# Productive performance, perching behavior, keel bone and other health aspects in dual-purpose compared to conventional laying hens

Julia Malchow,<sup>1</sup> Beryl K. Eusemann,<sup>2</sup> Stefanie Petow, E. Tobias Krause<sup>(D)</sup>, and Lars Schrader<sup>(D)</sup>

Institute of Animal Welfare and Animal Husbandry, Friedrich-Loeffler-Institut, 29223 Celle, Germany

**ABSTRACT** Several alternatives to avoid killing male day-old chicks are available. One of these alternatives is to keep dual-purpose chicken strains. The aim of this study was to compare dual-purpose hens (Lohmann Dual, LD) with conventional laying hens (Lohmann Tradition, **LT**) in terms of performance, animal welfare parameters such as keel bone state and foot pad dermatitis, and perching behavior. We expected a generally equal or even better performance of the dual-purpose hens except for laving performance. Four hundred female day-old chicks were housed in 6 pens (3 pens per strain) and reared until 54 wk of age. Each pen offered a littered area, elevated slatted manure pit, elevated wooden frame with perches or grids and nest boxes on the manure pit. The wooden frame was alternately equipped with perches or grids. The elevated manure pit as well as the elevated structure was accessible via ramp. Productive performance parameters like mortality, total number of eggs and body weight were assessed periodically. In week 49, 132 hens (66 hens per strain) were randomly selected for radiography of the keel bone and assessment of plumage and foot pad state. Perching behavior was analyzed via scan sampling during rearing and laying period, respectively. Statistical analyzes were done with Linear Mixed Effect Model and General Linear Mixed Model. LD had a higher radiographic density than LT hens (P = 0.0016), other keel bone parameters (fracture score, P = 0.36; deformation, P = 0.83) showed no differences. The vast majority of fractures occurring in both strains were located in the caudal part of the keel bone. During the laying period, usage of elevated structures was higher with grids compared to perches (P < 0.001) and in LD compared to LT (P = 0.01). Some animal welfare problems were less frequent in LD compared to LT hens while other problems did not differ between the 2 strains or were even more frequent in LD hens. Grids may be more suitable as resting area than perches and may possibly help to decrease the prevalence of keel bone damage.

Key words: keel bone damage, dual-purpose chicken, laying hen, perching, animal welfare

2022 Poultry Science 101:102095 https://doi.org/10.1016/j.psj.2022.102095

# INTRODUCTION

Currently, the avoidance of killing day-old male chickens from layer strains is increasingly discussed and there is a need for alternatives in Europe (2021). In Germany killing day-old chicks is forbidden since January 2022 (TierSchG, 2021) and in France it is also forbidden from 2022 onward but with a transitional period until December 2022 (Ministère de, 2021). Beside in-ovo sex determination (e.g., Krautwald-Junghanns et al., 2017) and rearing of male chickens (Koenig et al., 2012), a further possibility is to keep dual-purpose chicken breeds (Mueller et al., 2020) in which both sexes are reared: The males for meat and the females for egg production, with both having moderate performance compared to strains selected either for egg or meat production (Icken & Schmutz, 2013; Baldinger and Bussemas, 2021a, b). The dual-purpose strain is a hybrid, with a crossing between layer and broiler strains.

Some studies have already investigated the behavior, carcass composition, nutritional needs, and performance of the recently commercially available dual-purpose chickens (excluding backyard and "fancy" strains) compared to layer and meat strains (Alshamy et al., 2018; Giersberg et al., 2019a; Malchow et al., 2019; Röhe et al., 2019; Tiemann et al., 2020). Male dual-purpose chickens, for instance, are more active at the same live body weight than fast-growing broiler chickens (Malchow et al., 2019), but have a lower feed efficiency. Dual-purpose hens, on the other hand, lay fewer and

<sup>© 2022</sup> The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/(4.0/)).

Received April 26, 2022.

Accepted July 26, 2022.

<sup>&</sup>lt;sup>2</sup>Current address: The German Federal Institute of Risk Assessment, Unit Animal Welfare and Knowledge Transfer, German Center for the Protection of Laboratory Animals, Location Marienfelde, 12277 Berlin, Germany.

<sup>&</sup>lt;sup>1</sup>Corresponding author: julia.malchow@fli.de

smaller eggs but are more efficient in feed conversion and tend to have a lower prevalence of feather pecking and cannibalism (Giersberg et al., 2017; Rieke et al., 2021). Furthermore, the prevalence of foot pad lesions was found to be low in male as well as female dual-purpose chickens (Lambertz et al., 2018; Tiemann et al., 2020; Malchow and Schrader, 2021).

In addition to feather pecking and cannibalism, keel bone damage is a very important welfare issue in laying hens (EFSA, 2005; FAWC, 2013; Ali et al., 2020). There are 2 forms of keel bone damages: fractures and deviations. Keel bone fractures are characterized by complete or incomplete interruption of continuity of the bone tissue and severing of the bone which can lead to the formation of 2 or more fragments Casey-Trott et al. (2015). Keel bone deviation has been described by Casey-Trott et al. (2015) as "bone(s) with an abnormally shaped structure that has not resulted from a fracture but contains section(s) that vary from a theoretically perfect 2dimensional straight plane in either the transverse or sagittal planes. Additionally, indentations along the ventral surface can also be classified as a deviation."

Keel bone damages, that is, fractures and deviations, can be found in all housing systems for laying hens. The overall prevalence in cage systems, single and multi-tier systems, and systems with access to free-range ranged between 3% and 100% of hens within one flock (Donaldson et al., 2012; Petrik et al., 2015; Hardin et al., 2019; Jung et al., 2019; Thøfner et al., 2021). The presence of keel bone damage, especially fractures, can cause pain in laying hens (Nasr et al., 2012), which can negatively affect hens' mobility (Rentsch et al., 2019) and welfare (Dawkins, 2004; Riber et al., 2018). Keel bone damages can occur throughout the entire life period, but the highest prevalence was found in laying hens between 49 and 58 wk of age and at the end of laying period (reviewed by Rufener and Makagon, 2020). Causes of fractures and deviations are still not completely identified, but it is known that keel bone damages are a multifactorial problem (Harlander-Matauschek et al. 2015). The interplay between genetics, bone health and bone composition, laying performance (Habig et al., 2021), hormone balance (Eusemann et al., 2020), as well as age and housing system (reviewed by Rufener and Makagon, 2020) have been found to be influencing factors, amongst others.

Due to the high calcium demand for the eggshell and the fact that the skeleton serves as a source of calcium (Kerschnitzki et al., 2014), egg production itself as well as the selection for high laying performance seem to play a major role in the etiology of keel bone damages. In previous studies, fracture risk was at least 80% lower in hens in which egg production was suppressed compared to egg-laying control hens (Eusemann et al., 2018b; Eusemann et al. 2020). In addition, fracture prevalence has recently been found to be significantly lower in the unselected red jungle fowl, that is, the wild ancestor of the domestic chicken, compared to White Leghorn hens, a typical layer strain (Kittelsen et al., 2021). Furthermore, some high performing layer lines (320 eggs/yr) showed a higher prevalence of keel bone fractures and more severe deviations compared to their moderately performing counterparts (200 eggs/yr) (Eusemann et al., 2018a; Eusemann et al., 2020). However, this finding was not consistent throughout all investigated layer lines and depended on phylogenetic background of the hens (Eusemann et al., 2018a). In line with this, it has been shown that brown and white layer lines differ in prevalence of keel bone damage. In some studies, hens of brown layer lines showed more fractures while hens of white layer lines showed a higher prevalence or severity of deviations, respectively (Stratmann et al., 2015a; Heerkens et al. 2016b; Eusemann et al. 2018a; Habig et al. 2021). However, other authors found a higher prevalence and severity of deviations in hens of brown compared to white layer lines (Wahlström et al. 2001; Vits et al. 2005; Habig and Distl, 2013). In view of these differences between breeds differing in phylogenetic background and, in particular, laying performance, it seems likely that prevalence of keel bone damage would be lower in hens of a dual-purpose strain that show a relatively low laying performance compared to hens of a layer strain with a high laying performance.

Especially during night, pullets (Heikkilä et al., 2006) as well as laying hens (Olsson and Keeling, 2000; Campbell et al., 2016) are motivated to use elevated structures for roosting. For laying hens, the height of elevated roosting areas seems to be more important compared to the flooring of these areas, that is, whether they can grasp around perches or sit on flat plastic grids (Schrader and Müller, 2009). However, if perches and grids are offered at the same height, laying hens prefer perches for night-time roosting (Schrader and Müller, 2009; Schrader and Malchow, 2020). During the first 5 wk of age, male chickens of a layer strain did not show a clear preference for either perches or grids at night. Male chickens of both a fast-growing broiler strain and a dualpurpose strain in contrast, preferred grids compared to perches during night-time (Malchow et al., 2019). So far, there has only been one study on perching behavior of adult hens of a dual-purpose strain. Giersberg et al. (2019b) showed that hybrid laying (Lohmann Brown Plus) and dual-purpose hens (Lohmann Dual) used perches to a comparable frequency at night-time, but dual-purpose hens used the lower perches more often than the laying hens which clearly preferred the highest perches. This indicates that dual-purpose and laying pullets and hens may differ in their demands regarding roosting areas.

In our study, we compared pullets and hens of a laying strain (Lohmann Tradition) and a dual-purpose strain (Lohmann Dual) for plumage condition, foot pad lesions, keel bone damages, and perching behavior. In order to test for differences in preferences for roosting areas we offered elevated grids and perches in an alternating but balanced order. In addition, we compared the keel bone health by radiography and the plumage and foot pad condition of both strains. We hypothesized that due to the lower egg performance, dual-purpose hens would have a better keel bone state (less fractures. less deviations, higher radiographic densities) compared to commercial laying hens. Furthermore, we hypothesized that dual-purpose hens would have a better plumage due to less feather pecking, integument und foot pad condition than commercial laying hens due to the lower laying performance. Finally, we predicted that both strains would use elevated structures to the same extent

# MATERIALS AND METHODS

## Ethical Statement

The investigations were carried out in accordance with the German laws and with the approval of the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES # 33.19-42502-04-16/2108). All birds were controlled daily and had commercial food for either pullets or layer and water ad libitum available.

# **Birds and Housing Conditions**

The experiments were conducted at the Institute of Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institut, Celle, Germany.

A total of 400 female chickens of 2 different genetic strains with untrimmed beaks were kept within the same barn from the first day of age until the 54th wk of age: 200 Lohmann Dual hens (LD, dual-purpose, moderate laying performance~249 eggs/y (Damme et al., 2015); mean body weight at hatching ( $\pm$ SD): 37.0 g  $\pm$ 

0.7 g; mean weight at onset of laying (18th wk of age):  $1,253.8 \text{ g} \pm 148.4 \text{ g}$ ; mean body weight in the 54th wk of age: 1,834.7 g  $\pm$  180.1 g, mortality during the entire observation period:  $2.0\% \pm 1.9\%$ ) and 200 Lohmann Tradition hens (LT, conventional hybrid, high laying performance  $\sim 310 \text{ eggs/y}$  (Lohmann Breeders, 2020); mean body weight at hatch:  $39.7 \text{ g} \pm 0.3 \text{ g}$ ; mean weight at onset of lay (18th wk of age): 1,400.5 g  $\pm$  116.5 g; mean body weight in the 54th wk of age: 1,968.3 g  $\pm$ 194.0 g; mortality during the entire observation period:  $5.0 \pm 3.9$ ). The animals were obtained as day-old chicks from a commercial hatchery (Lohmann Tierzucht GmbH, Cuxhaven, Germany). At the beginning of the laying period (18th wk of age), 180 hens per strain were randomly allocated to 3 pens (60 chickens of one strain per pen). The remaining chickens were kept in a separate pen without further involvement in the study. In the rearing period, more chickens were kept in order to compensate eventual losses, for example, due to possible infections.

Each pen had an area of 10.9 m<sup>2</sup> (L [length] × W [width]: 3.5 m × 3.1 m; Figure 1). The area was divided into a littered area (L × W: 3.10 m × 1.13 m) and an elevated slatted manure pit (H [height] × L × W: 0.5 m × 3.10 m × 2.4 m, Figure 1). On top of the manure pit there was a nest box (area: L × W: 0.89 m × 0.57 m) as well as an elevated wooden frame (H × L × W: 0.8 m × 2.3 m × 1.2 m). In general, on the wooden frame, there were either plastic grids (mesh size: 42 mm × 22 mm, Big Dutchman International GmbH,



Figure 1. Simplified display of the experimental pen (view from above, area of  $3.5 \times 3.1 \text{ m} = 10.9 \text{ m}^2$ ): (a) - littered area ( $3.1 \times 1.13 \text{ m} = 3.5 \text{ m}^2$ ), (b) - feeding through, (c) - slatted manure pit ( $3.1 \times 2.37 \text{ m} = 7.3 \text{ m}^2$ ), (d) - nest box ( $0.89 \times 0.57 \text{ m} = 0.5 \text{ m}^2$ ), (e) - water dispenser, (f) - ramp, (g) - elevated structure (variable use for perches or grids; ( $1.2 \times 2.3 \text{ m} = 2.9 \text{ m}^2$ ), (h) - color camera for corner mount with IR-LEDs.

Vechta, Germany) or plastic perches (4 in total, mushroom shaped,  $H \times L \times W$ : 0.07 m  $\times$  2.3 m  $\times$  0.06 m, LUBING Maschinenfabrik Ludwig Bening GmbH & Co. KG, Barnstorf, Germany) enabling either 15 cm perch length per hen or 460 cm<sup>2</sup> grid area per hen. The perches were installed above the outer wooden frame and, thus, hens were able to reach the perches from the entire area below.

Until the age of 4 wk, chickens were only kept in the littered area without access to the elevated structures. From the fourth week of age onwards, the hens had access to the elevated manure pit as well as to the elevated structure by a plastic ramp (plastic grid). The elevated structure type  $(3 \times \text{grids and } 3 \times \text{perches})$  was randomly assigned to the 6 pens at the beginning of the study. Every 8 wk, the order of the assignment changed: In the pens with perches these were exchanged by grids and vice versa.

The light program was adapted according to the life period, that is, rearing period and laying period, and the official recommendations by using artificial lighting. A conventional complete feed for pullets (rearing period: 11.36 MJ ME/kg, 145 g/kg crude protein, 35.2 g/kg crude fat, 10 g/kg Ca, 4.6 g/kg P) and laying hens (laying period: 11.2 MJ ME/kg, 155g/kg crude protein, 52.4 g/kg crude fat, 35 g/kg Ca, 5.5 g/kg P) as well as water were ad libitum available during the entire observation period.

#### Measurements

**Productive performance** The mortality and number of total eggs, broken and shell-less ("wind-egg") eggs (during laying period) were daily recorded during the entire observation period. Laying performance was summarised in 4-wk periods (during the entire observation period: nine periods in total) to obtain an average value per laying month. In the last 3 d of a period, eggs were weighed per pen and assigned to appropriate categories. The 3 categories were small (<53 g), medium (53–63 g), and large (>63 g) eggs.

Body weights of 20 hens randomly selected from each pen was assessed in weeks 1, 4, 12, 16, 25, 30, 36, 39, 50, and 54 of age according to Knierim et al. (2016).

**Radiographic assessment of keel bone state** In the 49th week of age, a total of 132 hens in total were randomly selected for radiography (22 hens per pen  $\times$  6 pens; 66 hens per strain). The selected chickens were carefully carried to the barn anteroom where a mobile X-ray device was set up. According to Eusemann et al. (2018a), the chicken was placed on its left side on the digital flat panel detector (Thales Pixium 2430 EZ Wireless; Thales Electron Devices S.A., Vélizy-Villacoublay, France). Lateral radiographs of the sternum region were taken with 50.0 kV and 2 mAs using the X-ray device WDT Blueline 1040 HF (Wirtschaftsgenossenschaft Deutscher Tierärzte eG, Garbsen, Germany) and the X-ray suitcase Leonardo DR mini (Oehm und Rehbein GmbH, Rostock, Germany). In addition, an aluminum

step-wedge was positioned next to each hen for the determination of the radiographic density of the keel bone. The following paragraphs describe each of the assessment methods used to determine the keel bone state (Radiographic density, keel bone fractures, and severity of deviation) into more detail.

Radiographic density: To assess radiographic density of the keel bone, an aluminum step-wedge was radiographed together with each hen for calibration purposes. One person who was blind toward the genetic of the chicken evaluated all images for radiographic density as described by Eusemann et al. (2020), using the image processing program ImageJ (Version 1.48; National Institutes of Health, Bethesda, MD). The gray value of the background and of each step was measured after which a calibration curve was generated with a third degree polynomial function. Afterwards, the whole keel bone was circumscribed up to the insertion of the trabecula intermedia and keel bone radiographic density was assessed based on the calibration curve. The mean gray value was given as millimeters of aluminum equivalent (mm Al eq). Areas with callus formation or in which legs overlapped with parts of the keel bone were excluded from radiographic density assessment as they resulted in increased, nonrepresentative density measures.

Keel bone fractures: In order to determine the prevalence of a fracture as well as its severity, location, and age (callus formation), the assessment scheme developed by Rufener et al. (2018) was applied. The scoring system ranged from 0 (no fracture) to 5 (extremely severe) with intermediate tags for scores 1, 2, 3, and 4. One person first performed the e-learning tool of Rufener et al. (2018) until the observer criterion was reached. Afterwards, the same person blindly toward genetic of the chicken evaluated the radiographs. After a short time period, this procedure was repeated and an intra-observer-reliability was applied. A Pabak coefficient of 0.82 was obtained. To detect all details in the radiographs, the RadiAnt Dicom Viewer 2020.2 (Medixant, Poznań, Poland) was used. In addition to the assessment following Rufener et al. (2018) we recorded the localization of the detected fractures according to Baur et al. (2020), dividing the keel bone into three different regions: "A" cranial third, "B" middle, and "C" caudal third (Baur et al., 2020).

Severity of deviations: To estimate the severity of a deviation, the percentage of the deviated keel bone area (POD) was assessed with the program AxioVision (Version 4.3; Zeiss, Jena, Germany) as described by Eusemann et al. (2018a). The deviated area was estimated by circumscribing the deformed outline and connecting the start and end point of this outline with a straight line. The size of this area was calculated. The entire keel bone was then circumscribed up to the point where the trabecula intermedia begins and the size of its surface was calculated with AxioVision. Again, the start and end points of the deformed contour were connected with a straight line as an estimate for the size of the actual keel bone surface. Finally, POD was calculated by dividing the deviated area by the keel bone surface area, multiplied with one-hundred.

**Perching behavior** In each pen, a camera (Model VTC-E220IRP, color camera for corner mount with IR-LEDs; SANTEC BW AG, Ahrensburg, Germany, see Figure 1) covering the elevated structure, was installed on the ceiling and connected to a local computer that stored the video data on an external hard drive. Due to the development of the use of elevated structures of chickens throughout the rearing period, the data were analyzed for 2 d per week (usually Saturday and Sunday) by scan sampling and counting the hens on the elevated structures at the dark period (6 time points per day, that is, from 12 am to 05 am). For the laying period, that is, from the 22nd week of age onwards, the usage of elevated structures was analyzed for 2 d 2 wk before and for 2 d 2 wk after the elevated structure changed. This was also done by scan sampling and counting the birds on the elevated structures during the dark period (2) time points per day, i.e., 3 am and 6 pm). Fewer time points were chosen in the laying period because the number of birds on elevated structures during the night does not vary much between weeks of age in contrast to the rearing period (modified after Wichmann et al., 2007).

Plumage, integument and foot pads One hundred thirty-two randomly selected hens per time point (22 hens from each pen) were individually scored for feather damage, feather loss and integument injury at predefined time points after Knierim et al. (2016) during rearing (week 4, 12, 16, 18) and laying period (week 25, 37). 50). The assessment scheme was modified according to Sepeur et al. (2015) and Giersberg et al. (2017). For assessing the plumage condition (feather damage, feather loss) and integument injuries, the hens' body was divided into 5 body regions: head/neck, back, tail, wing and belly/chest and a 4 or 5 point-scale was used, respectively. The scores of all 5 body regions were summed up so that there was one score per hen and parameter which could range from 0 (i.e., no feather damage, feather loss or integument injuries at any body region, respectively) to 20 for feather damage and feather loss (i.e., score 4 at each body region) or to 15 for integument injuries (i.e., score 3 at each body region). The scoring scheme is described into detail in Table 1. The assessment of the foot pad condition was done visually for both feet per hen according to Heerkens et al. (2016a). The foot with the higher score was used for data analysis. The detailed scoring scheme is described in Table 1.

# Statistical Analysis

**Productive performance** The body masses of individuals were analyzed at 7 different ages stages (weeks 18, 25, 30, 36, 39, 50, 54), each time using a Linear Mixed Effect Model (**LME**) with respective body mass at that age as dependent variable, strain as explanatory factor and penID as random nesting factor. Residuals' normal distribution was checked using q-q-plots.

Laying rate data were available on pen level and calculated by number of eggs per period divided by number of hens per pen and number of days per period. Laying rate for period 1 was separately analyzed using a Linear Model (**LM**) as in this period differences of egg laying between individuals may affect the laying rate. The LM was calculated with the  $\log(x+1)$  transformed laying rates for period 1 as dependent variable and strain as explanatory variable. Periods 2 to 9 were analyzed in one LME with  $\log(x+1)$  transformed laying rates as dependent variable and strain, period and their interaction as explanatory variables, and penID as random nesting factor. Residuals of models were checked for residuals' normal distribution using q-q-plots. Relative number of broken and shell less eggs was analyzed for strain and period, respectively, using Kruskal-Wallis ANOVA's, as residuals were not normally distributed.

Keel bone state The radiographic density was analyzed on an individual level using a Linear Mixed Effect model with strain as explanatory factor and penID as random nesting factor. Bone density was  $\log(x+1)$ transformed to achieve a normal distribution of the residuals in the q-q-plot. Individuals' fracture scores were analyzed using a General Linear Mixed Model (GLMM) with Poisson distribution and strain as explanatory factor and PenID as random factor. Localization of fractures was not assessed statistically but descriptively, that is, the percentage of fractures within one of the keel bone regions ("A," "B," or "C") in relation to all fractures was assessed for each chicken strain. Occurrence of keel bone deviation (y/n-variable) was analyzed with a GLMM with binomial distribution and strain as explanatory factor and PenID as random factor. From the hens having a keel bone deviation (i.  $e_{i} = y$ ) the relative deviation, that is, POD, was analyzed using an LME with strain as explanatory factor and penID as random factor.

As explorative post-hoc analyses we analyzed the relationship between fracture scores (integer) and radiographic density, respectively, in a GLMM with Poisson distribution with fracture scores as dependent variable and radiographic density as explanatory variable and penID within strain as nesting random factor.

**Perching behavior** Usage of elevated structure elements was analyzed using the proportion of hens that were observed on average (per hour per pen) on the respective structure element (grid or perch) during the dark period. The relative usage of the structure elements in each pen was analyzed separately for rearing and laying period, each using an LME with structure type (grid, perch), strain and age and their 2 way-interactions as explanatory factors and penID as nesting random factor. For laying phase, data had to be log(x+1) transformed.

**Plumage, integument and foot pads** Plumage status, that is, summed feather damage scores, summed feather loss scores, and injuries as well as maximal foot pad scores, that is, the highest score of both feet, were analyzed separately for the end of the rearing (18th wk of age) and the end of the laying phase (50th wk of age) using GLMM with Poisson distribution and with strain as explanatory factor and penID as random factor.

Table 1. Scoring description of the assessment of plumage condition, integument damage and foot pad condition.

Score	Plumage cor	ndition			
	Feather damage	Feather loss	Integument damage	Food pad condition	
0	Negative results, intact and complete plumage	Negative results, intact and complete plumage	Negative result, no injuries	Negative results, no foot pad lesions	
1	≤25% of feathers destroyed at the spe- cific body part	≤25% absence of feathers at the specific body part	Individual injuries $<0.5 \text{ cm} (\text{length or diameter})$	Slight to moderate lesions	
2	$>25\% \le 50\%$ of feathers destroyed at the specific body part	$>25\% \le 50\%$ absence of feathers at the specific body part	Several injuries ${>}0.5~{\rm cm}$ ${\leq}1~{\rm cm}$	Severe lesions	
3	$>50\% \le 75\%$ of the feathers destroyed at the specific body part	$> 50\% \le 75\%$ absence at the specific body part	Injuries $>1$ cm	/	
4	${>}75\%$ of the feathers destroyed at the specific body part	> 75% absence of feathers at the specific body part	/	/	

For plumage condition, the chickens' body was divided into 5 regions, namely the head/neck, back, tail, wings, chest/belly modified after Sepeur et al. (2015) and Giersberg et al. (2017). Assessment of foot pad condition were modified after Heerkens et al. (2016a) and both feet were visually scored.

Models were calculated using R 4.0.3 (R Core Team, 2020) and the packages "nlme" (Pinheiro et al., 2020), "lme4" (Bates et al., 2015). *P*-values for GLMM's were calculated using the package "car" (Fox and Weisberg, 2019).

# RESULTS

## **Productive Performance**

Individual body weights significantly differed between strains. From week 18 until week 54, conventional LT laying hens were heavier at every single age than the LD hens (Table 2 for details).

Laying performance in the first period significantly differed between the 2 strains with higher laying rates in the LD hens compared to the LT hens (LM, factor strain  $F_{1,4} = 118.27, P = 0.0004;$  Figure 2). The laying performance between laying periods 2 and 9 was again significantly affected by strain, as well as period as temporal factor and their interaction, again with higher laying rates in the LT hens compared to the LD hens (LME, factor strain  $F_{1,4} = 39.36$ , P = 0.003; factor period  $F_{1,40} = 121.10, P < 0.0001;$  factor strain\*period  $F_{1.40} = 32.97, P < 0.0001;$  Figure 2). In the second and third laying period, laying performance did not significantly differ between strains. From the fourth period onward, laying performance decreased in both strains but more markedly in LD hens, resulting in significantly higher laying performance in LT compared to LD hens.

The relative amount of broken and shell-less eggs between laying periods 2 and 9 was again significantly affected by the factor period (Kruskal-Wallis-ANOVA,  $\chi 2 = 15.2$ , df = 7, P = 0.033), with having increased values at the beginning and at the end, while strain had no effect (Kruskal-Wallis-ANOVA,  $\chi 2 = 1.11$ , df = 1, P = 0.29).

Egg size categorization was only recorded on strain and laying period level, thus we present it descriptively. Over the entire considered periods 2 to 9, LD hens laid 3,098 eggs while LT hens laid 3,716 eggs, that is, about 20% more in total. As shown in Table 3, the  $\Delta$  number of all eggs (i.e., number of eggs from LT–number of eggs from LD) was positive in each laying period, showing that number of eggs was higher in the LT hens throughout. In addition,  $\Delta$  eggs classes were biased toward larger eggs being more often apparent in the LT hens than in the LD hens (Table 3).

# Keel Bone State (Radiographic Density, Fractures, and Deviations)

The radiographic density significantly differed between dual-purpose and conventional LT laying hens, with dual-purpose hens having a higher radiographic density (LME, factor strain  $F_{1,4} = 57.40$ , P = 0.0016; Figure 3). In contrast, neither the fracture scores (GLMM, factor strain  $\chi^2_1 = 0.85$ , P = 0.36; Figure 4) nor the occurrence of deviations (GLMM, factor strain  $\chi^2_1 = 0.30$ , P = 0.58) significantly differed between strains. In hens with a keel bone deviation, the percentage of deviated keel bone area (POD) did not significantly differ between strains (LME, factor strain  $F_{1,4} = 0.055$ , P = 0.83). On average, POD was 0.020 % ( $\pm 0.011$  S.D.) in the LD hens and 0.22 % ( $\pm 0.010$  S.D.) in the LT hens.

Table 2. Mean live body weight ( $\pm$ SD = standard deviation) of Lohmann Dual (LD) and Lohmann Tradition (LT) in detail from week 18 until week 54.

Age in weeks	$\begin{array}{c} {\rm Mean\ live\ body\ weight\ in\ g} \\ {\rm of\ the\ LD\ hens\ \pm\ SD} \end{array}$	$\begin{array}{c} {\rm Mean\ live\ body\ weight\ in\ g} \\ {\rm of\ the\ LT\ hens\ \pm\ SD} \end{array}$	F-value	df	P-value
18	$1254 \pm 148$	$1401 \pm 117$	39.874	1,4	0.003
25	$1542 \pm 144$	$1829 \pm 180$	92.958	1,4	< 0.001
30	$1651 \pm 183$	$1918 \pm 174$	26.133	1,4	0.007
36	$1735 \pm 154$	$1979 \pm 185$	28.264	1,4	0.006
39	$1734 \pm 179$	$1974 \pm 223$	39.962	1,4	0.003
50	$1776 \pm 164$	$1993 \pm 171$	26.506	1,4	0.007
54	$1835 \pm 180$	$1968 \pm 194$	15.277	1,4	0.02



Figure 2. Total number of eggs divided by the total number of hens within each period (laying performance  $[\%] \pm SD$ ). In one period, four weeks are combined (LD = Lohmann Dual, LT = Lohmann Tradition). In the first laying period LD hens had a higher laying performance than LT hens (factor strain P = 0.0004). Over the subsequent periods 2-9 LT hens had higher laying performance (factor strain\*period P < 0.0001).

Regarding the localization of fractures, all fractures within each affected hen were localized in only one of the 3 keel bone regions. None of the hens showed fractures in several regions. One hundred percent of fractures in LD and 85.7% of fractures in LT hens were found in the caudal third of the keel bone (area "C"). The remaining 14.3% of fractures in LT hens were observed in the middle part (area "B") of the keel bone.

An explorative post-hoc analysis revealed no clear significant relationship between fracture scores and radiographic density (GLMM, factor radiographic density  $\chi^2_1 = 2.26, P = 0.13$ ).

# Perching Behavior

The usage of the structure elements during rearing phase was affected by structure type (grid vs. perch), age and the interactions between structure type and strain as well as structure type and age (LME<sub>rearing</sub>, factor structure type  $F_{1,79} = 142.63$ , P < 0.0001; factor strain  $F_{1,4} = 0.05$ , P = 0.83; factor age  $F_{1,79} = 205.03$ ,

P < 0.0001; factor structure type\*strain  ${\rm F}_{1,79} = 13.83,$  P = 0.0004; factor structure type\*age  ${\rm F}_{1,79} = 14.70,$  P = 0.0003; factor strain\*age  ${\rm F}_{1,79} = 3.85,$  P = 0.053). In general, the usage of structure elements increased with age and was higher with grids compared to perches. During rearing phase, the average usage of elevated structures in LT was  $50.3 \pm 25.6\%$  for grids and  $22.4 \pm 18.4\%$  for perches while the average usage of elevated structures in LD was  $47.4 \pm 28.2\%$  for grids and  $21.7 \pm 10.4\%$  for perches.

The usage of the structure elements in the laying phase was affected by structure type, strain, age, the interaction between structure type and age and the interaction between strain and age (LME<sub>laying</sub>, factor structure type  $F_{1,85} = 102.52$ , P < 0.0001; factor strain  $F_{1,4} = 20.67$ , P = 0.01; factor age  $F_{1,85} = 8.85$ , P = 0.004; factor structure type\*strain  $F_{1,85} = 1.33$ , P = 0.25; factor structure type\*age  $F_{1,85} = 11.24$ , P = 0.001; factor strain\*age  $F_{1,85} = 27.5$ , P < 0.0001). With successive week of age, the usage of elevated structures increased. In addition, usage of elevated structures was higher with grids compared to perches and in LD

**Table 3.** Delta ( $\Delta$ ) egg numbers (difference: LT-LD) and egg size class distribution.

laying period	$\Delta$ number of all eggs	$\Delta$ number of eggs up to size class "S"	$\Delta$ number of eggs in size class "M"	$\Delta$ number of eggs from size class "L" and larger	
2	76	-311	221	98	
3	23	-186	-62	194	
4	70	-78	-142	238	
5	70	-36	-139	223	
6	94	-19	-141	210	
7	107	-13	-112	180	
8	89	-11	-118	180	
9	89	-6	-111	170	

Positive values indicate that a higher number of eggs from the respective category was found in LT compared to LD hens while negative values indicate the opposite. Egg class: "S" (weight < 53 g), "M" (weight = 53-63 g), and "L" (weight > 63 g).



Figure 3. Average radiographic density ( $\pm$ SE) of Lohmann Dual purpose (LD) hens and Lohmann Tradition (LT) laying hens) in the 49th week of age. LD hens had a significant higher radiographic density than LT hens (P = 0.0016). \* indicate a P - value < 0.05.

compared to LT hens. During laying phase, the average usage of elevated structures in LT was  $46.5 \pm 21.8\%$  for grids and  $27.3 \pm 6.1\%$  for perches while the average usage of elevated structures in LD was  $61.7 \pm 18.3\%$  for grids and  $34.0 \pm 7.7\%$  for perches.

# Plumage, Integument and Foot Pads

At the end of the rearing period, feather damage scores were slightly but significantly increased in the LT hens compared to the LD hens (GLMM, factor strain  $\chi^2_1 = 4.42$ , P = 0.036). Within the LT hens, 4.5% of the hens had a feather damage score of "2" and 27.3% had a feather damage score of "1" while the other LT hens had no feather damage. In the LD hens, feather damage score "2" was not present at all and only 10.6% had the damage score of "1" while no feather damage was seen in the remaining hens. Feather loss was not analyzed as it only occurred in a single bird at the end of the rearing period while injuries were absent in all birds.



Figure 4. Cumulative representation of the distribution of the fracture scores (0-5) on the individual hens of Lohmann Dual purpose hens (LD) and Lohmann Tradition (LT) laying hens in the 49th week of age (n= 66/strain). 0 = no fracture, 5 = extremely severely fractured keel bone, 1 to 4 = intermediate tags. No hen was found having score 5.

Feather damage at the end of the laying period, that is, at around the time when radiographs were taken, also significantly differed between strains, again with LT hens having higher damage scores than LD hens (GLMM, factor strain  $\chi^2_1 = 3.90$ , P = 0.048). Within the LT hens, 1.5% of the hens had a feather damage score of "6" and 10.6% had a feather damage score of "5," while none of these 2 scores were reached in the LD hens. Feather damage score "4" was seen in 24.2% of the LT and in 4.5% of the LD hens, score "3" in 40.9% of the LT and in 66.7% of the LD hens, score "2" was present in both strains at 19.7%, while feather damage score "1" was seen in 3.0% of the LT and in 7.6% of the LD hens. Feather damage score "0" was not present in any of the LT hens but in 1.5% of the LD hens.

Also feather loss scores were significantly higher in LT than in LD hens at the end of the laying period (GLMM, factor strain  $\chi^2_1 = 208.08$ , P < 0.0001). On average, the feather loss score, that is, the sum of the scores of all body regions, was 2 (median; first quartile: 1; third quartile: 3) in the LD hens and 10 (median; first quartile: 8; third quartile: 11) in the LT hens. Injuries did not significantly differ between strains at the end of the laying period (GLMM, factor strain  $\chi^2_1 = 0.05$ , P = 0.82).

Foot pads were fully intact in all LT and LD hens at the end of rearing. However, the maximal foot pad score of the feet at the end of the laying period significantly differed between the strains (GLMM, factor strain  $\chi^2_1 = 11.95$ , P < 0.0001), with LT hens having less foot pad lesions. 66.7% of the LT hens had a foot pad score of "0," that is, no lesions, while this was only the case in 24.2% of the LD hens. Score "1" was found in 34.8% of the LT hens and in 56.1% of the LD hens. and score "2" in 4.5% of the LT and in 19.7% of the LD hens.

#### DISCUSSION

Our results showed that the dual-purpose hens (LD) weighed less and had a lower laying performance compared to laying strain hens (LT). Furthermore, the dualpurpose hens had a higher radiographic density, but no lower prevalence of fractures and deviations of the keel bone compared to laying strain hens. During the laying period, the LD used the elevated structures more than LT hens.

## Productive Performance

The lower body weight of the dual-purpose line LD that we found compared to the layer line LT is in accordance with findings by Giersberg et al. (2019b) who compared LD hens with hens of the layer line Lohmann Brown plus (LB+). However, no difference in body weight was found between LB+ and LD hens in another study (Giersberg et al., 2017). The differences between the studies may be explained by the age at which body weight was assessed. In the present study, hens were repeatedly weighed between the 18th and 54th wk of age and Giersberg et al. (2019b) assessed body weight at 34

wk of age. In contrast, hens in the study by Giersberg et al. (2017) were weighed in the 70th wk of age. Thus, it is possible that body weight of LD hens is lower compared to hens of brown layer lines at a relatively young but not at a high age, indicating that body mass increase begins earlier in layer lines but is more pronounced later in life in dual-purpose hens. Accordingly to that, the difference in body weight decreased with increasing age in the current study (see Table 2). This decrease was most pronounced between the 40th and 45th wk of age. This may indicate that the difference in body weight between LD and LT was mainly caused by differences in body size. LD hens were smaller compared to LT hens (personal observations by J.M.). Later, this difference may have leveled off because muscle mass increased more in LD compared to LT hens. However, as the hens were only weighed until the 54th wk of age and neither body size nor muscle mass were assessed in the present study, no final conclusion can be drawn about these findings.

As expected, laying performance was lower in LD compared to LT hens from the fourth period onwards and, in total, LT hens laid about 20% more eggs than LD hens. This typical difference between dual-purpose strains and layer strains has also been found by others (Giersberg et al., 2017, 2019b).

Eggs of LD hens were also smaller compared to eggs of LT hens. This may be explained by both the lower body weight in LD hens as well as the intensive selection toward large eggs in layer lines but not in dual-purpose chickens. In accordance with our findings, Röhe et al. (2019) found a mean egg weight of 37 g to nearly 44 g in LD hens between weeks 23 and 52, which is equivalent to eggs of size class "S."

In contrast to egg number and size, the amount of broken and shell-less eggs did not differ between LD and LT hens in the current study. However, more broken and shell-less eggs were found at the beginning and at the end of the study in both strains.

## Keel Bone State

As hypothesized, LD hens had a higher radiographic density of the keel bone compared to LT hens. However, radiographic density was not significantly linked to the fracture scores. It is important to note that radiographic density is not identical to bone density. As we radiographed live hens and not isolated keel bones, the radiographic density may also have been influenced by soft tissue surrounding the keel bone. It is possible that LD hens had a higher amount of breast muscles as they have been bred for both egg and meat. This possible higher amount of breast muscles could then have led to a higher radiographic density. However, the amount of breast muscles has not been assessed in the present study. Alternatively, it is also possible that the higher radiographic density in LD did reflect a higher bone density but that bone density had no important influence on the risk of keel bone fracture. To get a clearer picture of differences in bone density, composition, and bone quality

between LD and LT hens as well as their impact on keel bone fractures, a detailed analysis of isolated bones would be required.

In contrast to our hypothesis, we did not find any significant difference between LD and LT hens concerning keel bone fractures and deviations. In a previous study, we were able to show that egg production plays a major role in the etiology of keel bone fractures (Eusemann et al., 2020). Moreover, several authors described differences in prevalence of keel bone fractures and deviations as well as bone strength between high and moderately performing layer lines (Hocking et al., 2003; Candelotto et al., 2017; Habig et al., 2017; Eusemann et al., 2018a, 2020; Habig et al., 2021). In addition, significantly more fractures and deviations were recently found in White Leghorn hens, that is, a breed which has intensively been selected for egg production, compared to Reg Jungle fowl hens, that is, the wild ancestor of the domestic chicken that has not been selected for any goal (Kittelsen et al., 2021). Thus, we had expected that LD hens, that showed a lower laying performance compared to LT hens, would have a lower prevalence of keel bone fractures and deviations. However, this was not the case. This could indicate that under the tested circumstances, LD hens are as susceptible to keel bone fractures and deviations as LT hens. There are several possible explanations for that unexpected finding.

Laying performance differed less between LD and LT hens compared to different strains in former studies. LT hens typically lay 310 eggs/y (Lohmann Breeders, 2020) and LD hens typically lay 249 eggs/yr (Damme et al., 2015). In comparison, the high performing layer lines that were assessed by Eusemann et al. (2018a, 2020) show a higher average laying performance compared to LT (320 eggs/yr) whereas the moderately performing layer lines show a lower average laying performance compared to LD (200 eggs/yr; Lieboldt et al., 2015). Thus, it is possible that in contrast to laying performance of the breeds assessed in former studies, the difference in laying performance between LD and LT hens was not large enough to have any influence on bone physiology.

Laving performance is only one characteristic of the strains. As LD and LT hens differ from each other in many ways, it is difficult to relate findings in these 2 strains to laying performance alone. As an example, LT hens are brown egg laying hens while LD hens are white egg laving hens. This characteristic has been found to influence the prevalence and severity of keel bone fractures and deviations, too, although the direction of this effect differs between studies and examined breeds (Wahlström et al., 2001; Vits et al., 2005; Habig and Distl, 2013; Stratmann et al., 2015a; Heerkens et al., 2016b; Eusemann et al., 2018a; Habig et al., 2021). One characteristic that did not differ between LD and LT hens was age at onset of lay. Both strains started to lay eggs in the 18<sup>th</sup> week of age. Gebhardt-Henrich and Fröhlich (2015) found that the younger hens were when laying their first egg, the higher was the probability of having a keel bone fracture at depopulation. A possible

explanation for this finding is the late ossification of the keel bone at about 35 wk of age (Buckner et al., 1949; Thøfner et al., 2021). At this age, hens have already been laying eggs for several weeks. Thus, the ossification process may be disturbed due to the competing calcium demand for the eggshell, which may possibly lead to a weak keel bone structure. Our findings support this hypothesis and the early onset of lay may possibly have a higher impact on prevalence and severity of keel bone damage than laying performance itself. The hypothesis is also supported by the fact that, comparable to findings by Thøfner et al. (2021), the vast majority of fractures in our study occurred at the caudal third of the keel bone in both strains. This part is the last one to ossify and, thus, may be more susceptible to fracture in this critical stage.

Another possible explanation for the missing difference between LD and LT hens in terms of keel bone fractures may be the design of the housing equipment. The housing equipment used in this study has been designed, for example, in shape and size, for layer strains rather than for dual-purpose chickens. LD hens were smaller and had smaller feet compared to LT hens (personal observations by J.M.). Thus, the diameter of the provided perches may have allowed for a good footing in LT hens but less for LD hens. That could have led to an increased number of collisions and falls in LD hens, resulting in keel bone fractures. In addition, usage of elevated structures was higher in LD compared to LT hens which may also have affected prevalence and severity of keel bone fractures and deviations. Consequently, these effects could have hidden a possible difference in bone health between LD and LT hens. However, falls and collisions were not assessed in the current study and, thus, this possible explanation remains uncertain. Again, in order to disentangle between internal risk factors for keel bone fractures and deviations such as bone quality, and external risk factors such as suitability of the housing equipment, a detailed analysis of the bones of both LD and LT hens would be required.

Lastly, it is worth noting that fracture prevalence and percentage of deviated keel bone area were comparatively low in both strains in the current study. 34.8% of the LD hens and 31.8% of the LT hens had a keel bone fracture and relative POD was 0.02% in LD and 0.22%in LT hens. Hens were in their 49th wk of age when being radiographed. In former studies, when assessing the keel bone of hens kept in floor housing systems at a similar age, different research groups found a total fracture prevalence of 86.5 % (49 wk of age; Heerkens et al., 2016b), 46.3% (50th wk of age; Petrik et al., 2015), 35.7% (untreated control hens of a layer line with a moderate laying performance) or 74.3% (untreated control hens of a layer line with a high laying performance), respectively (both: 50th wk of age; Eusemann et al., 2020), and 61.7% (51st wk of age; Eusemann et al., 2018a). The relatively low fracture prevalence in the current compared to other studies may be explained by the presence of ramps. Both Stratmann et al. (2015b) and Heerkens et al. (2016b) showed that providing pens with

ramps significantly reduced fracture prevalence, although that was only true at 60 wk of age but not at 66 wk of age in the study by Stratmann et al. (2015b). It is likely that the observed reduced prevalence of keel bone fractures was due to a decreased number of falls and collisions with furniture in pens with ramps (Stratmann et al., 2015b). Another possible explanation for the relatively low fracture prevalence and also for the low percentage of deviated keel bone area, that is, severity of deviation, in the current study may be the fact that perches and grids were alternately offered in each pen. Perches seem to play an important role in the etiology of keel bone fractures and deviations (Pickel et al., 2011; Wilkins et al., 2011; Stratmann et al., 2015a; Eusemann et al., 2018a) and it has been shown that both fracture prevalence and severity of deviation can be decreased by providing perches with a soft cushion (Stratmann et al., 2015a). It has been suggested that this effect is due to "an enlarged contact area between the keel bone and the perch" that leads to an "increased spread of pressure on the keel bone" (Stratmann et al., 2015a). This suggestion is supported by findings that peak force on the keel bone is indeed lower and contact area is indeed larger with soft, air-cushioned perches compared to perches without any cushion (Pickel et al., 2011). It is possible that peak force on the keel bone is also lower when the hens rest on grids compared to perches due to the larger contact area. This may have led to the comparatively low fracture prevalence and deviation severity in the current study in which hens had access to perches for 8 wk followed by 8 wk with grids and so on. The potential benefit from grids for keel bone health could be subject of further studies as it could be a promising way to decrease keel bone damage. There do not seem to be any arguments against replacing perches by grids from a behavioral point of view as hens in the current study used the grids more frequently compared to perches and a former study showed that height was more important to the hens than form of the elevated structure (i.e., perches or grids; Schrader and Müller, 2009).

The comparatively low fracture prevalence and severity of deviations possibly may have contributed to the unexpected missing difference between LD and LT hens. We can speculate that possibly a more challenging environment, for example, with more tiers and no ramps, where risk of fractures and deviations is higher, a possible, yet hidden difference between LD and LT may become apparent.

# **Perching Behavior**

During the rearing phase, both strains showed an increase of usage of elevated structures. However, LT used the elevated structures more than LD during the rearing period. In contrast, during the laying period, LD was recorded more often on the elevated structures compared to LT. Usage of grids was higher than usage of perches. In general, due to findings in other studies

(Giersberg et al., 2019b; Malchow et al., 2019), we had not expected strain differences in the usage of elevated structures. However, diurnal patterns (Brendler and Schrader, 2016) and design of the elevated structure (Schrader and Müller, 2009) need to be mentioned for elevated structure acceptance in rearing and laying phase. In this study, we only analyzed the dark period. Therefore, we expected a usage that more than 60%(Wood-Gush and Duncan, 1976; Olsson and Keeling, 2000; Campbell et al., 2016) of our hens would be found on elevated structures because perching at night should be a behavioral priority (Weeks and Nicol, 2006). However, this was not the case. One possible explanation may be the regular change of the elevated structures. It is possible that chickens did not accept the changing and looked for other roosting places on the manure pit. While chickens prefer higher positions for resting, it might also be important that the structure is the same for the entire period. LD are more flexible in behavior to new situations in a known environment (Giersberg et al., 2020), which may be a possible explanation for the higher usage of elevated structures in this strain. Thus, it is possible that these changes led to a comparatively low usage of elevated structures in LT hens which are not very flexible in behavior while LD hens showed a higher usage as they are more flexible in behavior and, thus adapted more easily to the new conditions (perches or grids, respectively). It is known that different strains differ in the usage of perches (Habinski et al., 2017; Ali et al., 2019) Thus, a higher motivation for perching in LD compared to LT could be another or additional explanation for these findings despite a possibly less suitable environment for LD as discussed above. In addition, perches were regularly replaced with grids and vice versa.

Design and arrangement of elevated structures plays an important role for perching (Schrader and Malchow, 2020). Especially during the night, a high position is strongly preferred by laying hens (Brendler et al., 2014). Our grids as well as perches were offered at the highest reachable point in each pen from the beginning of the observation onwards. In our study, ramps were offered from the littered area to the manure pit and also from the manure pit to the perches or grids. The ramps provided much easier access, which was pointed out by the higher usage of the passage area from the ramp to the elevated structures (personal observation by J.M.). However, the chickens were already in these pens from the first day of age onwards and got access to the elevated structures from the fourth week of age. It has been shown that at the beginning, pullets do not use the highest points for resting (Habinski et al., 2017). In this case, the manure pit could have been accepted as a resting place due to the height difference to the littered area. The elevated structures were not freely accessible due to less than optimal accessibility and blockages by conspecifics at the ramp. In addition, only part of the grids was accessible, using the ramp. The remaining grid area had to be approached by flight. The perches, on the other hand, could also be approached from other positions.

However, it seemed that there were several crashes in LD (personal observation by J.M.), possibly because the mushroom-shaped perches might not be suitable for their comparatively small feet, but this was not explicitly assessed in this study. There seems to be a link between perching during rearing phase and perching during laying phase (Heikkilä et al., 2006). Furthermore, the chickens do not build spatial skills to use the highest level in the pen. Colson et al. (2008) pointed out that providing food and water at higher levels during rearing led to an increased usage of higher tiers in adult chickens. All in all, grids were used more by both LD and LT compared to perches. This result may show that grids are more suitable for roosting in comparison to perches.

# Plumage, Integument and Foot Pads

In the rearing and laying phase, poorer plumage, including feather damage and feather loss, was found in LT compared to the LD hens. Especially in the laying phase, LT showed high feather damage and high feather loss, but only few injuries were assessed for both strains. In general, some studies showed differences of plumage condition and feather pecking between different strains (Niebuhr et al., 2006; de Haas et al., 2014). However, poorer plumage conditions were found in white compared to brown layer strains (Spindler et. al, 2013). In our study as well as the chickens from Giersberg et al. (2017) showed opposite results. The Lohmann Dual, a white strain, showed better plumage than the Lohmann Tradition, a brown layer strain. This difference between the studies may be explained by the fact that Spindler et al. (2013) compared different layer strains while in the current study as well as in the study by Giersberg et al. (2017), a layer strain was compared to a dual-purpose strain. Higher activity level (Kjaer et al., 2001; but see Krause et al., 2019) and more fearful behavior (Rodenburg et al., 2013) may support the prevalence of feather pecking. Conventional brown layers are more active (Rieke et al., 2021) and also more fearful (Giersberg et al., 2020) in comparison to dual-purpose hens. Thus, the better plumage condition in LD compared to LT hens may be explained by a lower prevalence of feather pecking in LD compared to LT hens.

Unfortunately, our pen set up and also other housing conditions could not totally prevent feather damage as well as feather loss. It has been shown that in conventional high-performing laying hens, other arrangements such as additional enrichment, besides pecking blocks, must be offered in order to prevent the behavioral disorder feather pecking from the rearing period until the laying period (Campbell et al., 2018).

Foot pad lesions were not found at the end of the rearing period in either strain, but were present at the end of the observation period. This is in accordance with Baldinger and Bussemas (2021b) who found no foot pad lesions during rearing but found lesions at the end of the laying period in all strains. In our study, LT hens showed fewer lesions in comparison to the LD hens. This was unexpected as Eusemann et al. (2018b) found that nonegg laying hens had better foot pad states than egg laying hens. Thus, we had suspected that the lower laying performance in LD compared to LT hens may be beneficial for foot pad health. Thus, it is clear that other factors affect the foot pad state as well. In this study, we did not distinguish between specific lesions such as bumble foot, and toe and claw damage. This may be considered in future studies.

#### CONCLUSION

The present study showed that under the tested circumstances, the dual-purpose strain Lohmann Dual (LD) with its moderate laying performance had a similar prevalence and severity of keel bone damage compared to the high performing commercial layer line Lohmann Tradition (LT). In both strains, the vast majority of fractures occurred in the caudal part of the keel bone. The dual-purpose chickens showed a better plumage condition compared to the laying strain, which may indicate a lower prevalence of feather pecking and cannibalism in these hens. The housing conditions, especially the furniture such as perches, that are used for layer lines, may not be appropriate for dual-purpose chickens in terms of size and shape. In general, elevated grids may be more suitable than perches as roosting places and could have a potential benefit for keel bone health. Taken together, the dual-purpose strain LD is not only an alternative to the killing of male day-old chicks, but may also have a positive role in reducing feather pecking and cannibalism. However, based on our findings, other animal welfare problems may be as prevalent in LD as in LT hens (keel bone fractures and deviations) or even be more severe in LD hens (foot pad lesions). These problems may possibly be decreased by adapting the housing conditions and furniture to the size and needs of dualpurpose chickens rather than using the same furniture as for the larger layer lines.

## ACKNOWLEDGMENTS

We thank 2 anonymous reviewers for their helpful comments. We are grateful to the "Integhof" project and associated working groups. We thank the staff for taking care of the chickens and the technicians for helping during the realization and data analyzing of the different methods of our research station.

Author contributions: JM, LS, BE, and SP designed the study. JM and SP collected the data. JM, SP, and ETK analyzed the data. All authors contributed to writing of the manuscript.

Funding: The study was supported by funds from the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank (grant number: 28RZ3-72.050).

## DISCLOSURES

The authors declare no conflicts of interest.

## REFERENCES

- Ali, A. B. A., D. L. M. Campbell, D. M. Karcher, and J. M. Siegford. 2019. Nighttime roosting substrate type and height among 4 strains of laying hens in an aviary system. J. Poult. Sci. 98:1935–1946.
- Ali, A. B. A., D. L. M. Campbell, and J. M. Siegford. 2020. A risk assessment of health, production, and resource occupancy for 4 laying hen strains across the lay cycle in a commercial-style aviary system. J. Poult. Sci. 99:4372–4384.
- Alshamy, Z., K. Richardson, H. Huenigen, M. Hafez, J. Plendl, and S. Al Masri. 2018. Comparison of the gastrointestinal tract of dual-purpose to a broiler chicken line: A qualitative and quantitative macroscopic and microscopic study. PloSOne 13:e0204921.
- Baldinger, L., and R. Bussemas. 2021a. Dual-purpose production of eggs and meat – Part 1: cockerels of crosses between layer and meat breeds achieve moderate growth rates while showing unimpaired animal welfare. Org. Agri. 11:489–498.
- Baldinger, L., and R. Bussemas. 2021b. Dual-purpose production of eggs and meat-part 2: hens of crosses between layer and meat breeds show moderate laying performance but chose feed with less protein than a layer hybrid, indicating the potential to reduce protein diets. Org. Agr. 11:73–87.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.
- Baur, S., C. Rufener, M. J. Toscano, and U. Geissbühler. 2020. Radiographic evaluation of keel bone damage in laying hens – morphologic and temporal observations in a longitudinal study. Front. Vet. Sci. 7:129.
- Brendler, C., S. Kipper, and L. Schrader. 2014. Vigilance and roosting behaviour of laying hens on different perch heights. Appl. Anim. Behav. Sci. 1578(Supplement C):93–99.
- Brendler, C., and L. Schrader. 2016. Perch use by laying hens in aviary systems. Appl. Anim. Behav. Sci. 182:9–14.
- Buckner, G. D., W. M. Insko Jr., A. H. Henry, and E. F. Wachs. 1949. Rate of growth and calcification of the sternum of male and female New Hampshire chickens having crooked keels. Poult. Sci. 28:289–292.
- Campbell, D. L. M., E. N. de Haas, and C. Lee. 2018. A review of environmental enrichment for laying hens during rearing in relation to their behavioural and physiological development. Poult. Sci. 98:9–28.
- Campbell, D. L. M., M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016. Perch use by laying hens in a commercial aviary. Poult. Sci. 95:1736–1742.
- 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.
- Casey-Trott, T., J. Heerkens, M. Petrik, P. Regmi, L. Schrader, M. Toscano, and T. Widowski. 2015. Methods for assessment of keel bone damage in poultry. Poult. Sci. 94:2339–2350.
- Colson, S., C. Arnould, and V. Michel. 2008. Influence of rearing conditions of pullets on space use and performance of hens placed in aviaries at the beginning of the laying period. Appl. Anim. Behav. Sci. 111:286–300.
- Dawkins, M. 2004. Using behaviour to assess animal welfare. Anim. Welfare 13:3–7.
- de Haas, E. N., J. E. Bolhuis, B. Kemp, T. G. Groothuis, and T. B. Rodenburg. 2014. Parents and early life environment affect behavioral development of laying hen chickens. PloS One 9: e90577.
- Donaldson, C., M. Ball, and N. Connell. 2012. Aerial perches and freerange laying hens: the effect of access to aerial perches and of individual bird parameters on keel bon einjuries in commercial freerange laying hens. Poult. Sci. 91:304–315.
- Damme, K., Urselmans, S., and E. Schmidt. 2015. Economics of dualpurpose breeds-a comparison of meat and egg production using dual purpose breeds versus conventional broiler and layer strains. Accessed August 25, 2022. www.ltz.de/de-wAssets/docs/

lohmann-information/Lohmann-Information2\_2015\_Vol.-49-2-October-2015\_Damme.pdf 49, 4-9. (2021/04/21).

- EFSA. 2005. Welfare aspects of various systems of keeping laying hens. EFSA J. 197:1–23.
- Eusemann, B., U. Baulain, L. Schrader, C. Thöne-Reineke, A. Patt, and S. Petow. 2018a. Radiographic examination of keel bone damage in living laying hens of different strains kept in two housing systems. PloS One 13:e0194974.
- Eusemann, B. K., A. Patt, L. Schrader, S. Weigend, C. Thöne-Reineke, and S. Petow. 2020. The role of egg production in the etiology of keel bone damage in laying hens. Fron. Vet. Sci. 7:81.
- Eusemann, B. K., A. R. Sharifi, A. Patt, A. Reinhard, L. Schrader, C. Thöne-Reineke, and S. Petow. 2018b. Influence of a sustained release deslorelin acetate implant on reproduction physiology and associated traits in laying hens. Front. Physiol. 9:1846.
- FAWC. 2013. An open Letter to Great Britain Governments: Keel Bone Fractures in laying Hens. Farm Animal Welfare Committee, London.https://assetspublishing.service.gov.uk/government/ uploads/system/uploads/attachment\_data/file/324505/FAWC \_advice\_on\_keel\_bone\_fractures\_in\_laying\_hens.pdf (2021/ 11/01).
- Fox, J., and S. Weisberg. 2019. An {R} Companion to Applied Regression. Third Edition Sage, Thousand Oaks CA. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.
- Gebhardt-Henrich, S. G., and E. K. Fröhlich. 2015. Early onset of laying and bumblefoot favor keel bone fractures. Animals 5:1192– 1206.
- Giersberg, M., N. Kemper, and B. Spindler. 2019a. Pecking and piling: the behavior of conventional layer hybrids and dual-purpose hens in the nest. Appl. Anim. Behav. Sci. 214:50–56.
- Giersberg, M., B. Spindler, and N. Kemper. 2017. Assessment of plumage and integument condition in dual-purpose breeds and conventional layers. Animals 7:97.
- Giersberg, M. F., B. Spindler, and N. Kemper. 2019b. Linear space requirements and perch use of conventional layer hybrids and dual-purpose hens in an aviary system. Fron. Vet. Sci. 6:231.
- Giersberg, M.F., B. Spindler, and N. Kemper. 2020. Are dual-purpose hens less fearful than conventional layer hybrids? Vet. Rec. 187: e35-e35.
- Habig, C., U. Baulain, M. Henning, A. M. Scholz, A. R. Sharifi, S. Janisch, H. Simianer, and S. Weigend. 2017. How bone stability in laying hens is affected by phylogenetic background and performance level. Eur. Poult. Sci. 81:200.
- Habig, C., and O. Distl. 2013. Evaluation of bone strength, keel bone status, plumage condition and egg quality of two layer lines kept in small group housing systems. Brit. Poult. Sci. 54:413–424 pmid:23906215.
- Habig, C., M. Henning, U. Baulain, S. Jansen, A. Scholz, and S. Weigend. 2021. Keel bone damage in laying hens – its relation to bone mineral density, body growth rate and laying performance. Animals 11:1546.
- Habinski, A. M., L. J. Caston, T. M. Casey-Trott, M. E. Hunniford, and T. M. Widowski. 2017. Development of perching behaviour in 3 strains of pullets reared in furnished cages. Poult. Sci. 96:519– 529.
- Hardin, E., F. Castro, and W. Kim. 2019. Keel bone injury in laying hens: the prevalence of injuries in relation to different housing systems, implications, and potential solutions. World Poult. Sci. J. 75:285–292.
- Harlander-Matauschek. 2015Rodenburg, T., V. Sandilands, B. Tobalske, and M. Toscano. 2015. Causes of keel bone damage and their solutions in laying hens. World Poult. Sci. J. 71:461–472.
- Heerkens, J. L. T., E. Delezie, B. Ampe, T. B. Rodenburg, and F. A. M. Tuyttens. 2016b. Ramps and hybrid effects on keel bone and foot pad disorders in modified aviaries for laying hens. Poult. Sci 95:2479–2488.
- Heerkens, J. L. T., E. Delezie, T. B. Rodenburg, I. Kempen, J. Zoons, B. Ampe, and F. A. M. Tuyttens. 2016a. Risk factors assicuated with keel bone and foot pad disorders in laying hens housed in aviary systems. Polutry Sci. 95:482–488.
- Heikkilä, M., A. Wichmann, S. Gunnarsson, and A. Valros. 2006. Development of perching behaviour in chicks reared in enriched environment. Appl. Anim. Behav. Sci. 99:145–156.

- Hocking, P. M., M. Bain, C. E. Channing, R. Fleming, and S. Wilson. 2003. Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. Brit. Poult. Sci. 44:365–373.
- Icken, W., and M. Schmutz. 2013. Lohmann dual-layer and broiler at the very same time. Poult. New Lohmann Tierzucht 2:8–10 (2020/ 10/06).
- Jung, L., K. Niebuhr, L. K. Hinrichsen, S. Gunnarsson, C. Brenninkmeyer, M. Bestman, J. Heerkens, P. Ferrari, and U. Knierim. 2019. Possible risk factors for keel bone damage in organic laying hens. Animal 13:2356–2364.
- Kerschnitzki, M., T. Zander, P. Zaslansky, P. Fratzl, R. Shahar, and W. Wagermaier. 2014. Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone 69:109–117.
- Kittelsen, K. E., P. Gretarsson, P. Jensen, J. P. Christensen, I. Toftaker, R. O. Moe, and G. Vasdal. 2021. Keel bone fractures are more prevalent in White Leghorn hens than in Red Jungle fowl hens—a pilot study. Plos One 16:e0255234.
- Kjaer, J. B., P. Sørensen, and G. Su. 2001. Divergent selection on feather pecking behaviour in laying hens (Gallus gallus domesticus). Appl. Anim. Behav. Sci. 71:229–239.
- EC. 2021. European Commission revision of the animal welfare legislation. Accessed August 25, 2022. https://ec.europa.eu/food/ani mals/animal-welfare/evaluations-and-impact-assessment/revi sion-animal-welfare-legislation en (2022/06/30)
- Knierim, U., Andersson, R., Keppler, C., Petermann, S., Rauch, E., Spindler, B., and R. Zapf. 2016. Tierschutzindikatoren: Leitfaden für die Praxis-Geflügel. KTBL e.V., Darmstadt, Germany.
- Koenig, M., G. Hahn, K. Damme, and M. Schmutz. 2012. Utilization of laying-type cockerels as "coquelets": influence of genotype and diet characteristics on growth performance and carcass composition. Arch. Geflueglkd. 76:197–202.
- Krause, E. T., L. Phi-van, A. Dudde, L. Schrader, and J. B. Kjaer. 2019. Behavioural consequences of divergent selection on general locomotor activity in chickens. Behav. Process. 169:103980.
- Krautwald-Junghanns, M., K. Cramer, B. Fischer, A. Förster, R. Galli, F. Kremer, E. Mapesa, S. Meissner, R. Preisinger, and G. Preusse. 2017. Current approaches to avoid the culling of dayold male chicks in the layer industry, with special reference to spectroscopic methods. Poult. Sci. 97:749–757.
- Lambertz, C., K. Wuthijaree, and M. Gauly. 2018. Performance, behavior, and health of male broilers and laying hens of 2 dual-purpose chicken genotypes. Poultry Sci. 97:3564-3576.
- Lieboldt, M. A., I. Halle, J. Frahm, L. Schrader, U. Baulain, M. Henning, R. Preisinger, S. Dänicke, and S. Weigend. 2015. Phylogenic versus selection effects on growth development, egg laying and egg quality in purebred laying hens. Eur. Poult. Sci. 2015:79.
- Lohmann Breeders, (2020). Lohmann Tradition Performance data. Lohmann Breeders. https://lohmann-breeders.com/strains/loh mann-tradition-alternative-housing/ (2021/04/21).
- Malchow, J., J. Berk, B. Puppe, and L. Schrader. 2019. Perches or grids? What do rearing chickens differing in growth performance prefer for roosting? Poult. Sci 98:29–38.
- Malchow, J., and L. Schrader. 2021. Effects of an elevated platform on welfare aspects in male conventional broilers and dual-purpose chickens. Front. Vet. Sci. 8:660602.
- Mueller, S., L. Taddei, D. Albiker, M. Kreuzer, M. Siegrist, R. E. Messikommer, and I. D. M. Gangnat. 2020. Growth, carcass, and meat quality of 2 dual-purpose chickens and a layer hybrid grown for 67 or 84 D compared with slow-growing broilers. J Appl. Poult. Res. 29:185–196.
- Nasr, M., C. Nicol, and J. Murrell. 2012. Do laying hens with keel bone fractures experience pain? PloSOne 7:e42420.
- Niebuhr, K., Zaludik, B., Baumung, I., Thenmaier, A., Lugmair, R., and J. Troxler. 2006. Untersuchungen zum Auftreten von Kannibalismus und Federpicken in alternativen Legehennenhaltungen in Österreich-Empfehlungen für die Praxis. Online Fachzeitschrift des Bundesministeriums für Land- Und Forstwirtschaft, Umwelt und Wasserwirtschaft: Vienna, Austria. 1-21.
- Ministère de l'Agriculture. 2021. Ministère de l'Agriculture et de l'Alimentation – Communiquè de presse: La France sera le premier pays au monde, avec l'Allemagne, à mettre fin à l'élimination des poussins mâles. Accessed August 25, 2022. https://agriculture.

gouv. fr/la-france-sera-le-premier-pays-au-monde-avec-lallemagne-mettre-fin-lelimination-des-poussins-males (2022/06/30).

- Olsson, I. A. S., and L. J. Keeling. 2000. Night-time roosting in laying hens and the effect of thwarting access to perches. Appl. Anim. Behav. Sci. 68:243–256.
- Petrik, M., M. Gierin, and T. Widowski. 2015. On-farm comparison of keel fracture prevalence and other welfare indicators in conventional cage and floor-housed laying hens in Ontario. Cananda. Poult. Sci. 94:579–585.
- Pickel, T., L. Schrader, and B. Scholz. 2011. Pressure load on keel bone and foot pads in perching laying hens in relation to perch design. Poult. Sci. 90:715–724.
- Pinheiro J., Bates D., DebRoy S., Sarkar D., R Core Team. 2020. \_nlme: linear and nonlinear mixed effects models\_. R package version 3.1-151. Accessed November 11, 2021. https://cran.r-project. org/web/packages/nlme/nlme.pdfhttps://svn.r-project.org/Rpackages/trunk/nlme/.
- R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rentsch, A., C. Rufener, C. Spadavecchia, A. Stratmann, and M. Toscano. 2019. Laying hen's mobility is impaired by keel bone fractures and does not improve with paracetamol treatment. Appl. Anim. Behav. Sci. 216:19–25.
- Riber, A., T. Casey-Trott, and M. Herskin. 2018. The influence of keel bone damage on welfare of laying hens. Fron. Vet. Sci. 5:6.
- Rieke, L., B. Spindler, I. Zylka, N. Kemper, and M. F. Giersberg. 2021. Pecking behavior in conventional layer hybrids and dual-purpose hens throughout the laying period. Fron. Vet. Sci. 8:660400.
- Rodenburg, T. B., M. M. van Krimpen, I. C. de Jong, E. N. de Hass, M. S. Kops, B. J. Riedstra, R. E. Nordquist, J. P. Wagenaar, M. Bestmann, and C. J. Nicol. 2013. The prevention and control of feather pecking in laying hens: identifying the underlying principles. World Poult. Sci. J. 69:361–373.
- Röhe, I., J. Urban, A. Dijkslag, J. Te Paske, and J. Zentek. 2019. Impact of an energy- and nutrient-reduced diet containing 10% lignocellulose on animal performance, body composition and egg quality of dual purpose laying hens. Arch. Anim. Nutr. 73:1–17.
- Rufener, C., S. Baur, A. Stratmann, and M. J. Toscano. 2018. A reliable method to assess keel bone fractures in laying hens from radiographs using a tagged visual analogue scale. Fron. Vet. Sci. 5:124.
- Rufener, C., and M. Makagon. 2020. Keel bone fractures in laying hens: a systematic review of prevalence across age, housing systems, and strains. J. Anim. Sci. 98:S36–S51.
- Schrader, L. and J. Malchow. 2020. The role of perches in chicken welfare. In: Nicol C, editor. Understanding the Behaviour and Improving the Welfare of Chickens. Oxford, England: Burleigh dodds Science Publishing (2020). p. 375–416.
- Schrader, L., and B. Müller. 2009. Night-time roosting in the domestic fowl: the height matters. Appl. Anim. Behav. Sci. 121:179–183.
- Sepeur, S., B. Spindler, M. Schulze-Bisping, C. Habig, R. Andersson, M. Beyerbach, and N. Kemper. 2015. Comparison of plumage condition of laying hens with intact and trimmed beaks kept on commercial farms. Europ. Poult. Sci 79:1612, doi:10.1399/ eps.2015.116.
- Spindler, B., M. Schulze-Hillert, and J. Hartung. 2013. Praxisbegleitende Untersuchungen zum Verzicht auf Schnabelkürzen bei Legehennen in Praxisbetrieben. Abschlussbericht, Hanover, Germany.
- Stratmann, A., E. Fröhlich, A. Harlander-Matauschek, L. Schrader, M. Toscano, H. Würbel, and S. Gebhardt-Henrich. 2015a. Soft perches in an aviary system reduce incidence of keel bone damage in laying hens. PloSOne 10:e0122568.
- Stratmann, A., E. K. F. Fröhlich, S. G. Gebhardt-Henrich, A. Harlander-Matauschek, H. Würbel, and M. J. Toscano. 2015b. Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. Appl. Anim. Behav. Sci. 165:112–123.
- Thøfner, I. C. N., J. Dahl, and J. P. Christensen. 2021. Keel bone fractures in Danish layhin hens: prevalence and risk factors. PLoS One 16:e0256105.
- Tiemann, I., S. Hillemacher, and M. Wittmann. 2020. Are dualpurpose chickens twice as good? Measuring performance and animal welfare throughout the fattening period. Animals 10:1980.

- TierSchG, (2021). Tierschutzgesetz (In German). https://www.gese tze-im-internet.de/tierschg/BJNR012770972.html (2022/06/30).
- Vits, A., D. Weitzenbürger, H. Hamann, and O. Distl. 2005. Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes. Poult. Sci. 84:1511–1519 pmid:16335118.
- Wahlström, A., R. Tauson, and K. Elwinger. 2001. Plumage condition and health of aviary-kept hens fed mash or crumbled pellets. Poult. Sci. 80:266–271 pmid:11261554.
- Weeks, C. A., and C. J. Nicol. 2006. Behavioural needs, priorities and preferences of laying hens. World Poult. Sci. J. 62:296–307.
- Wichmann, A., M. Heikkilä, A. Valros, B. Forkman, and L. J. Keeling. 2007. Perching behaviour in chickens and it relation to spatial ability. Appl. Anim. Behav. Sci. 105:165–179.
- Wilkins, L. J., J. L. McKinstry, N. C. Avery, T. G. Knowles, S. N. Brown, J. Tarlton, and C. J. Nicol. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet. Rec. 169:414.
- Wood-Gush, D. G. M., and I. J. H. Duncan. 1976. Some behavioural observations on domestic fowl in the wild. Appl. Anim. Ethol. 2:255–260.