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Effect of different dietary regimens at dry-off on performance, metabolism, and immune system in dairy cows

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ABSTRACT

Concentrate withdrawal and feed restriction are commonly used to reduce milk production and to facilitate dry-off, but may impair immune function in dairy cows. We investigated the effect of feed rations providing different amounts of nutrients in combination with feed restriction on performance, endocrine, and metabolic responses, as well as on leukocyte function before and after abrupt dry-off. Forty-three cows were studied from d 12 before until d 6 after dry-off (56 d before scheduled calving). Cows were fed experimental concentrates rich in crude protein (nitrogenic, n = 14), glucogenic precursors (glucogenic, n = 14), or lipids (lipogenic, n = 15). On d 3 before dry-off, total feed allowance was restricted to 50% in half of the animals of each dietary group, whereas feed allowance remained unchanged in the other animals. Performance parameters (milk yield, milk composition, and dry matter intake) were recorded, and daily blood and milk samples were taken and analyzed for various metabolic and endocrine parameters. Additionally, activity and mRNA abundance of several genes in leukocytes were measured at selected time points before and after feed restriction and dry-off, respectively. Feed restriction immediately resulted in a negative energy balance and decreased milk production. Concomitantly, concentrations of nonesterified fatty acids increased, whereas insulin, insulin-like growth factor-1, and glucagon decreased. After dry-off, energy balance turned positive and plasma nonesterified fatty acids decreased. Plasma glucose, insulin, and insulin-like growth factor-1 concentrations increased in all groups after dry-off. Glucose, insulin, and glucagon concentrations in plasma were higher in nonrestricted compared with restricted animals after dry-off. The experimental concentrate types marginally affected the investigated metabolic and endocrine factors, with the exception of elevated milk and plasma urea concentrations in cows fed the nitrogenic concentrate. Chemotactic and phagocytic activity of leukocytes were not affected by diets, feed restriction, or dry-off. Likewise, blood leukocyte mRNA abundance encoding for tumor necrosis factor α (TNF), heat shock protein family A (HSP70), and the glucose transporters (GLUT) 1 and 3 remained unchanged throughout the study period. Overall, the short-term negative energy balance induced by feed restriction was temporarily accompanied by metabolic adaptations, but did not alter the studied factors related to the immune system. Metabolic and endocrine adaptations supporting milk synthesis were continued during the first days after dry-off despite cessation of milking. Thus, the abrupt dry-off resulted in a short-term increase of glucose and triglyceride concentrations, with a delayed endocrine response to re-establish nutrient homeostasis in blood.
Key words: dry-off, diet composition, feed restriction, metabolism, immune system

INTRODUCTION

A long enough nonlactating period before calving, known as the dry-period, is important to renew mammary epithelial cells and maximize milk production in the subsequent lactation (Capuco et al., 1997; Kuhn et al., 2005). However, increasing milk production of modern dairy cows concomitantly results in greater milk yields at the scheduled time of dry-off, accompanied by an elevated intramammary pressure, milk leakage, and potential entry of mastitis pathogens through the teat canal (Odensten et al., 2007; Vilar and Rajala-Schultz, 2020). Hence, the beginning of the dry-period belongs to the most susceptible periods for IMI in dairy cows (Bradley and Green, 2004). In addition, the mastitis risk after parturition is greater in cows that were dried-off at higher milk yields (Rajala-Schultz et al., 2005). To facilitate dry-off, milk yield can be reduced by decreasing milking frequency (e.g., only once vs. twice milking/d; Kelly et al., 1998; Tucker et al., 2009) or restricting dietary energy and nutrient supply (Tucker et al., 2009; Ollier et al., 2014). A lower intramam-

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mary pressure and an earlier cessation of milk secretion were observed following a feed restriction in late lactating cows at dry-off (Blau et al., 2019). However, feed restriction negatively affects animal welfare (e.g., by inducing hunger or animal discomfort), and in addition to the intended decline in milk production, a transient negative energy balance (**NEB**) may occur. The forced energy deficiency is compensated by the mobilization of body reserves similar to the catabolic situation observed during early lactation (Valizaheh et al., 2008; Ollier et al., 2014; Zobel et al., 2015). A NEB leading to alterations in blood metabolites may affect the immune system because glucose is the preferred metabolic fuel for activated leukocytes, and BHB and nonesterified fatty acids (**NEFA**) have been shown to negatively influence leukocyte functions in blood (Suriyasathaporn et al., 1999; Ster et al., 2012; Ingvartsen and Moyes, 2013). Hence, the increased risk of mastitis around dry-off must be studied in view of the concomitant metabolic status of dairy cows. Earlier research showed that dietary nutrient composition in early lactation can alter concentrations of NEFA and BHB in plasma, particularly when glucogenic precursors or a ketogenic diet are fed (van Knegsel et al., 2007b,c). Furthermore, cows receiving a diet rich in glucogenic precursors had a greater immunoglobulin activity binding keyhole limpet hemocyanin and *Escherichia coli* derived LPS compared with cows fed a lipogenic diet (van Knegsel et al., 2007a). Additionally, diets varying in macronutrients were shown to modify metabolic and endocrine parameters in mid-lactating cows (Gross et al., 2021b). However, the effect of diet composition on the metabolic status and consequences for the immune function in dairy cows around dry-off were scarcely investigated.

Therefore, the objectives of the present study were to investigate the effect of feeding rations varying in nutrient composition in combination with a restricted feed allowance on performance parameters, endocrine and metabolic adaptations, as well as their effect on the immune system in dairy cows around dry-off. We specifically altered the dietary contents of carbohydrates, proteins, and lipids to provoke metabolic shifts that in turn modulate immune function. The short-term feeding period and the increase of experimental concentrates before dry-off were hypothesized to enhance changes of metabolic adaptation and immune cell function.

MATERIALS AND METHODS

Animal experiments were carried out in accordance with the Swiss law on animal protection and welfare, and approved by the cantonal committee of animal experimentation (approval no. 2018_35_FR).

Animals, Husbandry, and Experimental Design

Holstein dairy cows (n = 43) were selected from the dairy herd of the Agroscope research station (Posieux, Switzerland) and kept in a tiestall barn (after an acclimatization period of at least 7 d) with straw and sawdust bedding during the entire study period. All animals were clinically examined by a veterinarian before entering the study. At the start of the experiment, all cows were in late gestation, of similar BW (712 ± 11 kg; mean ± SEM) and milk yield (18.0 ± 0.4 kg of milk/d). Animals were divided into 3 dietary treatment groups according to their BW and milk yield. The experimental period lasted from 12 d before until 6 d after dry-off (scheduled at 56 d before expected parturition, Figure 1). Until abrupt dry-off, cows were milked twice daily (0500 and 1700 h) in a milking parlor, where milk yield and BW were recorded automatically.

Feeding Regimen and Feed Analyses

Formulation of diets followed the Swiss national feeding recommendations of Agroscope (2016). In addition to the ad libitum feeding of hay and independent from dietary treatments, an in-house concentrate supplement with a mineral and vitamin premix (referred to as premix; on a DM basis: 26.1% barley, 24.8% wheat, 24.8% corn kernels, 12.0% soybean meal, 5.0% corn gluten, 2.0% potato protein, 3.4% molasses, and 1.9% mineral and vitamin premix) considering the mineral requirements for lactating and dry cows, respectively, was fed at a rate of 1.5 kg/d. Experimental treatments involved 3 different types of additional concentrate supplementation on top of the basal diet (Figure 1): An nitrogenic concentrate (emphasis on an elevated CP supply, n = 14 cows; on a DM basis: 10.0% barley, 10.0% corn kernels, 54.8% soybean meal, 15.0% rapeseed meal, 10.2% dried sugar beet pulp), a glucogenic concentrate (emphasis on an elevated carbohydrate supply, n = 14 cows; on a DM basis: 10.0% barley, 19.9% wheat, 39.8% corn kernels, 10.0% soybean meal, 10.1% rapeseed meal, 10.2% dried sugar beet pulp), and a lipogenic concentrate (emphasis on an elevated lipid supply, n = 15 cows; on a DM basis: 19.5% barley, 19.5% corn kernels, 9.7% soybean meal, 9.9% rapeseed meal, 10.1% rapeseed cake, 16.4% rumen protected fat, and 14.9% dried sugar beet pulp). The formulation of experimental concentrates was not balanced in terms of energy and protein supply as the aim was to provide a high proportion of CP, carbohydrates, or lipids. Despite the focus on an unilateral supply of nutrients, however, animal health should not be comprised (e.g., avoidance of rumen acidosis, lack of other nutrients). Therefore, the experimental diets (nitrogenic, glucogenic, lipo-

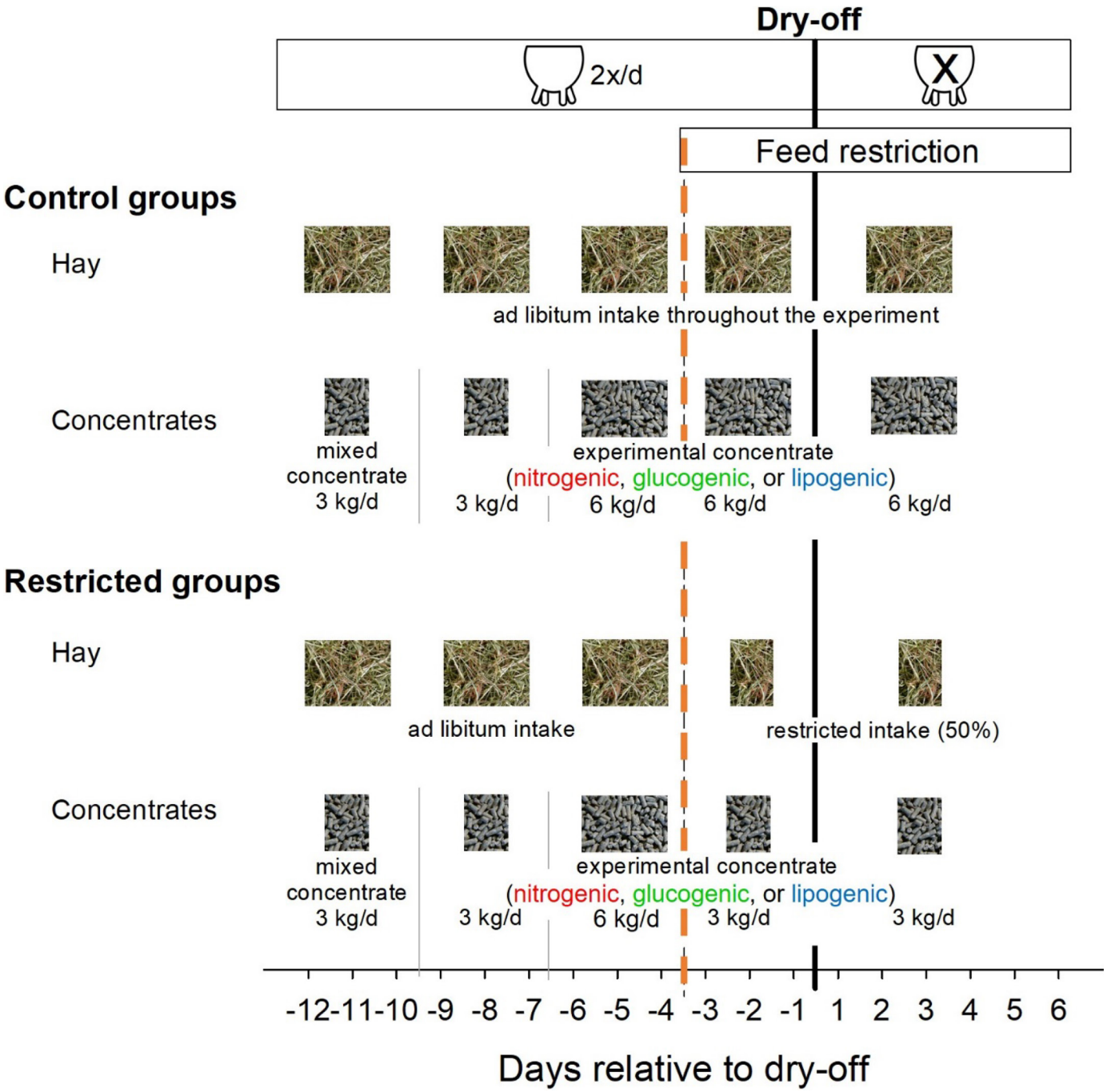

Figure 1. Experimental setup of the feeding trial with dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments.

genic) simultaneously contained a smaller proportion of other nutrients as well. Individual intakes of hay and concentrates were recorded daily. Feed samples of hay and concentrates were taken regularly from the different lots, and analyzed for chemical composition (DM, crude fat, CP, crude fiber, ADF, NDF, starch, water-soluble carbohydrates, and ash content) as described earlier by Heublein et al. (2017). The chemical composition of hay and concentrates fed in the present study is shown on Table 1. Experimental concentrates were fed in 3 equal portions daily. Figure 1 shows the experimental setup. Before the start of dietary treat-

Table 1. Chemical analysis and nutrient value of hay and concentrates fed during the experiment (data presented as means ± SD)

Item[1]	Hay (n = 5)	Concentrates			
		Premix[2] (n = 5)	Nitrogenic (n = 3)	Glucogenic (n = 3)	Lipogenic (n = 3)
DM (g/kg)	927 ± 25	890 ± 9	886 ± 8	889 ± 5	911 ± 9
Energy (MJ of NE_L/kg of DM)	5.44 ± 0.25	7.01 ± 0.03	8.21 ± 0.05	8.23 ± 0.04	9.04 ± 0.02
ADF (g/kg of DM)	258 ± 15	89 ± 5	93 ± 4	72 ± 2	122 ± 3
NDF (g/kg of DM)	445 ± 24	151 ± 5	173 ± 6	155 ± 7	213 ± 4
CA (g/kg of DM)	98 ± 16	135 ± 6	58 ± 4	31 ± 3	39 ± 1
CF (g/kg of DM)	228 ± 10	59 ± 2	66 ± 3	49 ± 2	64 ± 2
CP (g/kg of DM)	129 ± 18	317 ± 4	340 ± 9	157 ± 5	170 ± 8
Ether extract (g/kg of DM)	ND[3]	23 ± 2	24 ± 1	30 ± 3	178 ± 4
Starch (g/kg of DM)	ND	131 ± 19	144 ± 8	463 ± 5	271 ± 4
WSC (g/kg of DM)	ND	64 ± 12	73 ± 2	46 ± 9	51 ± 2

[1]CA = crude ash; CF = crude fiber; WSC = water-soluble carbohydrates.

[2]Premix = concentrate premix with mineral and vitamin supplement, fed to all cows at a rate of 1.5 kg/d (0.75 kg/d in restricted groups during feed restriction) independent of the specific dietary treatments.

[3]ND = not determined.

ments until d 10 before dry-off, cows were fed a mixture of the 3 experimental concentrates (1/3 nitrogenic, 1/3 glucogenic, 1/3 lipogenic; total amount: 3 kg/d) to ensure adaptation of the ruminal system. Beginning at d 9 before dry-off, dietary treatments started and cows received 3 kg/d of one specific concentrate (i.e., nitrogenic, glucogenic, or lipogenic), whose amount was increased to 6 kg/d at d 6 before dry-off. At d −3 relative to dry-off until d 6 thereafter, half of the animals of each dietary treatment group were only allowed to 50% of their individual previous hay and concentrate intake, whereas fed amounts of the respective control cows were not changed. Mineral supplementation was adjusted to the recommendations for nonlactating cows at dry-off in all animals.

Milk Sampling and Analyses

Milk samples were collected twice daily until dry-off and stored in a refrigerator (5°C) until further processing. Milk samples of the morning and evening milking were pooled on a daily basis, and contents of milk fat, protein, lactose, and SCC were analyzed using a MilkoScan-FT (Fossomatic) in the laboratory of Suisselab AG (Zollikofen, Switzerland). Milk urea was analyzed with a commercially available enzymatic kit (Urea FS 1.3101 99 10 021) from Randox Laboratories Ltd. on an autoanalyzer (Cobas Mira, Roche). Another aliquot of the pooled milk samples was frozen at −20°C for the later analysis of electrolytes (Na, K, and Cl) by ion-selective electrodes linked to the autoanalyzer.

Blood Sampling and Analyses

After morning milking and before feeding, blood samples were drawn daily from one jugular vein using evacuated tubes coated with EDTA for plasma preparation, and with clot activator for serum harvest, respectively (Vacuette, 9 mL with K_3EDTA, cat. no. 455036; Vacuette, 9 mL with serum clot activator, cat. no. 455092; Greiner Bio-One International GmbH). Samples in EDTA tubes were put immediately on wet ice, whereas serum tubes were kept at ambient temperature for around 2 h until centrifugation. After centrifugation at 2,000 × *g* (+4°C, 20 min), aliquots of 1.5 mL were frozen at −80°C until further analyses. In plasma, various metabolites were measured using commercially available enzymatic kits with an autoanalyzer (Cobas Mira, Roche): glucose (GLUC-PAP GL364), NEFA (NEFA FA115), and BHB (Ranbut RB1007) kits were obtained from Randox Laboratories Ltd., and kits for measurements of urea (Urea FS 1.3101 99 10 021), total cholesterol (Cholesterol FS 1.1350 99 10 021), and triglycerides (Triglycerides FS 1.5760 99 10 021) from DiaSys Diagnostic Systems GmbH.

Glucagon in plasma was measured by a commercially available RIA (catalog no. GL-32 K, EMD Millipore), whereas concentrations of insulin and IGF-1 were measured by RIA as described earlier by Vicari et al. (2008).

Blood Cells and Immunological Tests

At d −10, −7, −4, −1, +3, and +6 relative to dry-off, whole blood was drawn into tubes containing K_3EDTA and analyzed on an automatic analyzer (Vetscan HM5, Abaxis Inc.) to measure total leukocyte and neutrophil count.

A commercially available Phagotest-Kit (Celonic Deutschland GmbH) was used to determine blood leukocyte phagocytic activity. Heparinized whole blood was incubated with FITC-labeled *Escherichia coli* for

10 min at 37°C and as negative control at 0°C. Samples were washed, lysed, fixed, and the DNA stained before they were analyzed using flow cytometry on an imaging reader (Cytation 5 Cell Imaging Multi-Mode Reader, BioTek Instruments Inc.). The phagocytic activity (difference between test and control samples) and phagocytic activity per neutrophil granulocyte were calculated.

Leukocyte chemotaxis was analyzed with the CytoSelect 96-Well Cell Migration Assay (catalog no. CBA-104, Cell Biolabs Inc.) according to the manufacturer's instructions. In short, a cell suspension was placed in the upper chamber of a migration plate, then incubated for 2 h. Cells migrated through a polycarbonate membrane (3 μm pore size) toward a medium containing IL-8 as chemoattractant or without IL-8 as control. Migrated cells were then lysed and quantified using a fluorescent dye. Results were calculated as the logarithm base 2 of the difference between test and controls from d −1 and d +3 relatively to d −4 relative to dry-off.

Total RNA Extraction, Reverse-Transcription, and Quantitative PCR of Blood Cells

Blood cells were isolated from blood samples harvested at d −11, d −1, and d +6 relative to dry-off, with 0.5-mL RNAprotect Animal Blood Tubes, and the total RNA extracted using the RNeasy Protect Animal Blood Kit according to the manufacturer's instructions, including a digestion with DNase (catalog no. 76554 and 73224, Qiagen GmbH). The RNA concentration and purity were determined by spectrophotometry (NanoDrop One, Thermo Scientific) and only samples measuring at optical density A260/280 > 1.8 were used for further analysis (2 samples from different cows were therefore excluded from this study). The RNA was stored at −80°C until further processing. One hundred nanograms of RNA was reverse transcribed into cDNA with GoScript Reverse Transcriptase (catalog no. A5003, Promega Corporation) according to the manufacturer's protocol, using oligo-dT (Microsynth) and dNTP (catalog no. D7295, Sigma), and the cDNA was then stored at −21°C. The quantitative real-time PCR was performed in duplicates using SYBR Green on a C1000 Touch Thermal Cycler/CFX384 (Bio-Rad Laboratories). The reagent (10 μL) contained 1 μL of a gene-specific primer mix (forward and reverse primer each 0.5 μ*M*), 5 μL of GoTaq qPCR Master Mix (catalog no. A600A, Promega Corporation), 3 μL of nuclease-free water, and 1 μL of cDNA. Primers were commercially synthesized (Microsynth; for details see Table 2). Amplification conditions for 39 cycles were as follows: 50°C/2 min, enzyme activation at 95°C/10 min, denaturation at 95°C/15 s, and annealing at 60°C/1 min. Cycle thresholds (**Ct**) were automatically calculated (Bio-Rad CFX Maestro software 1.1), and all samples of a cow repeated if duplicates differed in one or more Ct. Mean values of duplicates were normalized with the arithmetic means of the Ct values of 2 reference genes: ubiquitin B (*UBB*) and tyrosine 3-monooxygenase (*YWHAZ*). While *UBB* is already an established reference gene in our research group (Pfaffl et al., 2003), *YWHAZ* was described as one of the most stable genes in bovine leukocytes (Spalenza et al., 2011; Vorachek et al., 2013; Crookenden et al., 2016) and both reference genes were not systematically influenced by the experiment. The Ct values of target genes were calculated as relative expression, multiplied by −1, so a higher Ct value indicates an increase in mRNA abundance.

Calculations and Statistical Analysis

All data presented in the manuscript are means ± standard error of the mean, except where denoted as standard deviation. Energy balance (**EB**) was calculated according to Agroscope (2016) expressing the difference between energy intake (intake multiplied with the energy content of the respective feed ingredients) and energy output (i.e., sum of requirements for maintenance, pregnancy, and milk production considering BW, stage of gestation, milk yield, and composition). The molar ratio of insulin:glucagon was calculated using the formula published by Muller et al. (1971).

For statistical analyses, the area under the curve of performance and metabolic parameters was calculated at 3-d intervals at an individual cow level, and evaluated using the statistical software SAS (version 9.4; SAS Institute Inc.) using the MIXED procedure with the Tukey-Kramer post hoc test adjusting for multiple comparisons. Fixed effects were the type of diet (nitrogenic, glucogenic, lipogenic), feeding level (restricted, control), and time (day relative to dry-off). The individual cow was considered as repeated factor. Changes within restricted groups following feed restriction were compared by a pairwise *t*-test (e.g., d −4 vs. d −1 relative to dry-off). Effects were assumed to be significant at $P < 0.05$, whereas tendencies toward significance were assumed when $0.05 \leq P < 0.10$.

RESULTS

Body Weight, Dry Matter Intake, and Energy Balance

Within the experimental period of 18 d, BW was not affected by dietary treatments, feed restriction, or dry-off (data not shown). Until d 4 before dry-off, the dietary treatments did not affect DMI (Supplemental

Table 2. Primer sequences for PCR, GenBank accession number, and size

Gene[1]	Primer sequence[1] (5′–3′)	GenBank accession no.	Size (bp)
SLC2A1[2]	F: GCTTCTCCAACTGGACTTCG R: ACAGCTCCTCAGGTGTCTTG	NM_174602	225
SLC2A3[2]	F: GGAAAACTTGCCGCCGATAG R: CGCCTCAGGAGCATTGATGA	NM_174603	223
SLC2A4[2]	F: GACTGGTACCCATGTACGTG R: CCGGATGATGTAGAGGTAGC	NM_174604.1	242
HSPA1A[3]	F: ACATGAAGAGCGCCGTGGAGG R: GTTACACACCTGCTCC	NM_203322.3	170
TNF[4]	F: CCACGTTGTAGCCGACATC R: CCCTGAAGAGGACCTGTGAG	NM_173966.3	155
UBB[5]	F: AGATCCAGGATAAGGAAGGCAT R: GCTCCACCTCCAGGGTGAT	NM_174133.2	426
YWHAZ[4]	F: CAGGCTGAGCGATATGATGAC R: GACCCTCCAAGATGACCTAC	NM_174814.2	141

[1]F = forward, R = reverse.

[2]Gross et al., 2015b.

[3]Caldeira et al., 2019.

[4]Griesbeck-Zilch et al., 2008.

[5]Designed with Beacon Designer 8.21 (Premier Biosoft International).

Figure S1, https://doi.org/10.48350/164806, Jermann et al., 2022). Whereas DMI in control cows receiving the nitrogenic and lipogenic concentrate did not change during the experimental period, DMI declined after dry-off in nonrestricted cows fed the glucogenic diet ($P < 0.05$; Supplemental Figure S1). Due to the limited feed allocation to feed-restricted cows, DMI was lower in feed-restricted compared with respective control cows from d −3 before dry-off until the end of the study ($P < 0.05$; Supplemental Figure S1). Energy balance was not affected by the different dietary concentrate types (Figures 2A–C). Concomitantly to DMI, EB turned immediately negative with the start of feed restriction in restricted cows, but turned positive again directly after dry-off ($P < 0.05$). Compared with feed-restricted cows, EB of control cows remained positive throughout the study, but further increased and remained at a higher level after dry-off in all dietary groups ($P < 0.05$; Figures 2A–C). During the feed restriction period lasting from d −3 until d +6 relative to dry-off, all feed-restricted groups had a lower EB compared with the respective control groups ($P < 0.05$), although EB turned positive after dry-off.

Milk Yield and Milk Composition

Milk yield (Figures 2D-F) was not affected by the different dietary concentrate types, but decreased in all feed-restricted groups from d −4 to d −1 relative to dry-off ($P < 0.05$). Milk yield did not change in the respective control groups during the feed restriction period. Milk protein content was not affected by the dietary treatments (Supplemental Figure S2, https://doi.org/10.48350/164806, Jermann et al., 2022). The milk fat yield decreased in all feed-restricted groups from d −4 to d −1 relative to dry-off ($P < 0.05$; data not shown). Since the milk yield decreased proportionally more than the milk fat yield, milk fat content (Supplemental Figure S3, https://doi.org/10.48350/164806, Jermann et al., 2022) increased after feed restriction in the nitrogenic and glucogenic group (d −4 vs. d −1 relative to dry-off, $P < 0.05$) and tended to increase in the lipogenic group ($P = 0.09$). The milk fat:protein ratio increased in all feed-restricted groups after the initiation of feed restriction ($P < 0.05$; Supplemental Figure S4, https://doi.org/10.48350/164806, Jermann et al., 2022). Milk lactose content was not affected by the dietary treatments, except for lower concentrations in the restricted lipogenic group compared with controls during the feed restriction period ($P < 0.05$; data not shown). The SCC in milk was not affected by dietary treatments and feed restriction (data not shown). Milk urea content increased markedly with increasing supply levels of the nitrogenic concentrate ($P < 0.05$; Figure 3A). No differences in milk urea concentration were observed between restricted and control cows of the nitrogenic treatment. Whereas milk urea content did not change in the nonrestricted glucogenic and lipogenic groups, it increased in the respective restricted groups ($P < 0.05$; Figures 3B and 3C). Consequently, elevated milk urea concentrations in restricted compared with nonrestricted cows during feed restriction from d −4 to d −1 before dry-off were observed in the glucogenic treatment ($P < 0.05$) and by tendency in the lipogenic treatment ($P = 0.06$). Concentrations of milk electrolytes (Na, K, Cl) were not affected by dietary concen-

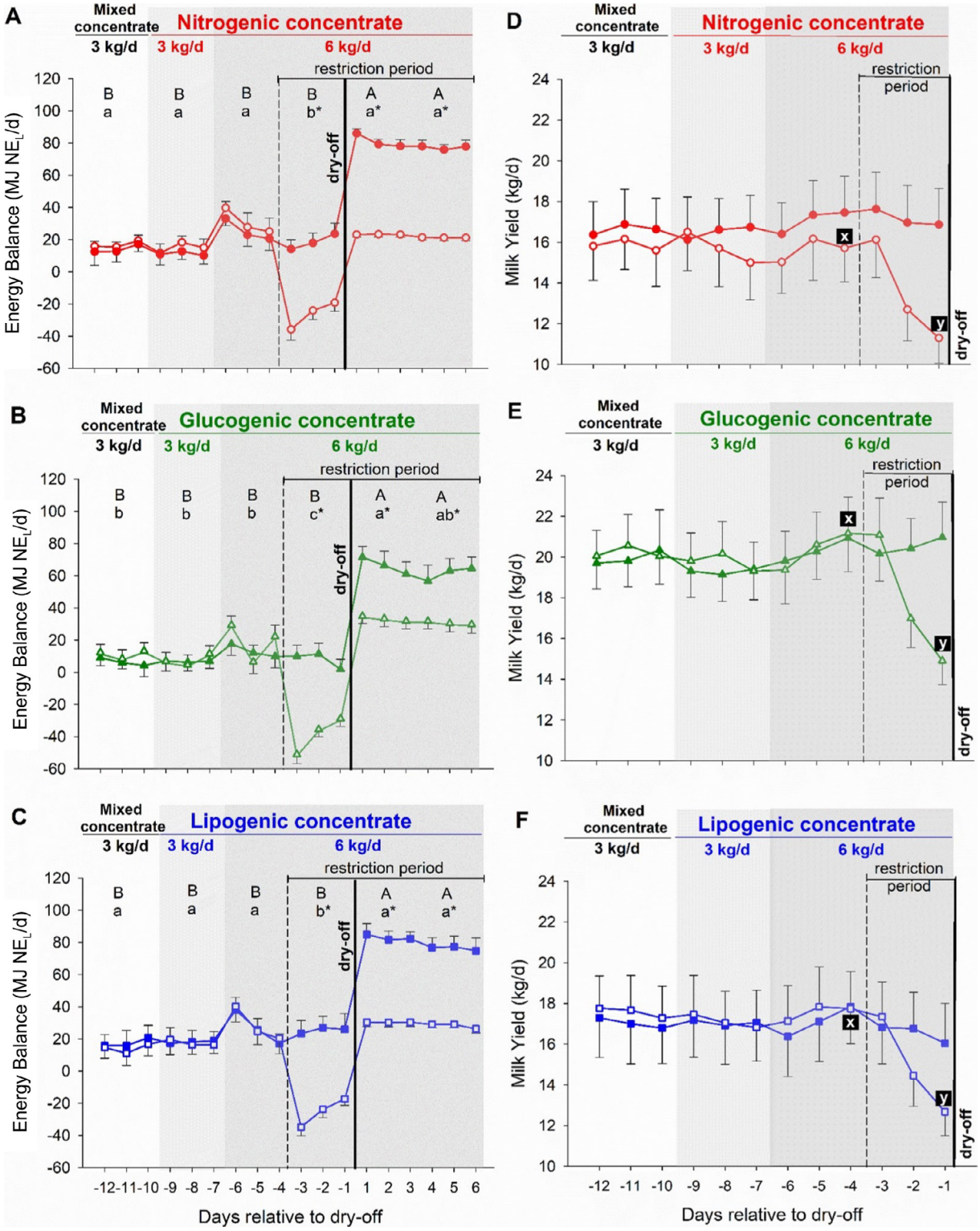

Figure 2. Energy balance (EB; panels A–C; from d −12 until d +6 relative to dry-off) and milk yield (panels D–F; d −12 until dry-off) in dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a–c) or control group (A,B) over time (3-d intervals). The letters x and y indicate a significant difference ($P < 0.05$) between d −4 and −1 relative to dry-off in the restrictive groups. A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *.

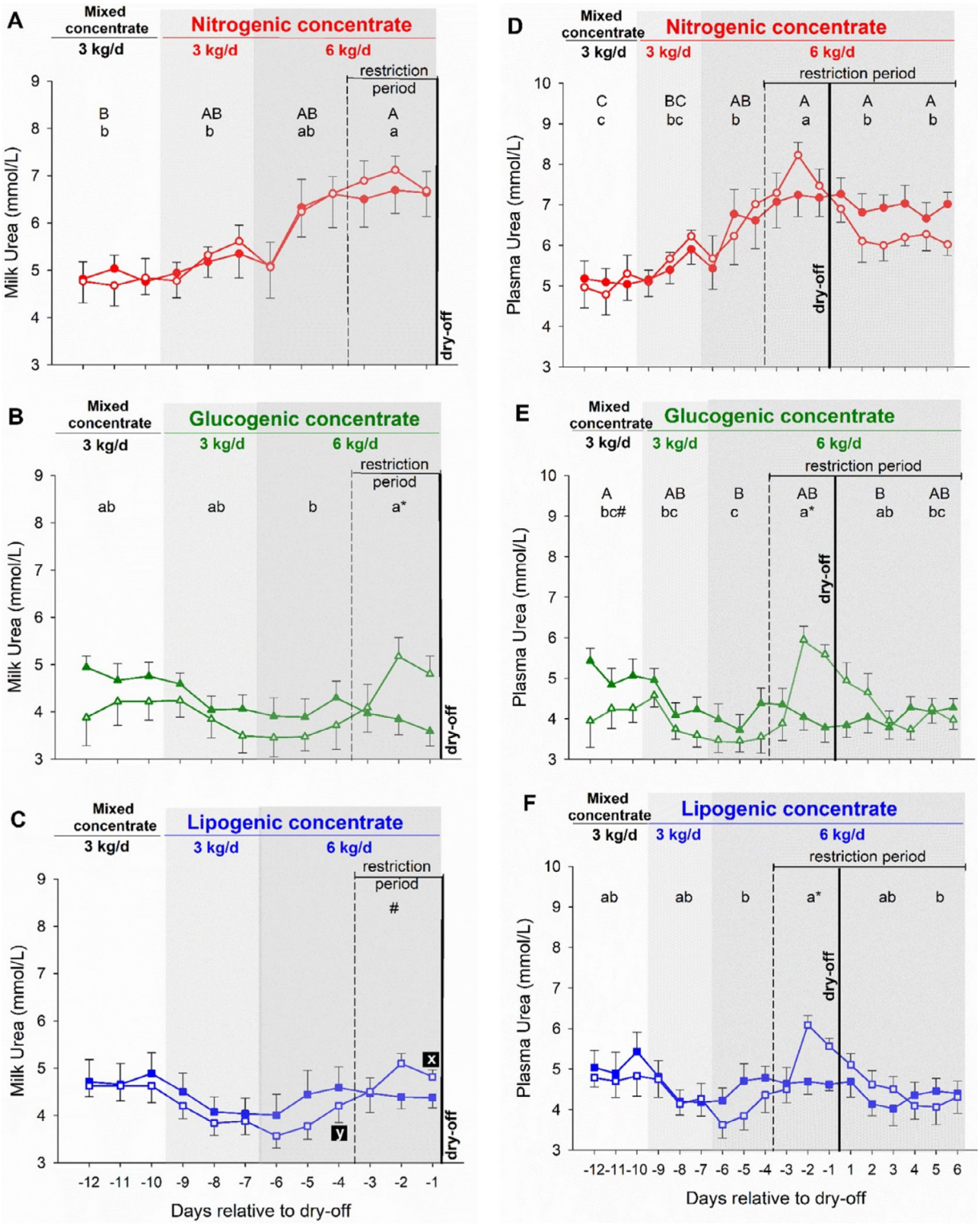

Figure 3. Urea concentrations in milk (panels A–C; d −12 until dry-off) and plasma (panels D–F; from d −12 until d +6 relative to dry-off) in dairy cows. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a–c) or control group (A–C) over time (3-d intervals). The letters x and y indicate a significant difference ($P < 0.05$) between d −4 and −1 relative to dry-off in the restrictive groups. A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *, whereas tendencies ($P < 0.10$) are marked with #.

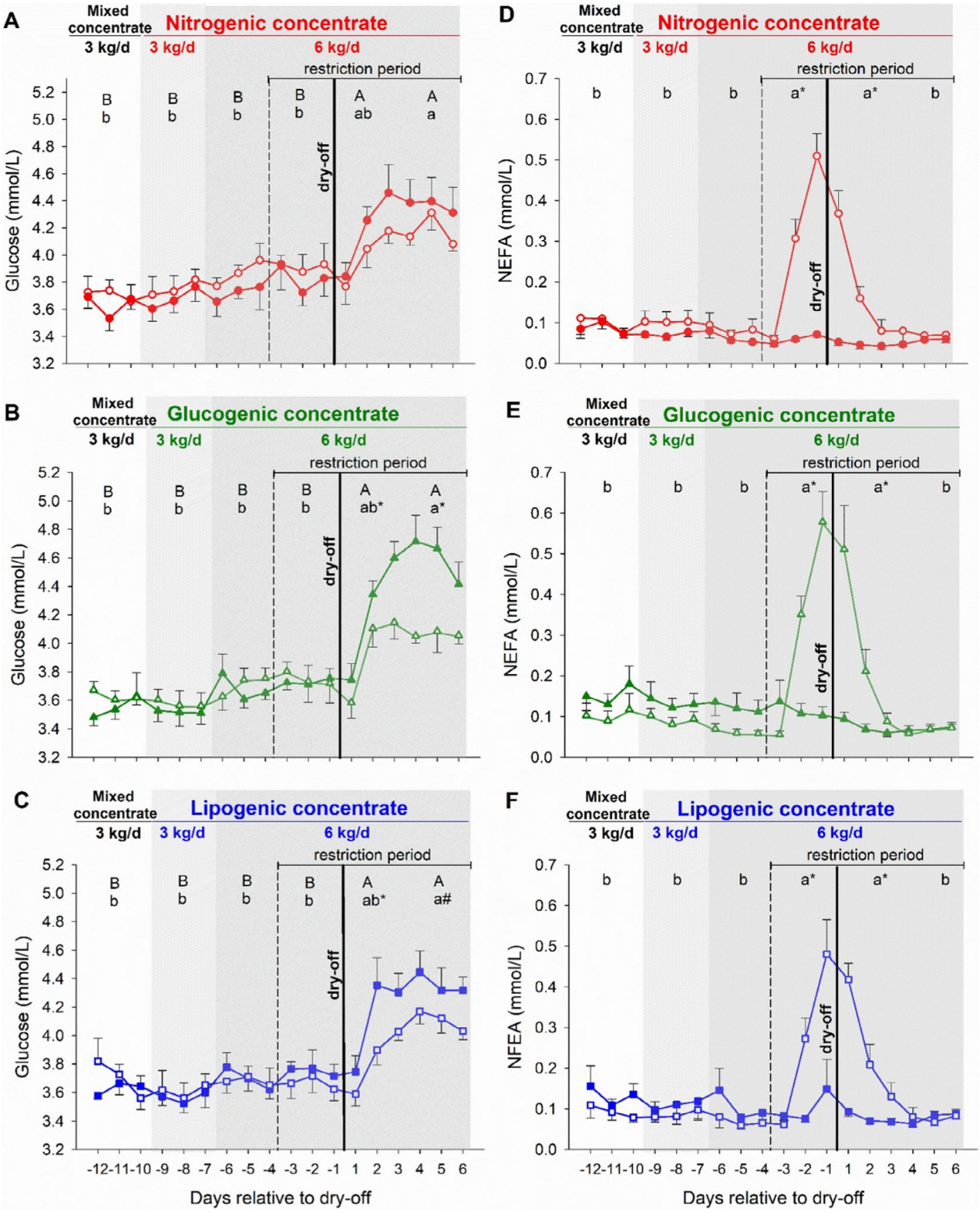

Figure 4. Plasma concentrations of glucose (panels A–C) and nonesterified fatty acids (NEFA; panels D–F) in dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a,b) or control group (A,B) over time (3-d intervals). A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *, whereas tendencies ($P < 0.10$) are marked with #.

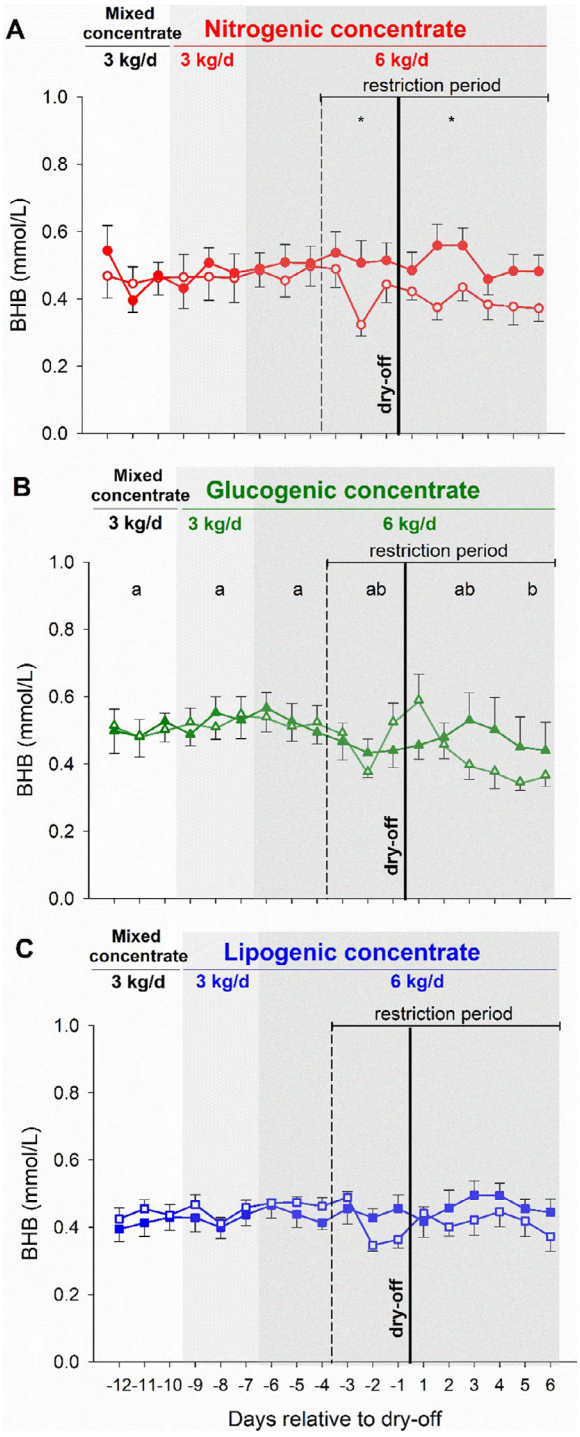

Figure 5. Beta-hydroxybutyrate concentrations in plasma of dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters (a,b) indicate a significant difference ($P < 0.05$) within the restricted group over time (3-d intervals). A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *.

trate types (Supplemental Figures S5–S7, https://doi.org/10.48350/164806, Jermann et al., 2022). After the initiation of feed restriction, potassium concentration in milk decreased in all restricted groups from d −4 to d −1 ($P < 0.05$; Supplemental Figure S6, https://doi.org/10.48350/164806). Milk potassium concentration was lower in restricted than control cows of the nitrogenic treatment during the restriction period ($P < 0.05$). Milk chloride concentration was greater in restricted compared with control cows of the lipogenic treatment after feed restriction ($P < 0.05$; Supplemental Figure S7).

Blood Plasma Metabolites and Endocrine Factors

Similar to the urea content in milk, plasma urea concentration increased concomitantly with increasing levels of the nitrogenic concentrate in both the control and restricted group until dry-off ($P < 0.05$; Figure 3D). Whereas plasma urea concentration remained elevated in the control group of the nitrogenic treatment after dry-off, urea concentration decreased to pre-restriction levels in the restriction group ($P < 0.05$). Compared with the respective control groups, plasma concentrations of urea increased and were higher in animals fed the glucogenic and lipogenic diet during the feed restriction phase before dry-off ($P < 0.05$; Figures 3E and 3F).

Glucose concentrations were not affected by dietary concentrate type or by feed restriction, but increased in all experimental groups after dry-off ($P < 0.05$; Figures 4A–C). Except for the nitrogenic group, glucose concentrations were higher in control compared with feed-restricted animals after dry-off ($P < 0.05$). Plasma NEFA concentrations were not affected by dietary type and did not change in control animals during the entire study period (Figures 4D–F). As expected, NEFA concentrations started to rise in all feed-restricted animals after the first d of feed restriction, peaked at 1 d before dry-off, and decreased within 3 d after dry-off to pre-restriction levels (Figures 4D–F). Compared with the respective control cows, NEFA concentrations were greater in feed-restricted cows between 3 d before until 3 d after dry-off ($P < 0.05$). Plasma BHB was not affected by feed restriction or dry-off in animals fed the lipogenic concentrate, but slowly decreased after feed restriction until d 6 after dry-off in animals fed the glucogenic concentrate ($P < 0.05$), and was lower in feed-restricted animals fed the nitrogenic concentrate from 3 d before until 3 d after dry-off ($P < 0.05$; Figures 5A–C). Cholesterol concentrations in plasma were not altered by dietary treatments, feed restriction, or dry-off in all control groups and the lipogenic restriction group, but were lowered in the restricted nitrogenic and

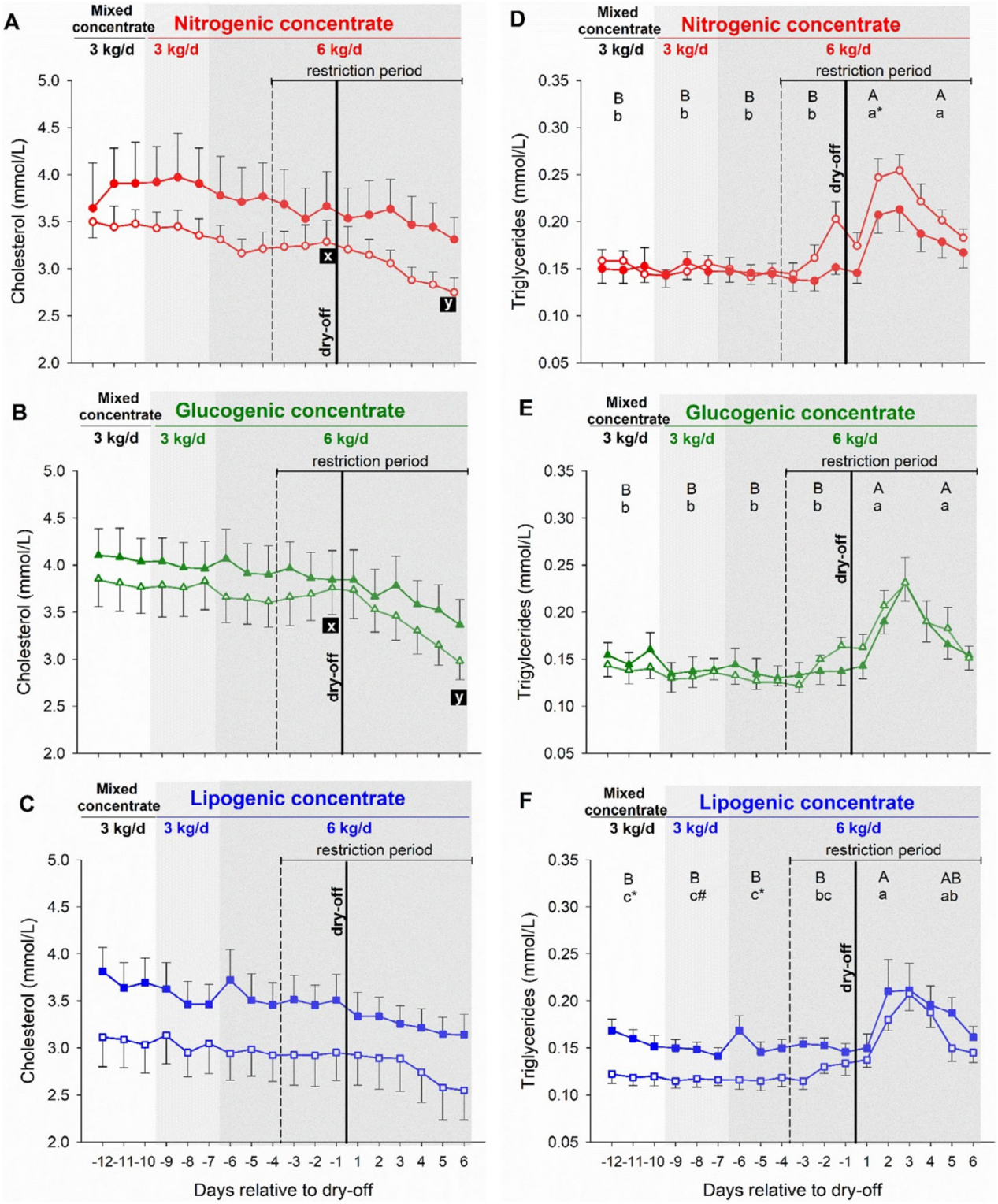

Figure 6. Cholesterol (panels A–C) and triglyceride concentrations (panels D–F) in plasma of dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a–c) or control group (A,B) over time (3-d intervals). The letters x and y indicate a significant difference ($P < 0.05$) between d −1 and +6 relative to dry-off in the restrictive groups. A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *, whereas tendencies ($P < 0.10$) are marked with #.

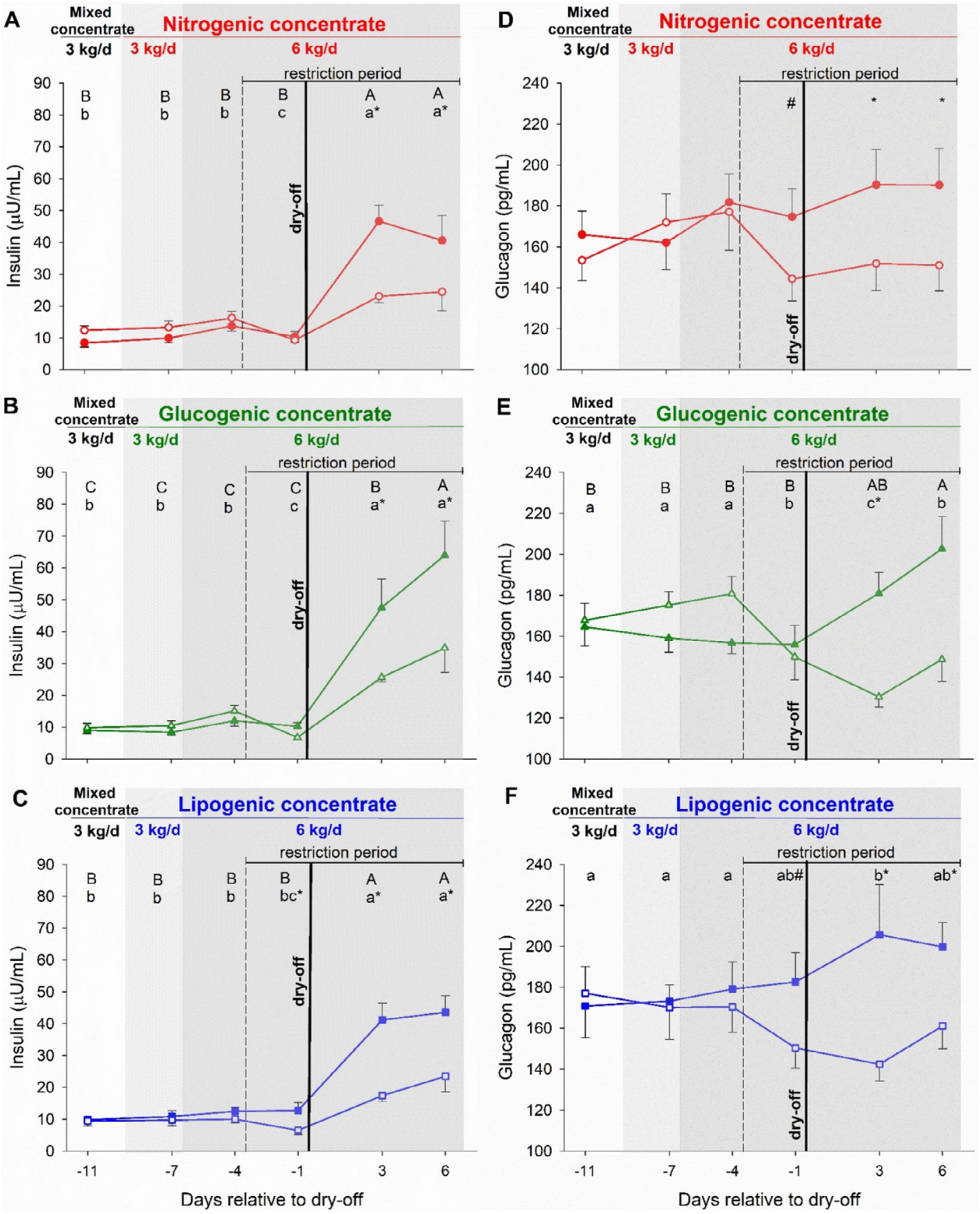

Figure 7. Concentrations of insulin (panels A–C) and glucagon (panels D–F) in dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a–c) or control group (A–C) over time. A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *, whereas tendencies ($P < 0.10$) are marked with #.

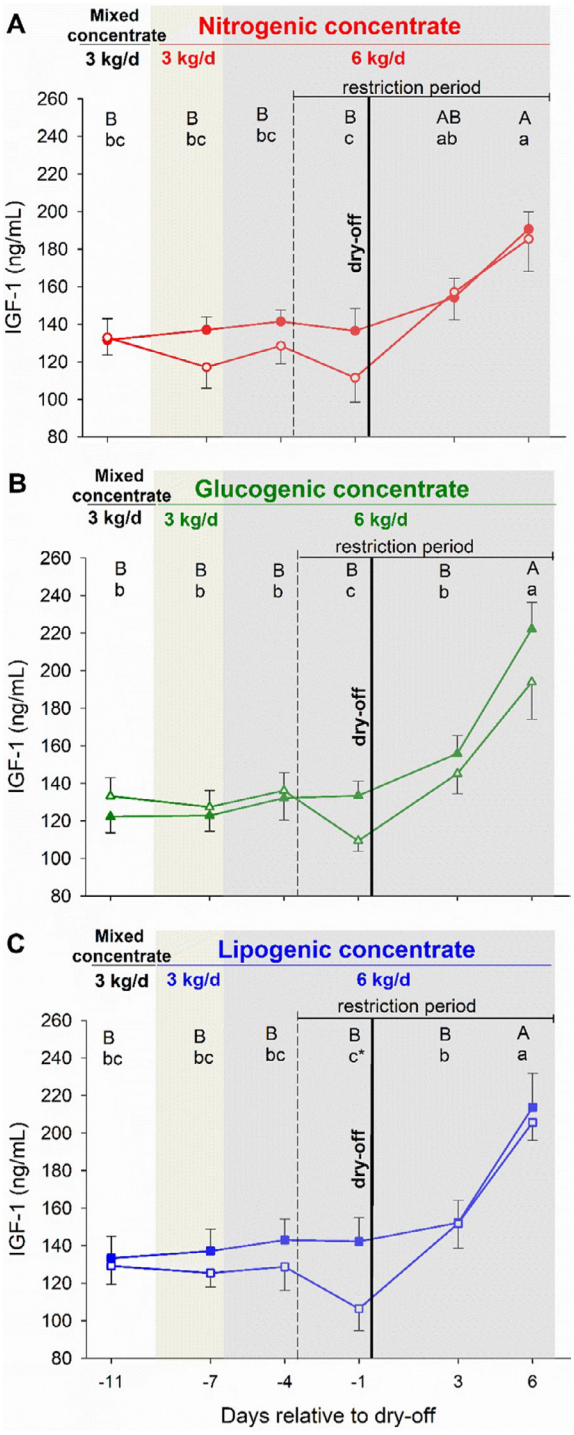

Figure 8. Concentrations of IGF-1 in plasma of dairy cows from d −12 until d +6 relative to dry-off. Cows were fed concentrates rich in CP (nitrogenic; n = 14), carbohydrates (glucogenic; n = 14), or lipids (lipogenic; n = 15). At d −3 before dry-off, feed restriction was applied to half of the cows in all dietary treatments. Data are means ± SEM. Filled symbols indicate the control groups; empty symbols represent the restricted groups of the respective dietary treatments. Different letters indicate a significant difference ($P < 0.05$) within the restricted (a–c) or control group (A,B) over time. A significant difference ($P < 0.05$) between the restrictive and respective control group at the same interval is marked with *.

glucogenic groups at d +6 versus d −1 relative to dry-off ($P < 0.05$; Figures 6A–C). Plasma concentrations of triglycerides (Figures 6D–F) were not affected by dietary type or feed restriction before dry-off, although the lipogenic control group consistently showed greater triglyceride concentrations from the very beginning of the study despite an identical feeding of the feed restriction group until the start of the feed restriction period (Figure 6F). Triglyceride concentrations increased from d 1 until d 3 after dry-off in all groups ($P < 0.05$), and slightly decreased thereafter. During the first 3 d after dry-off, triglyceride concentration was higher in the feed-restricted compared with the control group of the nitrogenic diet ($P < 0.05$; Figure 6D).

Insulin concentrations in plasma were not affected by the type of dietary concentrate (Figures 7A–C). Within animals assigned to the feed-restricted groups, feed restriction before dry-off resulted in a decrease of plasma insulin in the restricted nitrogenic and glucogenic groups ($P < 0.05$), and a tendency toward lower insulin concentrations in the restricted lipogenic group ($P = 0.07$). After dry-off, insulin concentrations increased in all groups (control and restricted groups) to higher levels compared with the concentrations measured before feed restriction until d 3 after dry-off, and even further until d 6 after dry-off in glucogenic control animals ($P < 0.05$; Figures 7A–C). However, all feed-restricted cows had lower insulin concentrations compared with respective control cows after dry-off ($P < 0.05$). Glucagon concentration was not affected by the dietary concentrate type (Figures 7D–F). Feed restriction led to a decrease of plasma glucagon from d −4 to d −1 relative to dry-off in the restricted glucogenic group ($P < 0.05$), and by tendency in the restricted nitrogenic ($P = 0.08$) and lipogenic group ($P = 0.09$, Figures 7D–F). After dry-off, glucagon concentration was lower in all feed-restricted compared with control cows ($P < 0.05$).

The molar insulin:glucagon ratio was not affected by diet and feed restriction before dry-off, but increased after dry-off in all groups ($P < 0.05$; data not shown). Feed-restricted animals of all 3 dietary treatments showed a lower insulin:glucagon ratio compared with control cows after dry-off until the end of trial ($P < 0.05$). Plasma IGF-1 concentrations were not affected by dietary types, but decreased after the start of feed restriction in restricted glucogenic animals ($P < 0.05$), and tended to decrease in feed-restricted nitrogenic ($P = 0.09$) and lipogenic cows ($P = 0.08$; Figures 8A–C). Plasma IGF-1 concentrations were lower in the restricted compared with the nonrestricted lipogenic group during feed restriction before dry-off ($P < 0.05$). After dry-off, IGF-1 concentrations increased in all cows to greater concentrations when compared with the levels observed during the lactation period without any dif-

ferences between feed restriction and control groups ($P < 0.05$; Figures 8A–C).

Blood Cells and Immunologic Tests

The number of total leukocytes in blood was not systematically influenced by dietary treatments or dry-off [8.15 (nitrogenic), 7.91 (glucogenic), 7.86 (lipogenic) ± 0.27 × 10^9/L; LSM ± SEM; $P > 0.05$]. The number of neutrophils in blood was not affected by dietary treatments or dry-off, except for a decrease in the glucogenic control group from d −4 until d +3 relative to dry-off (2.93 vs. 2.42 ± 0.16 × 10^9/L; LSM ± SEM; $P < 0.05$).

The chemotactic activity of the leukocytes was not affected by the experimental diets and did not change during feed restriction or the transition from lactation to nonlactation after dry-off (nitrogenic control: 0.15, nitrogenic restricted: 0.17, glucogenic control: 0.21, glucogenic restricted: 0.12, lipogenic control: 0.24, lipogenic restricted: 0.11 ± 0.15 relative fluorescent units; LSM ± SEM; difference between test and controls, $\log_2$).

The phagocytic activity of the leukocytes was not affected by feed restriction or dry-off, except for an increase on d 6 in both nitrogenic groups (+2.29 and +2.88 ± 1.17; mean fluorescence intensity; LSM ± SEM; $P < 0.05$). The phagocytic activity (mean fluorescence intensity) per neutrophil did not change throughout the experiment in all control (327.4) and the restricted lipogenic group (355.8), whereas there was an increase on d 6 in the restricted nitrogenic and glucogenic group (496.4 and 624.2 ± 121.3; LSM ± SEM; $P < 0.05$). The phagocytic activity and phagocytic activity per neutrophil were higher in control animals than feed-restricted ones on d 3 after dry-off in the nitrogenic groups ($P < 0.05$).

Gene Expression in Leukocytes

The mRNA abundance encoding for the glucose transporter (GLUT) 1 (coded by *SLC2A1*) was not affected by dietary treatments or dry-off, except for an increase after dry-off in the restricted nitrogenic group ($P < 0.05$). Compared with the respective control groups, gene expression of GLUT1 was higher after dry-off in restricted animals fed the nitrogenic (6.99 ± 0.15 vs. 6.30 ± 0.20; delta Ct, $\log_2$; $P < 0.05$) and tended to be higher in cows fed the glucogenic concentrate (6.94 ± 0.23 vs. 6.47 ± 0.22; delta Ct, $\log_2$; $P = 0.07$). The mRNA abundance of GLUT3 (coded by *SLC2A3*) and TNF did not change during the experiment (data not shown). Likewise, the mRNA abundance of heat shock protein family A (HSP70; coded by *HSPA1A*) did not change during the experiment, except for restricted lipogenic animals showing a lower HSP70 expression compared with controls on d −1 relative to dry-off (6.25 ± 0.06 vs. 6.98 ± 0.16; delta Ct, $\log_2$; $P < 0.05$). The mRNA abundance of GLUT4 (coded by *SLC2A4*) in blood leukocytes could not be detected. This finding was confirmed by a positive control with mammary epithelial cells expressing GLUT4 that was running in parallel to leukocyte samples on the same PCR plate.

DISCUSSION

The interaction between metabolic status and immune function is well recognized. However, both surplus and deficiency of energy and nutrients may also be detrimental for metabolism and consequently immune function. In the present study, we investigated how different dietary nutrient compositions and feeding levels affect performance, metabolic status, and factors related to the immune system around dry-off in dairy cows.

Effect of Dietary Treatments, Feed Restriction, and Dry-Off on Performance Parameters

Milk yield in dairy cows markedly increased during the last decades and nowadays cows are dried-off while still producing high amounts of milk. Feed restriction is one approach to reduce milk production and therefore to facilitate the dry-off process (Ollier et al., 2014). A recent meta study investigating studies with different intensity levels of feed restrictions carried out during different stages of lactation revealed that milk yield significantly decreased in 41 out of 44 studies (Leduc et al., 2021). Despite the immediate decline in milk production and the short duration of feed restriction until the cessation of milking in the present study (approximately 30% after 3 d of feed restriction), the calculated EB remained negative until dry-off, where it turned positive again. A reduced milk yield due to feed restriction can be associated with reduced arterial glucose provision to the mammary gland due to reduced plasma glucose concentrations, reduced arterial mammary blood flow, and a reduced mammary glucose uptake (Guinard-Flament et al., 2006, 2007). Feed restriction not only limits the energy supply, but also the availability of protein for the synthesis of milk and milk components. Thus, in early-lactating cows, AA supply was associated with milk yield (Larsen et al., 2015). To our knowledge, there are no data on the AA turnover in the mammary gland around dry-off. However, we speculate that the deliberately induced negative energy and nutrient balance before dry-off

also contributed to a reduced availability of AA and consequently to the lower milk yield. Although milk fat content increased during feed restriction, the total amount of secreted milk fat decreased. Hence, skim milk production relatively decreased to a greater extent than milk fat production. In line with the lower metabolic priority of the mammary gland at this stage of lactation, the adjustment of milk production contributed to the maintenance of glucose homeostasis. Therefore, the availability of glucose was not limiting for milk fat production, which was further supported by increased plasma NEFA due to enhanced lipolysis (Pullen et al., 1989; Gross et al., 2021a; Leduc et al., 2021). Milk protein and lactose contents did not show any major changes throughout our experiment, even though some studies showed partial alterations after an induced feed restriction depending on its severity, duration, and the lactational stage (Kvidera et al., 2017; Leduc et al., 2021). In terms of the relatively low milk yield and high concentrate amounts fed before the start of feed restriction, we assume that the intensity of the realized feed restriction and its short duration in our experiment might have been not severe enough to provoke changes in all milk components. In agreement with a recently conducted study in mid-lactation dairy cows using the same dietary concentrate types (Gross et al., 2021b), milk and blood urea concentrations increased with increasing supplementation of the nitrogenic concentrate. A urea surplus occurs when rumen microorganisms are exposed to high dietary CP intake relative to the concomitant energy supply (Hof et al., 1997; Nousiainen et al., 2004; Bach et al., 2005). Our nitrogenic treatment implied an elevated CP supply above the actual requirements that resulted in elevated milk and plasma urea concentrations. Our observations on the elevated urea levels during feed restriction in the glucogenic and lipogenic groups confirm earlier reports where feed allowance was restricted (Pires et al., 2019). In plasma, urea concentrations declined again after dry-off when a positive EB was established. However, the control group receiving the full amount of the nitrogenic diet still showed greater urea concentrations in plasma after dry-off, as more CP was provided compared with the restricted group. Albeit milk electrolytes did not consistently change in response to dietary treatments and feed restriction, potassium concentrations declined during the restriction phase, in agreement with Ollier et al. (2014). It seems that electrolytes, especially potassium, decreased and were substituted by other osmolaric active components in milk such as fat or urea because an inverse correlation between milk electrolytes and major organic constituents has been shown (Oshima and Fuse, 1977).

Effect of Dietary Treatments, Feed Restriction, and Dry-Off on Metabolic and Endocrine Factors in Plasma

Feeding lipogenic diets may lead to elevated glucose concentrations in plasma because glucose can be spared by increased dietary fat intake (Blum et al., 1985). In mid-lactating cows, glucose concentration in plasma was not altered by nitrogenic, glucogenic, or lipogenic concentrate supplementation (Gross et al., 2021b). Whereas our results are in agreement with observations from Lapierre et al. (1995) and Carlson et al. (2006), other researchers reported a decrease of plasma glucose concentration in feed-restricted animals (Nielsen et al., 2003; Kay et al., 2013). We assume that greater glycogen reserves and a greater gluconeogenic rate at later lactational stages are more efficiently compensating for disturbances of glucose homeostasis, whereas in early lactation homeorhetic processes enhance nutrient partitioning primarily in favor of the mammary gland, resulting in low glucose concentrations (Gross and Bruckmaier, 2019). Nevertheless, feed restriction reduces the amount of glucose that is taken up by the mammary gland, which explains the reduced milk yield in restricted animals (Guinard-Flament et al., 2006; Boutinaud et al., 2008). Glucose concentrations in the present study increased after the abrupt dry-off and were maintained at an elevated level during the following days. This finding supposes that metabolic and endocrine adaptations would allow continued milk synthesis during the first days after dry-off despite cessation of milking. Obviously, the rate of gluconeogenesis still oriented toward the needs of lactation. Consequently, the abrupt dry-off resulted in a short-term surplus of glucose as well as of triglycerides. The differences in plasma glucose concentration between restricted and nonrestricted cows after dry-off reflect the supply with glucose and glucogenic precursors provided by the different feeding levels that were continued until the end of the experiment.

In agreement with several other studies (Ferraretto et al., 2014; Kvidera et al., 2017), feed restriction led to a short-term decrease in insulin in the restricted groups before dry-off. Insulin is known to inhibit lipolysis (Gordon and Cherkes, 1958; Hayirli, 2006). Decreasing insulin concentrations enable lipolysis, which was confirmed by increased NEFA concentrations during feed restriction in our study. Insulin concentration followed the pattern of increased glucose levels after dry-off, particularly when abundant glucose is available (Yang et al., 1984), suggesting the initiation of counter-regulation for re-establishing of glucose homeostasis. Similar to insulin, IGF-1 concentration in plasma decreased during feed restriction before dry-off, which is

in agreement with observations of Herve et al. (2019). Concentrations of IGF-1 are indicative for nutrient partitioning and closely related to the EB (Ronge et al., 1988; Spicer et al., 1990; Lucy et al., 2001). After dry-off, both insulin and IGF-1 concentrations increased as the cow's EB improved. This finding is further supported by the fact that insulin levels were higher in nonrestricted compared with restricted animals. In contrast to insulin, IGF-1 concentrations did not differ between nonrestricted and restricted animals after dry-off. It seems that IGF-1 in supporting lactation is more important than the regulation of the concomitant nutritional status. In contrast to recent results with similar diets fed in mid-lactating cows (Gross et al., 2021b), feeding of the nitrogenic concentrate did not stimulate glucagon secretion. Although we expected an increase of plasma glucagon concentrations during feed restriction, we observed a decrease in glucagon concentration as shown earlier by Vicini et al. (1988). Glucose homeostasis could be maintained during feed restriction by reducing milk production. These observations lead to the speculation that the lower glucagon concentrations are indicating a lower gluconeogenesis during feed restriction before dry-off. Moreover, the elevated NEFA concentrations in restricted animals may have had an inhibitory effect on glucagon secretion (Madison et al., 1968). Furthermore, the improved body condition could be involved in altering the regulation of glucagon secretion as shown in obese mice (Stern et al., 2019). In terms of the advanced stages of lactation and pregnancy, Canniff et al. (2006) observed a decrease in glucagon secretion that could facilitate anabolic metabolic regulation to support fetal growth in cases of maternal malnutrition.

As expected and shown earlier by Ollier et al. (2014), feed restriction before dry-off resulted in an elevation of NEFA concentrations indicating lipolysis of adipose tissue. Concentrations of BHB in plasma did not increase after feed restriction in our experiment. This is in agreement with Bjerre-Harpøth et al. (2012), who observed an increase of BHB during feed restriction during early lactation but not in mid and late lactation. Furthermore, the hepatic oxidation of NEFA is obviously more capable at later lactational stages (Grum et al., 2002). Nonesterified fatty acids can be oxidized in the liver, exported to the circulation via very low-density lipoproteins, or stored as triglycerides in the liver (Grummer, 1993). Neither feed restriction nor dietary concentrate type affected plasma triglyceride concentrations in our study, although we expected an effect of the lipogenic diet on fat metabolism. However, triglyceride concentrations increased transiently after dry-off in all of the experimental groups, which can be explained by ceased mammary gland absorption of triglycerides for milk fat synthesis (Barry et al., 1963; Crociati et al., 2017). Thus, the homeostatic adaptation of metabolism lags behind the termination of the mammary secretory activity. An earlier study showed that cholesterol concentrations increased after feed restriction in mid-lactating cows (Gross et al., 2015a), which is in contrast to our results in late-lactating cows. The overall lower cholesterol concentrations in dry cows are thought to be related to the needs for steroid hormone synthesis and the fetus (Pysera and Opalka, 2000; Tucker et al., 2009). However, the present study does not allow us to differentiate between cholesterol fractions derived from hepatic export or peripheral transport back to the liver.

Effect of Dietary Treatments, Feed Restriction, and Dry-Off on Factors Related to the Immune System

Elevated concentrations of NEFA, BHB, and urea have previously been associated with impaired functions of leukocytes (Suriyasathaporn et al., 1999; Ster et al., 2012; Kowsar et al., 2016). Inflammatory conditions of leukocytes were preferentially observed at the time of dry-off, probably because of the leukocytes' involvement in the involution of the mammary gland (Mezzetti et al., 2020). Although TNF and HSP70 have important roles in the inflammatory response (Beutler and Cerami, 1989; Jacquier-Sarlin et al., 1994), their mRNA expressions were not affected by our experimental treatments. Gene expression of HSP70 was shown to be upregulated in leukocytes of early-lactating cows with an unfavorable metabolic profile (Wathes et al., 2021). As all cows enrolled in the present study were in healthy condition and had a low milk SCC before dry-off, the presented immune parameters were expressed at a basal level.

Increased glucose utilization is related to the activation of immune cells, partly facilitated by elevated expression of glucose transporters. However, alterations in glucose concentrations were not followed by changes in the investigated immune parameters and glucose transporters. The mRNA abundance encoding for GLUT1, GLUT3, and GLUT4 was described in bovine monocytes, and GLUT3 was also expressed in bovine polymorphnuclear leukocytes, whereas GLUT1 was minimally expressed (O'Boyle et al., 2012; Garcia et al., 2015). Although no significant changes occurred, we could detect the mRNA of GLUT1 and GLUT3 in leukocytes, but not of GLUT4. Eger et al. (2016) found no effect of insulin on glucose uptake of bovine monocytes or macrophages. This finding highlights the importance of an insulin-independent glucose uptake to ensure monocyte function even during periods of glucose shortages (e.g., the peripartum period), where

insulin resistance inhibits a major glucose uptake of peripheral tissues (Bauman, 2000; Eger et al., 2016). Although GLUT1 is insulin-independent, the responsiveness of GLUT3 to insulin of human leukocytes was dependent on the cell type and activation state (Maratou et al., 2007). Despite the increase of plasma insulin concentration in our study after dry-off, GLUT3 gene expression did not change. However, our experimental diets, feed restriction, and dry-off did not provoke an inflammatory status. Despite lacking effects on the mRNA expression of different factors, we cannot draw conclusions on the protein and cell surface expression of glucose transporters or the glucose consumption of leukocytes, which would finally affect their immune competence.

CONCLUSIONS

The different dietary concentrate types rich in CP, carbohydrates, or lipids had only a minor effect on the investigated metabolic and endocrine factors. The induction of a NEB by feed restriction led to an immediate decline in milk production, accompanied by elevated plasma NEFA and lowered insulin and IGF-1 concentrations. However, the short duration of the NEB before dry-off and the overall moderate performance level did not severely disturb metabolic homeostasis. Even though feed restriction lasted for another 6 d after dry-off, metabolic status improved after dry-off. Metabolic and endocrine adaptations supporting milk synthesis were continued during the first days after dry-off despite cessation of milking. The abrupt dry-off resulted in a short-term increase of plasma glucose and triglyceride concentrations, indicating a delayed endocrine response to re-establish nutrient homeostasis in blood. The overall good health status throughout the experiment could be confirmed by the lacking response of various factors related to the immune system, as no specific activation of the immune system beyond its basal status occurred. However, further research is warranted in terms of the potential of dietary composition supporting the immune system during high-risk periods such as dry-off.

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