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Authors: Pereira, A., Akbari Moghaddam Kakhki, R., and Kiarie, E.G.

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# The effects of different spacing allowances in the pullet phase on the eggshell and bone quality of hens in the laying phase

A. Pereira, R. Akbari Moghaddam Kakhki, and E.G. Kiarie

**Abstract:** Eggshell and bone quality were investigated at 72 wk of age (WOA) using Lohmann Brown and Dekalb White hens raised with a rearing spacing allowance (SA) of 247 and 299 cm<sup>2</sup>·bird<sup>-1</sup> from days 0 to 16 before being transferred to a common SA (755 cm<sup>2</sup>·bird<sup>-1</sup>) for lay cycle. Eggshell thickness, breaking strength, along with tibia and femur weight, breaking strength, length, and ash were measured at 72 WOA. Strain and SA did not interact on any above parameter ( $P > 0.05$ ). Hens reared in high SA during the pullet phase had heavier tibia and more femur ash, indicating rearing environment during the pullet phase influences the bone quality of hens in the late laying stage ( $P < 0.05$ ).

*Key words:* spacing allowance, pullet, laying hens, bone strength, furnished cage.

**Résumé :** La qualité de coquille d'œuf et d'os ont été évalué chez des poulets Lohmann Brown et Dekalb White de 72 semaines d'âge (WOA — « weeks of age ») élevés avec des allocations d'espacement (SA — « spacing allowance ») de 247 et 299 cm<sup>2</sup>·poulet<sup>-1</sup> pendant les jours 0 à 16 puis transférés à un SA commun (755 cm<sup>2</sup>·poulet<sup>-1</sup>) pendant le cycle de ponte. L'épaisseur de la coquille (EST — « eggshell thickness »), la force de rupture de coquille (ESBS — « eggshell breaking strength »), ainsi que les poids de tibia et fémur, la force de rupture (BS — « breaking strength »), la longueur, et les cendres ont été mesurés à 72 WOA. Il n'y avait pas d'interaction entre la lignée et la SA et les autres paramètres indiqués ci-dessus ( $P > 0,05$ ). Les poules élevées à grande SA pendant la phase poulette avaient des tibias plus pesants et davantage de cendres du fémur, indiquant que l'environnement d'élevage a une influence sur la qualité des os des poules dans l'étape tardive de la ponte ( $P < 0,05$ ). [Traduit par la Rédaction]

*Mots-clés :* allocation d'espacement, poulette, poule pondeuse, solidité des os, cage aménagée.

## Introduction

The rearing period in hens is critical for optimal development of structural bones that are pivotal in mitigating inevitable structural bone loss during the course of the laying cycle. Indeed, the skeleton is 95% developed by 12 wk of age (WOA), and at the surge of estrogen at sexual maturity, structural bone growth ceases. The interaction between rearing environment, management, health, and nutrition sets the stage for productivity and welfare in the layer house (Widowski and

Torrey 2018). Unlike conventional cages, alternative housing such as enriched cages and cage-free housing is characterized with large groups of birds. Physical activity during the rearing phase has been reported to be positively correlated with bone quality at the late stage of lay (Neijat et al. 2019). However, there is a lack of information on the influence of rearing spacing allowance (SA) in enriched cages on bone quality and eggshell quality in aged hens. Therefore, the purpose of this study was to investigate the effect of rearing SA on eggshell

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A. Pereira,\* R. Akbari Moghaddam Kakhki, and E.G. Kiarie.† Department Animal Biosciences, University of Guelph, Guelph, ON N1G 2W1, Canada.

**Corresponding author:** E.G. Kiarie (email: [ekiarie@uoguelph.ca](mailto:ekiarie@uoguelph.ca)).

\*Visiting undergraduate Doctor of Veterinary Medicine student, Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Paraná, Brazil.

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and bone quality in the late phase of the lay cycle of Lohmann Brown (LBR) and Dekalb White (DW) strains.

## Materials and Methods

The protocol was approved by the University of Guelph Animal Care Committee, and birds were cared for following the Canadian Council on Animal Care Guidelines (Canadian Council on Animal Care 2009). The present study was part of a larger project investigating the impact of rearing spacing and pullet development (Jensen 2019). For the purpose of the present study, a total of 1992 one-day-old pullets of either LBR or DW were placed in furnished cages (Clark Ag Systems, Ontario, Canada) at the University of Guelph's Arkell Poultry Research Station. Upon the arrival of pullets, birds were distributed into two SA: 247 cm<sup>2</sup> for 91 birds and 299 cm<sup>2</sup> for 75 birds for each strain to WOA (Jensen 2019). The cages were outfitted with platforms and terraces to increase opportunities for load-bearing exercises (e.g., jumping, perching, and flying). There were six replicate cages for each strain and SA (24 cages in total, each either 91 or 75 pullets for low and high SA, respectively). After the onset of lay, 30 pullets from each replication were selected and transitioned based on rearing treatment in furnished common cages, each containing 30 birds (754.8 cm<sup>2</sup>·bird<sup>-1</sup>) and in total 720 birds for either of the strain. More information about environmental conditions and vaccination programs has been described in Jensen (2019).

Birds were fed commercial fine crumble starter [0–6 WOA, 2900 kcal apparent metabolizable energy (AME), 21.0% crude protein (CP), 1.06% calcium (Ca), and 0.77% phosphorus (P)] then coarse crumbles (7–16 WOA, 2900 kcal AME, 18.0% CP, 1.00% Ca, and 0.78% P). Layer ration had 2875 kcal AME, 18.0% CP, 4.24% Ca, and 0.68% P. The house temperature and intensity of lux were set according to the birds' requirements. During the laying period, the birds received 14 h of incandescent light (20 lx) per day. For the current study, daily egg production was recorded from 68 to 72 WOA. All eggs laid in the last three days of week 72 were counted; 10 eggs from each replication were labeled and kept for eggshell analyses. In the early morning of the last day of week 72, 48 hens (two birds per cage) were weighed and necropsied for left tibia and femur bone samples.

Eggshell thickness (EST) and eggshell breaking strength (ESBS) were measured according to Mwaniki et al. (2018). Eggs were then cracked open, shells washed with water, dried for 24 h at 105 °C, and weighed. Tibia and femur samples were dried, weighed, and ash content measured according to Akbari Moghaddam Kakhki et al. (2018). Right tibia and femur samples were used for measuring breaking strength (BS) via an Instron material tester (Instron crop, Canton, MA, USA) with the crosshead speed of 2 mm·s<sup>-1</sup>, according to Khanal et al. (2019).

Dry weight, the ash content of tibia, and femur bone were normalized based on body weight (BW, Akbari Moghaddam Kakhki et al. 2018). Egg weight was considered as a covariate for analyzing data of EST and ESBS. Data were analyzed using GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) with the strain (LBR and DW), SA (247 and 299 cm<sup>2</sup>) associated interactions as fixed factors. The cage was the experimental unit. Significance was declared at  $P < 0.05$ .

## Results and Discussion

There was no interaction between strain and SA nor any main effect of SA on egg production, egg mass, egg weight, eggshell, eggshell thickness, or ESBS (Table 1;  $P > 0.05$ ). Dekalb white hens had 4.07% higher hen-day egg production compared with LBR hens ( $P < 0.001$ ). Conversely, LBR had 3.13 g heavier eggs relative to DW hens ( $P < 0.001$ ). According to the strains' guidelines, at 72 WOA, the average hen-day egg production (HDEP) is 81.6% and 84.8% for LBR and DW, respectively, and the average egg weight in the same age is 65.7 g for LBR and 64.8 g for DW (Lohmann Brown Guide 2017; Dekalb White Guide 2019). The corresponding HDEP and egg weight in the current study agree with guidelines, with the exception that LBR eggs were slightly heavier (67.31 g). This difference may be attributed to factors that were not evaluated; however, brown hens are generally heavier and lay heavier eggs than white strains (Scott and Silversides 2000). It is well documented that as the egg production cycle progresses, the eggs become heavier in concomitant with a decrease in eggshell quality (Akbari Moghaddam Kakhki et al. 2019). In the current study, there was no reduction in the eggshell quality in LBR; this may be explained by the lower HDEP and the more effective mobilization of Ca from bone sources for eggshell formation in LBR compared with DW. At the end of the cycle, there is a reduction of estrogen level, leading to a reduced capacity of the gut to absorb Ca from the diet. Thus, there is an increase in Ca demand for eggshell formation at the cost of bone resorption, which contributes to the reduction in bone quality at the end of the cycle (Akbari Moghaddam Kakhki et al. 2018).

Tibia BS, length, weight, and ash content were not affected by the interaction between strain and SA or the main effect of strain (Table 1;  $P > 0.05$ ). The main effect of SA on tibia characteristics was such that hens reared in 299 cm<sup>2</sup> cage SA had heavier ( $P = 0.042$ ) tibia compared with hens reared in 247 cm<sup>2</sup> cage SA. This showed that birds allowed to have smaller space to exercise and kept under more pressure from housing density during the rearing phase had a lower bone mass in their tibia at the end of their laying cycle. Femur BS, length, weight, and ash content were not influenced by the interaction between strain and SA or the main effect of strain (Table 1;  $P > 0.05$ ). Furthermore, the effects of strain and SA on bone measurements were not

**Table 1.** The effects of strain (Dekalb White vs. Lohmann Brown) and rearing cage spacing allowance (299 vs. 247 cm<sup>2</sup>) on eggshell and bone attributes at 72 wk of age.

Items	SA (cm <sup>2</sup> )	Group size during rearing	Group size during laying	Egg <sup>b</sup>					Tibia				Femur				
				HDEP (% <sup>a</sup> )	Weight (g)	Shell (%)	EST (mm) <sup>c</sup>	ESBS (kgf) <sup>c</sup>	Weight (g) <sup>d</sup>	BS (kgf) <sup>d</sup>	Length (mm) <sup>d</sup>	Ash <sup>d</sup>	Weight (g) <sup>d</sup>	BS (kgf) <sup>d</sup>	Length (mm) <sup>d</sup>	Ash <sup>d</sup>	
Strain																	
Dekalb	299	75 birds	30 birds	85.2	64.2	8.88	0.34	3.99	3.79	154.2	69.2	1.86	2.75	143.3	47.2	1.47	
Dekalb	247	91 birds	30 birds	83.7	64.2	8.77	0.33	3.94	3.46	149.3	68.1	1.73	2.41	123.5	45.7	1.22	
Lohmann	299	75 birds	30 birds	80.1	67.5	8.83	0.36	4.05	3.74	162.0	64.9	1.75	2.66	135.0	44.9	1.40	
Lohmann	247	91 birds	30 birds	80.7	67.1	8.85	0.36	4.06	3.50	148.0	70.9	1.65	2.63	119.4	47.3	1.21	
SEM				0.61	0.45	0.07	0.006	0.04	0.14	13.59	2.81	0.077	0.12	15.66	1.65	0.08	
<b>Main effects</b>																	
Strain																	
Dekalb				84.4a	64.18b	8.83	0.34	3.96	3.63	151.7	68.7	1.80	2.58	133.4	46.5	1.34	
Lohmann				80.4b	67.31a	8.84	0.36	4.08	3.62	155.0	67.9	1.70	2.64	127.2	46.1	1.30	
SA (cm <sup>2</sup> )																	
299				82.6	65.9	8.86	0.35	4.02	3.77a	158.1	68.0	1.81	2.70	139.1	46.1	1.43a	
247				82.2	65.6	8.81	0.35	4.00	3.48b	148.6	69.5	1.69	2.52	121.4	46.5	1.22b	
SEM <sup>e</sup>				0.43	0.32	0.05	0.004	0.02	0.10	9.12	1.99	0.054	0.08	9.90	1.17	0.05	
<b>Probabilities</b>																	
Strain				<0.001	<0.001	0.808	0.059	0.107	0.963	0.803	0.772	0.237	0.595	0.648	0.838	0.622	
SA				0.469	0.648	0.469	0.372	0.443	0.042	0.467	0.386	0.109	0.119	0.198	0.801	0.008	
Strain × SA				0.106	0.756	0.337	0.330	0.306	0.728	0.728	0.215	0.799	0.185	0.878	0.283	0.702	

**Note:** Within factor of analyses (strain and spacing allowance and interactions), least square means assigned different lowercase letters differ ( $P < 0.05$ ). HDEP, hen-day egg production; EST, eggshell thickness; ESBS, eggshell breaking strength; BS, breaking strength; SEM, standard error of the mean; SA, spacing allowance.

<sup>a</sup>Measured between 68 and 72 wk of age,  $n = 6$  cages per treatment.

<sup>b</sup>Egg weight used as covariate for eggshell parameters,  $n = 60$  (10 eggs per replicate).

<sup>c</sup> $n = 60$  (10 eggs per replicate).

<sup>d</sup>Values expressed on body weight,  $n = 12$  (two birds per replicate).

<sup>e</sup>SEM value was equal for the main effect of strain and SA,  $n = 12$  (two birds per replicate).

influenced by BW ( $P > 0.05$ ; data are not presented). The average BW for DW and LBR were 1745 and 1803 g, respectively. The lack of difference between the strains in tibia and femur quality contradicted previous findings showing that BW was positively correlated with bone attributes (Whitehead and Fleming 2000). In addition, Khanal et al. (2019) showed that LBR bones had higher ash content and were heavier than white strains housed in conventional cages. The current study used older hens housed in furnished cages, which might have attributed to the differences with the aforementioned studies. The femur of birds reared in 247 cm<sup>2</sup> cage SA had 0.21 g·kg<sup>-1</sup> BW lower ash than birds reared in 299 cm<sup>2</sup> ( $P = 0.008$ ), suggesting that birds allowed to have smaller space to exercise and kept under more pressure from housing density during the rearing phase had lower mineral content in their femur at the end of their laying cycle.

It is known that greater physical activity during rearing leads to bone quality improvement in hens (Neijat et al. 2019), perhaps linked to the reduction of structural bone loss (Whitehead and Fleming 2000). The lower tibia weight and femur ash content in hens reared in a smaller space may be due to reduced opportunities for exercise during the rearing phase. The larger space available allows hens to change positions on the perch and to exercise more, increasing their physical activity, which potentially increases the mineral content. Neijat et al. (2019) showed that pullets reared and kept until the end of the cycle in the aviary system had heavier medullary and pneumatic bones compared with conventional cages and enriched aviary systems. The increase in physical activity imposed by the environmental condition during the rearing phase can promote skeletal development (Neijat et al. 2019). The presence of perches in the cage may contribute to increasing the pullets' motivation to express their natural behavior in vertical spaces (Jensen 2019). However, for this to be achieved, there must be adequate space for the hens to better use the cage amenities.

In conclusion, LBR hens kept in enriched cages had lower egg production and heavier eggs compared with the DW hens at 72 WOA. In addition, the SA affected the tibia weight and femur ash content. Increasing rearing SA had positive effects on the tibia and femur attributes in older hens without any adverse effects on egg production and eggshell quality.

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