Examining the catching, carrying, and crating process during depopulation of end-of-lay hens

C. Gerpe,*[,1] A. Stratmann,* R. Bruckmaier,† and M. J. Toscano*

*Center for Proper Housing: Poultry and Rabbits (ZTHZ) Division of Animal Welfare, Vetsuisse Faculty, University of Bern, 3052 Zollikofen, Switzerland; and †Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern Switzerland

Primary Audience: Producers, Poultry Product Manufacturing Industry, Researchers

SUMMARY

Laying hen flocks undergo depopulation at end of lay, a difficult process that has been associated with injuries and is considered stressful to hens and human collectors. To date, most assessments of depopulations have been conducted within cage housing systems, layers, or noncage broilers, thus offering little relevance to noncage laying hen systems including aviaries. Given that the predominant housing systems in Switzerland are multitier aviaries and their growing popularity in general, our study was undertaken to establish a baseline for hen injuries and stress as well as the experience of human workers during depopulation. For the effort, a total of 15 individual farms were visited, and a battery of assessments was made on a total of 603 individual end-of-lay hens. In addition, potentially influential factors such as time of day, handling duration, or time into depopulation were recorded and analyzed. The results suggest that approximately 8.1% of hens sustain severe injuries (i.e., fractures and muscle damage) or exhibit a considerable stress reaction (i.e., increased corticosterone levels), whereas 90% of laying hens are only mildly affected.

Key words: depopulation, laying hen, animal welfare, fracture, stress, fearfulness

2021 J. Appl. Poult. Res. 30:100115
https://doi.org/10.1016/j.japr.2020.100115

DESCRIPTION OF PROBLEM

Laying hen flocks are depopulated typically between 65 and 90 wk of age. Despite limited reports of injuries such as fractures or increased stress levels during depopulation (Gregory and Wilkins, 1989; Gregory et al., 1990, 1992; Sandilands, 2011), the main cause, type, and frequency of injury and stress are relatively unknown and particularly acute for aviary systems.

In extreme cases, up to 24% of end-of-lay hens have been reported to sustain a fracture during the catching, carrying, and carting process (Gregory and Wilkins, 1989), with the type of the housing system likely having a large role in the amount and severity of injuries (Knowles and Wilkins, 1998; Whitehead and Fleming, 2000; Webster, 2004). In addition to bone injuries, damage to muscles is also likely as hens have collisions with pen furnishings, crates, or in response to being caught and held by workers. Hens are also likely to experience increased stress and fear during depopulation (Beuving and vonder, 1978; Freeman, 1982; Knowles, 1994; Sandilands et al., 2007; Herborn et al., 2015). Laying hens within commercial systems are unaccustomed to being handled, and even short or

[1]Corresponding author: Christopher-gerpe@gmx.ch

gentle handling treatments can evoke a stress and fear response (Scott and Moran, 1993; Edgar et al., 2013). Studies that assess stress responses during the depopulation procedure have only assessed corticosterone concentrations (Knowles et al., 1993; Sandilands et al., 2007) and primarily within cage systems.

The catching process and the subsequent conveyance to the crates are arguably the most detrimental steps of depopulation (Knowles and Wilkins, 1998), with other stages including loading onto the transport truck, transporting itself, offloading, and slaughter.

During commercial depopulations, hens are caught by one leg and bundled together in groups of 3–5 individuals and then handed over to people who carry them upside down by their legs to the crating station. The method of handling hens is likely detrimental. Catching hens by 2 legs (opposed to one) has been shown to result in reduced fracture rates (Gregory et al., 1992; Knowles, 1994), and carrying hens upright (vs. upside down) reduced stress (Broom, 1990). A better understanding of the different insults to welfare as a result of the depopulation process and further information on the potential causes are necessary to effectively improve animal welfare. The main objective of the current project was to benchmark the type and frequency of injury, consider their potential causes, and identify areas of improvement.

We aimed for a comprehensive approach to assess welfare of hens during depopulation that included the assessment of injuries, stress, and fearfulness to gain a better understanding of potential aversive effects of depopulation in aviary systems. Our efforts were guided by predictions that differences would exist between hens examined before (baseline) and after depopulation in terms of muscle injuries, stress, and fearfulness. We also predicted fresh skeletal injuries would be identified in depopulated hens. Variation in these responses would relate to factors such as the aviary system, worker experience, duration of handling, time into the depopulation process, lighting, and handling methodology. We used plasma creatine kinase concentration as a marker for muscle damage (Bollinger et al., 1989; Dabbert and Powell, 1993). Examined stress-related parameters were as follows: respiration rate, comb and cloacal temperature, and plasma corticosterone concentrations as well as tonic immobility (**TI**) tests to measure hens' fear levels. Hematocrit was assessed to gain a better understanding of the general health state of hens (Scanes, 2016; Pantaya and Utami, 2018). Elevated hematocrit values can indicate dehydration, whereas low values can result from chronic anemia (Fair et al., 2007). The capacity to respond toward a stressor such as depopulation may be altered by such factors. We also collected information on the barn infrastructure, flock, planning by the producer for depopulation, and animal handlers and their experience.

MATERIALS AND METHODS

Ethical Statement

Data collection on all visited farms was approved by the Veterinary Office of the Canton of Berne, Switzerland (cantonal license number: BE 89/16), as well as by the Canton of Solothurn, Aargau, Thurgau, St. Gallen, and Luzern. All corresponding ethical guidelines were followed. The study was conducted in commercial farms; thus, management and criteria for euthanizing animals was the responsibility of the producers.

Animals and Experimental Design

In total, 15 individual farms were visited. The depopulation on these farms was always limited to a single barn and was performed by the producers' employees, family, friends, and neighbors as there are no professional catching crews in Switzerland. Flock sizes ranged from 2,040 to 13,540 individuals. Overall, 603 hens were examined, ranging from 72 to 85 wk of age, and were of various hybrids (Table 1). Hens were kept in multitier aviary systems, except for one single-tier system. Nine different systems from 5 manufacturers were assessed including Natura (Big Dutchman, Vechta, Germany), Natura Bio (Big Dutchman, Vechta, Germany), Alterna 1750 (Farmer Automatic GmbH & Co. KG, Laer, Germany), Alterna 2000 (Farmer Automatic GmbH & Co. KG, Laer, Germany), Volito Voletage (Volito, VeenenDaal, The Netherlands), Bolegg Terrace (regular and Swiss version) (Vencomatic, Eersel, The Netherlands), and Glovital (Glovital AG, Arbon, Switzerland).

Table 1. Information regarding flock size, hybrid, hens caught per minute indicating depopulation speed, and collisions per farm.

Farm	Flock size	Hybrid	Animals caught per minute	Passive collisions per minute	Active collisions per minute
1	2910	Brown Nick (brown hybrid)	19.7	1.2	1.1
2	13540	Nick Chick (white Hybrid)	17	1.6	0.4
3	4000	Lohmann Selected Leghorn (white hybrid)	8.6	0.8	0.7
4	3700	Lohmann Selected Leghorn (white hybrid)	17.5	0.5	0.7
5	9000	Lohmann Selected Leghorn (white hybrid)	13.5	1.1	0.5
6	8400	Lohmann Selected Leghorn (white hybrid)	NA	NA	NA
7	3060	Brown Nick (brown hybrid)	NA	NA	NA
8	5200	Lohmann Brown (brown hybrid)	NA	NA	NA
9	5365	Lohmann Brown (brown hybrid)	12.8	0.7	1.1
10	2040	Lohmann Brown (brown hybrid)	12.4	0.7	1.3
11	6500	Lohmann Selected Leghorn (white hybrid)	19.2	0.3	1
12	6000	Lohmann Selected Leghorn (white hybrid)	9.5	1.2	1.7
13	5970	Super Nick (white hybrid)	16	0.9	0.8
14	9735	Nick Chick (white hybrid) and Super Nick (white hybrid)	10.4	1.1	0.8
15	7000	Nick Chick (white hybrid) and Lohmann Selected Leghorn (white hybrid)	10.5	0.3	1.4

Passive collisions include incidences of wing flapping or head movements that resulted in a contact with the pen furniture. Active collisions include incidences during which the catcher actively caused a collision of any part of the hens' body with the pen furniture. Per minute averages were calculated from 15 min of observations. On 3 farms (6–8), either we were asked by the producer not to video record the depopulation or none of the catchers volunteered to wear the head mount with the camera. The missing data on animals caught and passive/active collisions per minute are indicated by the term "NA."

For each farm, 2 sets of collections were performed: one before (i.e., baseline) and a second during the depopulation. To reduce handling, hens were divided into 2 subgroups (for both baseline and depopulation) that were examined simultaneously. In one subgroup (N = 313 hens; 131, baseline and 182, depopulation), respiration rate and TI were measured. In the second subgroup (N = 290 hens; 124, baseline and 166, depopulation), comb temperature, cloacal temperature, and blood samples were taken in that order. For each subgroup (4–17), hens were examined during baseline and depopulation per farm (Tables 2 and 3).

Baseline examinations were performed approximately 2 h before depopulation and ended after examining 10 individuals, resulting in 10 entries for each parameter before depopulation began. It was not always possible to examine 10 individuals as some depopulations started ahead of schedule. We considered performing baseline collections 24 h or 48 h before depopulation (i.e., the same time of the day) but decided against it as factors associated with farm preparations for the depopulation (e.g., fasting, removal of litter) could confound the comparison. Baseline hens were collected in a stratified manner from various locations (corridors, aviary blocks, and different perch heights) within the barn. Hens were caught individually using both hands and carried upright, holding them by their body with the wings pressed against the body to prevent wing flapping. Data collection began within 30 s of catching the hens. Examined hens were released before catching the next individual for examination.

During depopulation, hens were collected and examined throughout the entire process. Hens were collected from crates immediately after they were placed into crates at the packing location but before loading onto the transport truck. If more than one packing location was present, hens were gathered from the different stations in alternating fashion. In contrast to baseline collections, assessment of temperature and blood collection during depopulation started after hens were in the crates for 12 to 30 min, a time frame based on observations of these measures reaching their peak (Beuving and vonder, 1978; Voslarova et al., 2008; Edgar et al., 2013;

Table 2. List of the treatment means with the SE, the number of observation, and the p-value obtained from the analysis for each of the investigated parameters.

Measure (unit)	Baseline mean	Depopulation mean	Overall SE	Total no. of observations	p-value
Corticosterone (ng/mL)	3.972	4.333	0.177	273	0.041
Respiration rate (chest movement per min)	36.343	41.123	0.153	298	<0.001
Temperature cloaca (°C)	35.81	36.28	0.09	257	<0.001
Temperature comb (°C)	32.465	29.06	0.272	257	<0.001
LAT1 (s)	76.557	78.34	5.154	243	0.81
LATSU (s)	107.489	122.826	7.57	243	0.27
Creatine kinase (U/L)	1263.73	1322.007	99.563	252	0.019

The number of observations listed does not always match the number provided in the Material and methods section. Owing to technical issues such as low quality of thermal images or the contamination of blood samples, some individuals were excluded from the analysis.

Herborn et al., 2015). Respiration rate and TI tests were taken within a window of 30 min that began immediately after newly crated hens were collected from the crating stations, which served as the next individuals to be assessed.

Hens examined during depopulation were euthanized on farm using a lethal injection of pentobarbital (Esconarkon, 1.5 mL/kg; IP; Streuli Pharma AG, Uznach, Switzerland) and cervical dislocation if needed. Cervical dislocation was used in case hens were still breathing after coronal reflexes were determined to have ceased in response to stimuli. Dislocation was only necessary in very few instances (<10). Euthanized hens were transported to our laboratory facility where, if necessary, they were frozen at −35°C to allow radiograph imaging at a later date.

At the conclusion of the depopulation, each producer was asked to fill out a questionnaire regarding basic information concerning the farm. Collected data included information about the flock (evidence of disease or illness within the current flock, mortality rates, and laying performance), barn infrastructure (type of aviary system [i.e., model], barn length, and barn

Table 3. Overview of the number of hens examined per farm, measure, and treatment.

Farm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Baseline/depopulation																
Respiration rate																
Baseline	4	9	11	10	9	10	7	5	9	6	11	11	9	11	9	131
Depopualtion	11	10	10	10	12	12	10	11	12	10	10	16	15	17	16	182
Temerpatures																
Baseline	2	7	9	10	10	9	6	5	8	5	11	10	10	11	10	123
Depopualtion	5	5	5	11	13	13	10	10	13	8	11	14	11	15	12	156
TI latencies																
Baseline	2	8	8	9	8	4	4	5	4	2	4	5	8	9	8	88
Depopualtion	6	8	8	9	10	9	9	6	7	8	8	14	14	15	13	144
Corticosterone																
Baseline	2	7	11	9	10	9	6	5	8	5	11	10	9	10	6	118
Depopualtion	6	6	3	12	13	13	10	10	13	9	11	14	12	14	9	155
Creatine kinase																
Baseline	2	7	11	9	10	9	6	5	8	5	11	10	9	10	10	122
Depopualtion	6	6	3	12	13	13	9	10	13	9	11	12	12	14	9	152
TI inductions																
Baseline	4	9	11	10	9	10	7	5	9	6	11	11	9	11	9	131
Depopualtion	11	10	10	10	12	12	10	11	12	10	10	16	15	17	16	182

The last column "total" summarizes the total sample size per measure and treatment. As multiple measures were recorded, the sum of the "total" column does not match the actual number of examined individuals which is provided in the Material and methods sections.

width), and the depopulation procedure itself (estimated depopulation duration, number of workers recruited, and recommendations given to the workforce, e.g., number of hens per bundle, catching hens by one or 2 legs, and so on). Catchers were asked to complete a separate questionnaire to evaluate their formal qualifications in regard to poultry and handling of animals, experience regarding the depopulation procedure, and information on their physical condition.

Blood Collection and Processing

For blood collection, hens were restrained by one person, while another drew approximately 3 mL of blood from a wing vein using a 5-mL syringe with a sterile needle (23 G × 1 0.6 × 25 mm AGANI; TERUMO, Tokyo, Japan). Most blood samples were collected within 5 min (73% of all samples) and transferred to tubes containing EDTA (product code: 49.355.001; Sarstedt, Nümbrecht, Germany). Hematocrit was measured from whole blood, which was extracted from the syringe by a capillary tube (Mikro-Hämatokrit-Kappilaren Brand, Huberlab product code: 3.3531.10; Aesch, Switzerla) and immediately centrifuged for 10 min using a hematocrit centrifuge (2011; Hettich, Switzerland). Hematocrit values were calculated immediately after centrifuging by assessing the proportion of the erythrocyte column height in relation to the total sample height using a ruler. The EDTA tubes were closed and stored in ice until processing 7–10 h later. Plasma was isolated after samples were centrifuged for 10 min at 1,000 g and divided into 6 aliquots depending on the amount of plasma available. Corticosterone was analyzed using the Corticosterone HS (High Sensitivity) EIA kit from (analytical sensitivity 0.17 ng/mL, AC-15F1; Immunodiagnostic Systems Holdings, Tyne and Wear, United Kingdom). Creatine kinase levels were assessed using a kit (AXON00005; Axon Lab AG, Baden-Dättwil, Switzerland).

Temperature

Temperature was measured at the comb using a thermal camera (C2; FLIR, Wilsonville, OR) and the cloaca using an infrared thermometer (Beurer FT70; Beurer GmbH, Ulm, Germany). The thermal camera was positioned using a custom-made mount on a tripod, 30 cm above the comb and calibrated before each visit, to account for the reflecting temperature of the surroundings. To obtain the thermal image, one person restrained the hens sideways on a table by holding both legs of the hen with one hand and the neck (or in isolated cases the beak) with the other hand. Three images were taken per hen.

The maximum temperature of the comb was determined for each image using the accompanying software program "FLIR Tools," (FLIR, Wilsonville, OR, USA) and the average of the 3 images was calculated for analysis. For cloacal temperature, the thermometer was inserted approximately 5 cm into the cloaca. Both temperature measurements took less than 60 s in total duration.

Tonic Immobility

To induce TI, the head was placed under a wing, and the hen was picked up, with one hand applying pressure on the back (dorsal) and another hand applying on the chest (ventral). Hens were then inverted, slowly raised, and lowered 3 times in a steady motion before being released on the ground. Induction was successful if hens remained motionless for up to 10 s; otherwise, the procedure was repeated up to a total of 3 induction trials.

Hens that could not be induced were recorded as failed inductions and not included in the analysis of latencies. If successful, we recorded the number of induction trials, the latencies to the first head movement and for the hen to stand up. The latency to the first head movement (**LAT1**) started when the hens were placed on the ground after the induction procedure and ended as soon as a head motion was detected or the 5-min mark was reached. Individuals that remained motionless for 5 min after induction were excluded from the analysis of the latency to stand up. The latency to stand up (**LATSU**) is the sum of the latency to the first head movement and the time until the hen stood up or the maximum score of 300 s (5 min).

Respiration Rate

Respiration was assessed by counting chest movements for 20 s and extrapolated to determine

respiration rate per minute. One complete up-and-down movement equaled a count of one with the possibility of half counts. In depopulated hens, respiration rate was assessed immediately after removing hens from crates.

Video Recordings

The catching process was recorded using video cameras with infrared lenses (Panasonic HX-A1 M; Panasonic, Osaka, Japan) combined with an infrared light (IR-torch C16, Nitecore; SYSMAX Innovations Co. Ltd., Guangzhou, Guangdong, China). Both devices were fixed on the head of the catchers either via a head mount (Panasonic VW-HMA1; Panasonic, Osaka, Japan) or an elastic band. Video analysis of the video recordings were made of 3, continuous 5-min blocks in the middle of each of the three-thirds of the depopulation procedure, that is, a total of 15 min of recorded video.

The collected video was examined by one person to quantify the number of hens handled per minute by the catcher and the number of active (wherein the animal collides with the pen furniture as a direct result of the catching process) and passive (wherein actions from the hens such as wing flapping or movements of the head result in collisions) collisions. On farms where multiple catchers volunteered to wear head cameras (n = 6 farms), an average of the mentioned variables of interest was calculated to create farm-level values that were then used for analysis. Collection of video footage for 4 farms was not possible owing to several reasons, including lack of volunteers, cameras falling off, or the catcher removing the camera before reaching the final third of the depopulation.

Radiograph Imaging

Selection of examined bones was based on the available literature (Gregory and Wilkins, 1989; Gregory et al., 1990) and our own preliminary observations of the depopulation process. Examined bones included the sternum, tibia, femur, pubis, ischium, pygostyle, furcula, ulna, radius, humerus, metacarpus, and phalanges.

Depopulated hens that were designated to be radiographed were killed on farm and stored at the University of Bern's Radiology Department at −35 °C unless imaging could be conducted the next day, in which case hens remained at room temperature. Frozen hens were defrosted approximately 36 h before examination and then placed on a freestanding table. The mobile X-ray (GIERTH TR 90/30; GIERTH X-RAY int. GmbH, Riesa, Germany) was placed on the table opposite the hen, to allow a quick exchange of individuals after images were taken. The operating voltage ranged from 52 to 56 kV, and exposure time was set between 0.1 s and 0.3 s, depending on the size of the animal and the quality of the image obtained. The protocol was adapted from previous works (Clark et al., 2008; Richards et al., 2011). Images were made from 2 different angles, one with the hens placed laterally at 90° and a second with the hens placed ventrodorsal, taking the image in the cranial direction at 45°. Images were taken of all individuals before changing the setting to the second angle.

Fractures were categorized as fresh when there were no signs of callus formation and a sharp line was present indicating a break. Fractures that occurred within a few days before are therefore still likely to fall into this category and lead to an overestimate of fracture prevalence caused by depopulation. Radiographs of baseline animals were not taken owing to limitations in the number of animals that could be transported to our facilities after depopulation. Owing to the low incidence of fractures, some bones were grouped for statistical analysis. Fractures of the humerus and the tibia were combined as "leg fractures" and the humerus, ulna, radius, metacarpus, and phalanges were combined in the category "wing fracture." Only the presence or absence of fractures in the examined bones was evaluated and not the severity or type of fracture.

Other Recorded Variables

First, corridor lengths were taken from the producer questionnaires, and the distance was used as a proxy for handling time. Second, we wrote down the exact time before and after the examination of each hen. For the analysis, we took the first time point for each hen and assigned it to one of 3 blocks: evening (until 23:00), middle of the night (until 02:00), and

early morning (until 06:00). This categorical variable with 3 levels is referred to as time of day and was chosen owing to concerns of farmers that depopulation too early in the evening and or in the morning is more stressful for the hens. On average, depopulations lasted approximately 1 h and 40 min (mean ± SE = 102.20 ± 24.09 min), with 5 spanning across the blocks of evening and middle of the night and from the middle of the night to early morning. Depopulation of other farms either took place only in the evening (2), the middle of the night (1), or the early morning (2). Finally, the duration for each depopulation was divided into thirds (beginning, middle, and end) to see whether injury rates and stress measures remained constant over the course of the depopulation.

Statistical Analysis

All analyses were carried out using R (Version 2.01, R Core Team, 2018, Vienna, Austria). Except for the number of inductions during TI testing, which was considered a categorical response and analyzed using multinomial logistic regression (packages: nnet [Venables and Ripley, 2002], reshape2 [H, 2007], and ggplot2 [Bates et al., 2015]), all other response variables were classified as continuous and analyzed using linear mixed-effect models (package lme4 [Wickham, 2016]).

If visual assessment of model residuals did not follow a normal distribution, raw data were transformed either by exponentiating to the power of 3, applying natural log or natural log base 10. Model selection was conducted following the stepwise backward approach using ANOVA with a *P*-value >0.05 as an exclusion criterion (Nathaniel, 2016). If all independent variables were insignificant, the overall means and standard errors of the raw data are presented.

To compare baseline and depopulated hens, we first ran a linear mixed-effect model for each of the dependent variables (respiration rate, cloacal and comb temperature [in one model], corticosterone, creatine kinase, TI latency to first head movement [LAT1], and TI latency to upright [LATSU]), with the independent variable "treatment" (levels: baseline and depopulation). Farm was included as a random factor (dependent variable ~ treatment, random = Farm). The temperature model included location as a factor with 2 levels (comb and cloaca). Environmental temperature was also included as a farm-level covariate as it was suspected to affect the comb temperature.

To examine the effects of the independent variables, barn length (continuous: in m), time of day (categorical: until 23:00, 02:00, and 06:00), and time point of depopulation (categorical: beginning, middle, end), a second set of models was run using data from the depopulation only (i.e., excluding baseline animals). The models included the same dependent variables as previously mentioned, with the addition of collisions per minute that were assessed using the video recordings of the catchers' head cameras. Barn length was not expected to influence the number of collisions; therefore, the model did not include barn length. Farm was always included as a random factor. *Post hoc* analysis using Tukey (package multicomp [Hothorn et al., 2008]) was used to explore the relationship of the different factor levels of statistically significant independent variables.

The number of inductions for TI was analyzed using a multinomial logistic regression, calculating the likelihood of hens to require 1–3 induction attempts or fail the induction, and followed the same two-model approach as described previously.

RESULTS AND DISCUSSION

Observed Injuries and Potential Causes

Approximately 8.1% of hens (n = 26) exhibited skeletal injuries such as bone damage or dislocated joints that appeared to have resulted from depopulation (Table 4). We anticipated that collisions with pen furniture during initial hen catching would be a major source of fractures. Various elements such as feeders, drinkers, or perches can get in the way when catching hens. Based on recordings from head cameras, collisions did occur, although predominantly involving the wings and not the bones, where most fractures were identified, that is, the sternum, pubis, and furcula. More so, although we did not quantify impact energy, the collisions recorded by the head cameras seemed to be relatively minor. Given the unexpected findings

Table 4. Overview of the examined bones and joints with the number of fresh fractures and the number of dislocated joints.

Bones and joints	Number of animals affected
Leg bones (tibia and femur)	2
Ischium	2
Pygostyle	3
Wing bones (humerus, radius, ulna, alula, metacarpals and phalanges)	3
Furcula	5
Pubis	7
Sternum	9
Dislocated wing	0
Dislocated leg	3

As several individuals had more than one injury, numbers cannot be summed up to calculate the total of affected hens (26 of 319).

of the head camera video in combination with the observed rates of fractured bones, it is likely that mechanisms other than collisions with the barn furnishings during the catching process are responsible for the injuries observed.

Focusing on fractures to the sternum, and given the high occurrence of fractures throughout the laying period, this bone seems to be particularly susceptible to fractures (Fleming et al., 2004; Wilkins et al., 2004; Käppeli et al., 2011) and is a welfare concern (Harlander-Matauschek et al., 2015). Although collisions throughout lay are believed to play a role, it has also been hypothesized that the cause of fractures in the laying period could be pathological ones, wherein fractures would stem from an inherent bone weakness or disease state (Webster, 2004; Toscano et al., 2020). If bones are weakened enough, fractures caused by the depopulation procedure may occur even in the absence of collisions or at least as a result of those with low energy. Alternatively, excessive force from the pectoralis minor and major involved in wing flapping (rather than collisions with the barn furnishings) applied to the sternum beyond its ultimate capacity may result in fractures independent of contact with the pen furniture.

It is unlikely that wing flapping would cause fractures of the pubis and furcula, which could also be of a pathological nature although fractures of these bones are not as widespread as in the sternum (Wilkins et al., 2004). Fractures of the pubis and furcula (as well as the sternum) might also occur during the crating process as birds can make contact with the crate frame and floor and as a result of pressure applied by workers or conspecifics. The pubis, furcula, and sternum are all particularly susceptible as these bones are relatively exposed during this part of the depopulation process. We observed directly (i.e., without video) various methodologies of placing the hens in the crates, which were typically worker or farm specific. In some instances, hens were dropped into the crates, whereas on other occasions, hens were lowered directly onto the crate floor. Some workers were observed to place the bundles of hens from each hand consecutively, whereas others placed bundles from both hands at the same time. Crating 2 bundles of hens at a time might increase the chance of contact with the lid or the crate frame and potentially result in skeletal injuries. In addition, placing the last hens in the crate sometimes required squeezing hens together, which could result in enough pressure to cause the observed injuries. Our main focus was on the catching phase of the depopulation, and thus, we conducted (only) casual observations of the crating process. Further research should consider the crating and carrying process in greater detail to better understand the source of bone injuries and potential risk factors such as quality of lighting, the aviary system, or time of day that might influence handling of hens. Although we had attempted to assess the relationship between these risk factors and injury, the low occurrence of fractures and high variation between farms did not allow a meaningful analysis.

Comparing Affected Bones and Fracture Rate With the Existing Body of the Literature

As discussed, a considerable amount of fresh fractures that could have resulted from the depopulation procedure was identified in the present study (Table 4). To expand on the potential causes of fracture that were already discussed, it is also interesting to note that their locations seem to conflict with previous reports. The most affected bones in the present study were the sternum, pubis, and furcula. In contrast, others have reported a high proportion

of injury in the legs (Gregory et al., 1992) or wings (Gregory and Wilkins, 1989; Gregory et al., 1990; Sandilands et al., 2007), whereas we observed a lower incidence of fractured legs and wings. The explanations for injuries suggested in these previous efforts are likely also valid for those in the present study in that the weak bones of end-of-lay hens are prone to break as a result of rough handling. However, no study, including the present one, was able to definitively link the various fractured bones caused by depopulation with the specific cause complicating any effort to explain the different ratios of bones affected. Genetics may be partly responsible for varying results in terms of susceptibility to fractures as genetic lines (Budgell and Silversides, 2004; Candelotto et al., 2017) or even the same line over the course of just a few generations may differ significantly (Bishop et al., 2000; Whitehead and Fleming, 2000). Variation in overall fracture rates caused by depopulation and bone-specific incidences might depend on genetic predisposition.

The type of housing system also influences bone health and consequent susceptibility to fractures, with previous studies that investigated depopulation reporting the highest incidences of fractures in caged systems (Gregory et al., 1990; Sandilands et al., 2007). Aviary systems, such as those in this study, encourage hens to move through the system in vertical and horizontal directions to reach the various resources such as feed, nest boxes, or litter (Fröhlich and Oester, 2001). Load-bearing movements, such as walking, wing flapping, and wing-assisted movements, that help to preserve cortical bone structure via bone remodeling (Wilson et al., 1993; Shipov et al., 2010) are increased in all cage-free systems compared with cage systems (Black and Hughes, 1974). As a result, hens within cage-free systems have stronger bones than those in cages (Knowles et al., 1993; Hansen, 1994; Gregory and Devine, 1999; Leyendecker et al., 2005; Regmi et al., 2016). Intensity and frequency of these load-bearing movements may still vary between cage-free systems (Nørgaard-Nielsen, 1990), which might contribute to varying fracture susceptibilities but likely to a less extent than overall cage systems. The resulting predispositions in terms of bone strength as a result of load-bearing activity within different housing systems may therefore influence injury rates during depopulation. Reported fracture rates as a result of depopulation are indeed lower in cage-free systems (Gregory et al., 1990; Sandilands et al., 2007), although these studies might be less relevant to modern hen genetics. Further exploration of favorable traits in aviaries on bone health and the effects on old as well as new fractures observed at depopulation may help to further reduce bone damage in laying hens.

Differences between housing systems would also affect the ability of workers to collect hens during depopulation and thus could contribute to different rates of bone types being fractured relative to other studies. The majority of hens in aviaries are readily accessible on the top perches although a portion of the flock will require removal from more confined spaces (within the system). Depopulation of hens from caged systems is relatively uniform but requires the removal through cage openings which bears the risk of collisions.

Finally, differences between the presented and previously reported fracture rates could be the result of methodological differences of the present and previous studies to assess bone damage, specifically X-ray and dissections. To our knowledge, with the exception possibly of the keel (Gebhardt et al., 2019), no study exists that compared fracture detectability rates of these methodologies in laying hens (Casey-Trott et al., 2015).

Evidence of Muscle Injuries

In addition to bone injuries, hens during depopulation exhibited plasma creatine kinase concentrations 5.8% higher than those at baseline ($\chi^2 = 5.53$, df = 1, $P = 0.019$), which suggested the occurrence of muscle injuries. Pulling hens by the legs, excessive wing flapping, or collisions during handling or during escape attempts may lead to muscle injuries.

The increased creatine kinase concentration during depopulation is moderate in regard to previous reports regarding different catching and handling methods in mallards (Bollinger et al., 1989; Dabbert and Powell, 1993), which suggests the injuries were not severe. When examining concentrations of individual hens, some hens had extreme values, with scores that

exceeded the average by a multiple of 10, although these extremes also occur in baseline animals. The combination of relatively extremely high values for a limited number of samples suggests only a small proportion of hens are likely to sustain severe muscle damage during depopulation, similar to the results with regard to fractures.

Stress-Related Measures and Fearfulness

Plasma corticosterone concentrations were 16% higher during depopulation (χ^2 = 4.18, df = 1, P = 0.041) than at baseline. Temperatures of the cloaca and the comb also differed between baseline and depopulation (χ^2 = 540.64, df = 4, $P < 0.001$). Cloacal temperature was +0.9 °C higher and comb temperature was -1.9 °C lower in depopulated hens. In addition, respiration rate of depopulated hens was 5 chest movements per minute higher (χ^2 = 45.92, df = 1, $P < 0.001$) than that examined during baseline. These observed differences were in line with our predictions that the depopulation procedure would elicit a stress response. The elevated levels of corticosterone in depopulated hens were indicative of the stress response through activation of the hypothalamic–pituitary–adrenal axis and secretion of corticosterone (Ellis et al., 2006; Cockrem, 2007). The stress response initiates a state of high alertness and promotes the supply of glucose and free fatty acids (Adriaan Bouwknecht et al., 2007; Chrousos, 2011). Although in agreement with the expected reaction as a result of the received handling during depopulation, the magnitude of the response was relatively low compared with previous efforts examining different handling treatments in poultry (Beuving and vonder, 1978; Knowles and Broom, 1993; Kannan and Mench, 1996; Korte et al., 1997; Herborn et al., 2015).

Hematocrit values did not indicate a response owing to depopulation. The proportion of erythrocytes that is considered normal has a relatively wide range and lies between 26 and 36% (Medway and Kare, 1959; Suchý et al., 2004; Weigle, 2007; Scanes, 2016; Pantaya and Utami, 2018). The average hematocrit value in the present study (mean ± SE: 31.8 ± 3.2%) was well within this range, indicating hens were in good condition. The relatively small change in corticosterone levels in response to depopulation in the present study might therefore be a result of the short handling times, which ranged from a few to approximately 80 s compared with the handling treatments described in published findings, which were typically much longer. The relatively small differences between baseline and depopulation concentrations could also be explained by elevated corticosterone levels in baseline hens. Although most blood samples were sampled within 5 min (73% of all samples), handling (required during baseline and depopulation collections) might have caused a measurable increase in corticosterone concentrations. Generally, it is considered that it takes approximately 3 min to detect such changes and that blood samples collected within 5 min should not greatly influence the response concentrations (Romero and Reed, 2005), although the potential influence of handling during baseline collections should still be considered. The same concern (i.e., handling required during baseline) may be made in regard to the other measures.

To provide context for the current work, comparable handling procedures include the following: manually restraining laying hens for up to 8 min or holding one or multiple birds by one leg (upside down) for a maximum of 3 min (Beuving and vonder, 1978; Knowles and Broom, 1993; Kannan and Mench, 1996; Korte et al., 1997). Corticosterone concentrations of these studies reported increases, with a minimum of 6.7% and mean of 53.6%. Additional confounding factors for corticosterone comparisons between baseline and treatment could also be diurnal differences, hybrid, or age, which are factors that are known to associate with fluctuation in corticosterone concentrations (Beuving and Vonder, 1977; Johnson and Van Tienhoven, 1981).

The observed shift of lower comb and higher cloacal temperatures is a result of reduced blood supply to peripheral areas of the body and reallocation to central areas such as the brain and heart muscle (Goldman, 1963; Caraffa-Braga et al., 1973; Yamamoto et al., 1987). The increased blood flow in these tissues ensures adequate energy and oxygen is received for stress-related physiological challenges. The

observed increase in respiration rate further supports the hypothesis that the hens were stressed owing to the depopulation procedure. The differences in baseline and depopulation temperatures were similar to values reported in hens that were subjected to gentle, short, sometimes repeated handling bouts (Cabanac and Guillemette, 2001; Edgar et al., 2013; Herborn et al., 2015). Handling during depopulation is arguably more intensive, but the lack of a response with a greater magnitude may indicate a ceiling effect.

Based on the physiological parameters that we assessed, we conclude that the depopulation resulted in an active stress response, although it was relatively modest in comparison with published findings on handling procedures that would be considered innocuous. In that sense, we believe hens are able to effectively cope with the depopulation procedure, although future research should include assessments focusing on the acute response at the actual point of catching. To ensure a comprehensive assessment, long-term consequences should also be evaluated such as the persistence of the response on immune function and the reaction norm of the examined animals opposed to overall averages (Moberg, 1985; Cockrem, 2007).

The Effect of Depopulation on Fearfulness of Hens

The analysis of TI inductions revealed that baseline hens were more likely to require 3 attempts ($z = 2.5$; $P < 0.05$) or fail the inductions ($z = 2.5$; $P < 0.05$) than depopulated hens (Figure 1). This suggests that depopulated hens were more fearful than baseline hens. In contrast, both TI latencies, LAT1 ($\chi^2 = 207.93$, df = 1, $P = 0.81$, overall raw mean ± SE = 74.5 ± 5.4 s) and LATSU ($\chi^2 = 224.73$, df = 1, $P = 0.27$, overall raw mean ± SE = 117.1 ± 8.4 s), were similar during baseline and depopulation. It is possible that loud noises caused by the depopulation procedure had an effect on latencies and thus masked potential differences. Carried out in varied and difficult settings (i.e., whatever space the producer could make available on site), we sought to minimize the effect of site variation by performing tests in separated rooms either within the barn or adjacent structures. Hens that appeared to react to a loud noise by lifting their head or standing up were excluded from the analysis.

Individual Differences in Depopulation Methodology

Previous reports have suggested that the skills and abilities of workers may have a strong influence on the process of depopulation, but the aspect was never explored in detail. Based on responses to the questionnaires and video analysis, we were able to explore differences in handling of hens between workers. A statistical analysis of worker- or farm-specific methodologies in the current work was not feasible owing to the relatively low sample size and high variability of the mentioned factors; hence, we can only provide a subjective analysis.

From the producer's questionnaire, we learned that all catchers and people at the crating stations in the present study were experienced and handpicked by producers. The task of catching hens and managing crating stations is considered crucial by producers, not only in terms of welfare but also that the depopulation runs without incidents and is completed within a reasonable time frame. Despite all catchers being experienced (performed at least 4 depopulations), they varied in speed (Table 1) and the catching and handling method, as assessed by video analysis. The difficulty in accessing hens adjacent to feeders and drinkers was expressed by workers in the surveyed Swiss farms and a professional catching crew (Van den Broek Poultry Service BV) based in the Netherlands, who were visited for evaluating different approaches. Surveyed persons communicated that handling is affected by a multitude of factors that explain the wide variety of handling methodologies that were observed between farms. Potentially influential factors included the physical arrangement of a particular aviary system, the position of the worker within the system, and personal preferences.

Farm-Specific Variation and Environmental Factors

Factors such as time of day, the time into the depopulation, or handling duration are factors that were suspected to affect hen welfare in this

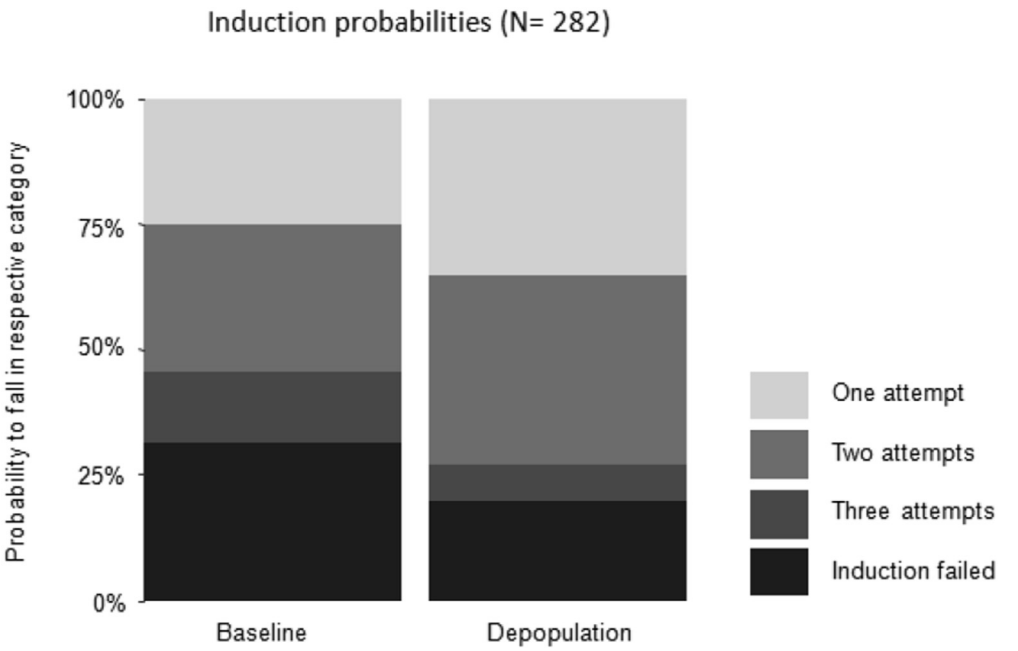

Figure 1. Calculated probabilities for baseline and depopulated hens to be induced into TI after one, 2, and 3 attempts or failing. Baseline hens were more likely to require 3 attempts (z = 2.5; $P < 0.05$) or to fail (z = 2.5; $P < 0.05$) TI induction than depopulated hens. Abbreviation: TI, tonic immobility.

study. In contrast with the assumption, all measures were unaffected except for TI latencies. Both latencies, LAT1 and LATSU, were independent of time of day and barn length but increased by 4.7% (χ^2 = , df = 1, $P = 0.06$) and 5.47% (χ^2 = , df = 1, $P = 0.02$) between the first and last third of the depopulation (post hoc analysis, LAT1: z = 1.9, $P = 0.09$; LATSU: z = 2.19, $P = 0.08$), which suggests that hens become increasingly fearful toward the end of the depopulation. The last batch of hens is usually made up of individuals that had escaped catchers previously or in difficult to reach locations, which would probably contribute to this difference. There was no indication that the number of collisions per minute changed during the course of depopulation ($\chi^2 = 3.62$, df = 2, $P = 0.164$).

Many surveyed producers stated that they perceived hens to be more stressed and difficult to catch when depopulations were performed in the early evening or morning. In this study, we did not find any evidence for such an effect of time of day. It is possible that rather than the time of day, the light conditions are responsible for the negative effects described by farmers. Some of the examined depopulations took place at times that the sun would still be up or have started to rise in the spring and summer months. The presented data set only includes depopulations conducted during the months from October to March; therefore, all depopulations were performed when it was dark outside.

Handling duration is influenced by the experience of handlers and barn factors, specifically the distance that people need to cover between catchers and crating stations. Handling duration has been shown to influence the intensity of the observed stress response (Craig and Craig, 1985; Cockrem et al., 2010), although we did not find an effect on the stress-related measures when analyzing the effect of average walking distances per farm despite occasional waiting lines at the stations. The risk of injury, on the other hand, is likely to remain unaffected by handling time as hens usually remain physically calm as soon as they are handed over to the people carrying them, with collisions and wing flapping unlikely to occur except for the crating process. The creatine kinase results do support that assumption, but more detailed analysis is needed to investigate

the effect of handling duration. Instead of using barn length as a proxy, measuring actual walking distances and the time from when hens are caught until they are crated would be helpful. Collecting these measures continuously throughout the depopulation might furthermore facilitate the disentanglement of the influence of handling duration and time into the depopulation process.

CONCLUSIONS AND APPLICATIONS

To conclude, our data suggest the following:

1. The depopulation from aviary systems leads to injuries such as fractures or muscle injuries and causes stress in depopulated hens.
2. The results in regard to fearfulness were inconclusive, although there was a measurable response regarding when in the depopulation hens were collected.
3. Although injury rates were relatively low compared with those in other studies and stress-related responses did not appear to be physiologically threatening or severe, there remains potential to improve welfare during depopulation.
4. The considerable variability in terms of handling methodologies, lighting, and planning of the procedure paired with the highly variable results obtained on farms suggests refinement of practices could lead to improved hen welfare.
5. We advocate for future research focusing on the point of collection and carrying.
6. Future efforts need to identify best practices so that farmers can be informed about the benefits of certain methodologies and make adjustments accordingly.

ACKNOWLEDGMENTS

The authors want to thank Suzanne Petit for her aid with organizing and conducting the radiographs and Urs Geissbühler from the Division of Clinical Radiology from the University of Bern for the support with the evaluation of the radiographs. The authors also thank Chantal Philipona for her assistance with the coordination and conduct of the blood analysis. The authors' special gratitude goes to Reto Strässle of GalloCircle, who helped to identify and establish contact with producers, as well as to GalloSuisse and the Swiss animal protection organization (STS) for their support. Finally and most importantly, the authors want to thank all the producers who participated in this study and the Federal Food Safety and Veterinary Office for funding the project (grant: 2.16.07).

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Adriaan Bouwknecht, J., B. Olivier, and R. E. Paylor. 2007. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. Neurosci. Biobehav. Rev. 31:41–59.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. 67:1–48.

Beuving, G., and G. M. vonder. 1978. Effect of stressing factors on Corticosterme levels in t of laying hens. Gen. Comp. Endocrinol. 35:153–159.

Beuving, G., and G. M. A. Vonder. 1977. Daily rhythm of corticosterone in laying hens and the influence of egg laying. J. Reprod. Fertil. 51:169–173.

Bishop, S. C., R. H. Fleming, H. A. McCormack, D. K. Flock, and C. C. Whitehead. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. Br. Poult. Sci. 41:33–40.

Black, A. J., and B. O. Hughes. 1974. Patterns of comfort behaviour and activity in domestic fowls: a comparison between cages and pens. Brit. Vet. J. 130:23–33.

Bollinger, T., G. Wobeser, R. G. Clark, D. J. Nieman, and J. R. Smith. 1989. Concentration of creatine kinase and Aspartate Aminotransferase in the blood of Wild mallards following Capture by three methods for Banding. J. Wildl. Dis. 25:225–231.

Broom, D. M. 1990. Effects of handling and transport on laying hens. Worlds Poult. Sci. J. 46:48–50.

Budgell, K. L., and F. G. Silversides. 2004. Bone breakage in three strains of end-of-lay hens. Can. J. Anim. Sci. 84:745–747.

Cabanac, A. J., and M. Guillemette. 2001. Temperature and heart rate as stress indicators of handled common eider. Physiol. Behav. 74:475–479.

Candelotto, L., A. Stratmann, S. G. Gebhardt-Henrich, C. Rufener, T. Van De Braak, and M. J. Toscano. 2017. Susceptibility to keel bone fractures in laying hens and the role of genetic variation. Poult. Sci. 96:3517–3528.

Caraffa-Braga, E., L. Granata, and O. Pinotti. 1973. Changes in blood-flow distribution during acute emotional stress in dogs. Pflügers Arch. Eur. J. Physiol. 339:203–216.

Casey Trott, T., J. L. T. Heerkens, M. T. Petrik, P. Regmi, L. Schrader, M. J. Toscano, and T. M. Widowski. 2015. Methods for assessment of keel bone damage in poultry. Poult. Sci. 71:461–472.

Chrousos, G. P. 2011. The Concepts of stress and stress system Disorders. Jama 267:1244.

Clark, W. D., W. R. Cox, and F. G. Silversides. 2008. Bone fracture incidence in end-of-lay high-Producing, Noncommercial laying hens identified using radiographs. Poult. Sci. 87:1964–1970.

Cockrem, J. F. 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148:169–178.

Cockrem, J. F., E. J. Candy, S. A. Castille, and D. G. Satterlee. 2010. Plasma corticosterone responses to handling in Japanese quail selected for low or high plasma corticosterone responses to brief restraint. Br. Poult. Sci. 51:453–459.

Craig, J. V., and J. A. Craig. 1985. Corticosteroid levels in white Leghorn hens as affected by handling, laying-House Environment, and genetic Stock. Poult. Sci. 64:809–816.

Dabbert, C. B., and K. C. Powell. 1993. Serum Enzymes as indicators of Capture Myopathy in mallards (Anas Platyrhynchos). J. Wildl. Dis. 29:304–309.

Edgar, J. L., C. J. Nicol, C. A. Pugh, and E. S. Paul. 2013. Physiology & Behavior Surface temperature changes in response to handling in domestic chickens. Physiol. Behav. 119:195–200.

Ellis, B. J., J. J. Jackson, and W. T. Boyce. 2006. The stress response systems: Universality and adaptive individual differences. Dev. Rev. 26:175–212.

Fair, J., S. Whitaker, and B. Pearson. 2007. Sources of variation in haematocrit in birds. Ibis (Lond. 1859) 149:535–552.

Fleming, R. H., H. A. Mccormack, L. Mcteir, C. C. Whitehead, H. A. Mccormack, L. Mcteir, C. C. Whitehead, and C. C. Whitehead. 2004. Incidence, pathology and prevention of keel bone deformities in the laying hen. Br. Poult. Sci. 45:320–330.

Freeman, B. M. 1982. Transportation of poultry. Worlds Poult. Sci. J. 40:19–30.

Fröhlich, E. K. F., and H. Oester. 2001. From battery cages to aviaries: 20 years of Swiss experiences. Page 176 in M. G. H. Sambraus and A. Steiger, (Eds.), Das Wohlergehen von Legehennen Eur. Anal. und Schlussfolgerungen. Internationale Gesellschaft für Nutztierhaltung IGN und den Verlag Universität. Kassel, 28.

Gebhardt-Henrich, S. G., C. B. Rufener, and A. Stratmann. 2019. Improving intra- and inter-observer repeatability and accuracy of keel bone assessment by training with radiographs. Poult. Sci. 98:5234–5240.

Goldman, H. 1963. Effect of acute stress on the pituitary gland: endocrine gland blood flow. Endocrinology 72:588–591.

Gregory, N. G., and C. D. Devine. 1999. Body condition in end-of-lay hens: some implications. Vet. Rec. 145:49.

Gregory, N. G., and L. J. Wilkins. 1989. Broken bones in domestic fowl: handling and processing damage in end-of-lay battery hens. Br. Poult. Sci. 30:555–562.

Gregory, N. G., L. J. Wilkins, S. D. Austin, C. G. Belyavin, D. M. Alvey, and S. A. Tucker. 1992. Effect of catching method on the prevalence of broken bones in end of lay hens. Avian Pathol. 21:717–722.

Gregory, N. G., L. J. Wilkins, S. D. Eleperuma, A. J. Ballantyne, and N. D. Overfield. 1990. Broken bones in domestic fowls: effect of husbandry system and stunning method in end-of-lay hens. Br. Poult. Sci. 31:59–69.

H, W. 2007. Reshaping data with the reshape package. J. Stat. Softw. 21:1–20.

Hansen, I. 1994. Behavioural expression of laying hens in aviaries and cages: Frequencies, time budgets and facility utilisation. Br. Poult. Sci. 35:491–508.

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. 71:461–472.

Herborn, K. A., J. L. Graves, P. Jerem, N. P. Evans, R. Nager, D. J. Mccafferty, and D. E. F. Mckeegan. 2015. Skin temperature reveals the intensity of acute stress. Physiol. Behav. 152:225–230.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in general Parametric models. Biometrical J. 50:346–363.

Johnson, A. L., and A. Van Tienhoven. 1981. Plasma concentrations of corticosterone relative to photoperiod, oviposition, and ovulation in the domestic hen. Gen. Comp. Endocrinol. 43:10–16.

Kannan, G., and J. A. Mench. 1996. Influence of different handling methods and crating periods on plasma corticosterone concentrations in broilers. Br. Poult. Sci. 37:21–31.

Käppeli, S., E. Fröhlich, A. Pfulg, M. H. Stoffel, E. Fröhlich, A. Pfulg, and M. H. Stoffel. 2011. Prevalence of keel bone deformities in Swiss laying hens. Br. Med. J. 52:531–536.

Knowles, T. G. 1994. Handling and transport of spent hens. World`s Poult. Sci. J. 50:60–61.

Knowles, T. G., and D. M. Broom. 1993. Effect of catching method on the concentration of plasma corticosterone in end-of-lay battery hens. Vet. Rec. 133:527–528.

Knowles, T. G., D. M. Broom, N. G. Gregory, and L. J. Wilkins. 1993. Effect of bone strength on the frequency of broken bones in hens. Res. Vet. Sci. 54:15–19.

Knowles, T. G., and L. J. Wilkins. 1998. The problem of broken bones during the handling of laying hens–a review. Poult. Sci. 77:1798–1802.

Korte, S. M., G. Beuving, W. I. M. Ruesink, and H. J. Blokhuis. 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiol. Behav. 62:437–441.

Leyendecker, M., H. Hamann, J. Hartung, J. Kamphues, U. Neumann, C. Sürie, and O. Distl. 2005. Keeping laying hens in furnished cages and an aviary housing system enhances their bone stability. Br. Poult. Sci. 46:536–544.

Medway, W., and M. R. Kare. 1959. Blood and plasma volume, hematocrit, blood specific Gravity and Serum Protein Electrophoresis of the Chicken. Poult. Sci. 38:624–631.

Moberg, G. P. 1985. Biological Response to Stress: Key to Assessment of Animal Well-Being? Springer, New York, NY.

Nathaniel, D. P. 2016. YaR Rr ! the Pirates' S Guide to R. 1. Chapter 14. Accessed Dec. 2020. https://bookdown.org/ndphillips/YaRrr/. Bookdown. San Francisco. CA.

Nørgaard-Nielsen, G. 1990. Bone strength of laying hens kept in an alternative system, compared with hens in cages and on deep-litter. Br. Poult. Sci. 31:81–89.

Pantaya, D., and M. M. D. Utami. 2018. The blood haematological profile on laying hens that treated by different levels of yeast supplementation. IOP Conf. Ser. Earth Environ. Sci. 207:1–6.

Regmi, P., N. Smith, N. Nelson, R. C. Haut, M. W. Orth, and D. M. Karcher. 2016. Housing conditions alter properties of the tibia and humerus during the laying phase in Lohmann white Leghorn hens. Poult. Sci. 95:198–206.

Richards, G. J., M. A. Nasr, S. N. Brown, E. M. G. Szamocki, J. Murrell, F. Barr, and L. J. Wilkins. 2011. Use of radiography to identify keel bone fractures in laying hens and assess healing in live birds. Vet. Rec. 169:279.

Romero, L. M., and J. M. Reed. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. - A. Mol. Integr. Physiol. 140:73–79.

Sandilands, V. 2011. Papers: influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet. Rec. 169:411–412.

Sandilands, V., L. Baker, S. Brocklehurst, and N. H. C. Sparks. 2007. The welfare effects of different methods of depopulation on laying hens. Defra AW0235:1–23.

Scanes, C. G. 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poult. Sci. 95:2208–2215.

Scott, G. B., and P. Moran. 1993. Fear levels in laying hens carried by hand and by mechanical conveyors. Appl. Anim. Behav. Sci. 36:337–345.

Shipov, A., A. Sharir, E. Zelzer, J. Milgram, E. Monsonego-Ornan, and R. Shahar. 2010. The influence of severe prolonged exercise restriction on the mechanical and structural properties of bone in an avian model. Vet. J. 183:153–160.

Suchý, P., E. Straková, B. Jarka, J. Thiemel, and V. Večerek. 2004. Differences between metabolic profiles of egg-type and meat-type hybrid hens. Czech J. Anim. Sci. 49:323–328.

Toscano, M. J., I. C. Dunn, J.-P. Christensen, S. Petow, K. Kittelsen, and R. Ulrich. 2020. Explanations for keel bone fractures in laying hens: are there explanations in addition to elevated egg production? Poult. Sci. 99:4183–4194.

Venables, W. N., and B. D. Ripley. 2002. Random and mixed effects. Pages 271–300 in Modern applied statistics with S-Plus. Springer.

Version 2.01, R. C. T. 2018. Version 2.01. R Core Team, NY.

Voslarova, E., P. Chloupek, P. Vosmerova, J. Chloupek, I. Bedanova, and V. Vecerek. 2008. Time course changes in selected biochemical indices of broilers in response to pre-transport handling. Poult. Sci. 90:2144–2152.

Webster, A. B. 2004. Welfare implications of avian osteoporosis. Poult. Sci. 83:184–192.

Weigle, B. 2007. Gesundheitsstatus von Legehennen in Klein- und Großvolierenhaltung im Vergleich. Accessed Dec. 2020. https://edoc.ub.uni-muenchen.de/7298/1/Weigl_Birgit.pdf.

Whitehead, C. C., and R. H. Fleming. 2000. Osteoporosis in cage layers. Poult. Sci. 79:1033–1041.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer Verlag, NY.

Wilkins, L. J., S. N. Brown, P. H. Zimmerman, C. Leeb, and C. J. Nicol. 2004. Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. Vet. Rec. 155:547–549.

Wilson, S., B. O. Hughes, M. C. Appleby, and S. F. Smith. 1993. Effects of perches on trabecular bone volume in laying hens. Res. Vet. Sci. 54:207–211.

Yamamoto, J., M. Nakai, and T. Natsume. 1987. Cardiovascular responses to acute stress in young-to-old spontaneously hypertensive rats. Hypertension 9:362–370.