Strain differences and effects of different stocking densities during rearing on the musculoskeletal development of pullets

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ABSTRACT There are few published studies on the effect of stocking density (**SD**) of pullets, particularly between different genetic lines. The objectives of this study were to determine if strain or SD affects musculoskeletal development of pullets and determine any impact on the productivity and keel bone health of adult hens. Lohmann Selected Leghorn Lite (**LSL**), Dekalb White (**DW**), and Lohmann Brown (**LB**) pullets were reared at 4 different SD (247 cm^2/bird, 270 cm^2/bird, 299 cm^2/bird, and 335 cm^2/bird) in large cages furnished with elevated perches and a platform. At 16 wk of age, the keel bone, the muscles of the breast, wings, and legs, and the long bones of the wings and legs were collected to compare keel bone development, muscle growth, and bone breaking strength (**BBS**) between strain (adjusted for bodyweight) and SD treatments. Stocking density did not have an effect on the metasternum length, height, or area of the keel bone, the weights of the bicep brachii, pectoralis major or pectoralis minor, or the BBS of any of the selected bones. However, strain differences were found for all keel bone characteristics, all muscle weights, and the majority of BBS measures. The keel metasternum, height, and overall area of the keel bone were found to be smaller in LB pullets compared with LSL and DW pullets ($P < 0.0001$); however, cartilage length and overall percentage of the cartilage present on the keel bone was greatest in LB pullets ($P < 0.0001$). Leg muscles were heaviest in LB pullets ($P < 0.05$); however, breast muscles were heavier in LSL and DW pullets ($P < 0.0001$). Lohmann Brown pullets had lower BBS of the tibia ($P < 0.0001$) and femur ($P < 0.0001$) compared with LSL and DW pullets, whereas DW pullets had greater BBS of the humerus ($P = 0.033$). Additionally, there was a higher prevalence of keel bone fractures at 50 wk of age in LB hens compared with DW ($P = 0.0144$). Overall, SD during rearing used in this study had little impact on the musculoskeletal growth of pullets; however, significant differences were found between strains which may reflect strain-specific behavior. Additionally, differences in keel bone development between strains may lead to differences in keel bone damage in adult hens.

Key words: pullet, keel bone, musculoskeletal growth, rearing stocking density, strain

2020 Poultry Science 99:4153–4161
https://doi.org/10.1016/j.psj.2020.05.046

INTRODUCTION

Over the last few years, more attention has been drawn to the rearing phase of commercial laying hens to proactively address concerns that arise during the adult stage, such as bone weakness and keel bone damage. Widowski and Torrey (2018) stress the importance of early life experiences on birds', in particular developing layer pullets', ability to adapt to changes in their environment, and hence be better able to handle stresses later in life when the hens' environments can be complex and varied. It is widely recognized that differences in rearing environment, such as providing early access to perches (Enneking et al., 2012; Hester et al., 2013) or providing a more complex rearing environment to allow increased opportunities for exercise (Casey-Trott et al., 2017a), can play a significant role on bone strength and can have lifelong effects on the structural bones of hens. In particular, pullets reared in aviary systems have superior bone characteristics at 16 wk of age compared with pullets reared in conventional cages (Regmi et al., 2015; Casey-Trott et al., 2017a), which has been shown to carry out until the end-of-lay (Regmi et al., 2016; Casey-Trott et al., 2017b). These differences in musculoskeletal characteristics were likely attributed to more opportunity to exercise and to perform load-bearing activities in the aviary system, such as running, jumping, wing-flapping, and flying. Therefore, these activities during rearing may be crucial

Received January 29, 2020.

Accepted May 22, 2020.

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to lay down a solid framework of bone to prevent bone weakness and subsequent fractures in the production period (Regmi et al., 2015). However, owing to very limited research on stocking density (**SD**) for pullets, it is unknown if differences in SD during rearing would similarly have a significant effect on musculoskeletal development. Therefore, the first objective of this study was to determine if pullets reared at different SD in furnished rearing cages had an effect on musculoskeletal development.

There is also very limited research on musculoskeletal development between different strains, particularly differences in keel bone development. Strain comparisons of bone characteristics, including the keel bone, have mostly been performed in adult hens during the laying period or at the end-of-lay. Previous studies have indicated that brown strains have greater bone breaking strength (**BBS**) of the humerus (Riczu et al., 2004; Vits et al., 2005; Habig and Distl, 2013), the tibia (Habig and Distl, 2013), and the femur (Riczu et al., 2004) compared with white strains of laying hens. Additionally, studies comparing keel bone damage between different genetic lines throughout the production cycle and at the end-of-lay have described significant differences between brown and white strains of laying hens. Previous studies have shown that brown hens have significantly more frequent and more severe keel bone deviations (Vits et al., 2005; Habig and Distl, 2013) and more keel bone fractures (Heerkins et al., 2016; Eusemann et al., 2018) in comparison to white strains. Stratmann et al. (2015) found higher keel bone damage in ISA brown hens at the start of production (18 wk) compared with Dekalb White (**DW**) hens, but at the end of production found that these results reversed. Descriptions of keel bone development and musculoskeletal growth in different strains of pullets have not been previously described and may provide insight into the development and occurrence of keel bone damage in adult hens. Therefore, the second objective of this study was to determine if there were strain differences in musculoskeletal development between Lohmann Brown (**LB**), Lohmann Selected Leghorn Lite (**LSL**), and DW pullets. We hypothesized that 1) pullets reared at a lower density would have superior musculoskeletal qualities compared with pullets reared at a higher density because of more space and opportunities to move and exercise and 2) larger strains of birds would be more affected by density than smaller strains, and 3) LB pullets would have stronger bones than the white strains but a higher proportion of keel bone damage as adult hens.

MATERIALS AND METHODS

Animal Use Approval

All procedures undertaken in this research project were approved by the University of Guelph Animal Care Committee under Animal Utilization Protocol #3607 and in accordance with guidelines from the Canadian Council on Animal Care.

Pullet Housing and Management

This study was conducted at the Arkell Poultry Research Station (Arkell, ON, Canada) where 48 Farmer Automatic Pullet Combi-Cages (Clark Ag Systems; Caledonia, Ontario, Canada) (Figure 1) were distributed between 2 rooms. All chicks were obtained at 1 D of age from a commercial hatchery. Dekalb White (**DW**) chicks hatched 3 D later than Lohmann Brown (**LB**) and Lohmann Selected Leghorn Lite (**LSL**) chicks. One thousand two hundred sixty-four birds of each strain were used, totaling to 3,792 chicks. Chicks of each strain were divided into cages of 4 different stocking densities (**SD**), where SD was manipulated through adjusting the number of birds per cage. Stocking density was therefore confounded with group size. The initial SD were double the finishing SD, as number of birds per cage was split in half between top and bottom tiers at 6 wk of age. Following the split, the SD and number of birds per cage were 247 cm^2/bird, 91; 270 cm^2/bird, 83; 299 cm^2/bird, 75; and 335 cm^2/bird, 67. The dimensions (239 cm W × 55 cm D × 84 cm H) and total floor space of each cage (22,439 cm^2) was held constant, and there were 3 to 4 replicates per strain. The feeder space was held constant at 2,729 cm^2. A platform (6,065 cm^2) and 3 length-wise circular perches were provided in each cage to allow perching opportunities.

Birds used in this study were reared as part of another research project and managed in accordance with a protocol determined by Jensen (2019) from day 1 to 16 wk of age. During the first week, the pullets received an intermittent lighting schedule of 4 h of light followed by 2 h of darkness. Lighting was reduced gradually between 2 and 7 wk from 18 h down to 10 h of light per day to 16 wk. Chicks were started at 32°C, and the temperature gradually reduced to ∼20°C by 4 wk. Standard crumble diets were fed ad lib, pullet starter (0–42 D), grower (42–112 D), and layer breeder (112 to end of trial). The Arkell Poultry Research Station Standard Operating Procedures vaccination protocol was followed.

Sample Collection and Measurement

At 16 wk of age, 2 birds per replicate cage were randomly selected and euthanized by a nonpenetrating captive bolt (Zephyr—EXL, Bock Industries Inc.

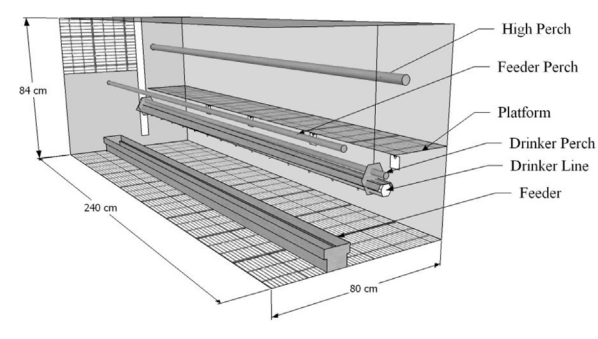

Figure 1. Side view of rearing cages where pullets were housed. From Figure 1 in Habinski et al. 2017.

Animal Welfare Division, 128 North Front Street, Philipsburg, PA 16866 USA). This amounted to 8 pullets per treatment–strain combination. Before euthanasia, pullets were restrained in a Velcro wrap to prevent bone breakage because of wing flapping during convulsions. Carcasses were stored at −20°C.

Before sample collection, carcasses were thawed and weighed. Bone and muscle tissue collection was performed as per Casey-Trott et al. (2017a). The pectoralis major, pectoralis minor, and combination of all leg muscles (of the femur: iliotibialis, sartorius, semitendinosus, semimembranosus, quadriceps femoris, ambiens, adductor longus; of the tibiotarsus: gastrocnemius, tibialis anterior, peroneus longus, flexor perforans et perforates II & III) were removed from the right side of each pullet, and the bicep brachii was removed from the left side of each pullet. Immediately after removal, all muscles were weighed.

Following muscle collection, the left radius and humerus and right tibia and femur were removed to test BBS (N). The keel bone was also removed to measure length of the keel metasternum (mm), height from the ventral surface to the Carina apex (mm), and length of the cartilage (mm), measured from the tip of the cartilaginous tip to the start of ossified bone. These measurements were taken immediately following extraction, and measurements were taken as per Casey-Trott et al. (2017a). These measurements were taken with Fisher Science Education Traceable Digital Carbon Fiber Calipers (Fisher Scientific, Toronto, Ontario, Canada). Total percentage of cartilage on the keel bone was also calculated using the length of the keel metasternum and the length of the cartilage. Area of the keel bone was calculated as the area of a triangle (area = base x height × ½). For a more precise and accurate measure, area of the keel bone was additionally calculated using ImageJ 1.46r program (U.S. National Institutes of Health, Bethesda, MD). A digital image of each keel bone was taken from a distance of 19.4 cm between camera lens and bone. Images were uploaded to ImageJ. The scale used was 10pixels/mm. All keel bones were carefully outlined, and the area was calculated by the ImageJ program (mm^2).

Three-Point Bone Breaking Strength

An Instron system (Model #5969; Instron Material Testing, Norwood, MA) with Bluehill Universal software was used to measure BBS of the femur, tibia, radius, and humerus. Each bone was positioned in the same orientation on a cradle support with posts 5 cm apart. A 50 kN load cell at a speed of 100 mm/minute was used to apply force from the 3-point bending test fixture (10 mm anvils and 50 mm in length) to the mid-point of the bone shaft. The maximum force that was required to break the bone was recorded (N).

Layer Housing and Management

At 16 wk of age, DW and LB pullets that were reared at SD of 247 cm^2/bird and 299 cm^2/bird were transferred to Farmer Automatic Enrichable/Enriched Housing System Cages (Clark Ag Systems; Caledonia, ON, Canada) (Figure 2) at the Arkell Poultry Research Station. Owing to limited facilities and space, the LSL strain was not used in this study past 16 wk of age. Two cage sizes were used, large (358 × 122 cm) and small (178 × 122 cm). Refer to Widowski et al. (2017) for description of cages. Stocking density was held at 748 cm^2/bird, such that group sizes were either 30 or 60 birds. There were a total of 24 cages distributed between 2 rooms, with 3 tiers and 2 rows of cages in each room. Each room contained 6 large cages (3 DW and 3 LB) and 6 small cages (3 DW and 3 LB). Strain and rearing SD were balanced across room and tier.

All hens were fed layer ration as course crumbles (18% crude protein, 4.24% calcium, 0.68% phosphorus). Birds were exposed to 11 h of light per day during week 16 and 17, at an intensity of 5 lux. From 18 to 20 wk of age, birds were exposed to 12 h of light per day, at an intensity of 30 lux. From 20 wk to the end-of-lay, hens were exposed to 14 h of light per day, at an intensity of 30 lux. The temperature of both rooms was kept at approximately 20.0°C.

Keel Bone Assessments of Hens

At 35 and 50 wk of age, 20% of birds in each cage were randomly selected to evaluate keel bone damage, including keel bone deviations and fractures. Two trained individuals scored keel bone deviations and fractures through palpation of the keel bone. A keel bone deviation was identified if abnormal curvature of the keel bone deviated from a theoretically perfect 2-dimensional straight plane in either the transverse or sagittal planes (Casey-Trott et al., 2015). A keel bone fracture was identified if, on palpation, a bony callus was present on the ventral or lateral surfaces of keel bone, which is a result of the regenerative healing process that takes place after the fracture occurs (Casey-Trott et al., 2015). Birds were scored as either positive or negative for a deviation or a fracture.

Statistical Analysis

All statistical analyses were completed using SAS statistical software, version 9.4 (SAS Institute, Inc., Cary, NC). The level of statistical significance of difference was set a $P < 0.05$.

All muscle weights (g/kg), keel bone measurements (mm/kg), and BBS (N/kg) were adjusted for pullet body weight before analysis. To assess the effects of rearing SD and strain on the muscle and keel bone characteristics, generalized linear mixed model analyses (Proc GLIMMIX) were performed. Bone breaking strength data were analyzed using a generalized linear model procedure (Proc GLM). For all analyses, strain, SD, and strain by SD interaction were included in the model, and means were separated using the method of Least Squares. A correlation (Proc CORR) was performed to determine the strength of the association between the

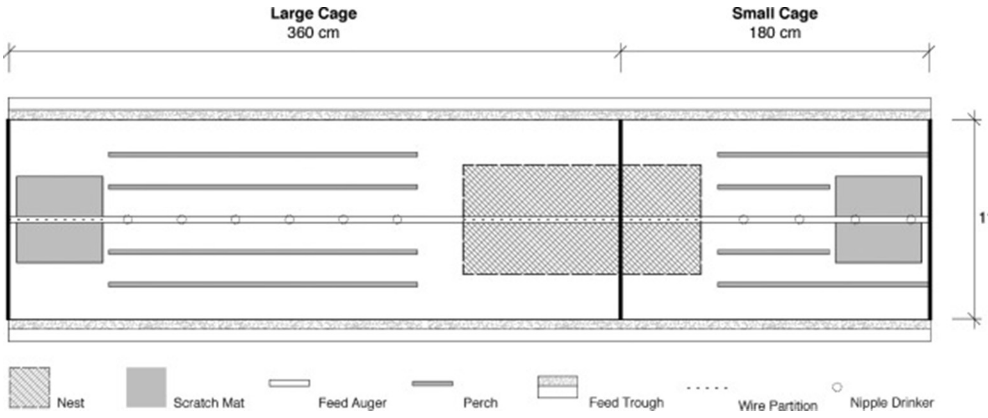

Figure 2. Top view of 1 tier of cages for adult housing: 1 large cage (left) and 1 small cage (right). The legend depicts all resources in the cages. Adapted from Figure 1 in Widowski et al., 2017.

2 methods used for measuring keel bone area (area of triangle vs. imageJ analysis). Proportion of birds in a cage scoring positive for keel deviation and fracture at 35 and 50 wk of age were analyzed using mixed model analyses of variance (Proc Mixed) for effect of strain, rearing SD, and their interaction. Cage size and location were initially included and subsequently removed from the model because they were not significant. Age was included in the model as a repeated measure.

RESULTS

Muscle and Bone Analyses

There was no difference in mean BW at 16 wk of age ($P = 0.6678$) between rearing SD of 247 cm^2 (1.3 kg ± 0.02), 270 cm^2 (1.3 kg ± 0.02), 299 cm^2 (1.3 kg ± 0.02), and 335 cm^2 (1.3 kg ± 0.02) per bird. There was a difference in mean BW ($P < 0.0001$) between LB (1.57 kg ± 0.01 SE), DW (1.24 kg ± 0.01), and LSL (1.17 kg ± 0.01). There was no interaction between treatment groups and strain of birds ($P = 0.8352$).

Stocking density during rearing did not affect musculoskeletal characteristics, except for leg muscle weights and amount of cartilage on the keel (see Tables 1 and 2). Pullets reared at the lowest SD had the shortest cartilage length ($P = 0.023$) and the lowest percent of cartilage on the keel bone ($P = 0.010$). Pullets at the lowest SD also had the heaviest leg muscles; however, the results were not linear, and this was only significantly different than birds reared at the second lowest SD. Stocking density had no effect on BBS.

Table 1. Comparison of keel bone characteristics at 16 wk of age for different strains of pullets reared in furnished cages at different stocking densities.

Treatment	Keel bone characterisitics[1] (±SE)					
	Metasternum[2] length (mm/kg)	Height[3] (mm/kg)	Area[4] (mm^2/kg)	Area[5] (mm^2/kg)	Cartilage[6] length (mm/kg)	Cartilage[7] percentage (%)
SD (cm^2/bird)						
247	70.2 (0.96)	23.6 (0.39)	1,088.7 (19.79)	1,950.5 (32.48)	22.1 (0.77)^{a,b}	32.1 (0.98)^{a}
270	70.2 (1.11)	24.0 (0.45)	1,093.3 (22.86)	1,937.2 (37.50)	22.6 (0.89)^{a}	32.8 (1.13)^{a}
299	71.9 (0.96)	24.4 (0.39)	1,122.3 (19.79)	1,950.5 (32.48)	22.0 (0.77)^{a,b}	31.3 (0.98)^{a,b}
335	70.2 (0.96)	23.4 (0.39)	1,068.0 (19.79)	1,979.1 (30.23)	19.3 (0.77)^{b}	28.0 (0.98)^{b}
Strain[8]						
LB	59.8 (0.87)^{c}	20.5 (0.35)^{b}	958.8 (17.84)^{b}	1,724.8 (28.59)^{b}	23.9 (0.70)^{a}	39.8 (0.88)^{a}
DW	77.8 (0.87)^{a}	25.8 (0.35)^{a}	1,164.5 (17.84)^{a}	2,109.4 (29.27)^{a}	22.1 (0.70)^{a}	28.4 (0.88)^{b}
LSL	74.2 (0.87)^{b}	25.3 (0.35)^{a}	1,156.1 (17.84)^{a}	2,055.3 (28.59)^{a}	18.5 (0.70)^{b}	25.0 (0.88)^{c}
P-values						
SD	0.559	0.268	0.299	0.712	0.023	0.010
Strain	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Strain × SD interaction	0.855	0.962	0.687	0.362	0.847	0.834

[a-c]LSmeans within column and within SD or Strain rows with different superscripts are significantly different ($P < 0.05$).
Abbreviation: SD, stocking density.
[1]All keel bone characteristics were adjusted for pullet BW.
[2]Length measured on the dorsal metasternum surface parallel to the cranial region of the sternal notch ending at the caudal border of the keel metasternum tip.
[3]Height measured from the ventral surface of the metasternum to the peak of the Carina apex.
[4]Area of keel estimated using the formula for area of a right triangle: Area = (metasternum length × height) × ½
[5]Area of keel using ImageJ.
[6]Cartilage length measured on the dorsal metasternum from the line of distinction between the end of ossified bone tissue and initiation of cartilage tissue to the end of the caudal tip of the keel metasternum.
[7]Percent cartilage= (cartilage length/metasternum length) × 100.
[8]LB = Lohmann Brown; DW = Dekalb White; LSL = Lohmann Selected Leghorn-Lite.

Table 2. Comparison of muscle weights between SD and strain of pullets at 16 wk of age.

Treatment	Muscle weights, g/kg[1] (±SE)			
	Bicep brachii	Pectoralis major	Pectoralis minor	Leg muscle group
SD (cm[2]/birds)				
247	2.0 (0.03)	42.9 (0.45)	15.8 (0.19)	84.9 (0.65)[a,b]
270	2.1 (0.04)	43.7 (0.53)	15.9 (0.22)	85.7 (0.75)[a,b]
299	2.0 (0.03)	43.8 (0.45)	15.8 (0.19)	83.4 (0.65)[b]
335	2.1 (0.03)	43.4 (0.45)	15.9 (0.19)	86.1 (0.65)[a]
Strain[3]				
LB	2.1 (0.03)[a]	41.1 (0.41)[c]	14.5 (0.17)[b]	88.9 (0.58)[a]
DW	2.1 (0.03)[a,b]	45.4 (0.41)[a]	16.8 (0.17)[a]	83.2 (0.58)[b]
LSL	2.0 (0.03)[b]	43.8 (0.41)[b]	16.3 (0.17)[a]	82.9 (0.58)[b]
P-value				
SD	0.883	0.505	0.914	0.035
Strain	0.014	<0.0001	<0.0001	<0.0001
Strain × SD interaction	0.552	0.171	0.210	0.573

[a-c]LSmeans within column and within SD or Strain with different superscripts are significantly different ($P < 0.05$).
Abbreviation: SD, stocking density.
[1]All muscle weights were adjusted for pullet BW.
[2]Leg muscle group comprised of all femur and tibiotarsus muscles of the femur: iliotibialis, sartorius, semitendinosus, semimembranosus, quadriceps femoris, ambiens, adductor longus; of the tibiotarsus: gastrocnemius, tibialis anterior, peroneus longus, flexor perforans et perforates II & III.
[3]LB = Lohmann Brown; DW = Dekalb White; LSL = Lohmann Selected Leghorn-Lite.

Strain differences were found for all keel bone characteristics and all muscle weights (Tables 1 and 2), but there were no SD by strain interactions. When adjusted for body weight, length of the metasternum, height, and area of the keel bone were smaller in LB pullets compared with LSL and DW ($P < 0.0001$). However, LB pullets had a higher percentage of cartilage on the keel bone than both DW and LSL pullets, and DW pullets had a greater percentage of cartilage than LSL pullets ($P < 0.0001$). Additionally, leg muscles were heavier in LB pullets compared with both LSL and DW pullets ($P < 0.0001$), and bicep muscles of LB pullets were greater than LSL pullets ($P = 0.014$). However, DW and LSL pullets had heavier pectoralis major and minor muscles than LB pullets ($P < 0.0001$). Strain differences were also found in all BBS measures except the radius (Table 3). When adjusted for BW, LB pullets had lower BBS for the femur ($P < 0.0001$) and tibia ($P < 0.0001$) compared with DW and LSL. Dekalb White pullets had greater BBS of the humerus ($P = 0.033$) compared with LB and LSL, but this was mainly because of an interaction ($P < 0.03$) between strain and SD. Dekalb White pullets reared at 246 cm^2 and 299 cm^2 per bird had greater BBS than all other strain by SD combinations.

The correlation between keel bone area calculated by triangulation from measurements by calipers and those determined by tracing a digital image using imageJ for values from all 3 strains combined was 0.735 ($P < 0.0001$). When strains were analyzed separately, correlation coefficients were lower (DW 0.3906, $P = 0.0298$; LSL 0.3492, $P = 0.0542$; LB 0.5392, $P = 0.0010$), but still significant for all 3 strains. This is in contrast to visual inspection, as the keels of the white strains have a shape more closely resembling a triangle (Figure 3). Overall, however, the 2 methods gave largely the same results.

Table 3. Comparison of maximum BBS between SD and strain of pullets at 16 wk of age.

Treatment	Maximum bone breaking strength, N/kg[1] (±SE)			
	Femur (±SE)	Tibia (±SE)	Humerus (±SE)	Radius (±SE)
SD (cm[2]/birds)				
247	137.0 (4.07)	157.1 (3.33)	102.3 (3.08)	24.8 (0.56)
270	135.8 (4.70)	156.5 (3.84)	96.6 (5.56)	23.7 (0.64)
299	140.7 (4.07)	160.9 (3.33)	104.7 (3.08)	25.6 (0.56)
335	131.4 (4.07)	163.2 (3.33)	98.7 (3.08)	23.6 (0.56)
Strain[2]				
LB	115.4 (3.67)[a]	131.2 (3.00)[a]	97.2 (2.78)[a]	23.5 (0.50)
DW	148.6 (3.67)[b]	170.0 (3.00)[b]	106.8 (2.78)[b]	25.3 (0.50)
LSL	144.7 (3.67)[b]	177.1 (3.00)[b]	97.7 (2.78)[a]	24.5 (0.50)
P-values				
SD	0.449	0.480	0.321	0.058
Strain	<0.0001	<0.0001	0.033	0.056
Strain × SD interaction	0.537	0.068	0.031	0.312

[a-c]LSmeans within column and within SD or Strain rows with different superscripts are significantly different ($P < 0.05$).
Abbreviations: BBS, bone breaking strength; SD, stocking density.
[1]All maximum bone breaking strength values were adjusted for pullet BW.
[2]LB = Lohmann Brown; DW = Dekalb White; LSL = Lohmann Selected Leghorn-Lite.

Keel Bone Scores

At 35 wk of age, there was no difference in proportion of sampled birds with keel bone deviations ($P = 0.145$) or keel bone fractures ($P = 0.557$) between LB and DW hens (Table 4). No difference in keel bone scores was found between hens reared at different SD.

At 50 wk of age, a greater proportion of LB hens had keel bone fractures compared with DW hens ($P = 0.022$), but no difference was found for keel bone deviations (Table 4). No difference in keel bone scores was found between hens reared at different SD. Between 35 and 50 wk of age, hens showed significantly more keel bone deviations ($P = 0.0065$) and fractures ($P = 0.0216$).

DISCUSSION

Stocking Density Effects on Muscle Growth, Keel Bone Characteristics, and BBS

Stocking density had very little effect on the muscle growth, except on leg muscles. Pullets reared at the lowest SD were found to have the heaviest leg muscles, likely because of more space and opportunity to walk and run. However, the leg muscle weights were not linear with respect to other SD, nor did there appear to be a threshold for a difference in muscle weight. Therefore, this result is difficult to explain.

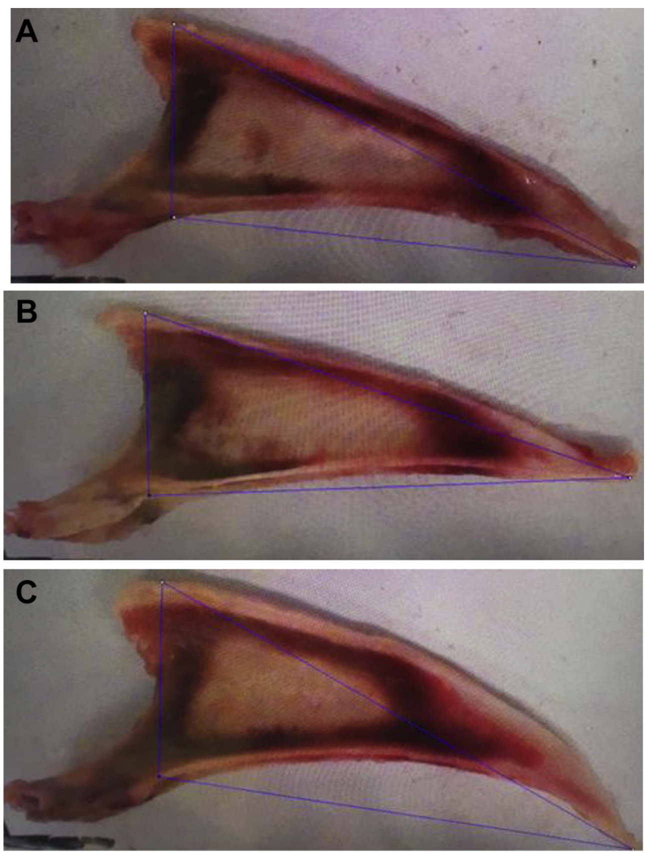

Figure 3. Digital images taken of keel bones from (A) LSL, (B) DW, and (C) LB pullets at 16 wk of age. The blue lines outline how the area of a triangle was calculated for each strain. Abbreviations: LSL, Lohmann Selected Leghorn Lite; DW, Dekalb White; LB, Lohmann Brown.

Stocking density also had very little effect on keel bone characteristics. Although no difference in size of the keel bone was found, the proportion of cartilage on the keel bone was greatest in pullets reared at the highest SD. However, these results are inconsistent with Casey-Trott et al. (2017a), who found that pullets reared in an aviary system had greater percent of cartilage on the keel bone compared with pullets reared in conventional cages. Differences found between Casey-Trott et al. (2017a) and the current study could be because of the greater differences in overall space and behavioral opportunities between an aviary system and a furnished cage. In the present study, no other effects of SD were found on the skeletal growth of pullets, including BBS. There are no previous reports on the effects of SD in pullets, although there are several reports for laying hens. Nicol et al. (2006) found no difference in bone strength at the end of the laying period between hens housed at 3 different SD (7, 9, 12 birds/m^2) in noncage systems, where hens have substantial opportunities for load-bearing exercise. Widowski et al. (2017) found no effects of housing laying hens at 520 vs. 748 cm^2 per hen in large furnished cages at different group sizes on BBS at 70 wk. No further inferences can be suggested on the overall skeletal development of pullets reared at the different SD used in this study. This may be because of either not providing large enough differences between the different SD to allow for behavioral differences or providing too high of SD in all treatments, thereby not providing enough space or opportunity for exercise. Casey-Trott et al. (2017a) suggested exercise to be the key driving factor for the relatively large and significant musculoskeletal differences between aviary and conventionally reared pullets.

However, a furnished cage does not provide the same opportunities for exercise (i.e., running, jumping, flying) as an aviary does. Campbell et al. (2019) carried out an extensive multifactorial review that focused on investigating various types of environmental enrichments during rearing and how they relate to behaviors and physiological development. Differences in rearing cage systems and enrichments provided were determined to be an important component for optimal laying hen performance. For example, with conventional cages, providing perches may offset the reduced amount of space available to birds and improve bone loading and bone mineralization during rearing (Enneking et al., 2012). As such, determining the best environment for the laying hen depends on what was offered to the birds during rearing to optimize skeletal development and avoid bird injuries.

Jensen (2019) performed live behavior analyses on pullets used in the current study for the first 16 wk and found that pullets reared at the highest density performed the least locomotive behavior but found no other significant behavioral differences between SD. There were few observations of wing-flapping. Therefore, it is likely that the space provided for all treatments in the current study limited the expression of behavior.

Table 4. Proportion of birds scoring positive for keel deviation and fracture at 35 and 50 wk of age.

Treatment	Keel bone damage (±SE)			
	35 wk deviations	35 wk fractures	50 wk deviations	50 wk fractures
Strain[1]				
LB	0.18 (0.03)	0.10 (0.03)	0.34 (0.06)	0.25 (0.04)[a]
DW	0.11 (0.02)	0.08 (0.03)	0.27 (0.05)	0.10 (0.03)[b]
Rearing SD				
299 cm^2/bird	0.14 (0.03)	0.08 (0.02)	0.33 (0.05)	0.19 (0.04)
247 cm^2/bird	0.15 (0.03)	0.11 (0.04)	0.28 (0.06)	0.16 (0.05)
P-values				
Strain	0.145	0.557	0.220	0.022
SD	0.673	0.670	0.776	0.753
SD × Strain interaction	0.335	0.427	0.595	0.133

[a-c]LSmeans within column and within SD or Strain rows with different superscripts are significantly different ($P < 0.05$).

Abbreviation: SD, stocking density.

[1]LB = Lohmann Brown; DW = Dekalb White; LSL = Lohmann Selected Leghorn-Lite.

Strain Differences in Muscle Growth, Keel Bone Characteristics, and BBS

To our knowledge, this is the first study to compare musculoskeletal growth between brown and white strains of pullets.

Strain differences were found for the majority of muscle weights, keel bone characteristics, and BBS measures. Lohmann Brown pullets had heaviest leg muscle weights but smallest breast muscle weights compared with LSL and DW pullets. The anatomical differences between brown and white strains may reflect differences seen in their behavior; brown and white strains of birds have been found to use space and resources differently (Ali et al., 2016). White strains of birds have been found to use more aerial space and perches (Ali et al., 2016; Kozac et al., 2016), perform more wing-assisted incline running (Leblanc et al., 2018), and overall, demonstrate better navigational skills compared with brown strains (Scholz et al., 2014). Furthermore, Jensen (2019) found that the LB pullets in the current study perched significantly less than DW and LSL pullets. The act of perching, in particular, requires an intact balancing mechanism, which involves a combination of visual and muscular responses (LeBlanc et al, 2015). A key muscular response during balancing attempts is the use of the pectoralis muscles, which supports the strain differences found in pectoralis muscle weights.

Lohmann Brown pullets also had significantly different keel bone development in comparison to the white strains of pullets. Dekalb White and LSL pullets had significantly larger keel bones and lower percentage of cartilage on the keel. These results suggest that the keel bone of brown birds follows a significantly slower rate of development and ossification. Additionally, this may have had an effect on the strength of the keel bone and the development of keel bone damage in adult hens. Contrary to visual inspection, the correlation between the triangulation and image tracing for determining keel bone area was higher for LB than for the white birds. Given that there could be different relationships for different strains of birds, it is recommended to use image tracing for determining keel area because it is more exact.

There are no previous reports in the literature comparing BBS in different strains of birds at the end of the rearing stage. In the current study, both the femur and tibia were stronger in white pullets than LB pullets when corrected for BW, but there were no differences in BBS of the radius. The humerae of DW pullets were found to have greater BBS in comparison to both LSL and LB pullets. However, this was primarily because of an interaction with SD in which BBS of DW in 2 of the SD treatments differed from all other strain by SD combinations. Values were highest for DW pullets reared at highest and second to lowest SD, and this difference may be because of sampling or statistical artefact. Most comparisons of bone strength between brown and white strains of hens have been made during production or at the end-of-lay. Habig and Distl (2013) found that at the end-of-lay, LB hens had greater bone strength of the humerus and tibia compared with LSL hens (both housed in large furnished cages). Similarly, Riczu et al. (2004) found that during production, brown hens had significantly higher BBS of the humerus and femur compared with white hens (both housed in conventional cages). Vits et al. (2005) found that BBS of the humerus, but not the tibia, was greater for brown than white strains (both housed in furnished cages), but this varied with rearing system of the hen. Finally, comparing bone strength of end-of-lay hens housed in aviary systems, Gebhardt-Henrich et al. (2017) found the tibia of white birds to be stronger than those of browns. Thus, differences in strength of different bones between strains can also depend on housing system. It should also be noted, however, that some authors adjust for BW or bone size in their statistical analysis of BBS (i.e., Vits et al., 2005; Gebhardt-Henrich et al., 2017; the current study), whereas others do not (i.e., Riczu et al., 2004; Habig and Distl, 2013).

Keel Bone Damage at 35 and 50 Wk

No differences in keel bone damage between strain or SD were seen at 35 wk of age. However, at 50 wk, a significantly higher prevalence of keel bone fractures were seen in LB compared with DW hens. Similarly, Eusemann et al. (2018) found no differences in keel bone damage at 35 wk of age between brown and white strains of hens; however, at 51 and 72 wk, brown hens were found to have significantly more keel bone fractures than white strains. Additionally, Heerkens et al. (2016) found that at 29, 39, and 49 wk of age, ISA brown hens had significantly higher fractures compared with DW birds. One suggestion to explain a higher prevalence of keel bone fractures in brown hens is their lower navigational skills and accuracy of safe landings (Scholz et al., 2014). This may be attributed to a higher wing load or larger BW (Scholz et al., 2014).

In this study, SD had very minimal effects on the musculoskeletal development of pullets; however, significant strain differences were found. At 16 wk of age, the keel bone of LB pullets had significantly more cartilage compared with white strains of pullets, suggesting a slower development of the keel bone. These findings may predispose LB hens to more keel bone damage in adults, which was found in the current study at 50 wk of age. Previous studies have suggested that brown birds suffer more keel bone damage because of higher body mass, lower navigational and motor skills, and strain specific behaviors (Scholz et al., 2014; Eusemann et al., 2018). Hardin et al. (2019), in a review, compared the prevalence of keel damage in 3 housing systems (conventional, enriched, or cage free), as well as genetic line and age. They concluded that there is a high level of variability within studies making comparison difficult. Fleming et al. (2004) acknowledged the potential for genetic selection by selecting birds for stronger keel bones. Candelotto et al. (2017) determined a strong relationship between the susceptibility of keel fracture and genetic lines because their impact test study was conducted in a controlled environment. That being said, genetic differences in keel fractures are most likely multifactorial and interactive. This would indicate future studies to identify if there is a strong correlation between keel bone development of different strains of pullets and keel bone damage in adult hens.

ACKNOWLEDGMENTS

This project was supported by funding from the Egg Farmers of Canada. The Ontario Agri-Food Alliance provided in-kind support. Tina Widowski holds the Egg Farmers of Canada Research Chair in Poultry Welfare.

Conflict of Interest Statement: All authors declare that there is no conflict of interest.

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