virus in semen for a period of time followed by intermittent shedding of virus in semen for months before ultimate resolution of the viral infection that occurred within the blood-testis barrier.

Depending on the geographic location for swine, appropriate control measures should be considered to prevent the contamination of semen with Aujeszky's disease virus, classical swine fever virus, foot and mouth disease virus, Japanese encephalitis virus, porcine reproductive and respiratory disease virus, swine vesicular disease virus, porcine circovirus 2, porcine enterovirus, porcine parvovirus, and rubulavirus. For cattle, appropriate control measures should be considered for bovine herpes virus 1, BVDV, bluetongue virus, and Schmallenberg virus.

Procedures to prevent the spread of these reproductive pathogens includes careful health monitoring of animals, vaccination (in some cases), biosecurity procedures, biocontainment procedures, and timely assays to ensure the absence of virus from collected semen samples. Biosecurity to prevent disease transmission between operations and biocontainment to prevent disease transmission within an operation focus on (1) the specific location and ventilation of farming operations, (2) movement of people, (3) mitigation of risks associated with rodents and insects, (4) ensurance of an uncontaminated water source, and (5) prevention of transmission via fomites. For the sake of facilitating viable commerce, diagnostic methods must be very rapid to prevent the spread of swine pathogens in chilled boar semen as it is used within a few days after collection. Alternatively, the longer term storage of cryopreserved bull semen does somewhat diminish the speed of results necessary for critical diagnostic assays on semen.

When considering the disease status of herds, the absence of evidence of disease cannot be considered the evidence of absence of disease unless thorough, careful, and sensitive methods have been actively employed to detect disease. For some viruses, validation of a seronegative status weeks after collection of semen can provide a valid result to demonstrate the freedom of semen from contaminants. However, in cases of infection of boars with porcine reproductive and respiratory disease virus and bulls with persistent infections with BVDV, a seronegative status does not ensure that semen is free of virus.

**Conclusions** In conclusion, understanding the specific characteristics of viral diseases can facilitate the consistent application of appropriate diagnostic assays and meaningful control measures of minimal constraint to prevent disease transmission while facilitating the efficient collection, distribution, and utilization of preserved semen from boars and bulls.



### Infectious laryngotracheitis - an update on current approaches for the prevention of an old disease

N. Wolfrum

National Reference Centre for Poultry and Rabbit Diseases (NRGK), Section of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland

Email: [nina.wolfrum@vetbakt.uzh.ch](mailto:nina.wolfrum@vetbakt.uzh.ch)

**Introduction** Infectious laryngotracheitis (ILT) is a worldwide occurring respiratory disease of chicken which was described for the first time already in 1925. The disease can occur in all age groups with the earliest infections described in three week old animals. Affected are broilers as well as layers. Depending on the virus strain mild symptoms from nasal discharge, expectoration of mucus and watery eyes to severe forms resulting in death by asphyxiation are observed. The mortality rate varies from 5-70%. But even reduced weight gain or decreased egg production, which goes along with an ILTV infection, cause massive economical losses every year. The most efficient way of protection is immunization. Currently, several vaccines are available. These include live attenuated vaccines as well as vector based vaccines derived from fowl pox or turkey herpesvirus. Furthermore, some alternative methods were experimentally tested. These tests include a new vaccination approach developed in the National Reference Centre for Poultry and Rabbit Diseases, which utilizes virus like particles displaying ILTV glycoproteins. Initial *in vivo* tests showed an immune response, but challenge experiments need to be done to reveal a possible protective effect.

Worth mentioning is the fact that currently available vaccines do not protect from latency, which can be described as ‘sleeping’ virus. Chickens latently infected with ILTV do not show acute symptoms, but virus can be ‘reactivated’, e.g. due to stress. Particularly, vector based vaccines do not confer a robust protection from infection but rather prevent or minimize disease symptoms. The protection conferred by live attenuated vaccines is more robust but these kind of vaccine strains bear the risk of recombination with other vaccine strains or field viruses, which can lead to new pathogenic virus variants, that can cause vaccination induced laryngotracheitis.

**Conclusions** Despite biosafety measures and various vaccination programs, ILTV continues to have a dramatic effect on commercial poultry systems and will require continued efforts to deliver effective solutions, including new vaccination approaches. The presentation will give an overview about ILTV, its prevalence and will discuss current vaccination strategies.

### Impact of the poultry red mite (*Dermanyssus gallinae*) on poultry production systems

O. A. E. Sparagano

Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong

Email: [Olivier.sparagano@cityu.edu.hk](mailto:Olivier.sparagano@cityu.edu.hk)

**Introduction** The poultry red mite or PRM (*Dermanyssus gallinae*) is a worldwide problem for the poultry production industry. Prevalence rates above 80% are common in many countries (Sparagano et al, 2009). Complete development of *D. gallinae*, from egg to adult through one larval stage and two nymphal stages, typically occurs over two weeks. They usually feed for short periods and retrieve in cracks and crevices to digest blood contrary to other poultry mites.

**Impact of poultry production systems** Economic costs associated with both control and production losses due to *D. gallinae* have been estimated at €130 million per year for the EU egg industry. There is a relationship between *D. gallinae* infestation and hen mortality; some reports record a tenfold increase in death rates following severe infestation. Although causal factors may vary, in extreme cases *D. gallinae* numbers may be so high that hens become severely anemic, with mortality resulting from exsanguination. At a sublethal level, mite feeding may result in significant stress to hens, causing an increase in circulating corticosterone and adrenaline and a decrease in  $\beta$ - and  $\gamma$ -globulins. Bird sleep patterns can be disrupted by the need for increased preening, and changes in head-scratching and feather-pecking behavior could also be seen. Increases in aggressive feather-pecking and cannibalistic behaviors have been reported. As blood feeders PRM mites could induce anemia with economic losses related to smaller egg size, less eggs/hen/year, higher mortality and can be responsible for the transmission of bacterial and viral diseases. These mites can survive for weeks if not months without a blood meal and can therefore reappear between flocks.

**Control methods** *D. gallinae* has typically been controlled using synthetic acaricides and some recent formulations can be used in the presence of the poultry birds to avoid delaying mite control procedures. Biopesticides and plant-derived products are now emerging as control alternative in organic poultry production systems (George et al, 2014). Biological control approaches using PRM predators (such as *Androlaelaps casalis*, *Hypoaspis aculeifer*, *Hypoaspis miles*, and *Stratiolaelaps scimitus*) seems reducing the mite infestation but in some specific systems in which predators can hunt easily the mites. However, recent work on genomics and proteomics focusing on vaccine development seems to be a possible future option (see Sparagano et al, 2014; Sparagano, 2017 for a review). To counter-react acaricide resistance reports have shown that P450 complexes could be a successful target (Graham et al, 2016).

**Conclusions** *D. gallinae* is a serious threat to laying hens and egg production systems in many parts of the world, and acaricide resistance and changes in pesticide and hen welfare legislation are set to exacerbate this issue in many countries. As the role of *D. gallinae* as a disease vector becomes better understood, its pest status increases commensurately (transmission of Salmonella and Avian Influenza have been reported). Recent reports of *D. gallinae* infestations in a range of alternative hosts further contribute to this status. Should existing trends continue, *D. gallinae* could soon be problematic for other domestic fowl, pets, and even humans, as it is for poultry birds. Hazard Analysis and Critical Control Point (HACCP) method can help preventing *D. gallinae* establishment in all types of poultry production systems (Mul and Koenraadt, 2009). Integrated Pest Management (IPM) should become a norm to avoid mites being able to develop counter mechanisms to the control methods presented above.

### References

- George, DR, Finn, R., Graham, K., Sparagano, OAE. (2014). Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. *Parasites and Vectors*, 7: 28. doi: 10.1186/1756-3305-7-28.
- Graham, Kirsty, M., Olivier AE Sparagano, Robert D Finn. (2016) Isolation of the monooxygenase complex from *Rhipicephalus* (*Boophilus*) *microplus* – clues to understanding acaricide resistance. *Ticks and Tick-borne Diseases* 7(4):614-23. doi: 10.1016/j.ttbdis.2016.01.012.
- Mul MF, Koenraadt CJM. 2009. Preventing introduction and spread of *Dermanyssus gallinae* in poultry facilities using the HACCP method. *Experimental and Applied Acarology* 48:167–81.
- Sparagano, Olivier, Aleksandar Pavlicevic, Takako Murano, Antonio Camarda, Hamid Sahibi, Ole Kilpinen, Monique Mul, Rick van Emous, Sophie Le Bouquin, Kristian Hoele, Maria Assunta Cafiero (2009). Prevalence and key figures for the poultry red mite *Dermanyssus gallinae* infections in poultry farm systems. *Experimental and Applied Acarology*, 48 (1-2), 3-10.
- Sparagano, OAE, George, DR, Harrington, D, Giangaspero, A (2014). Biology, epidemiology, management and risk related to the poultry red mite, *Dermanyssus gallinae* (de Geer, 1778). *Annual Review of Entomology*, 59, 447-466.
- Sparagano OAE (2017). Transcription factors as a target for vaccination against ticks and mites. (2017) *Adv Protein Chem Struct Biol.*; 107: 275-282.

# **Porcine Reproductive and Respiratory Syndrome virus: Phylogenetic analysis of circulating strains in the Republic of Ireland from November 2016 to June 2017.**

R. Fitzgerald<sup>1</sup>, P. J. Collins<sup>2</sup>, M. J. McNameey<sup>2</sup>, J. A. Calderón Díaz<sup>3</sup>, J. Moriarty<sup>4</sup>, E. G. Manzanilla<sup>3,5</sup>, F. C. Leonard<sup>5</sup>, H. McGlynn<sup>1</sup> and H. O'Shea<sup>1</sup>

<sup>1</sup>Cork Institute of Technology, Bishopstown, Cork, Republic of Ireland; <sup>2</sup>Agri-Food and Biosciences Institute Stormont, Belfast, Northern Ireland; <sup>3</sup>Teagasc PDD, Moorepark West, Cork, Republic of Ireland; <sup>4</sup>CVRL, Department of Agriculture Food and the Marine, Backweston Laboratory Campus, Celbridge, Kildare, Republic of Ireland; <sup>5</sup>University College Dublin, Belfield, Dublin, Republic of Ireland

Email: [rose.fitzgerald@mycit.ie](mailto:rose.fitzgerald@mycit.ie)

**Introduction** Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is a small, enveloped virus, classified in the Arteriviridae family and causes significant economic losses to the porcine industry worldwide. To date, there are two geographically distinct genotypes, referred to as type 1 (PRRSv-1; European) and type 2 (PRRSv-2; North American), with nucleotide (nt) similarity less than 60%. Within PRRSv-1, four genetically distinct subtypes (1 – 4) have been identified, with more diverse strains circulating in Eastern Europe than in Western and Central Europe at present. This study is the first to undertake phylogenetic analysis of PRRSv strains currently circulating in the Republic of Ireland (ROI).

**Materials and Methods** A longitudinal study was conducted on 7 farrow-to finish Irish commercial farms (4 vaccinated and 3 unvaccinated) located in the ROI from November 2016 to June 2017. Between 10 to 17 pigs (n = 83) from each farm were randomly selected 24 to 72 hours after birth, individually tagged, and serum and nasal samples were collected one week after weaning (time-point 1; T/P1) and at 4-week intervals thereafter (T/P2-T/P5). This generated 5 sampling T/Ps per pig, with only sera collected at slaughter (T/P5), yielding a total of 269 nasal and 396 serum samples. Serum samples were initially analyzed by enzyme-linked immunosorbent assay (ELISA) using IDEXX PRRS X3 Ab Test (sample-to-positive value (S/P) ≥ 0.40). All samples were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR), to distinguish between PRRSv-1 and PRRSv-2, using two distinct primer-sets. A total of 41 field strains were selected for amplification of open reading frame (ORF) 5 as it encodes the most variable structural glyco-protein (GP) 5, with 24 of the amplified products (22 serum and 2 nasal) being sequenced. Using MEGA v7.0, sequence alignment was undertaken using ClustalW on the 24 ROI field strains, 5 licensed vaccines and 46 strains sourced from GenBank, with the construction of a phylogenetic tree. A matrix of pairwise comparison based on the ORF5 nt sequence between ROI, United Kingdom (UK) and vaccine strains was also conducted.

**Results** A total of 188 samples returned a sero-positive result (49%) following ELISA analysis with an S/P range of 0.40 - 3.36. Sero-positivity was calculated at 64% at T/P1 and increased to 80% at all other T/P's. PRRSv-1 RNA was detected in 47 (11%) samples returning a cycle threshold (Ct) range of 24.8 to 38.82. All samples analyzed were negative for PRRSv-2. Prevalence rate fluctuated over time, peaking at T/P2 (Table 1). Comparison of PRRSv-1 nt identity values within and between ROI farms was undertaken and ranged from 88-99.8% within farms and 86-95.1% between farms (Table 2). These strains were also compared to vaccine (82.1-98.4%) and UK strains (83.5-98.6%) (Table 2). Phylogenetic analysis (Fig. 1) shows that all the ROI strains are located within PRRSV-1 subtype 1. Eleven of the sequences (green icons) are clustered with two vaccine strains (black diamonds). Six sequences, representing 2 separate herds (pink/blue icons) are nested within the same clade, with the remaining 6 strains, (red icons) situated on a separate, well defined cluster.

**Table 1** PRRSv-1 qRT-PCR from seropositive pigs by T/P

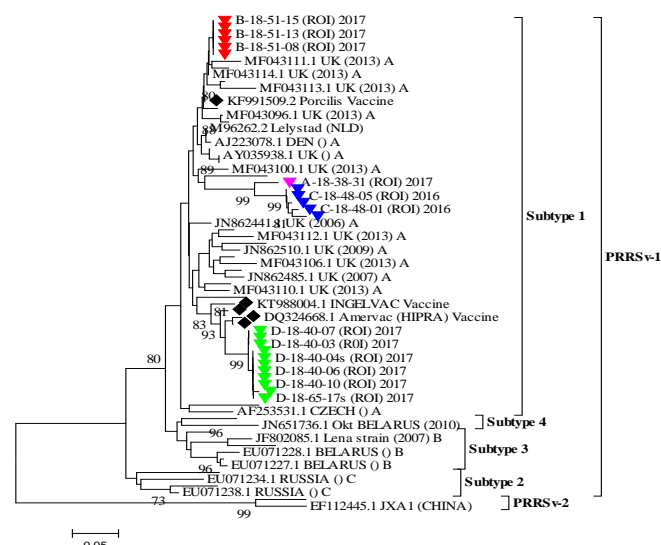
T/P	Number Tested	Number Positive	% Positive
1	89	3	3.37
2	94	27	28.72
3	91	16	17.58
4	103	1	0.97
5*	51	0	0

\*only serum samples available

**Table 2** Comparison of nt identity of ORF5 sequences

ORF5 sequences	Nt identity value (%)
ROI – ROI (between farms)	86.3 – 95.2
ROI – ROI (within farms)	99.1 – 99.8
ROI – Porcilis Vaccine	89 (blue) – 98.4 (red)
ROI – PYRSVAC	86.3 (blue) – 97.8 (green)
ROI – AMERVAC	82.1 (blue) – 93.4 (green)
ROI – INGELVAC	84 (blue) – 91.1 (green)
ROI – UNISTRAN	86.5 (blue) – 98.5 (green)
ROI – UK strains	83.5 – 98.6

**Conclusion** The ROI PRRSv strains identified in this study clustered into 3 distinct groups within the PRRSv-1, subtype 1, along with other Western European and vaccine strains. A relatively high genetic variability among ROI PRRSv-1 was observed in this study. All strains detected in this study were from vaccinated farms. This may pose challenges for the control of PRRSv through vaccination in ROI.



**Figure 1** Phylogenetic analysis of PRRSV ORF5 nt sequences using MEGA v7.0's ClustalW alignment program, neighbour-joining method, bootstrap values of 1000, and rooted with VR-2332 PRRSV-2 sequence. Irish porcine strains are identified with different coloured icons, each representing a distinct farm. Vaccine strains are represented by black diamonds.

### Normalization of Deviance in BRD Diagnosis: Part 1. Knowing Your Why

S. Barringer

Diamond V Mills<sup>1</sup>, Cedar Rapids, Iowa, USA

Email: [sbarringer@diamonddv.com](mailto:sbarringer@diamonddv.com)

**Introduction** Simon Sinek's book "Start With Why", contains some basic principles that can help direct veterinarians and animal health professionals to make core decisions. For years the dominant influence has been practices focusing on vaccinating for key diseases and mitigating impact. If that tactic failed then AIF advancements would keep us ahead of existing and emerging pathogens. While both those tactics have served us well, there were obvious second and third order impacts that could have, but were not anticipated. WHY we became veterinarians has as much to do with Public Health as it does animal welfare and productivity. These presentations are meant to look at the Behavioral Science of animal health as well as the Biological Science. Specifically as it relates to BRD (Bovine Respiratory Disease) and WHY we should explore and implement alternatives to AIF's.

**Materials and Methods & Results** The rate-limiting organ in the female dairy calf in the first year is the lung. The lung is the most likely organ to become diseased and break the whole system of growth and health. After the first 365 days then the uterus and mammary gland share center stage.

Genetic Selection for health, milk feeding practices and making our diets consistent have changed even in the past five years. Calf morbidity and mortality have been significantly reduced by professional calf raisers. Yet we still have a rate limiting problem. In the first year of a bovine's life the rate limiting organ that prevents a calf from reaching its potential is the **lung**.

Recent studies have confirmed that a true pneumonia event in the first year of life impacts both milk production and reproductive efficiency. Pregnancy rates drop and milk production is significantly impaired. A recent publication in Journal of Dairy Science indicated that if a calf had a lung consolidation at 60 days of age as diagnosed by a one lung ultra sound exam she had increased age at first calving and a significantly reduced first insemination rate success.

Other studies have shown decreased milk production in first lactation when a lung ultra sound exam reveals lung scar tissue.

If a calf has pneumonia it seems intuitive that the damage from that lung would impair its performance later in life. However, in spite of leaps in areas of reproduction and mammary organ surveillance and protection, the calf lung, (the most important rate limiting organ) is largely ignored.

**Conclusions** In conclusion, New ideas and tools emerge every year to address calf health but adaptation and evaluation never get the emphasis they deserve. The industry by and large understands the idea of getting heifers to gain and a few places routinely weigh now. Start asking these questions. 1. Can I feed them to protect the lung? 2. Are there tools that can evaluate my success? 3. Are there alternatives to AIF use? The answer to these questions is YES. Begin to challenge abnormal/normal.

**References (summarized in Part II)**

### Normalization of Deviance in BRD Diagnosis Part 2. Lung Ultra Sonography - New tool for invivo diagnostics

S. Barringer

Diamond V Mills<sup>1</sup>, Cedar Rapids, Iowa, USA

Email: sbarringer@diamonddv.com

**Introduction** The rate limiting organ in the female dairy calf in the first year is the lung. The lung is the most likely organ to become diseased and break the whole system of growth and health. After the first 365 days then the uterus and mammary gland share center stage. Lung ultrasonography allows an objective rapid non-invasive survey of our calf raising system. It answers the question: Did I take care of the most important organ?

**Materials and Methods** Lung Ultra Sonography - Avoiding Abnormal Normal:

Evidence is published and not refuted that our ability to detect pneumonia in the prey mindset of a Bovine is flawed. They are hard wired to avoid detection from predators, i.e. humans. Getting fidelity of diagnosis empowers us to make correct AIF decisions as well as interpretation of research.

The ultrasound provides a quick and non-invasive way to view the lungs of the calf

**Results** What can one learn from lung ultrasonography?

The purpose of the lung ultrasound in a calf is to look for evidence of pneumonia.

The linear ultrasound probe that most practitioners use for rectal ultrasound fits well between the rib spaces of calves and can penetrate to a depth of up to 13cm. Therefore the same ultrasound used to preg. check cows can be used to scan the thorax of calves.

Basic technique of the lungs ultrasound exam begins with the selection of calves to scan. Ultrasound of lungs is not to be used for diagnosing acutely sick pneumonia calves; decisions on which calves to treat for pneumonia should still be based on physical exam findings. The ultrasound is used to look for evidence of previous pneumonia that has caused damage to the lung parenchyma or pleural space. I recommend selecting calves two to six months of age. It can be based on a history of pneumonia treatment, calves that are smaller than their peers or calves that have been through a period of stress; such as shipment, pen movements or comingling of new groups or groups of calves when assessing the performance of a vaccine or treatment protocol. Scanning all calves, when practical, will achieve the highest fidelity of surveillance

Lung ultrasound provides the producer with the information that this calf previously had pneumonia and she has scar tissue that will limit her performance both reproductively and milk production. In addition, lung ultrasonography allows us to generate epidemiologic evidence for the success of our vaccination and treatment programs as well as identify calves that we can predict will have sub-optimal performance.

There are likely additional uses for the data generated from lung ultrasonography. It is an easy noninvasive rapid diagnostic technique using equipment that most veterinarians already have on their truck. As research continues, lung ultrasound scores can be a predictor of productivity and longevity of a dairy cow. Additionally, if an objective tool (lung ultrasonography) were utilized to assess lung quality from the contract heifer-raiser, the industry could transition from a yardage model to a quality model?

Respiratory Disease has been a challenge to the dairy industry for decades. Using the new technology of the lung ultrasound facilitates accurate objective impact of vaccinations, treatment protocols and management changes. Armed with real epidemiologic data decisions can be made to move forward with sound strategies to prevent and minimize pneumonia.

**Conclusions** Raising a heifer to her first drop of milk is a significant economic cost. While labor and feed will continue to be the key drivers in managing that cost, morbidity and mortality are significant. Interventions using new technologies can provide an approach to decrease both the direct cost of mortality and the indirect cost of lost productivity due to a pneumonia event. The technology of using lung ultrasonography to assess program effectiveness is a tool for serious evaluation.

**Acknowledgements:** Dr. Liz Adams DVM and Dr. Terri Ollivet are pioneers in developing scoring systems, and research to support lung ultrasonography. They provided direct data and insight into this abstract.

#### References

Know the Cost of Raising Replacement Heifers: J.W. Schroeder, Dairy Specialist NDSU Extension Service  
Heifer Economics

<http://extension.psu.edu/animals/dairy/health/nutrition/heifers/economics/heifer-economics-1>

A case Study of Quality Milk Milk Assessments at a Calf Ranch

DA Moore, J Taylor, ML Hartman, WM Sischo Department of Veterinary Clinical Sciences College of Veterinary Medicine, Washington State University, Pullman, WA

1996: Braun U et al. *Ultrasonography of the lungs, pleura and mediastinum in healthy cows*

1997: Braun U et al. *Ultrasonographic findings in cattle with pleuropneumonia*

1998: Rabeling B et al. *Ultrasonographic findings in calves with respiratory disease*

2003: Flock M. *Diagnostic Ultrasonography in cattle with thoracic disease*

2004: Jung C. *Thoracic ultrasonography technique in newborn calves and description of normal and pathological findings*

2009: Vet Clinics of North America. Marie Babkine and Laurent Blond. Ultrasonography of the Bovine Respiratory System and its Practical Application

2016 A. G. V. Teixeira, J. A. A. McArt, and R. C. Bicalho<sup>1</sup> Journal of Dairy Science Thoracic ultrasound assessment of lung consolidation at weaning in Holstein dairy heifers: Reproductive performance and survival

2017 E. A. Adams<sup>\*1</sup> and S. Buczinski Ultrasonographic assessment of lung consolidation postweaning and survival to the first lactation in dairy heifers

2017 T. R. Dunn,<sup>\*</sup> T. L. Ollivett,<sup>†</sup> D. L. Renaud,<sup>\*</sup> K. E. Leslie,<sup>\*</sup> S. J. LeBlanc,<sup>\*</sup> T. F. Duffield,<sup>\*</sup> and D. F. Kelton<sup>\*1</sup> The effect of lung consolidation, as determined by ultrasonography on first-lactation milk production in Holstein dairy calves

### Swiss farmers ethnoveterinary knowledge about the use of medicinal plants to treat and prevent livestock diseases in 19 German speaking cantons

M. Walkenhorst<sup>1</sup>, A. Maeschli<sup>1</sup>, B. Meier<sup>2</sup> and M. Hamburger<sup>3</sup>

<sup>1</sup>Research Institute of Organic Agriculture (FiBL), Frick, Switzerland; <sup>2</sup>Unit of Phytopharmacy and Natural Product Research, Institute of Chemistry and Biotechnology, Zurich University of Applied Sciences, Wädenswil, Switzerland; <sup>3</sup>Pharmaceutical Biology, Pharmazentrum, University of Basel, Switzerland

Email: michael.walkenhorst@fibl.org

**Introduction** Veterinary phytotherapy, the use of medicinal plants and herbal medicine to treat and prevent animal diseases, receive a rising interest of veterinarians during the last decade namely in German speaking countries. The need to reduce the amount of antimicrobials in livestock production as well as animal owner's demands might have promoted this development. However, even if medicinal plants were the main tool of veterinary pharmacology until the end of the first half of the last century, recent veterinary research in this issue is scarce. Beside (a) historical books and (b) recent *in vitro*, *in vivo* and clinical studies for human medicine, ethnoveterinary research is an important starting point to re-activate veterinary herbal medicine.

**Materials and Methods** Five projects (Stucki et al., 2019; Mayer et al., 2017; Bischoff et al., 2016; Disler et al., 2014; Schmid et al., 2012) have been conducted from 2011 – 2014. A total of 183 interviews were conducted with 242 persons in 19 German speaking cantons. Swiss farmers provided detailed information on species, part, manufacturing, indication, administration and dosing of medicinal plants. Finally, 1128 homemade herbal-remedies based on one single plant species were reported connected with 1466 use reports (UR). Dosages were measured per kg metabolic body weight in case of orally administered remedies. For external administration the concentration in the finished product was determined.

**Results** Swiss farmers mentioned far more than 100 plant species to treat or prevent animal diseases. The most often described indications were skin diseases and sores (516 UR), followed by disorders of the gastrointestinal tract and metabolism (412 UR, Table 1). Most of the remedies were reported to be administered either externally (660 UR) or orally (705 UR), mainly to treat cattle (1172 UR). *Matricaria recutita* L. (160 UR), *Calendula officinalis* L. (110 UR), *Urtica dioica* L. (71 UR), *Symphytum officinale* L. (62 UR), *Coffea* ssp. L. (60 UR), *Rumex obtusifolius* L. (52 UR), *Hypericum perforatum* L. (48 UR), *Arnica montana* L. (46 UR), *Linum usitatissimum* L. (46 UR) and *Picea abies* (L.) H. Karst. (44 UR) were the ten most often mentioned plant species, representing nearly half of all use reports.

**Table 1 Indication and dosing of the 5 most often mentioned plant species**

Plant species (number of use reports (UR))	Plant part	Administration	Indication <sup>1</sup>	Dosage (median), [human dose] <sup>2</sup>
<i>Matricaria recutita</i> L. (160 UR)	flowers	oral	QA,(QR)	0.2 [0,4-0.21]
		external	QD,(QG)	0.5 [0.75-3.5]
<i>Calendula officinalis</i> L. (110 UR)	flowers	external	QD	1 [5-7]
<i>Urtica dioica</i> L. (71 UR)	herb	oral	QA, GS, QG,QD)	0.4 [0.13-0.21]
<i>Symphytum officinale</i> L. (62 UR)	root	external	QD, QM, MA	4.4 [16-18]
<i>Coffea</i> ssp. L. (60 UR)	seed	oral	QA, (QG, GS)	0.2 [one cup: 0.085-0.11]

<sup>1</sup> QA: alimentary tract disorders and metabolic dysfunctions; QD: dermatologic diseases and sores; QM: musculoskeletal system (including hematomas and edema in the connective tissue); QG: genitourinary system and sex hormones (including peri-partum preparation); QR: respiratory tract diseases; MA: mastitis; GS: general strengthening; <sup>2</sup>dosage (both human and animal) in g (dry) plant equivalent: /kg metabolic body weight in case of oral administration; /100g final product in case of external administration

**Conclusions** There are still Swiss farmers, which have a broad and detailed knowledge about the use of medicinal plants to treat livestock diseases. Several of the most often mentioned uses and plant species correspond to historical veterinary textbooks as well as recent recommendations for human medicine. The results of Swiss ethnoveterinary research might stimulate further research to gain detailed knowledge about effects and mechanisms of action of medicinal plants in livestock.

#### References

Stucki K, Dal Cero M, Vogl CR, Ivemeyer S, Meier B, Maeschli A, Hamburger M, Walkenhorst M: Ethnoveterinary contemporary knowledge of farmers in pre-alpine and alpine Regions of the Swiss cantons of Bern and Lucerne compared to ancient and recent literature – is there a tradition?. *Journal of Ethnopharmacology*, 2019; 225-244; doi: 10.1016/j.jep.2018.12.022; Mayer M, Zbinden M, Vogl CR, Ivemeyer S, Meier B, Morena M, Maeschli A, Hamburger M, Walkenhorst M: Swiss ethnoveterinary knowledge on medicinal plants – a within-country comparison of Italian speaking regions with north-western German speaking regions. *Journal of Ethnobiology and Ethnomedicine*, 2017 Jan 3;13(1):1 doi: 10.1186/s13002-016-0106-y; Bischoff T, Vogl CR, Ivemeyer S, Klarer F, Meier B, Hamburger M, Walkenhorst M: Plant and natural product based homemade remedies manufactured and used by farmers of six central Swiss cantons to treat livestock. *Livestock Science*, 2016; 189, 110-125; Disler M, Ivemeyer S, Hamburger M, Vogl C, Tesic A, Klarer F, Meier B, Walkenhorst M: Ethnoveterinary herbal remedies used by farmers in four north-eastern swiss cantons (St. Gallen, Thurgau, Appenzell Innerrhoden and Appenzell Ausserrhoden). *Journal of Ethnobiology and Ethnomedicine* 2014; 10:32.; Schmid K, Ivemeyer S, Hamburger M, Vogl C, Klarer F, Meier B, Walkenhorst M: Traditional use of herbal remedies in livestock by farmers in three Swiss cantons (Aargau, Zurich and Schaffhausen). *Forschende Komplementärmedizin* 2012.

## Vaccination and challenge against *Staphylococcus aureus* in dairy cows using antigens expressed during bovine intramammary infections

C. Ster<sup>1</sup>, M. Allard<sup>2</sup>, J. Côté-Gravel<sup>2</sup>, S. Boulanger<sup>2</sup>, P. Lacasse<sup>1</sup> and F. Malouin<sup>2</sup>

<sup>1</sup>Agriculture and Agri-Food Canada, Sherbrooke Research & Development Centre, Sherbrooke, QC, Canada; <sup>2</sup>Département de biologie, Faculté des Sciences, Université de Sherbrooke, QC, Canada

Email : celine.ster@canada.ca

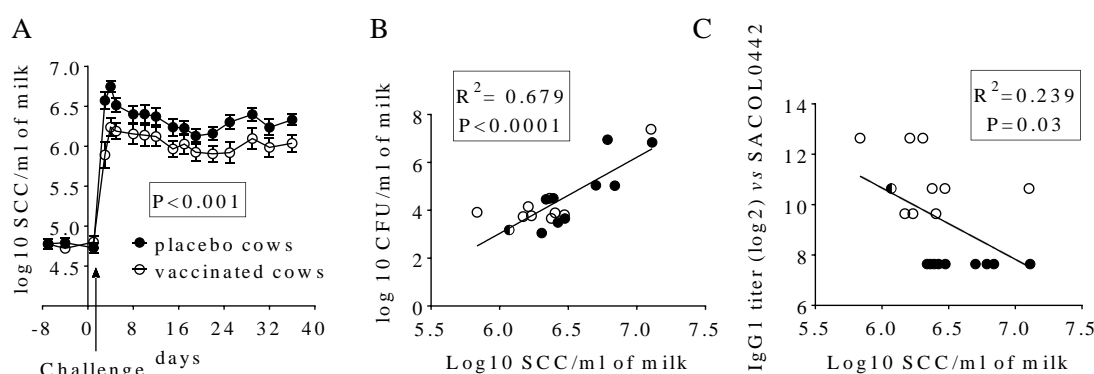
**Introduction** *Staphylococcus aureus* is one of the most important pathogens causing bovine intramammary infections (IMIs) worldwide. Standard antibiotherapy is not very effective and despite intensive research for the development of a vaccine, no efficient product is available. This lack of success could be attributed in part to the choice of antigens or the low expression of the targeted *S. aureus* antigens during infection. To tackle this drawback, Allard *et al* (2013) listed genes that were highly expressed by *S. aureus* during bovine IMIs. Among that list, six antigens were selected for the composition of a vaccine against *S. aureus*. The aims of this study were to analyze the immune response induced by this vaccine and to evaluate its benefits after a *S. aureus* challenge in cows.

**Materials and Methods** Six protein antigens (300 µg of each) highly expressed by *S. aureus* during bovine IMIs (SACOL0029, SACOL0442, SACOL0720, SACOL1867, SACOL1912, SACOL2565) were combined to Emulsigen®-D (30 % v/v, MPV Technologies, USA), CpG ODN 2007 (1 mg, VIDO-InterVac, Saskatoon, Canada) and indolicidin (4 mg, Chempep Inc., FL, USA). Healthy Holstein dairy cows in mid lactation were vaccinated with this vaccine composition (n=9) or saline (n=10). Cows were vaccinated twice 10 weeks apart. Four weeks after the second immunization, 3 out of 4 of the quarters were challenged with 63 colony forming units (CFU) of *S. aureus*. Quarter milk production, *S. aureus* CFU/ml of milk and quarter somatic cell counts (SCC) were monitored over a 36-day post-challenge period. The immune response to the vaccination was monitored before and after the challenge and total IgG, IgG1 and IgG2 titers were measured by ELISA. This project was approved by the Agriculture and Agri-Food Canada local institutional animal care committee and conducted in accordance with the guidelines of the Canadian Council on Animal Care. Data were analyzed as repeated measurements using the MIXED procedure of SAS (SAS institute Inc., Cary, USA).

**Results** Vaccination induced a humoral response in cows for all antigens (Total IgG, IgG1 and IgG2, Table 1). After the bacterial challenge, the SCC of the cows that were vaccinated were significantly lower ( $P < 0.001$ ) than the SCC of the cows that received saline indicating a lower inflammatory response (Figure 1A). There was a correlation between SCC and CFU in milk (Figure 1B), and between serum IgG1 antibody titers and SCC for some antigens (Figure 1C).

**Table 1** Ranges of titers for total IgG, IgG1 and IgG2 obtained four weeks after the second immunization against each antigen.

	SACOL0029	SACOL0442	SACOL0720	SACOL1867	SACOL1912	SACOL2385
IgG titers	800-12 800	3 200-25 600	6 400-102 400	6 400-204 800	6 400-204 800	3 200-25 600
IgG1 titers	400-12 800	800-6 400	3 200-25 600	6 400-51 200	6 400-51 200	1 600-25 600
IgG2 titers	200-1 600	400-3 200	400-1 600	1 600-1 280	400-6 400	200-3 200



**Figure 1** Evolution of SCC after a challenge with *S. aureus* (A), correlation between SCC and CFU in milk (B) and correlation between SCC and IgG1 titres against SACOL0442 (C). In panel A, data presented are LSmeans  $\pm$  sem for the vaccinated cows (open symbols) and for the cows that received saline (closed symbols). In panels B and C, data presented are the means of SCC and CFU for the 3 infected quarters for each cow over the challenge period and the IgG1 titers against SACOL0442 four weeks after the second immunization for the vaccinated cows (open symbols) and for the cows that received saline (closed symbols). The hybrid symbol (half open/closed), represents a placebo cow that had low CFU and SCC (in B) and high IgG1 titers vs SACOL0442 (in C).

**Conclusions** The vaccine led to a humoral response against each *S. aureus* antigen. Despite a rigorous challenge with *S. aureus* directly inoculated in 3 of 4 quarters, vaccinated cows showed low inflammation (low milk SCC) and a correlation was found between SCC and CFU in milk over the challenge period suggesting that vaccinated cows had generally lower milk CFU. The correlation between IgG1 titers and SCC for some antigens suggests that these antibodies might be implicated in the observed response to the challenge. This vaccine needs further research to optimize its composition and to evaluate its efficacy against naturally occurring *S. aureus* IMIs.

### References

Allard, M., Ster, C., Jacob, C.L., Scholl, D., Diarra, M.S., Lacasse, P., Malouin, F., 2013. Vet Microbiol 162, 761-770.

## *Staphylococcus aureus* related to bovine mastitis in Switzerland: Clonal diversity, virulence gene profiles, and antimicrobial resistance

N. Käppeli, M. Morach, S. Corti, C. Eicher, R. Stephan and S. Johler

Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Switzerland

Email: sophia.johler@uzh.ch

**Introduction** *Staphylococcus aureus* can be associated with subclinical, acute, chronic, and toxic cases of bovine intramammary infections, leading to considerable financial losses for the dairy industry in Switzerland and worldwide. In addition, milk products are one of the most common food categories implicated in staphylococcal food poisoning in humans. Detailed information on the population structure, as well as the virulence and resistance characteristics of *S. aureus* originating from bovine mastitis milk is needed to allow for source attribution and risk assessment of *S. aureus* in a food poisoning context and to improve therapeutic approaches in cattle. Our objective was to assess the population structure, phenotypic resistance patterns, and virulence and resistance gene profiles of *S. aureus* isolates from bovine mastitis milk in Switzerland.

**Materials and Methods** A total of 58 *S. aureus* strains were characterized. DNA microarray was used to test for the presence or absence of virulence and resistance genes. In addition, minimum inhibitory concentrations of various antimicrobial agents were determined by microdilution. To assess the population structure of the isolates, we determined clonal complexes (CC) using DNA microarray hybridization profiles and performed multilocus sequence typing and *spa* typing.

**Results** The strains were assigned to 7 clonal complexes, 10 sequence types, and 11 *spa* types, with CC705 (43%), CC97 (33%), and CC20 (12%) representing the most common lineages and t529 (43%) and t267 (21%) representing the most common *spa* types. Only one isolate was assigned to CC8, a clonal lineage linked to high within-herd prevalence of mastitis. A total of 14% (n = 8) of strains were classified as resistant to penicillin, and one strain each was classified as oxacillin and pirlimycin resistant. Although no clinical breakpoints are available for the combination of kanamycin/cefalexin, growth of all strains was inhibited by the lowest combination of kanamycin/cefalexin concentrations tested (4 µg/mL of kanamycin and 0.4 µg/mL of cefalexin). One strain assigned to CC20, ST389, and t2094 exhibited resistance to penicillin, oxacillin, and pirlimycin as well as intermediate susceptibility to erythromycin and high minimum inhibitory concentration for several antimicrobial agents, for which no breakpoints were available.

Antimicrobial agent	minimal inhibitory concentrations of antimicrobial agents in µg/ml										MIC 50%	MIC 90 %
	0.125	0.25	0.5	1	2	4	8	16	32	64		
Penicillin	50	1			2	2	1	2			0.125	2
Ampicillin						56	2				4	4
Cefazolin						57				1	4	4
Cefoperazone					52	6					2	4
Cefquinom				58							1	1
Oxacillin				57				1			1	1
Pirlimycin				57				1			1	1
Erythromycin	38	17	2	1							0.125	0.25
Marbofloxacin		48	10								0.25	0.5
						4/2	8/4	16/8	32/16			
Amoxicillin / clavulanic acid						57		1			4/2	4/2
						4/0.4	8/0.8	16/1.6	32/3.2			
Kanamycin* / cefalexin						58					4/0.4	4/0.4

**Table 1** Overview of MIC for the 58 *S. aureus* isolates tested. Numbers indicate the number of strains exhibiting the corresponding MIC value. Light blue areas indicate the tested concentration range on the microdilution plate. Values above this range denote MIC values greater than the highest concentration tested. If available, MIC breakpoints are indicated using vertical lines (red = human breakpoint, blue = bovine breakpoint) separating resistant and susceptible strains, with dotted lines indicating the differentiation between susceptible and intermediate. Breakpoints were obtained from the CLSI guidelines 2017 for human and 2015 for veterinarian breakpoints. MIC50 and MIC90 represent the concentration of antimicrobial agent inhibiting growth of 50 or 90% of strains, respectively. In the case of kanamycin/cefalexin, the MIC breakpoint is indicated for kanamycin only (marked by an asterisk).

**Conclusions** Our findings stress the crucial need for susceptibility testing before selecting antimicrobial agents for treatment of bovine mastitis, even though the local distribution of lineages may be well known. In addition, further veterinary clinical breakpoints are urgently needed to allow for accurate susceptibility classification and to improve therapeutic recommendations.



### **Eradication program of *Staphylococcus aureus* genotype B in dairy cows in Canton Ticino (Switzerland)**

L. Sesso<sup>1</sup>, M. Vaccani<sup>1</sup>, L. Bacciarini<sup>2</sup>, A. Steiner<sup>1</sup>, M. Bodmer<sup>1</sup> and H. U. Graber<sup>3</sup>

<sup>1</sup>Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>2</sup>Veterinary Cantonal Office, Bellinzona, Switzerland;

<sup>3</sup>Agroscope, Food Microbial System, Group Microbiological Safety of Foods of Animal Origin, Bern, Switzerland

Email: [Lorenzo.Sesso@ti.ch](mailto:Lorenzo.Sesso@ti.ch)

**Introduction** *Staphylococcus aureus* is globally the main pathogen responsible for contagious mastitis in dairy cows. In Switzerland several genotypes of the bacterium have been identified but the *Staphylococcus aureus* genotype B (GTB) is the most problematic due to its contagiousness and its ability to spread quickly in the herd. Intramammary infection with *S. aureus* GTB causes a decrease in the quantity and quality of the milk, an increase in the use of antibiotics and consequently has a strong economic and health impact due to the possible increase in bacterial resistance. In Canton Ticino, an alpine area, located in southern Switzerland, a voluntary eradication plan for *S. aureus* GTB in dairy farms was launched in December 2017. The aim of the project was to reduce the prevalence of *S. aureus* GTB within dairy cow population by 95%, during the first three years of the project.

**Materials and Methods** In Canton Ticino, 168 dairy farms participated voluntarily in the eradication project with an average total number of 3470 dairy cows. Foremilk samples were collected for real time quantitative PCR analysis monthly, during milking by trained personnel. All cows were subjected to at least three analytical controls in the period between December and June of each phase of the project. All animals that accessed seasonal communal alpine pastures in the Canton Ticino were tested before entering. In order to prevent transmission, the animals were milked following a precise milking order updated after each analytical control: GTB-negative cows, unknown GTB status cows, GTB positive cows. PCR-positive cows were either slaughtered or treated according to expected cure. Treatment was performed either during the dry off period or during lactation using a five day treatment protocol based on the results of resistance testing. Epidemiological data were analysed descriptively and the prevalences at the beginning were compared with the prevalences 16 months later using the  $\chi^2$  test.

**Results** At the beginning of the project, the within herd prevalence and between-herd prevalence were 9.9% (n=343 positive cows) and 36.9% (n=62 positive herds), respectively. After 16 months of the project, within herd prevalence and between-herd prevalence were 0.3% (n=11 positive cows) and 2.4% (n=4 positive herds), respectively. The difference of both prevalence values at the different time points is statistically significant ( $p < 0.01$ ). In 82% of the project herds, penicillin-resistant GTB strains were found, while in 18%, the strains were sensitive to penicillin. The percentage of animals cured with antibiotic therapy was 91%. Seventy-five cows were slaughtered because cure rate was considered to be too low.

**Conclusions** Based on the current results, the eradication of *S. aureus* GTB, in a complex territorial area, where communal alpine pastures are present, is possible. This project design can be easily adapted for the eradication of *Staphylococcus aureus* in larger areas. However, qualified personnel managing all the information and communicating it to the producers plays an essential role for the success of such a program.

Occurrence and significance of *Dichelobacter nodosus* in sheep, cattle, goats and South American camelids in Switzerland

F. Ardüser<sup>1</sup>, G. Moore-Jones<sup>2</sup>, S. Gobeli Brawand<sup>3,5</sup>, S. Dürr<sup>4</sup>, A. Steiner<sup>1</sup>, M.-P. Ryser-Degiorgis<sup>2</sup> and P. Zanolari<sup>1</sup>

<sup>1</sup>Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>2</sup>Centre for Fish and Wildlife Health, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>3</sup>Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Bern, Switzerland; <sup>4</sup>Veterinary Public Health Institute, Vetsuisse-Faculty, University of Bern, Liebefeld, Switzerland; <sup>5</sup>Federal Food Safety and Veterinary Office (FSVO), Bern, Switzerland

Email: flurin.ardueser@vetsuisse.unibe.ch

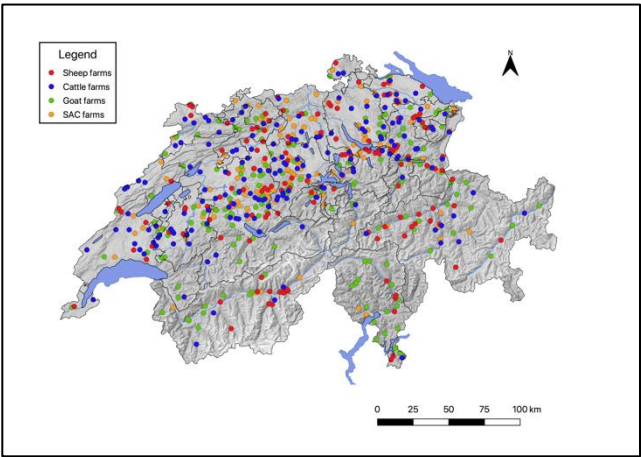
**Introduction** Footrot is a contagious foot disease caused by the bacterium *Dichelobacter nodosus* (*D. nodosus*) and affects sheep worldwide. Due to substantial economic and welfare impact, various countries developed control programs to combat footrot. The aim of this cross-sectional study was to estimate the national prevalence of virulent and benign *D. nodosus* in Switzerland in the four domestic ruminant species sheep, cattle, goats and South American camelids (SAC) to detect potential host populations and to propose targeted disease control measures. Risk factors of infection with the virulent strain of *D. nodosus*, based on a survey carried out among farmers, were investigated on animal and herd level.

**Materials and Methods** Overall, 613 farms and 2920 animals were investigated in Switzerland during May 2017 and June 2018 applying a two-stage cluster sampling strategy. Firstly, the farms were randomly selected and the farmers were asked by phone for participation in the study. Secondly, a four-feet sample of the interdigital space of five randomly selected animals were taken on the farm (Greber et al., 2017). A Real-Time PCR method for simultaneous detection of virulent and benign strains of *D. nodosus* was used for the first time in such a large study (Stäuble et al., 2014).

**Results** On animal level, the prevalence of virulent *D. nodosus* in sheep was estimated at 16.9%. In cattle and goats no virulent *D. nodosus* was detected and in SAC an prevalence of 0.2% was observed. On farm level, a prevalence of virulent *D. nodosus* of 16.2% for sheep and a prevalence of 1.5% for SAC herds was estimated. For the benign *D. nodosus*, the prevalence in sheep was 6.3%, in cattle 88.4%, in goats 2.3% and in SAC 0.9%. The prevalence for benign *D. nodosus* in sheep farms was 2.8% and in cattle farms 100%. In goat and SAC farms, the prevalence was 6.6% and 7.4%, respectively. Adult sheep were at higher risk of infection for virulent *D. nodosus* compared to lambs and yearlings. On herd level, risk factors for infection with virulent *D. nodosus* in sheep were earlier presence of footrot, winter compared to summer and autumn, and goat contact on pasture. Liming pastures had a protective effect on *D. nodosus* infection.

	Animals sampled	Farms visited
Sheep	690	142
Cattle	849	170
Goats	790	166
SAC	591	135
Total	2920	613

**Table 1** Sampled farms and analyzed animals in the prevalence estimation study for *Dichelobacter nodosus* in sheep, cattle, goats and South American camelids (SAC) in Switzerland.



**Figure 1** Sheep, cattle, goat and South American camelid (SAC) farms sampled in Switzerland between 2017 and 2018.

**Conclusions** Since the Swiss control program only targets the virulent strains of *D. nodosus*, it was concluded that cattle, goats and SAC do not play a role in footrot epidemiology in Switzerland. Due to the wide distribution of sheep infected with virulent *D. nodosus* among the country, a national strategy for the control program makes sense and is necessary. The findings of this study are crucial for assessing targeted disease control measures in Switzerland.

References

Greber, D., Locher, I., Kuhnert, P., Butty, M.-A., Holdener, K., Frey, J., Schüpbach-Regula, G., Steiner, A., 2017. Pooling of interdigital swab samples for PCR detection of virulent *Dichelobacter nodosus*. J. Vet. Diagnostic Investig. 30, 205–210. <https://doi.org/10.1177/1040638717733508>  
Stäuble, A., Steiner, A., Frey, J., Kuhnert, P., 2014. Simultaneous detection and discrimination of virulent and benign *Dichelobacter nodosus* in sheep of flocks affected by foot rot and in clinically healthy flocks by competitive real-time PCR. J Clin Microbiol 52, 1228–1231. <https://doi.org/10.1128/jcm.03485-13>

# Development of a multivalent peptide vaccine for prevention of important infections in dairy cows

D. V. Bran-Barrera<sup>1</sup>, E. Brouillette<sup>1</sup>, C. Ster<sup>2</sup> and F. Malouin<sup>1</sup>

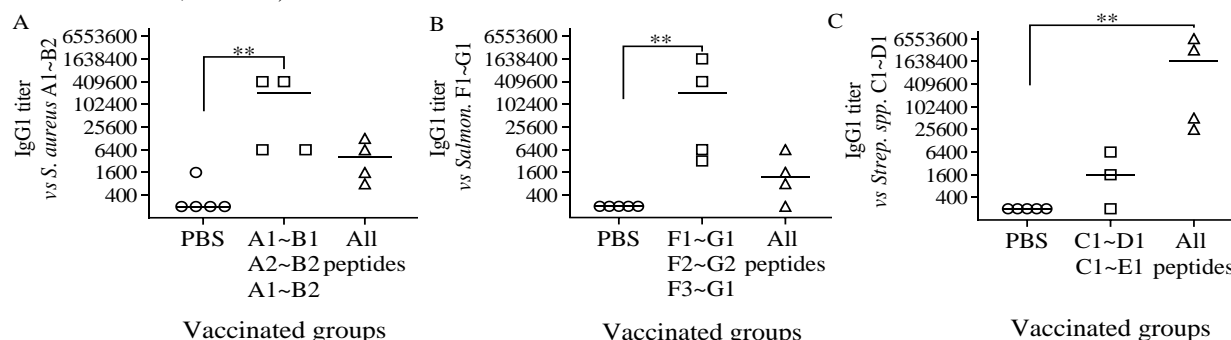
<sup>1</sup>Biologie, Sciences, Université de Sherbrooke, QC, Canada; <sup>2</sup>Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, QC, Canada

Email: Diana.Vanessa.Bran-Barrera@USherbrooke.ca

**Introduction** Bovine mastitis is an inflammation of the mammary gland resulting from a bacterial infection caused by various pathogens such as *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. Bovine mastitis is the most frequent and expensive disease in dairy cows in Canada (Aghamohammadi *et al.*, 2018). Also, *Salmonella* Dublin can cause a severe systemic disease in dairy cows and represents an important public health concern. Currently available vaccines offer minimal benefits. Our laboratory has developed a vaccine composed of *S. aureus* proteins that are highly expressed during intramammary infections (Allard *et al.*, 2013). We showed that these full-length protein antigens could be substituted with immunogenic peptides to stimulate a similar immune response. The aim of this study was to develop a multivalent peptide vaccine that will protect dairy cows against subsequent infections caused by *S. aureus*, *Strep. spp.* and *Salmonella* Dublin.

**Materials and Methods** For each targeted species, at least two proteins implicated in virulence were identified as vaccine candidates. Conserved sequences within strains of the targeted species were predicted with the SEED server. Subcellular localization and transmembrane helices of each target proteins were predicted with CELLO and TMHMM respectively. Potential immunogenic epitopes of 14 or 16 amino acids were predicted with BCPreds and peptides consisting of two epitopes of different proteins were connected by a linker. A total of seven proteins were selected for the multivalent vaccine: two *S. aureus* proteins (A and B), three *Strep. spp.* proteins (C, D and E) and two *Salmonella* proteins (F and G). In total, eight peptides were chemically synthesized for vaccination. CD-1 mice were divided into five groups (n=5/group) for vaccination: a group was immunized with *S. aureus* peptides (A1~B1, A2~B2 and A1~B2), one group with *Strep. spp.* peptides (C1~D1 and C1~E1), one group with *Salmonella* peptides (F1~G1, F2~G2 and F3~G1) and one group with all peptides (multivalent vaccine). A control group received PBS. Each mouse received three subcutaneous immunizations of the peptide mixture (50 µg of each peptide, according to their group) at two-week intervals and the multivalent group received 20 µg of each peptide also at two-week intervals. Pre-immune and immune serums were collected before and after each immunization for analysis of the immune response (IgG, IgG1 and IgG2a) by ELISA against each of the peptides used for immunization or against the full-length target proteins. Results for each group were compared to the PBS control group using a one-way ANOVA followed by a Kruskal-Wallis multiple comparisons test (GraphPad Prism v8.0.1). For IgG1, titers were log<sub>2</sub> transformed for statistical analysis since they are not normally distributed. The animal experiments were carried out according to the guidelines of the Canadian Council on Animal Care and the institutional ethics committee on animal experimentation.

**Results** A strong total IgG response against each individual peptide was observed in groups immunized with the *S. aureus* peptides (response vs A1~B2;  $P \leq 0.05$ ) and with the *Salmonella* peptides (response vs F1~G1,  $P \leq 0.001$  and vs F3~G1,  $P < 0.0001$ ). For the *Strep. spp.* peptides, a strong total IgG response was only observed in mice immunized with the multivalent vaccine (response vs C1~D1,  $P < 0.0001$ ; vs C1~E1,  $P \leq 0.01$ ). The sensitivity of the ELISA was not sufficient to detect the IgG response against the full-length proteins in some serums. A significant IgG1 response was observed in groups immunized with the *S. aureus* peptides (for example Fig. 1A, response vs A1~B2, median IgG1 titer of 208 000,  $P \leq 0.01$ ) and with the *Salmonella* peptides (for example Fig. 1B, response vs F1~G1, median IgG1 titer of 208 000,  $P \leq 0.01$ ). As for the IgG1 response against the *Strep. spp.* peptides, a significant response was only observed in mice immunized with the multivalent vaccine (for example Fig. 1C, response vs C1~D1, median IgG1 titer of 1 664 000,  $P < 0.01$ ).



**Figure 1** Serum IgG1 titers against *S. aureus* A1~B2 (A), *Salmonella* F1~G1 (B), and *Strep. spp.* C1~D1 (C) from the indicated vaccinated mouse groups. Each symbol represents one mouse. Horizontal lines represent medians. \*\*,  $P \leq 0.01$ .

**Conclusions** This study identified a set of immunogenic antigens for vaccination. More work is needed to determine the multivalent peptide composition that will induce a strong antibody response against all target pathogens. A multivalent vaccine would represent a great asset for the dairy industry worldwide.

## References

Aghamohammadi *et al.*, 2018. Herd-level mastitis-associated costs on Canadian dairy farms. *Front. Vet. Sci.* 5:100. Allard *et al.*, 2013. The expression of a putative exotoxin and an ABC transporter during bovine intramammary infection contributes to the virulence of *Staphylococcus aureus*. *Vet. Microbiol.* 162:761-770.

## Ulvan activates chicken heterophils and monocytes through Toll-Like Receptor 2 and Toll-Like Receptor 4

N. Guriec<sup>1</sup>, F. Bussy<sup>2</sup>, C. Gouin<sup>2</sup>, O. Mathiaud<sup>3</sup>, B. Quero<sup>3</sup>, M. Le Goff<sup>2</sup> and P. Nyvall Collén<sup>2</sup>

<sup>1</sup>Brest University and Brest medical School, Brest, France; <sup>2</sup>Amadeite SAS, Bréhan, France; <sup>3</sup>R&D Breizh, Moustoir'Ac, France

Email: FBussy@olmix.com

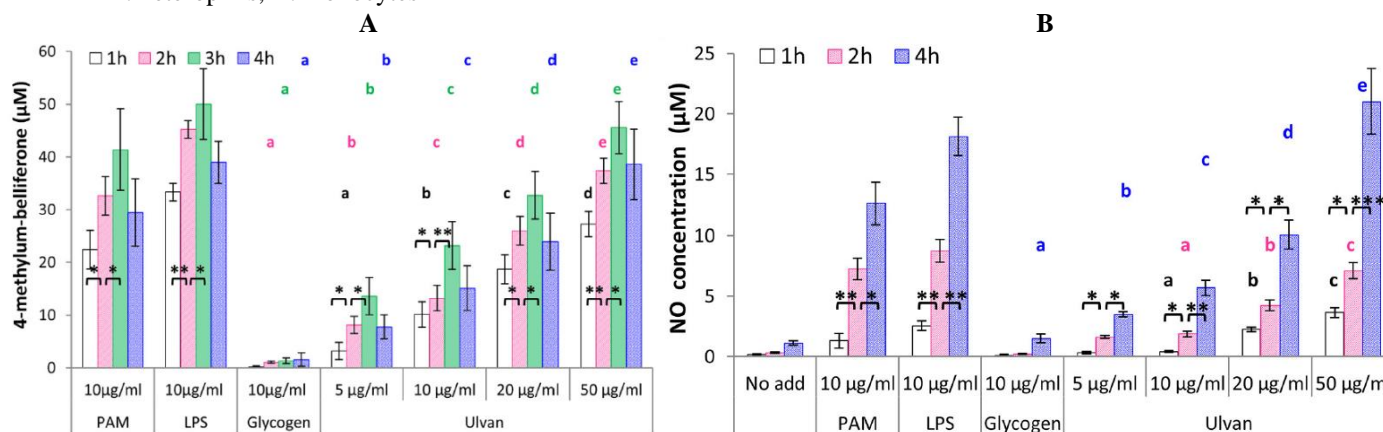
**Introduction** Responsiveness to invasive pathogens, clearance via the inflammatory response, and activation of appropriate acquired responses are all coordinated by innate host defenses. Toll-like receptor (TLR) ligands are potent immune-modulators with profound effects on the generation of adaptive immune responses. This property is being exploited in TLR-based vaccines and therapeutic agents in chickens. However, for administering the TLR agonist, all previous studies used *in ovo*, intra-muscular or intra-venous routes that cannot be performed in usual farming conditions, thus highlighting the need for TLR ligands that display systemic immune effects when given orally.

**Materials and Methods** Green tide algae *Ulva* sp. was collected on the beach at Plestin les Grèves (Bretagne, France). The algae were washed in fresh water, drained, and deep frozen. For extraction, the algae were thawed; wet ground, and liquid and solid phases were separated as part of an industrial process. Sterile peripheral blood was obtained during routine follow-up of 28 days of age animals. Blood from three chickens was pooled for each *in vitro* and *in vivo* experiment and cells purified as previously described [Farnell 2003]. Production of an oxidative burst by heterophils was quantified as described previously [He 2003] in five independent experiments carried out in triplicate. The concentration of nitric oxide (NO) in monocytes conditioned media (four experiments in duplicate) was determined in duplicate with Griess' reagent using a standard nitrite concentration curve.

**Results** We demonstrated that an ulvan extract of *Ulva armoricana* is able to activate avian heterophils and monocytes *in vitro*. Activation results in heterophils oxidative burst and degranulation, and NO production by monocyte. Using specific inhibitors, we evidenced that ulvan is a new ligand for TLR2 and TLR4, that acts in a time- and dose-dependent manner. Moreover, activation of heterophils as well as of monocytes leads to release of pro-inflammatory cytokines, through partly cell-specific pathways.

**Figure 1** Ulvan activates heterophils and monocytes *in vitro* in a time- and dose-dependent manner

A: heterophils, B: monocytes



Furthermore, when given orally to animals as a single dose at day 0, ulvan induces heterophils and monocytes to be activated *in vivo*, directly and/or indirectly without any modification of the broilers' behavior or weight. A transient release of pro-inflammatory cytokines occurs in a dose-dependent manner, with IFN $\alpha$ , IFN $\gamma$  and IL1 $\beta$  to be secreted. Plasma concentrations rose as soon as day 1 and started to decline at day 2, returning towards baseline levels at day 3.

**Conclusions** We report for the first time that ulvan activates TLR4 and TLR2 on avian heterophils and monocytes resulting in cellular activation *in vitro*. When given orally, a single dose of the ulvan extract stimulates heterophils and monocytes, directly and/or indirectly. The release *in vivo* of IL1 $\beta$ , IFN $\alpha$  and IFN $\gamma$  suggest a Th1 orientation of the immune response. The protective effect ulvans may confer will however also be dependent on the pathogens' virulence and on their adaptability, as for any prophylactic agent.

### References

- Farnell, M.B., et al. *Dev. Comp. Immunol.* (2003) 27:423–9.  
He, H., et al. *Comp. Biochem. Physiol.* (2003) Part A 135:177–84.

### Comparison of lung lesions at slaughter, their associated pathogens and serology in 9 farrow-to finish Irish commercial pig herds

L. O'Brien<sup>1</sup>, M. Costa da Rodrigues<sup>2</sup>, R. Fitzgerald<sup>3</sup>, J. Moriarty<sup>4</sup>, H. O'Shea<sup>3</sup>, F. Leonard<sup>1</sup>, J. Calderon Diaz<sup>2</sup>, E. Garcia Manzanilla<sup>2</sup> and M. McElroy<sup>4</sup>

<sup>1</sup>School of Veterinary Medicine, University College Dublin, Ireland; <sup>2</sup>Pig Development Department, Teagasc, Moorepark, Fermoy, Cork, Ireland; <sup>3</sup>Bio-Explore, Department of Biological Sciences, Cork Institute of Technology, Cork, Ireland; <sup>4</sup>Pathology Division, Department of Agriculture, Food and the Marine, Backweston Campus, Celbridge, Kildare, Ireland

Email: [lorna.obrien@ucd.ie](mailto:lorna.obrien@ucd.ie)

**Introduction** Respiratory disease is a major problem affecting pig production globally, resulting in significant economic losses due to reduced feed intake and growth rates, increased on-farm morbidity and mortality rates as well as enhanced treatment costs. While data has been collected and published regarding porcine respiratory disease in other countries, little is known about the prevalence and effects of respiratory disease on Irish pig farms. The main objective of this study was to investigate respiratory disease in Irish pigs, with a view to understanding the pathogens involved and the manifestation of the disease in the form of lung lesions at slaughter.

**Materials and Methods** Nine farrow-to-finish commercial pig herds participated in this study. A total of 282 lungs were assessed at slaughter by a trained veterinarian using the CEVA lung program. A sample of lung tissue representing the dominant gross lesion was collected for all animals and processed for histopathological examination. Bacterial culture and RT-PCR was also performed on each tissue sample to detect the following respiratory pathogens: *Actinobacillus pleuropneumoniae* (APP), *Pasteurella multocida* (*P. multocida*), *Streptococcus suis* (*S. suis*), *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), *Haemophilus parasuis* (*H. parasuis*), swine influenza virus (SIV), porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV). Serological analysis was performed on blood samples collected at slaughter for the detection of antibodies specific for APP, *M. hyopneumoniae*, SIV and PRRSV using IDEXX Ab ELISA kits. Vaccination strategies for each farm were also collected to aid interpretation. All statistical analysis was performed using SAS 9.4 where Chi-square and Fisher's Exact Test were used to determine probability (P) values where  $P < 0.05$  was deemed significant.

**Results** Pleurisy was found to be the most common gross lung lesion with dorsocaudal pleurisy and cranial pleurisy observed in 35.1% and 38.3% of lungs respectively. Enzootic pneumonia-like lesions (20.6%), scars (18.5%), abscesses (2.8%) and APP-like lesions (2.5%) were also observed. The most common respiratory pathogens detected were *M. hyopneumoniae* (72%), PCV2 (23.7%), *H. parasuis* (13.1%), *P. multocida* (8%), PRRSV (6%), *S. suis* (4.7%) and APP (3.2%). SIV was not detected in any animal. Histopathological examination was performed on 224 of the 282 samples where the subsequent findings were divided into 5 broad categories; alveolar interstitial infiltrates ( $n=155$ ), pleurisy ( $n=120$ ), bronchointerstitial pneumonia ( $n=23$ ), bronchopneumonia ( $n=19$ ) and bronchiolitis ( $n=18$ ). Most of the lungs (54%) displayed more than one morphological pattern suggesting multifactorial aetiology. Microscopic examination detected additional lesions of pleurisy ( $n=63$ ) than had been found upon gross examination. A positive association was found between APP-like gross lesions and microscopic pleurisy lesions ( $P=0.02$ ) however no association was observed between those pleurisy lesions and APP serology or the presence of APP pathogen. The most common microscopic lesion observed were alveolar interstitial infiltrates (69.2%), however they were not found to be associated with any of the gross lesions, respiratory pathogens or serology at slaughter. The presence of *M. hyopneumoniae* was associated with enzootic pneumonia ( $P < 0.0001$ ) and bronchopneumonic lesions ( $P < 0.01$ ) as well as scars ( $P=0.01$ ) where it was detected in 86.8% of lesions. No correlation was observed between serology at slaughter with the presence of lung lesions.

**Conclusions** Pleurisy was the most common abattoir lung lesion affecting the pigs in this study with additional cases detected microscopically than had been detected grossly. It was not found to be associated with any single respiratory pathogen therefore further studies are required to determine the aetiology. The presence of *M. hyopneumoniae* was found to be significant regarding enzootic pneumonia and scar lesions.

## Somatic cell count in milk of ewes and possible effect on milk yield and composition

V. Tančin<sup>1,2</sup>, K. Tvarožková<sup>2</sup>, L. Mačuhová<sup>1</sup>, M. Uhrinčat<sup>1</sup> and M. Vrškova<sup>1</sup>

<sup>1</sup>NPPC, Research Institute for Animal Production Nitra, Lužianky, Slovakia; <sup>2</sup>Slovak University of Agricultural in Nitra, FAFR, Dept of Veterinary Science, Nitra, Slovakia

Email: tancin@vuzv.sk

**Introduction** Welfare of animals is one of the most important factor influencing the animal breeding especially with special attention to the consumer demands. Many factors of breeding systems affect the animal welfare directly and also indirectly through production diseases. In dairy animals the udder health (mastitis) could be considered as important factor for welfare from the point of pain, infection, or death. Somatic cells count (SCC) in milk represent epithelial cells and leukocytes and they are considered from many aspects as an indicator of udder health and generally is used for detection of subclinical mastitis (González-Rodríguez et al., 1995). However, there is still a big discussion among scientists about the physiological level of SCC in udder of ewes for detection of udder health (Idriss et al., 2015; Persson et al., 2017). The aim of the work was to evaluate the possible effect of SCC on milk yield and composition under practical conditions. Also the effect of farm and breeds on SCC was evaluated too.

**Materials and Methods** The study was performed at four dairy farms with differed breeds (Lacaune – LC, Tsigai – T) and crossbreds (Improved Valachian x LC ewes - IV/LC) under Slovakian usual practical conditions in April, May, June and July. The milk samples were collected once per month during evening milking. Totally 2200 samples were collected. In all farms the ewes were on pasture during the day and housed in stable during the night. Basic milk composition was done by MilkoScan FT120 (Foss, Hillerød, Denmark) and somatic cells count were determined using a Fossomatic 90 instrument (Foss Electric, Hillerød, Denmark) after heat treatment at 40 °C for 15 min. For statistical analysis SAS program (ver. 8.2; SAS Institute, 2001) was used. Somatic cells count was evaluated using logarithm values (log<sub>10</sub>SCC). According to individual SCC in milk of animals, the dairy ewes were divided into five SCC groups (SOMATIC) (Low = SCC < 200 000 cells.ml<sup>-1</sup>, Middle = SCC between 200 000 – 400 000 cells.ml<sup>-1</sup>, Higher = SCC between 400 000 – 600 000 cells.ml<sup>-1</sup>, High = SCC between 600 000 – 1 000 000 cells.ml<sup>-1</sup>, Mastitis = SCC > 1 000 000 cells.ml<sup>-1</sup>).

**Results** The distribution of milk samples into SCC groups depended on farm, month of sampling and breed and in Mastitis group (over 1 000 000 cells.ml<sup>-1</sup>) ranged from 2% to 40%. LC had higher percentage of samples in Mastitis group as compared with IV/LC within one farm and the highest if compared with other breeds/farms. The effect of farm management (farm 1 and 4) with the same breed (TS) is also important factor contributing to the SCC in individual ewes (Mastitis group 6.2% and 14.6 %, resp.). Also SCC in mentioned farms significantly differed (4.42±0.06 logSCC.ml<sup>-1</sup>, and 5.41±0.05 logSCC.ml<sup>-1</sup>, resp.). Factor SCC group reduced milk yield but significant difference was observed in ewes of Mastitis group as compared with ewes in Low SCC one (421±13.12 ml, 511±6.31 ml, resp.). Also lactose content significantly reduced with increasing of SCC (table 1). Though significant effect of SCC group on protein and fat content was calculated there was not clear trend of changes with increasing of SCC.

**Table 1** The effect of SCC group on milk yield and milk composition

Variable	SCC groups										P
	Low		Middle		Higher		High		Mastitis		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
Milk yield, ml	511 <sup>a</sup>	6.31	449 <sup>bc</sup>	12.33	453 <sup>bc</sup>	17.43	462 <sup>ab</sup>	19.45	421 <sup>c</sup>	13.12	<0.0001
Fat, %	6.29 <sup>a</sup>	0.06	6.31 <sup>a</sup>	0.09	5.89 <sup>b</sup>	0.12	6.21 <sup>ab</sup>	0.14	6.22 <sup>a</sup>	0.10	0.0301
Protein, %	5.61 <sup>a</sup>	0.03	5.61 <sup>a</sup>	0.05	5.41 <sup>b</sup>	0.06	5.56 <sup>ab</sup>	0.07	5.67 <sup>a</sup>	0.05	0.0112
Lactose, %	4.86 <sup>a</sup>	0.02	4.84 <sup>ab</sup>	0.02	4.77 <sup>c</sup>	0.03	4.78 <sup>bc</sup>	0.04	4.55 <sup>d</sup>	0.03	<0.0001

<sup>a-d</sup> significantly different at P < 0.05

**Conclusions** In conclusion, SCC negatively influences milk yield and lactose content. Breeds and farms affected SCC but management could be the most important. Low percentage of ewes in Mastitis SCC group could significantly contribute to the possible development of SCC limits for sheep milk quality.

**Acknowledgements** Supported by the Ministry of Education Science Research and Sports of the Slovak Republic/the Slovak Research and Development Agency (Project No. APVV-15-0072).

## References

- González-Rodríguez, M. C., Gonzalo, C., San Primitivo, F., C. 1995. Relationship between somatic cell count and intramammary infection of the half udder in dairy ewes. *Journal Dairy Science*. vol. 78. no. 12. p. 2753-2759.
- Idriss, S. E., Tančin, V., Margetin, M., Tančinová, D., Sláma, P., Havlíček, Z. 2015. Frequency of distribution of somatic cell count in dairy ewe's milk. *Journal of Microbiology, Biotechnology and Food Sciences*. vol. 4. no. 3. p. 148-151.
- Persson, Y., Nyman, A. K., Söderquist, L., Tomic, N., Waller, K. P. 2017. Intramammary infections and somatic cell count in meat and pelt producing ewes with clinically healthy udders. *Small Ruminant Research*. vol. 156. pp. 66-72.

### Frequency of occurrence coagulase negative staphylococci and their antibiotics resistance in Lacaune breed

K. Tvarožková<sup>1</sup>, V. Tančin<sup>1,2</sup>, I. Holko<sup>3</sup>, L. Mačuhová<sup>2</sup>, M. Uhrinčat<sup>2</sup> and M. Vršková<sup>2</sup>

<sup>1</sup>Slovak University of Agriculture, Department of Veterinary Science, Nitra, Slovak Republic; <sup>2</sup>Research Institute for Animal Production Nitra, NPPC, Lužianky, Slovak Republic; <sup>3</sup>Vetservis, s.r.o. Kalvária, Nitra, Slovak Republic

Email: tancin@vuzv.sk

**Introduction** Mastitis is health and economic problem in dairy ewes. Subclinical mastitis is a major cause decreased of milk yield and losses of quality (Leitner et al., 2004). The incidence of subclinical mastitis is from 2 to 40% (Tančin et al., 2017). The most common pathogens cause mastitis are coagulase negative staphylococci (CNS) (Bergonier et al., 2003; Zafalon et al., 2018). The aim of this study was evaluated frequency of occurrence CNS on three farms with Lacaune breed and found out antimicrobial resistance on selected antibiotics.

**Materials and Methods** The experiment was carried on three dairy farms with Lacaune breed. Totally 304 milk samples was taken two times in one lactation period from 152 ewes. To study were included ewes without symptoms of clinical mastitis. The first squirts of milk were discarded, subsequently the udder halves were cleaned with 70% alcohol and approximately 5 ml of milk from each udder halves was taken in sterile tube for bacteriological examinations. The inoculum of each sample of milk was inoculated on the plates with 5% blood agar. All plates were incubated aerobically at 37 °C and examined after 24 and 48 hours. Colonies were identified by cell morphology, Gram staining, catalase test, hemolysis patterns, esculin hydrolysis and more detailed reactions for detection of pathogens. Contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*) were classified as positive if one or more colony-forming unit (CFU) were found. Other pathogens were classified as positive if at least five CFU were found. Samples were classified as contaminated if three and more pathogens were isolated from one milk samples and growth of contagious pathogens were not identified. Susceptibility testing was performed and evaluated according to the recommendations of CLSI (CLSI, 2012) on Mueller-Hinton agar after 24 hours incubation at 37 °C. CNS were tested with six different antibiotics: amoxicillin-clavulanic acid (AMC) (20 µg-10 µg), tetracycline (TE) (30 µg), enrofloxacin (ENR) (5 µg), trimethoprim-sulfamethoxazole (STX) (1.25 µg-23.5 µg), neomycin (N) (30 µg), lincomycin (MY) (2 µg). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for isolated CNS and recorded as susceptibility, intermediate and resistant. CNS recorded as intermediate were classified as resistant. Mathematical analysis was done by Microsoft Excel program.

**Results** Our results showed that 70.39% milk samples were classified as negative from total of 304 examined milk samples. Positive classified was 28.29% of milk samples and classified as contaminated were 1.32% of milk samples. In 8.1% from positive samples were found two pathogens. The most frequent pathogens isolated from the milk samples were CNS. CNS presented 91.4% from all positive samples. Antimicrobial susceptibility of CNS isolates to AMC, TE, MY, ENR, SXT, N antibiotics are shown in Table 1.

**Table 1** Antibiotics resistance results of CNS in Lacaune ewes

	AMC	TE	MY	ENR	SXT	N
Susceptibility	83,6%	76,7%	52,1%	86,3%	94,5%	31,5%
Intermediate	5,5%	1,4%	20,5%	8,2%	4,1%	50,7%
Resistant	11,0%	21,9%	27,4%	5,5%	1,4%	17,8%
Total resistant	16,4%	23,3%	47,9%	13,7%	5,5%	68,5%

**Conclusions** CNS were the most frequency pathogens isolated from milk samples of Lacaune breed. The most effective antibiotics were trimethoprim-sulfamethoxazole, enrofloxacin and amoxicillin-clavulanic acid. CNS showed the highest resistance to neomycin.

**Acknowledgements** Supported by the Ministry of Education Science Research and Sports of the Slovak Republic/the Slovak Research and Development Agency (Project No. APVV-15-0072).

### References

- Bergonier, D., De Crémoux, R., Rupp, R., Lagriffoul, G., Berthelot, X. 2003. Mastitis of dairy small ruminants. *Veterinary Research*, 34 (5), 689-716.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
- Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A., Silanikove, N. 2004. Changes in milk composition as affected by subclinical mastitis in sheep. *Journal of Dairy Science*, 87(1), 46-52.
- Tančin, V., Baranovič, Š., Uhrinčat, M., Mačuhová, L., Vršková, M., Oravcová, M. 2017. Somatic cell count in raw ewes milk in dairy practice: frequency of distribution and possible effect on milk yield and composition. *Mljekarstvo*, 67 (4), 253-260.
- Zafalon, L. F., Santana, R. C. M., Esteves, S. N., Júnior, G. A. F. 2018. Somatic cell count in the diagnosis of subclinical mastitis in sheep of different breeds. *Semina: Ciências Agrárias*, 39 (4), 1555-1564.



### The effect of vaccination on calf growth, mortality and farm economy on a Finnish beef farm – A case study

A. Wainio

Emovet Ltd

aino.wainio@emovet.fi

**Introduction** Bovine respiratory disease (BRD) is the most common pathology for which antibiotics are used and which causes economic loss in beef production. Prevention of BRD consists of boosting herd resistance by good management, reducing animal stress and immunization in combination with improving biosecurity. Published data on vaccination against BRD in cattle is contradictory and field trials are hard to execute because control groups and randomization are often difficult to produce. The multitude of causative factors and micro-organisms in BRD complicate the interpretation of results. To the author's knowledge no economic benefit of vaccines against BRD has been shown previously. The aim of this study was to evaluate the effect of BRD vaccination on calf growth, mortality and farm economy in one farm in two consecutive years without and with vaccination.

**Materials and Methods** 1450 calves were included into this study. The study was done in a farm rearing dairy-bred heifer and bull calves for beef in Eastern Finland. Six times per year the farm buys 120 calves at the age of two weeks from different farms via slaughterhouse. Each group is indoor housed divided into two sub-groups of 60. During the first 8 weeks calves are fed with 8l/d milk replacer plus grass silage and barley/concentrate mix ad libitum. After weaning at the age of 10 weeks, calves are moved to another barn (240 calves, groups 4 x 60, two partitions of different age groups) where they are fed grass silage and barley/concentrate mix ad libitum. Calves are sold out to another farm at 5,5 months of age. Calves are weighed at arrival and departure from farm. In 2014 an outbreak of severe respiratory disease occurred in weaned calves, increasing mortality and antimicrobial usage. A set of 4 nasopharyngeal swabs from untreated calves was taken at the time of outbreak and analyzed by PCR (*M. bovis*, respiratory viruses) and culture (other respiratory bacteria) at Finnish Food Authority. Results showed *M. bovis*, *H. somni*, *P. multocida*, *Ureaplasma diversum* and *Mannheimia haemolytica*, together with RS- and coronavirus. After sampling, the farm didn't want to start vaccination until January 2017. The situation was monitored yearly by nasopharyngeal swabs. A vaccination program was implemented against *M. haemolytica*, RS- and PI3-viruses (Bovilis Bovipast RSP, Intervet International B.V.). Calves received two vaccinations according to manufacturer's instruction; i.e. 5 ml sc twice. First vaccination was given about 2 weeks after arrival on the farm and the booster 4 weeks later. Production data from year 2016 were used as control data (no vaccination) and data from year 2017 as study data (vaccinated calves). Data was checked for completeness and possible outliers and analyses of correlations were performed with General Linear Model.

**Results** The average growth of calves on farm was (g/day, means  $\pm$  SD)  $1032 \pm 6,031$  in non-vaccinated (n=767) and  $1103 \pm 5,690$  in vaccinated (n=683) calves, respectively. The average growth on farm was in correlation with vaccination, sex, breed type (dairy or beef cross) and calf weight on arrival. All those variables were highly statistically significant ( $p < 0,0001$ ).

The causes for calf mortality are shown in figure 1. Respiratory disease attributed to 20 culls or deaths in 2016 and 7 in 2017 ( $p = 0,0116$ ). A total of 35 calves were culled or dead in 2016 and 22 in 2017 ( $p = 0,0796$ ).

The production report from the slaughterhouse shows an increased income of 26€ per calf in 2017 compared to 2016 related to better growth. This corresponds with a total increased income of 17758€. Rough costs related to animal health are given in Table 1. Total costs were 2994€ lower in 2017 than 2016 making the operating result in 2017 20752€ more beneficial as compared with the previous year.

	2016	2017
Disposal of carcasses (€, Vat 0%)	-2395	-1225
Vet + medication (€, Vat 0%)	-13394	-11570
Total	-15789	-12795

**Table 1** Costs related to animal health



**Figure 1** Causes of death and number of dead calves per year

**Conclusions** It is likely that vaccinating calves against *Mannheimia haemolytica*, RS- and PI3-viruses helped this farm to control mortality caused by BRD while increasing daily growth. Implementing a vaccination program has had a positive effect on farm economy and may do so on farms with similar problems with weaned calves.



### Bovine mammary chemerin: mRNA expression, regulation, and effect on primary bovine mammary epithelial cells

C. R. Baumrucker<sup>1</sup>, A. L. Macrina<sup>1</sup>, O. Wellnitz<sup>2</sup> and R. M. Bruckmaier<sup>2</sup>

<sup>1</sup>Department of Animal Science, The Pennsylvania State University, University Park, PA, USA; <sup>2</sup>Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland

Email: craigb12244@gmail.com

**Introduction** Chemerin is an adipokine and chemokine. It primarily serves as a ligand for the G protein-coupled Chemerin-like receptor 1 (CMKLR1 & GPR1), through autocrine or paracrine pathways to regulate adipogenesis, metabolism, and inflammation. Chemerin is mainly produced by adipocytes, immune cells, and hepatocytes, but other cells also synthesize this protein. While it appears to regulate insulin sensitivity and adipocyte differentiation, reports also suggest a role in reducing inflammation (Mattern et al., 2014). One report (Suzuki et al., 2015) suggested that bovine chemerin (bChemerin) was expressed in bovine mammary epithelial cells and regulates lactogenesis, but no subsequent reports have followed. We hypothesized that bovine mammary epithelial cells synthesize and secrete bChemerin and express the G protein-coupled receptors. We further hypothesized that tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and lipopolysaccharide (LPS) regulate bChemerin to act as an autocrine factor to dampen epithelial inflammation signaling.

**Materials and Methods** We had a synthetic bovine Chemerin 9-mer produced that mimics the active sequence for G-protein-coupled receptor binding with a modification that provides for protease resistance. We also created a recombinant bovine Chemerin (rbChemerin) with a 6 HIS side chain on the N terminal end and utilized an expression construct in E. coli (pEC-28+) to produce mg quantities of the protein for epithelial bioactivity testing *in vitro*. Polyacrylamide gel electrophoresis, Western blotting and mass spectrometry confirmed the identity of the recombinant protein (rbChemerin). A commercial ELISA kit (Novateinbio, Woburn, MA catalog #BG-BVN10481, Bovine Chemerin CHE ELISA) was used to detect bChemerin in milk. Primary bovine mammary epithelial cells (pMEC) were isolated from mammary glands as described by Wellnitz and Kerr (2004), and passages were stored at -80 C until subsequent use. Cells (Pass 5-7) were treated with 2% FBS for a 24 h pre-incubation prior to a 6h experimental treatment. Stock 1 mg/mL concentrations of rbChemerin and Chemerin-9mer were determined by the theoretical extinction coefficients for experimental dilutions. Cells were treated with LPS or TNF $\alpha$  with and without rbChemerin or Chemerin-9mer for 6h. Total RNA then was extracted using the Direct-zol RNA MiniPrep (Zymo Research Corp., Irvine, CA). Quantitative real-time PCR analysis was performed with the Sensimix DNA kit (Quantace, Biolabo, Chatel St. Denis, Switzerland) on a Rotor-Gene 6000 (Corbett Research, Sydney, Australia). Primers for the house-keeping (GAPDH & UBQ) and target genes were synthesized commercially (Microsynth, Balgach, Switzerland). Threshold values (cycle threshold; Ct) were calculated by Rotor Gene software version 1.7.75 (Corbett Research, Bath UK). Target gene Ct values were normalized to housekeeping genes ( $\Delta$ Ct) according to following equation:  $\Delta$ Ct = Ct (mean of two housekeeping genes) – Ct (target gene).

**Results and Discussion** Microgram quantities of Chemerin were detected in colostrum and milk and increased in milk with time and production. Two known agents of inflammation (LPS and TNF $\alpha$ ) in mammary cells *in vitro* were used to create cellular inflammation. Various concentrations of rbChemerin and Chemerin-9-mer were tested to determine the effect on expression of Chemerin, CMLKR1, serum amyloid A3 (SAA3), and TNF $\alpha$ . LPS, but not TNF $\alpha$ , stimulated the expression of SAA3 (>8 cycles), and only the chemerin-9mer increased SAA3 mRNA expression over that of LPS. Chemerin and CMLKR1 mRNA expression was detected in pMEC, but was very low and did not change with treatment. Interestingly, TNF $\alpha$  application did not alter pMEC expression of TNF $\alpha$  mRNA expression, but the application of rbChemerin and Chemerin 9-mer did increase its expression (>2 cycles). Due to concern with the low level of Chemerin and CMLKR1 expression in the pMEC *in vitro*, we tested the effect of lactogenic hormones on gene expression. Insulin, cortisol, and prolactin together stimulated bChemerin and CMLKR1 mRNA expression (>1.5 cycles).

**Conclusions** The data suggest that while chemerin mRNA was detected in mammary cells, it is not acting in an autocrine mechanism to alter LPS signalling. Interestingly, the findings suggest a possible role in TNF $\alpha$  expression mechanisms. The finding that lactogenic hormones stimulate Chemerin and CMLKR1 mRNA expression *in vitro* supports the finding of chemerin appearance in mature milk. The significance of these findings requires more experimentation.

#### References

- Mattern, A., T. Zellmann, and A. G. Beck-Sickinger. 2014. Processing, signaling, and physiological function of chemerin. *IUBMB. Life* 66:19-26.
- Suzuki, Y., S. Haga, D. Katoh, K. H. So, K. C. Choi, U. S. Jung, H. G. Lee, K. Katoh, and S. G. Roh. 2015. Chemerin is a novel regulator of lactogenesis in bovine mammary epithelial cells. *Biochem. Biophys. Res. Commun.* 466:283-288.
- Wellnitz, O., and D. E. Kerr. 2004. Cryopreserved bovine mammary cells to model epithelial response to infection. *Vet Immunol. Immunopathol.* 101:191-202.

## Examining the Microbial Composition of Animals Experimentally Infected with *Mycobacterium avium* subsp. *paratuberculosis*

C. Matthews<sup>1,2</sup>, A. M. Walsh<sup>1</sup>, P. D. Cotter<sup>1,3</sup> and J. M. O'Mahony<sup>2</sup>

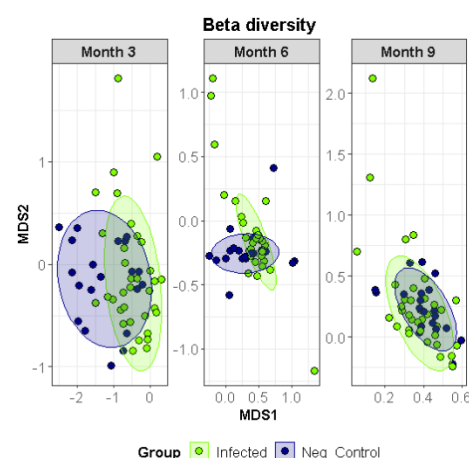
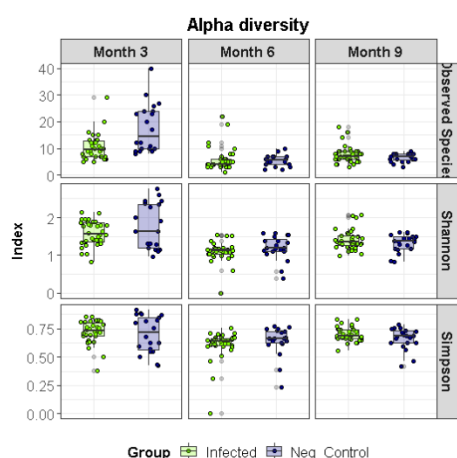
<sup>1</sup>Teagasc Food Research Centre, Fermoy, Cork, Ireland; <sup>2</sup>Cork Institute of Technology, Bishopstown, Cork, Ireland; <sup>3</sup>APC Microbiome Ireland, University College Cork, Ireland

Email: Chloe.Matthews@teagasc.ie

**Introduction** *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease in ruminants, a chronic enteritis, causing weight-loss and diarrhoea, higher susceptibility to other diseases, economically impacting farmers worldwide (Rathnaiah *et al.*, 2017). The disease results in a decrease in milk yield (reported in both clinical and sub-clinical stages of infection), adverse effects of reproductive performance and increased culling rates. Gross pathological changes in cattle with Johne's disease include thickening and corrugation of the intestinal wall (resulting in the inability to absorb nutrients), dilation of lymphatic vessels and enlargement of mesenteric lymph nodes (Britton *et al.*, 2016). Understanding community structure is an important part in the recognition of how microorganisms are affected by the environment, by other microorganisms in that environment and how they in turn affect the host organism. Since the advance of DNA sequencing technologies, research on microbial ecosystems have been transformed. These high through-put DNA sequencing technologies have reformed the identification of microorganisms, and overcome limitations associated with culture dependent approaches. It is through these approaches, that we examine the microbiome of animals experimentally infected with MAP. The objective of the study was to examine the microbiome in order to determine microbial composition that may contribute to disease susceptibility or resistance.

**Materials and Methods** This study employed a subset of samples derived from the original experiment carried out by Britton *et al.* (2013) with an exposed group (n=35) and a control group (n=20), across three time points (months 3, 6 and 9 post-inoculation). DNA extractions were carried out on 165 fecal swabs using the Qiagen PowerSoil Pro kit (Qiagen Inc.). The foam tip of each fecal swab was cut under sterile conditions into initial bead beating tubes supplied in the extraction kit. Extractions were performed as per protocol. Extracted DNA was quantified using the Qubit High Sensitivity DNA assay (Biosciences, Dublin, Ireland). Whole-genome shotgun libraries were prepared using the Illumina Nextera XT DNA Library Preparation Guide. Samples were sequenced on the Illumina Nexseq platform in the Teagasc sequencing facility (Teagasc, Food Research Centre, Moorepark, Co. Cork, Ireland), using the high output reagent cartridge V2 300 cycle kit, following standard Illumina sequencing protocols. Compositional assignment was carried out using MetaPhlAn2. Statistical analysis was carried out in R studio, R version 3.5.3 (2019-03-11) (RStudio, Inc.) MDS was performed using the "vegan" package. Data visualisation was performed using the "ggplot2" package. PERMANOVA analysis was performed using the "Adonis" function. LEfSe (Segata *et al.*, 2011) was used to determine differentially abundant species.

**Results** Alpha diversity analysis showed the number of observed species reduced over time, with Shannon and Simpson indices showing a similar pattern. Beta diversity analysis showed significant differences ( $p > 0.01$ ) between the groups over time. The results showed that the groups became more similar over time with month 3 showing the groups to be most different and month 9 showing them to be most similar. LEfSe analysis showed differentially abundant species between the exposed group and the controls. A number of species were found to be significantly different ( $p > 0.01$ ) between the groups.



**Figure 1** Shows Alpha diversity between groups over time

**Figure 2** Shows Beta diversity between groups over time

**Conclusions** In conclusion, the results show that Shotgun metagenomic analysis can be used to determine the microbial composition of animals infected with Johne's disease. The results may give way to novel diagnostic and therapeutic approaches. However, more research would be needed in order to further investigate this possibility.

## References

- Britton, L. E. *et al.* (2016) 'Potential application of emerging diagnostic techniques to the diagnosis of bovine Johne's disease (paratuberculosis)', *Veterinary Journal*. Elsevier Ltd, 209(May 2015), pp. 32–39. doi: 10.1016/j.tvjl.2015.10.033.
- Rathnaiah, G. *et al.* (2017) 'Pathogenesis, Molecular Genetics, and Genomics of *Mycobacterium avium* subsp. *paratuberculosis*, the Etiologic Agent of Johne's Disease', *Frontiers in Veterinary Science*, 4(November), pp. 1–13. doi: 10.3389/fvets.2017.00187.

### Nutrition in early life imprints metabolic health

K. Huber

Institute of Animal Science, Faculty of Agricultural Sciences, University of Hohenheim, Stuttgart, Germany

Email: [korinna.huber@uni-hohenheim.de](mailto:korinna.huber@uni-hohenheim.de)

**Introduction** Modern animal production is mainly based on economic principles; animal welfare and ecological issues are less considered. Short time spans between birth and weaning, rapid rearing of young farm animals and an early use for reproduction are common. Such an acceleration of physiological developmental processes is against the evolutionary conserved principle of growth and development of mammalian species. To maintain high producing healthy farm animals, it is mandatory to have metabolic *generalists* which are able to allocate resources to production, reproduction and health even under non-adequate environmental conditions (Saviato et al. 2015). Developmental time is a crucial resource for proper allocation of energy and nutrients within the body; however, this is widely neglected in modern animal farming (Huber 2017).

Plastic time windows are known in the early pre- and postnatal period what enable long-term imprinting of metabolic pathways, thereby determining health and productive life span. Rapid growth of calves is commonly used as indicator for well-being and health; however metabolic imprinting in early life may influence the process of growth leading to impairments of cellular and tissue functions. Growth itself is still observed, but body weight gain could be based on protein or fat accretion. As observed in many studies, compensatory growth (or catch up growth) early in life was mainly based on fat accretion at the expense of body protein (DeBlasio et al. 2006; Symonds et al. 2007). Besides body composition, metabolic imprinting in early life also determined cellular functions. Adipose, liver and muscle tissues expressed insulin resistance in individuals when undernourished during intrauterine life (Symonds et al. 2007;) However, underlying pathomechanisms of imprinting are not fully clear yet.

Metabolic disorders occur when cellular processes are impaired. A core process for all body cells is cellular respiration based on oxidative phosphorylation (OXPHOS) by mitochondria. Hypothesis of our research was that cellular core processes were imprinted by nutrition in early life in Holstein female calves with long-lasting effects for the productive life span.

**Methods** In different experimental settings using female calves, heifers and adult dairy cows (Holstein breed) EDTA-plasma was sampled to be analysed by liquid chromatography/mass-spectrometry in a targeted metabolomics approach. Up to 188 hydrophilic small-size metabolites were detected quantitatively using the Absolute IDQ p180 panel (Biocrates Life Science AG, Innsbruck; Austria) according to the manufacture's protocol. The metabolites belonged to 5 compound classes: acylcarnitines, proteinogenic and modified amino acids, biogenic amines, glycerophospho- and sphingolipids and hexoses. Metabolite profiles of animals were visualized by bioinformatics tools using MetaboAnalyst version 4.0 (<https://www.metaboanalyst.ca>) and statistics was performed using graphpad.prism version 7.0 (<https://www.graphpad.com>)

**Results and Discussion** Holstein dairy cows with high plasma acylcarnitine concentrations – what were discussed to be markers of well-developed mitochondrial functionality - expressed extended productive life span. Classical markers such as non-esterified fatty acids, beta-hydroxybutyrate, glucose and insulin did not announce cows at risk. Furthermore, markers indicating anti-inflammation were low in cows with extended productive life span (Huber et al. 2016). Presuming that a healthy metabolic phenotype is associated with well-functioning mitochondria, aim of research was to evaluate if early nutrition of female calves could have an impact on metabolite profiles, especially on acylcarnitines. Restricted feeding within the first month of postnatal life significantly decreased acylcarnitine concentrations in plasma during acute underfeeding; however, this metabolite profile was also maintained until the first lactation (Kenez et al. 2018). Inflammatory markers were higher in calves and consecutively in respective heifers fed restrictive during early life. Thus early nutrition imprinted metabolic pathways most likely related to respiratory chain and mitochondrial functionality.

**Conclusions** Management of early nutrition might offer opportunities to improve metabolic health in later life of dairy cows. Metabolomics approaches appeared to have a huge potential to identify novel pathways of importance for health and a longer productive life span.

### References

- DeBlasio et al. (2017) *Am J Physiol Regul Integr Comp Physiol*. 292, R875-R886.
- Huber (2017) *Animal* <https://doi.org/10.1017/S1751731117002051>.
- Huber et al. (2016) *SciRep* 6:24642 | DOI: 10.1038/srep24642.
- Kenez et al. (2018) *J Dairy Sci* 101, 8446-8460.
- Saviato et al. (2015) *Genet Sel Evol* 47:2; DOI 10.1186/s12711-014-0073-5.
- Symonds et al. (2007) *Reprod Fertil Develop* 19, 53-63.

### Denormalising poor dairy youngstock management – dealing with ‘farm-blindness’

J. F. Mee

Animal and Bioscience Research Department, Moorepark Research Centre, Teagasc, Ireland

Email: [john.mee@teagasc.ie](mailto:john.mee@teagasc.ie)

Farm-blindness may be defined as ‘*a perception by farmers that what they see every day on their own farm is normal, particularly when it is not; a new normal*’. At the core of farm-blindness is lack of recognition of/underestimation of problems due to desensitization resulting from the experience of the everyday or slowing changing trends; ‘bad becomes normal’. We can become inured to the abnormal by the anaesthetic of the familiar. Pluralistic ignorance may play a role in farm blindness whereby the farmer is ignorant of what other farmers are doing in calf management and how their calves are performing. Examples of farm-blindness cited in the animal health literature include nematode control practices, lameness and cow welfare. Evidence of farm-blindness in youngstock management may be found in failure to implement recommended practices, e.g. prompt colostrum feeding, or failure to recognise high calf morbidity or mortality rates (Mee, 2013a). Farmers’ youngstock management hazard ranking perceptions differ significantly from those of their veterinarians and agricultural advisors (Boersma et al., 2013), hence, the latter need to recognise this asymmetry when addressing the core issue of farm blindness. Some of the reason for this asymmetrical perception may be a lack of formal recording of problems. Thus, a Canadian study found that calf mortality was underestimated by 20-50% by dairy farmers and that 94% of farmers did not perceive calf mortality as a problem even though the perinatal mortality rate was 8.8% (Vasseur et al., 2010). Farm blindness is a unique problem with perinatal mortality. If the calf dies before the legally required age to tag the calf its birth/death may go unrecorded and so unnoticed. In dairy herds where the cow is likely to lactate independent of the calf death, the loss may be perceived as secondary to the onset of lactation and so the farmer may be blind to its occurrence/importance. Numerous surveys have been conducted internationally in the last decade which highlight areas where improvements in poor dairy youngstock management can be made. For example, on many dairy farms, (e.g. on 62% of both Austrian and Swedish farms, on 58% of Irish dairy and on 41% of US dairy farms), calving boxes are used for sick cows; on many dairy farms the calving box is not cleaned out after each calving, (e.g. 83% of farms in the USA, 70% in Ireland and 42% in Austria). In the majority of studies internationally calves are not immediately (<30 mins) separated from their dam (‘snatch calving’) and between 12 and 90% of farmers do not carry out umbilical antiseptics. Poor colostrum management practices are also common; a large percentage of farmers do not milk cows out immediately after calving, (e.g. 92% of UK farmers, 61% of Irish farmers and 36% of Canadian farmers); 56-89% of dairy farmers internationally do not measure colostrum quality and 86-98% do not measure calf serum Ig status for failure of passive transfer (FPT), but, 20-82% of farmers feed pooled colostrum. These throughput measures are reflected in output measures such as failure of passive transfer (12-46% of calves in international studies) and young calf mortality rates (1-20%). Thus there is huge scope for improvement in poor youngstock management at farm-level in all dairying countries internationally. Denormalising poor youngstock management can be achieved by firstly creating awareness of the existence of the problem [e.g. through national calf health campaigns; CalfCare (Ireland), KalfOK (The Netherlands)], secondly, by providing farmers with nationally-relevant peer benchmarks against which to compare their own youngstock performance (e.g. national FPT or calf mortality rates) and thirdly, by effectively communicating current recommended best practises to farmers (e.g. colostrum management), (Mee, 2013b). A critical concept veterinarians need to grasp regarding the latter is the principle that with individual animal cases the treatment protocol is under the control of the veterinarian. However, with herd problems, such as youngstock illnesses, the resolution of the issue resides with the farmer, thus veterinary communication can be equally as important as veterinary care. Denormalising poor dairy youngstock management and promoting good youngstock management is a pillar of sustainable agricultural systems (‘social license to farm’) and as such is critical to avoiding reputational risk to the dairy industry.

### References

- Boersma, J., Noordhuizen, J. and Lievaart, J. (2013) Hazard perception of Dutch breeders and veterinarians related to dairy young stock rearing. *J. Dairy Sci.* 96: 5027-5034.
- Mee, J.F. (2013a) Explaining unexplained bovine stillbirth: How to deal with farm blindness. *The Vet. J.* 197: 120-121.
- Mee, J.F. (2013b) Why do so many calves die on modern dairy farms and what can we do about calf welfare in the future? *Animals*, 3: 1036-1057.
- Vasseur, E., J. Rushen, A. de Passielle, R. Lefebvre, and D. Pellerin. (2010). An advisory tool to improve management practices affecting calf and heifer welfare on dairy farms. *J. Dairy Sci.* 93:4414-4426.

### Effects of milk replacer composition on gut and lung health and performance in veal calves

A. de Greeff<sup>1</sup>, A. J. M. Jansman<sup>2</sup>, L. Ruuls<sup>1</sup>, S. A. Vastenhouw<sup>1</sup>, D. Schokker<sup>2</sup>, M. Smits<sup>1,2</sup>, E. van der Wal<sup>3</sup>, P. Mölder<sup>4</sup> and N. Stockhofe<sup>1</sup>

<sup>1</sup>Wageningen Bioveterinary Research, Lelystad, The Netherlands; <sup>2</sup>Wageningen Livestock Research, Wageningen, The Netherlands;

<sup>3</sup>VanDrie Group, Mijdrecht, The Netherlands; <sup>4</sup>Denkavit Nederland BV, Voorthuizen, The Netherlands

Email: [astrid.degreeff@wur.nl](mailto:astrid.degreeff@wur.nl)

**Introduction** Various stressors have an impact on health and disease susceptibility of fattening veal calves. Transport, environmental and husbandry changes have an impact on the health of calves and especially on the occurrence of infectious diseases in the first 10 weeks of age. Respiratory diseases and pneumonia account for the major disease burden from arrival to 6 weeks after arrival at a veal farm. At time of birth the immune system is immature and has to develop in the following period of life. It is well-known that commensal microbiota in the gastrointestinal tract shape the immune system of the intestine in the post-natal period and modulates the competence of the system in later life. The commensal bacteria not only regulate the local mucosal defences against intestinal pathogens, but also regulate immune responses at extra-intestinal sites such as the lungs. The microbiota population in the intestinal tract is considered to be determined by early post-natal colonization and by diet composition. In the present study the effect of changes in the composition of milk replacer (CMR) on microbiota composition and immunological development in the gut and lungs of veal calves is studied. Here, we compare the effects of a plant based CMR (pCMR) to a milk/dairy protein based CMR (mCMR) on gut and lung health parameters as well as on zootechnical performance of veal calves.

**Materials and Methods** First the effects of CMR composition on overall health of calves, the mucosal and immune system of the lung and small intestine and the microbiota composition in the gut of thirty young calves from 1 to 49 days of age were evaluated. Effects of CRM on the immune system and health parameters were assessed by daily health recordings, repeated analysis of haematological parameters, immunological and functional assays on bronchoalveolar fluid (BALF) cells (FACS analysis, phagocytosis assays and oxygen radical formation measurement) and lung pathogen detection in BALF by PCR. After euthanasia on day 49, necropsy was performed and samples taken from lung and jejunal tissue for histological and molecular analysis (microarray and PCR) and from jejunum digesta for microbiome analysis. Subsequently, in a second study the effects of CMR composition on health parameters and production characteristics were studied during a whole production cycle. One hundred calves were followed during 196 days and health recordings, production parameters as well as lung and gut inspection at the slaughterhouse were used to compare the long term effects of both CMRs. BALF was collected and analysed similar to the first study.

**Results** The microbial populations in the jejunal digesta were significantly affected by the two different diets. The diversity of the microbial population, expressed as Shannon diversity, was higher in pCMR compared to the mCMR fed calves. The comparison of the bacterial populations in the jejunum demonstrated that the species composition was significantly different between both treatments. Intestinal gene expression was also affected by the CMR: expression of the investigated mucin genes muc-1, muc-2 and muc-5 were upregulated in calves on pCMR. Whole genome expression analysis indicated that genes involved in immunological/inflammatory pathways were upregulated in jejunal tissue of calves from the pCMR group. No histological differences were observed in small intestinal tissue between both groups.

Composition of CMR also induced changes in the lungs. Gene expression analysis of lung tissue and BALF showed significantly higher gene expression levels for TLR-5, IL-6, cyclooxygenase-1 and proliferation marker p53 in the lungs of mCMR fed calves. In BALF cells, increased levels of TLR-5 and IL-6 were observed at day 42 in the mCMR fed group. No functional changes in phagocytosis capacity of BALF nor in base line production of radical oxygen species were found both groups. Occurrence of clinical disease was not different between treatments.

The performance study showed body weight and body weight gain of calves fed mCMR were significantly higher than of pCMR fed animals. At slaughter the mean body weight was 3.6 kg higher in the group fed mCMR. Feed conversion was significantly lower in the group fed with mCMR from day 0 - 126. No differences with regard to medical treatments were observed between both groups. Slaughterhouse recordings showed that both groups had a similar profile of lung lesions, the majority being mild. A more detailed analysis of 20 calves per group showed that in 40 to 60 % of the animals pneumonia related lesions were found. Data on gene expression in lung tissue obtained at slaughter showed, in agreement with results of the first study, that expression of TLR-5, IL-6 and P53 was higher in mCMR fed calves.

**Conclusions** Composition of CMR not only change microbiota diversity and composition in the gut and gene expression in small intestinal tissue, but also affects immunological development of the lungs. Early life differences in dietary protein sources in CMR induced differences in the immune system in the lungs as reflected by a higher expression of the pathogen recognition receptor TLR-5 in lung tissue and alveolar cells at 49 and 196 days after the introduction of the experimental diets. Together with the functional activity of alveolar cells, a potential positive effect of mCMR on the immune system is suggested, but its functional significance is unknown so far. These effects might be induced by the changes in diversity and composition of the microbiome in the jejunum. Together, these contrasts in lung and gut development might lead to changes in health supporting capacity of veal calves related to diet composition. No effects of diet composition on the occurrence of general disease manifestations, especially respiratory disease, and required medical treatment were observed. Further investigations on the influence of CMR composition on the intestinal microbiome and the local and systemic immune system are required to understand the mechanisms involved and to exploit the knowledge for improved health management. Via manipulation of diet composition at early age, health benefits can be achieved in relation to modulation of intestinal and respiratory diseases.

## Influence of selection for growth and *in ovo* feeding on chicken body weight and immune organ size in the first two weeks of life

H. Abdel Aziz<sup>1</sup>, S. E. Ulbrich<sup>1</sup>, S. Mueller<sup>2</sup> and S. Thanner<sup>1</sup>

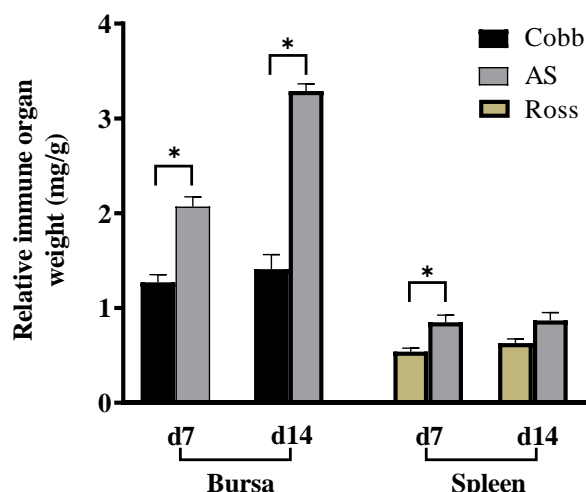
<sup>1</sup>ETH Zurich, Animal Physiology, Institute of Agricultural Sciences, Zurich, Switzerland; <sup>2</sup>Aviforum, Zollikofen, Switzerland

Email: hisham.abdel@usys.ethz.ch

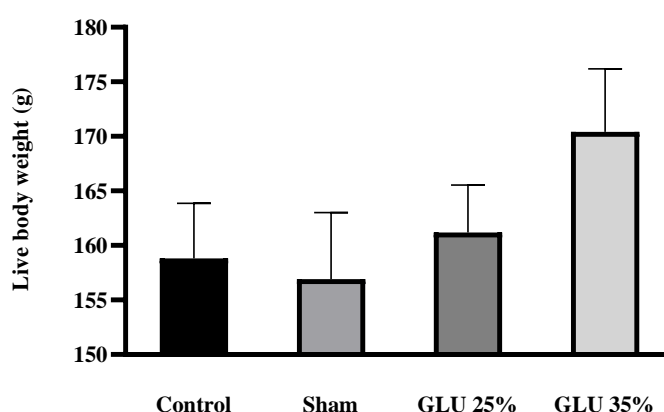
**Introduction** The innate immune system is the first line of defense against pathogens and infections. In chickens, this system is important in limiting the microbial invasion at early age post-hatch until the adaptive immune system is able to express sufficient response to eliminate an infection. The knowledge on the impact of the selection for growth and meat production of today's modern broiler chickens on immune response is scarce. Besides, nutrient supply in the egg becomes limited during embryogenesis because of the high metabolic rate of the fast-growing broiler embryos. This limitation may affect the adequate development of the immune system. To gain more knowledge about the fast-acting innate immune response in chickens, we investigated the variation in the weight of the immune organs between fast- and slow-growing breeds of chickens. In addition, we examined the extent to which the body and the immune organ weights are affected by *in ovo* feeding of nutrients during embryogenesis.

**Materials and Methods** In the first experiment, we investigated the effect of breed on the weight of immune organs post-hatch. Two fast-growing broiler hybrids (Ross 308 and Cobb) and three local slow-growing breeds (Appenzeller Spitzhaube "AS", Appenzeller Barthuhn "AB", and Schweizerhuhn "SH") were used. Fertile eggs (189) were incubated for 21 days until hatching. On day 7 and day 14 after hatching, we recorded the relative weight to the body weight of the bursa of fabricius (bursa) and spleen ( $n \geq 5$  for each breed). The second experiment included only the Ross 308, as a model breed of a fast-growing hybrid to investigate the effect of *in ovo* feeding on the weight of immune organs post-hatch. Fertile eggs (141) were allocated into four groups based on a completely randomized design. The *in ovo* feeding administration took place on day 7 of incubation in the yolk sac. As *in ovo* feeding substances, we used two concentrations of glucose (GLU 25% and GLU 35%), both dissolved in phosphate buffered saline (PBS). The treatment groups were as follows: Control (negative control; non-injected), Sham (positive control; injected with 150  $\mu$ l PBS), GLU 25% (injected with 150  $\mu$ l of a 25% glucose solution dissolved in PBS), and GLU 35% (injected with 150  $\mu$ l of a 35% glucose solution dissolved in PBS). After hatching, we recorded the live body weight and the bursa and spleen weight for each group ( $n \geq 5$ ) on day 7 and day 14. One-way ANOVA was used to test the statistical differences in SPSS version 24. Student-Newman-Keuls and Bonferroni Post Hoc tests were used to separate means, and differences were considered statistically significant at  $P < 0.05$ .

**Results** On both sampling days (day 7 and 14 of age), the relative weight of the bursa [mean (mg/g)  $\pm$  SEM] was higher ( $P = 0.001$ ) in the local slow-growing breed AS ( $2.07 \pm 0.104$  day 7 and  $3.29 \pm 0.075$  day 14) than the fast-growing broiler hybrid Cobb ( $1.27 \pm 0.082$  day 7 and  $1.41 \pm 0.153$  day 14), respectively. On day 7 of age, the relative weight of spleen was higher ( $P = 0.001$ ) in the AS breed ( $0.85 \pm 0.074$ ) than the Ross hybrid ( $0.54 \pm 0.037$ ). Moreover, on day 14 of age, the relative spleen weight was numerically higher ( $P > 0.05$ ) in the AS breed ( $0.87 \pm 0.082$ ) than the Ross hybrid ( $0.63 \pm 0.043$ ) (Figure 1). *In ovo* feeding of GLU 35% lead to a numerically higher ( $P > 0.05$ ) live body weight ( $170.4 \text{ g} \pm 5.78$ ) compared to the Control group ( $158.8 \text{ g} \pm 5.07$ ) on day 7 of age (Figure 2). On day 7, the weight of the bursa and spleen were numerically higher ( $P > 0.05$ ) in the *in ovo* treated groups than the Sham and Control groups.



**Figure 1** Relative immune organ weight [organ weight (mg)\*live weight (g)<sup>-1</sup>] of 2 hybrids (Cobb, Ross) and a local breed (AS) (\* $P = 0.001$ )



**Figure 2** Effect of *in ovo* feeding on the live body weight (g) on day 7 of age

**Conclusions** The preliminary results indicate that the selection programs for growth might have contributed to a decline of the relative immune organs size, which thereby may affect the response of the immune system. The impact of breed and *in ovo* feeding on the innate immune molecular markers is still under investigation and could further elucidate the possibility of the immune response being affected post-hatch.



### The effect of split-suckling in the postnatal period on piglet performances

M. Vandaele, C. Van Kerschaver, J. Degroote and J. Michiels

Department of Animal Sciences and Aquatic Ecology, Laboratory for Animal Nutrition and Animal Product Quality, Ghent University, Belgium

Email: [Mario.Vandaele@UGent.be](mailto:Mario.Vandaele@UGent.be)

**Introduction** During the last decades, due to strong selection for hyperprolificity, the number of piglets born alive each parturition has increased tremendously. During the same time, however, the proportion of low birth weight piglets has also accrued, bringing along challenges in rearing supernumerary piglets as well as litters with light and disadvantaged piglets. In order to cater these challenges several management tools and techniques are tested and applied. One particular, and less common technique in Flanders' sow farming is denominated as split-suckling (Michiels et al., 2013). Split-suckling means that not all piglets have access to the udder at all times; i.e. the litter is split in two or more groups being nursed alternatively or only the heaviest piglets are enclosed temporarily. In literature split-suckling is particularly recommended for safeguarding sufficient colostrum intake. In that light, the objective of this study was to determine the effect of split-suckling in the postnatal period on piglet performances (growth and survival). The study was conducted in a commercial Flemish sow farm with the preconceived hypothesis that split-suckling has a positive effect on the likelihood of survival and growth performances for low birth weight piglets.

**Materials and Methods** The experiment included 3 treatments: a control (CON) as well as two split-suckling treatments, whereby split-suckling was applied either during the first day of life (SS1d) or the three first days of life (SS3d). Split-suckling started after parturition was completed with the isolation of heavy birth weight piglets born in the first part of parturition. After three hours these piglets were reunited with the sow and at the same time heavy birth weight piglets born in the second part of parturition were subsequently isolated during three hours. In this way, first colostrum intake of piglets with high birth rank number was ensured. This isolation cycle was repeated resulting in a total of 12 hours of split-suckling to privilege colostrum intake of low birth weight piglets. At all times, a minimum of 10-12 piglets had access to the udder. Cross-fostering was not allowed during the first three life days. Treatments were allocated to sows to balance for parity and the number of live born piglets. Data of 28 sows and 469 piglets were collected, starting from parturition to weaning. Piglets were weighed at birth ( $T_0$ ) and 24 hours ( $T_{24}$ ) after birth first born piglet to estimate colostrum intake by the model of Theil et al. (2014). Thereafter piglets were weighed 72 hours ( $T_{72}$ ) after birth first born piglet and the day before weaning to determine growth performances. Weaning age was  $21.6 \pm 1.1$  days. In addition piglet mortality was monitored. Sow with her litter was considered the experimental unit. Treatment CON, SS1d and SS3d included 7, 11 and 10 sows, respectively. Data were statistically processed using IBM SPSS Statistics 24. Kolmogorov-Smirnov and Levene's test were adjusted to consider whether data could be analysed parametrically. When these criteria were fulfilled GLM-analysis was done with treatment as fixed factor and number of live born piglets and average birth weight as covariate. Only average daily gain at  $T_{72}$  was non-parametric, so a Welch-test was adjusted.

**Results** No significant differences between treatments for colostrum intake, growth performances and mortality were observed. Estimation for colostrum intake averaged 394 g in CON, 373 g in SS1d and 382 g in SS3d (SEM = 9). Piglets gained at  $T_{24}$  69, 58 and 68 g, respectively (SEM = 7 g). At  $T_{72}$  average daily gain of piglets in SS3d (82 g) was smaller than in CON (109) and SS1d (101g) (SEM = 7 g), but not statistically different. This is a direct consequence of the daily isolation of heavy piglets in SS3d. Mortality percentages at  $T_{72}$  for CON, SS1d and SS3d were 12.73, 12.80 and 15.69% respectively (SEM = 1.58 %). It seems that split-suckling did not improve performances. However other influences had a possible confounding effect. The higher number live born piglets in SS3d ( $18.4 \pm 2.1$ ) compared to SS1d ( $15.6 \pm 2.1$ ) and CON ( $15.9 \pm 3.2$ ) ( $P < 0.05$ ), in combination with the prohibition to cross-foster before  $T_{72}$  and the fact that sows were housed in farrowing crates without balanced floors, could have caused higher mortality. On the other hand piglet birth weight, which is a determining factor for performances, was smaller in CON ( $1.039 \pm 0.107$  kg) than in SS1d ( $1.170 \pm 0.165$  kg) and SS3d ( $1.230 \pm 0.207$  kg) ( $P = 0.09$ ). Furthermore, an effect of split-suckling is more likely to be expected on piglet level than when data are evaluated on sow level. Effects on growth may arise from the negative impact of isolation in heavy piglets and the increased access to suckle colostrum for non-isolated piglets during split-suckling. So a distinction between light and heavy birth weight piglets for data analysis on piglet level can be appropriate to investigate the effect of split-suckling within a litter. More detailed data-analysis is pending.

**Conclusions** No effect of split-suckling on colostrum intake, growth performances and mortality was observed. Further research on a larger scale and more detailed data-analysis is needed, important hereby to realize is the influence of random factors such as piglet birth weight next to number of live born piglets and parity. Ideally these factors are taken into account in future experimental designs.

### References

- J. Michiels, H. Vandenberghe, S. Van Gansbeke, J. Degroote, D. Fremaut, B. Ampe, 2013. Handling of supernumerary piglets in Flanders, a survey among pig breeders. In Proceedings of the 64<sup>th</sup> Annual Meeting of European Federation of Animal Science, August 26-30 2013, Nantes, France, 399.
- P.K. Theil, C. Lauridsen, H. Quesnel, 2014. Mechanistic model to predict colostrum intake based on deuterium oxide dilution technique and impact of gestation and lactation diets on piglet intake and sow yield of colostrum. Journal of Animal Science 92, 5507-5519.

## A

Abd Rahim S.	165
Abdalla E.	115
Abdel Aziz H.	225
Ader P.	66
Ahmadpour A.	106
Akthar I.	175
Alaedin M. T.	99, 100
Alkhawagah A. R.	177
Allard M.	210
Alsaad M.	42, 43
Amadori M.	104
Ammer S.	73
Amundson L. A.	188
Andersson B.	50
Antanaitis R.	83, 108
Antane V.	184
Ardüser F.	213
Arfiana A.	200
Aschenbach J. R.	194
Au M.	44
Auer W.	79
Ayrle H.	31

## B

Bacciarini L.	212
Baes C. F.	115
Bapst B.	120
Barenco A.	125
Barringer S.	207, 208
Bartel A.	80
Bäßler S.	109
Bauer C.	134
Baulain U.	52
Baumgartner C.	80
Baumrucker C. R.	220
Becker J.	146
Bee G.	124
Behr B.	44
Beier J.	117
Benavides-Reyes C.	53
Benedet A.	116
Bennewitz J.	117
Berckmans D.	74
Berends H.	143
Berger A.	79
Bergman J.	183
Bernabucci U.	101
Bernhard J.	41
Bessei W.	117
Betnér S.	149
Bewley J. M.	76
Bieber A.	31
Bilcik B.	58
Bionaz M.	104

Björkman S.	170
Blim S.	160
Bodmer M.	155, 212
Bohlouli M.	120
Bollwein H.	114, 178
Bonfatti V.	116
Borchardt S.	131, 180
Bosman A. L.	33
Bostedt H.	160
Boulanger S.	210
Boyle L. A.	85, 152
Braconnier M.	45
Bradford B.	88
Bran-Barrera D. V.	214
Braun H.-S.	194
Brodard I.	43
Brouillette E.	214
Bruckmaier R. M.	73, 106, 111, 112, 113, 114, 125, 144, 145, 150, 220
Brückmann R.	169
Brügemann K.	120
Brunner N.	113
Bucher E.	42
Burgers E. E. A.	148
Burla J.-B.	168
Bussy F.	136, 215
Byrne N.	154

## C

Calderón Díaz J. A.	206, 216
Canelas Raposo J.	113
Carp – van Dijken S.	96, 105, 195
Carson C. A.	33
Ceccarelli D.	65
Cerna A.	46
Cerri R. L.	180
Chamberlain A. J.	116
Chernitskiy A.	32, 201
Choe E.	202
Choi W.	202
Cieslak A.	194
Clear O.	152
Coleman D. N.	92
Collen P.	136
Collins P. J.	206
Connelly M. K.	190
Cornelison A.	35
Corti S.	211
Costa da Rodrigues M.	216
Costa M. R.	152
Côté-Gravel J.	210
Cotter P. D.	221
Counotte G.	96, 140, 195
Crenshaw T. D.	188
Cross A.	63



## D

Daetwyler H. D.	116
Dame-Korevaar M. A.	65
Dänicke S.	93, 159
Daniel J. B.	199
Daros R. R.	167
Dawkins M. S.	82
de Greeff A.	224
de Jong I. C.	193
De Koning D. J.	50, 51
de Oliveira G.	115
De Vlieghe S.	23
Deckert A.	33
Degroote J.	226
Deiss R.	42
Dennier S.	70
Derno M.	102
DeVries T. J.	164
Dijkstra J.	165, 191
Diskin M. G.	174
Dogan V.	60
Doherr M.	80
Dohme-Meier F.	73
Dolezal M.	39
Domig K. J.	28
Dominguez-Gasca N.	53
Döpfer D.	37
Dove C. R.	35
Drillich M.	79, 181
Drögemüller C.	121, 122, 123, 125
Dörig A.	75
Ducos A.	122
Duffield T. F.	133
Dunn I. C.	50, 51
Dürr S.	213
Durrer M.	31
Dwyer C.	153

## E

Ebert F.	46, 159
Egger-Danner C.	27
Eggerschwiler L.	73
Ehling-Schulz M.	181
Eicher C.	211
Elsabagh M.	98
Elsasser T. H.	63
Emamgholi-Begli H.	115
Emanuelson U.	149
Endres A.-C.	72
Engel B.	97
English L.	86
Erasmus M.	40
Erhard M.	131
Eriksson H. K.	167
Eusemann B. K.	53
Ezz M. A.	175

## F

Fadul M.	42
Failing K.	134, 160
Falker-Gieske C.	117
Farmer C.	126
Fiedler A.	44
Firth C. L.	27
Fischer E. A. J.	65
Fitzgerald R.	206, 216
Fleischer P.	158, 182
Fleming R. H.	50, 51
Fleuren M.	183
Forsberg L.	149
Freitag J.	189
Frieten D.	99, 100
Furrer R.	45

## G

Garcia Manzanilla E.	216
Gass E.	80
Gebhardt-Henrich S. G.	45, 54
Geiger A. J.	127
Geiger S.	194
Ghaffari M. H.	99, 100
Giang E.	151
Giller K.	161
Gimsa U.	114, 169
Givens M. D.	203
Gnott M.	101, 102
Gnott T.	137
Gobeli Brawand S.	213
Goddard M. E.	116
Goharshahi M.	79
Goossens K.	141
Gorka P.	95, 103
Goselink R. M. A.	148, 191
Gouin C.	215
Gow S.	33
Graber H. U.	212
Grahofer A.	122, 171, 179
Greber D.	123
Greer A. L.	151
Grimm P.	43
Groeger S.	113
Gross J. J.	111, 112, 113, 125, 144, 145
Grossen-Rösti L.	111, 112
Grotentraast R.	140, 195
Grünberg W.	192
Guggenbühl B.	161
Guriec N.	215
Güz B. C.	193
Gygax L.	168

## H

Hachenberg S.	44
Häfliger I. M.	121, 122, 123
Hahne F.	24, 25

Hall M.	196
Halle I.	52
Hamburger M.	209
Hammon H. M.	101, 102, 137
Hamscher G.	24, 25
Hansen K.	192
Harlander A.	56
Harlizius B.	118
Härtle S.	61
Hartnack S.	45
Hasseler W.	72
Hasunuma T.	98
Hayes B. J.	116
Hayirli A.	60
Heinsius J.	56
Heitmann S.	47
Hendriks W. H.	165, 191
Hennig-Pauka I.	198
Hensel O.	78
Héritier R.	111
Hermenau G.	159
Hernandez L. L.	185, 190
Heuvelink A. E.	22
Heuwieser W.	73, 131, 180
Higginson V.	133
Hinz K.	55
Hirter N.	123
Ho P. N.	116
Hoerr F. J.	62
Hofacre C.	28
Hofs I.	100
Holko I.	218
Hop G.	96
Hoppe S.	78
Hötzel M. J.	163
Huber K.	109, 222
Hufe P.	46, 159
Huitu O.	156
Humer E.	166
Hund A.	39

## I

Ichijo T.	69
Iffland H.	117
Illek J.	138
Imakawa K.	175
Ishitzuka N.	67
Iwamoto E.	67, 69
Iwersen M.	79

## J

Jaakson H.	94
Jagannathan V.	122
Jagusiak W.	95, 103
Jansen S.	52
Jansman A. J. M.	224
Jensch S.	24, 25
Johler S.	211

Johnston L. J.	84
John-Wiedmer J.	72
Jores J.	43
Junker K.	96
Juozaityenė V.	83
Jürgensen L.	139

## K

Kadek R.	138
Kaltenegger A.	166
Kanitz E.	169
Kanska I.	184
Käppeli N.	211
Karcher D. M.	187
Karis P.	94
Käsbohrer A.	27, 28
Kaske M.	139, 178
Kašná E.	158, 182
Kaspar H.	21
Kasper C.	124
Kaspers B.	61
Katagiri S.	173
Kawashima K.	98
Keady T.	153
Keanthao P.	191
Keil N.	73
Kelton D. F.	133
Kemp B.	97, 147, 176
Kemper N.	24, 47, 55, 78, 129
Kenéz Á.	109
Kennelly J.	154
Kenny D. A.	174
Kessler E. C.	144, 145
Kickinger F.	79
Kim D.	202
Kim W. K.	187
Kim Y.-H.	67, 69
Kindmark A.	51
Kjaer J. B.	56
Klein-Jöbstl D.	79
Klinsoda J.	68, 198
Knol E. F.	118
Koch C.	99, 100, 109, 160
Köhler O.	192
Kok A.	147, 148
König S.	120
Kostal L.	58, 59, 81
Kowalski Z. M.	95, 103
Kratzer G.	45
Kreuzer M.	114, 161
Kubasová T.	62
Kuhla A.	107
Kuhla B.	102, 107
Kuhnert P.	43
Kulig B.	78
Kumprechtová D.	138
Kushibiki S.	67, 69, 98
Kwieciński P.	26

Kwon I. Y. 56

## L

Lacasse P. 210  
Lam T. J. G. M. 22, 176  
Lange M. K. 134  
Langendijk P. 183  
Le Goff M. 136, 215  
Leal L. 135, 143  
Léger D. F. 33  
Leonard F. C. 206, 216  
Letko A. 122, 123  
Lettmann S. 61  
Li A. 56  
Li L. 62  
Li Y. Z. 84  
Lidauer L. 79  
Liesegang A. 189  
Ling K. 94  
Lipkens Z. 23  
Liu Z. 116  
Locher I. 43  
Lonergan P. 174  
Loor J. J. 92  
Lopes Pinto F. 51  
Lopreiato V. 92  
Lorenz I. 29  
Lošytė G. 108  
Luke T. D. W. 116

## M

Ma J. 176  
MacLeod I. M. 116  
Macrina A. L. 220  
Mačuhová J. 157  
Mačuhová L. 157, 162, 217, 218  
Madueira A. M. 180  
Maes D. 77  
Maeschli A. 209  
Magnusson U. 20  
Makagon M. 49  
Makino H. 67, 69  
Malašauskienė D. 83, 108  
Malouin F. 210, 214  
Männer K. 66  
Manzanilla E. G. 152, 206  
Manzhurina O. 32  
Manzocchi E. 161  
Marcato F. 97  
Marchewka J. 57, 85  
Marey M. A. 175  
Martín-Tereso J. 135, 143, 165, 199  
Masaki T. 67, 69  
Mateus-Vargas R. H. 24, 25  
Mathiaud O. 215  
Mathur P. 118  
Matthews C. 221  
May D. 159

Mayasari N. 200  
Mayrhofer S. 28  
McAloon C. 152  
McCarthy M. C. 154  
McCormack H. A. 51  
McCormack H. M. 50  
McElroy M. 216  
McEwen S. A. 33  
McGlynn H. 206  
McMenemy M. J. 206  
Mee J. F. 85, 86, 153, 154, 223  
Meese S. 114  
Meier A. 45  
Meier B. 209  
Meile A. 179  
Metzler-Zebeli B. U. 68, 198  
Mevissen M. 31  
Mevius D. J. 65  
Meylan M. 30, 146  
Mezzetti M. 91, 104  
Michiels J. 226  
Mikulková K. 138  
Minuti A. 91, 104  
Miyamoto A. 175  
Mizuguchi H. 67, 69  
Mölder P. 224  
Molenaar R. 193  
Moore-Jones G. 213  
Morach M. 211  
Moriarty J. 206, 216  
Mueller S. 225  
Mugnai C. 177  
Mülling C. K. W. 34  
Munari C. 177  
Münger A. 73  
Murphy J. P. 86  
Muscher-Banse A. S. 189, 192  
Muskens J. 140, 195

## N

Nagano M. 173  
Nägele F. 155  
Nathues H. 31, 122, 179  
Navalinskaitė I. 83  
Neubauer V. 166  
Neuenschwander T. F.-O. 125  
Neumayer J. 72  
Nguyen T. T. T. 116  
Niemimaa J. 156  
Niozas G. 178  
Norton T. 152  
Nouri M. 106  
Nowak T. 26  
Nyvall Collén P. 215

## O

O'Brien L. 216  
O'Grady L. 154

O'Mahony J. M.	221
O'Shea H.	206, 216
Obitsu T.	98
Obritzhauser W.	27
Öhlschuster M.	79
Olde Riekerink R. G. M.	22
Oliviero C.	170
Ollagnier C.	87
Onken F.	80
Oppermann Moe R.	57
Opsomer G.	89, 141
Ots M.	94
Otwinowska-Mindur A.	95
Overesch G.	30
Özcan A.	159

## P

Panagiotis P.	181
Park J.	202
Patra A. K.	194
Patt A.	53
Pechová A.	158, 182
Peltoniemi O. A. T.	170
Perreten V.	146
Pessoa J. C.	152
Peter V.	122
Petersen H.	47
Petow S.	53
Phyn C.	116
Piccioli-Cappelli F.	104
Pichová K.	58, 59, 81
Piepers S.	23
Plenio J. L.	180
Pless P.	27
Plesse J.	132
Pocevičienė E.	83
Poljak Z.	33, 151
Pontiggia A.	73
Ponzio P.	177
Post L.	94
Preuß S.	117
Prokopavičiūtė Ž.	83
Prowse-Wilkins C. P.	116
Pryce J. E.	116
Pucken V.-B.	155

## Q

Quander N.	31
Querengässer F.	80
Quero B.	215

## R

Rajaeerad A.	192
Rambo Z.	35
Randall L. V.	36
Rauch E.	131
Rautenschlein S.	62

Regmi P.	187
Reid-Smith R. J.	33
Reinländer U.	181
Reisinger N.	166
Renaud D. L.	133
Ribeiro E. S.	90
Richard O.	122
Ringa-Karahona G.	184
Ro Y.	202
Robison C. I.	187
Rochfort S. J.	116
Rodenburg T. B.	58
Rodriguez-Navarro A. B.	50, 51, 53
Röll S.	61
Römer A.	178
Röntgen M.	114
Rossow H.	156
Roth N.	28
Roth Z.	71
Ruchti S.	45
Rufener C.	54
Ruiz-Ascacibar I.	124
Russell R. W.	130, 142
Ruuls L.	224
Rychlik I.	62, 64
Ryser-Degiorgis M.-P.	213

## S

Sabatowicz M.	95, 103
Sadri H.	99, 100
Safonov V.	32, 110, 201
Salman L. B.	200
Sanchez-Rodriguez E.	53
Sanginabadi M.	106
Santman-Berends I. M. G. A.	22
Santos J. E. P.	90, 186
Sargeant J. M.	151
Sato S.	67, 69, 98
Sauerwein H.	99, 100, 137
Schären M.	46, 159
Scheu T.	109, 160
Schiavone A.	177
Schlegl R.	181
Schmitz-Hsu F.	125
Schmutz M.	50
Schnepel N.	189, 192
Schnyder P.	30
Schokker D.	224
Schönecker L.	30
Schonewille J. T.	191
Schouler C.	61
Schouwey S.	155
Schrader L.	53
Schrapers K. T.	194
Schuberth H. J.	128
Schuenemann G. M.	131
Schuh K.	99, 100
Schüller L.	73

Schulz J.	24, 25
Schüpbach-Regula G.	30, 146, 155
Schupp D.	160
Schusser B.	61
Schwarm A.	114
Schwarz S.	21
Schweer W.	35
Schwinn A.-C.	125
Seefried F. R.	121
Sematovica I.	184
Seppä-Lassila L.	156
Sesso L.	212
Setyowati E. Y.	200
Shabunin S.	32
Sharifi A. R.	52
Sheldon I. M.	172
Shiels D.	153
Shimada M.	175
Simianer H.	52
Simola M.	156
Sipos S.	171
Sipos W.	171
Skalná Z.	59, 81
Šlosárková S.	158, 182
Smits M.	224
Sohrt J.	139
Sparagano O. A. E.	205
Spargo J. T.	196
Spindler B.	47, 55
Springer B.	27
St Charles K.	84
St. Clair E. V.	130, 142
Starke A.	46, 101, 102, 159
Stauder A.	166
Stefaniak T.	101, 137
Stegeman J. A.	65
Steiner A.	41, 42, 43, 146, 168, 212, 213
Steinhöfel I.	178
Stephan R.	211
Ster C.	210, 214
Stevens M.	23
Stockhofe N.	224
Stoll P.	124
Stracke J.	47, 55
Stratmann A.	54
Stumpf M.	44
Sugino T.	98
Sujová V.	138
Sunduimijid B.	116
Sutter F.	131
Svensson C.	149
Swalve H.	159
Swartz T.	88
Szumacher-Strabel M.	194

## T

Tait-Burkard C.	119
-----------------	-----

Tančin V.	157, 162, 217, 218
Televičius M.	83, 108
Tetens J.	117
Thaller G.	160
Thanner S.	225
Tinkle A.	35
Tippenhauer C. M.	180
Torrison J.	35
Toscano M. J.	48
Trevisi E.	91, 101, 104, 137
Tröscher A.	101, 102, 111, 137
Tsousis G.	178
Tuchscherer A.	137, 169
Tuchscherer M.	169
Tuominen P.	156
Tvarožková K.	217, 218

## U

Uhrinčať M.	157, 162, 217, 218
Uken K. L.	137
Ulbrich S. E.	114, 225
Ullrich E.	46
Ulrich R.	53

## V

Vaccani M.	212
Vahjen W.	66
van den Brand H.	97, 193
van den Broek K.	96
van der Drift S.	105, 140, 195
van der Eijk J.	58
van der Goot J. A.	65
van der Wal E.	224
Van Hese I.	141
van Hoeij R. J.	147, 176
Van Kerschaver C.	226
van Kneysel A. T. M.	147, 148, 176
van Krimpen M.	193
van Laar H.	165
van Reenen K.	97
Van Saun R. J.	130, 142, 196, 197
van Staaveren N.	56
Vandaele L.	141
Vandaele M.	226
Van der Jagt C. J.	116
Vasdal G.	57
Vastenhouw S. A.	224
Vélez J.	134
Velge P.	61
Velkers F. C.	65
Velthuis A.	96
Ventsova I.	110
Vidondo B.	41
Vieira-Neto A.	186
Vogel L.	101, 102, 137
Volkman N.	78
von Brömssen C.	149
von Keyserlingk M. A. G.	163, 167

von Konigslow T. E.	133
Vostroilov A.	110
Vötterl J. C.	68, 198
Vršková M.	162, 217, 218

## W

Wagener K.	181
Wainio A.	219
Walkenhorst M.	31, 209
Wall H.	51
Wall S. K.	112
Walsh A. M.	221
Wang M.	116
Waurich B.	159
Weary D. M.	163, 167
Webster J.	38
Wechsler B.	168
Weck R.	46
Weigele H.	168
Weigend A.	52
Weigend S.	52
Wellnitz O.	112, 114, 150, 220
Wickström H.	149
Wiedemann S.	178
Wilkens M. R.	189, 192
Wilms J.	135, 143, 199
Wilson M.	35
Wilson P. W.	50
Winder C. B.	133
Wippermann W.	46, 159
Wirges J.	28
Wittich J.	159
Wöckel A.	46, 159
Wódz K.	26
Wolfrum N.	204
Wolthuis-Fillerup M.	97
Wood B. J.	115
Würbel H.	45

## X

Xiang R.	116
----------	-----

## Y

Yanagawa Y.	173
Yoon I.	134
Yousef M. S.	175

## Z

Zanolari P.	213
Zarrin M.	106
Zavadilová L.	158, 182
Zebeli Q.	68, 166, 198
Zentek J.	66
Zieger P.	134
Zitz U.	28
Zobel G.	38