ANIMAL WELL-BEING AND BEHAVIOR

Opportunities for exercise during pullet rearing, Part I: Effect on the musculoskeletal characteristics of pullets

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ABSTRACT Increased load-bearing exercise improves bone quality characteristics in a variety of species, including laying hens. Providing increased opportunities for exercise during the pullet rearing phase, a period of substantial musculoskeletal growth, offers a proactive approach to reducing osteoporosis by improving bone composition. The main objective of this study was to determine whether differing opportunities for exercise during rearing influences pullet musculoskeletal characteristics. Two flock replicates of 588 Lohmann Selected Leghorn-Lite pullets were reared in either standard, conventional cages (Conv) or an aviary rearing system (Avi) from day-old chicks until 16 wk of age. The keel bone and the muscles and long bones of the wings and legs were collected at 16 wk to measure muscle growth differences between rearing treatments and quantify bone quality characteristics using quantitative computed tomography (QCT) and bone breaking strength (BBS) assessment. Keel bone characteristics and muscle weights were adjusted for BW and analyses for QCT and BBS included BW as a covariate. At 16 wk of age, rearing system had an effect on the majority of keel bone characteristics ($P < 0.05$). The length of the keel metasternum, caudal tip cartilage length, and the overall percentage of cartilage present on the keel at 16 wk was greater in the Avi pullets compared to the Conv pullets ($P < 0.01$). Wing and breast muscle weights of the Avi pullets were greater than the Conv pullets ($P < 0.001$), but leg muscle weights were greater in the Conv pullets ($P = 0.026$). Avi pullets had greater total bone density, total cross-sectional area, cortical cross-sectional area, total bone mineral content, and cortical bone mineral content than Conv pullets for the radius, humerus, and tibia ($P < 0.001$). Avi pullets had greater BBS compared to the Conv pullets for the radius, humerus, and tibia ($P < 0.01$). Increased opportunities for exercise offered by the aviary rearing system increased muscle and bone growth characteristics in pullets at 16 wk of age.

Key words: Pullet, Keel bone, Musculoskeletal growth, Exercise, Rearing system

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INTRODUCTION

Osteoporosis is described as the development of fragile, brittle bones from loss of bone mass and deterioration of the microarchitecture of the bone leading to a greater susceptibility to fractures (Saraff and Hogler, 2015). In laying hens, osteoporosis poses an animal welfare concern because of its high prevalence and association with fractures during and at the end of the laying period (Newman and Leeson, 1997; Fleming et al., 1998; Knowles and Wilkins, 1998).

As part of a naturally occurring process, the skeletal frame of avian species routinely supplies calcium for production of the egg shell in the form of readily-available calcium stores in the medullary bone (Mueller et al., 1964; Miller et al., 1984). Unlike the structural forms of bone tissue (cortical and trabecular), medullary bone is a non-structural, woven bone tissue that contributes only marginally to the overall strength of a bone (Fleming et al., 1998; Whitehead, 2004). At the onset of sexual maturity in laying hens, and as long as estrogen levels remain high, these medullary

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stores are replenished daily by dietary calcium (Fleming et al., 1998; Beck and Hansen, 2004), whereas structural bone ceases development prior to the onset of lay and is not replenished unless estrogen levels drop and the hen pauses egg production (Hurwitz, 1965; Hudson et al., 1993). Osteoclasts primarily mobilize calcium from the medullary bone stores during lay; however, because osteoclasts are indiscriminate, they can also mobilize structural bone in areas where medullary bone is sparse (Fleming et al., 2006). Since structural bone is not replenished while the hen is in lay, areas of erosion in the structure can result in increased risk of fracture.

In the 1950s, initial investigations into the causes of severe bone loss in laying hens suggested the importance of exercise, noting that the problem was highly prevalent in hens housed in confined, conventional (i.e., battery) cages, yet was rarely seen in hens housed in open, barn-floor systems (Couch, 1955; Grumbles, 1959). Although osteoporosis is present across all housing systems with current commercial breeds, exercise still seems to be an essential component to reducing severe levels of osteoporosis (Knowles and Broom, 1990; Abrahamsson and Tauson, 1995; Fleming et al., 2006). Housing adult laying hens in modified furnished-cage systems (Jendral et al., 2008), aviaries (Newman and Leeson, 1998; Leyendecker et al., 2005), or free-range systems (Shipov et al., 2010) compared to conventional cages has demonstrated effects on bone characteristics; however, the most marked improvements to bone health are predominantly seen in housing systems where the opportunities for load-bearing exercise are greatest (Whitehead, 2002).

Because the detrimental effects of osteoporosis are most obvious after long periods of egg production, minimal research efforts have targeted the pullet rearing stage as an important part of the prevention of osteoporosis in laying hens. In humans, osteoporosis is increasingly considered to be a pediatric disease, as the precursors for it begin in early childhood (Bailey et al., 1999; Chesnut, 1989). Stimulating the development of bone mass through routine load-bearing exercise during pre-pubertal growth in humans has become a key area of interest for preventing osteoporosis, especially for females (Burrows, 2007; Chesnut, 1989), and routine weight-bearing activity in adolescence is considered a key component to increasing peak bone mass into adulthood (Welten et al., 1994; Vincente-Rodriguez, 2006).

The same approach might hold true for laying hens: building a stronger pre-lay skeleton may result in sufficient calcium reserves and structural bone strength to reduce depletion later in life. The pullet growing phase, prior to sexual maturity, represents a critical period for structural bone growth. In the last few weeks before the onset of lay, the diameters of the long bones increase by approximately 20% (Riddell, 1992), and then as estrogen levels begin to rise, structural bone deposition and longitudinal growth of long bones ceases. During this stage, cross-sectional growth occurs by periosteal apposition as osteoblasts add mineralized tissues to the outer periosteal surface (Rauch, 2007), and constant endosteal resorption allows for increased space for future medullary bone production within the bone cavity (Whitehead, 2004). This increase in bone diameter in pullets may be critical to enhancing life-long bone strength, as the increase in bone diameter in humans has been shown to be positively correlated with the overall bending strength of a bone (Rauch, 2007).

To date, few studies have addressed the effect of exercise in pullets on long term bone health (Enneking et al., 2012; Hester et al., 2013; Regmi et al., 2015, 2016). These pullet studies either focused on minimal opportunities for loading exercise with the addition of perches to conventional cages (Enneking et al., 2012; Hester et al., 2013) or only allowed access to increased exercise at >6 wk of age (Regmi et al., 2015).

The overall objective of this study was to assess the effects of different opportunities for exercise during the pullet rearing period. Pullets were reared in one of 2 housing systems: a rearing aviary system allowing for diverse load-bearing exercise available in the form of running, jumping, perching, wing-flapping, and flight allowed starting at 1 d of age, or a standard, conventional rearing system offering limited opportunities for exercise throughout the rearing period. In this paper, we present results on keel bone and musculoskeletal characteristics in a sample of pullets obtained from 2 consecutive flocks at 16 wk. Results on effects of these 2 rearing treatments in combination with adult housing system from a larger population of 4 consecutive flocks on prevalence of keel fractures during lay (Casey-Trott et al., 2017a) and on bone characteristics at end-of-lay (Casey-Trott et al., 2017a) are reported elsewhere.

MATERIALS AND METHODS

Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947).

Pullet Housing and Management

Two consecutive flocks of Lohmann Selected Leghorn Lite (LSL-Lite) chicks were obtained at 1 d from a commercial hatchery. From each flock, 960 of the chicks were placed in standard conventional cages (**Conv**) (Ford Dickinson, Mitchell, Ontario, Canada) with 16 pullets/cage during wk 0–6 and a space allowance of 145 cm^2/pullet, followed by 8 pullets/cage during wk 6–16 with a space allowance of 290 cm^2/ pullet; total cage area = 2,322 cm^2. The remaining 756 chicks from each flock were placed in a single Farmer Automatic Portal Pullet rearing system (**Avi**) (Clark Ag Systems, Caledonia, Ontario, Canada) with system space allowance of 285 cm^2/pullet during wk 0–6, and system + outer platforms + litter space allowance of 754 cm^2/pullet during wk 6–16. The aviary system was selected to allow maximum opportunities for exercise starting at

Figure 1. Images of the Farmer Automatic Portal Pullet rearing system (A and B) and conventional cage rearing system (C). Image A depicts the area inside the system (System Area: 183,272 cm^2) equipped with feeders, waterers, perches, a suspended platform (32,371 cm^2) in the centre of the system (Total System Area: 215,643 cm^2). Image B depicts 9 platforms (Platform Area: 118,887 cm^2) on the outer edge of the system, which are opened up at 6 wk of age to allow for access to the litter portion (Litter Area: 235,767 cm^2; Total Aviary Area: 570,297 cm^2) of the aviary enclosure seen in the bottom corner of the image. Image C depicts the conventional cage rearing system (Total Cage Area: 2,322 cm^2).

1 d of age with access to the floor area of the system (183,272 cm^2), perches, and a suspended platform (32,371 cm^2) that was gradually raised vertically in accordance with the age of the pullets to encourage hopping and flight to access vertical space (Total System Area: 215,643 cm^2). At 6 wk of age, additional space was added by opening the sides of the system to allow for access to the litter area (235,767 cm^2) and 9 elevated terraces (118,887 cm^2) on the outer edge of the system, increasing the total system area to 570,297 cm^2 (Figure 1).

Both the Conv and Avi pullets for both flocks were fed identical, 21% crude protein, 1.06% calcium, 0.47% available phosphorus starter diets (as crumbles) from 0–6 or 7 wk (see below) and identical 18% crude protein, 1.01% calcium, 0.45% available phosphorus grower diets (as crumbles) from 6 or 7–16 wk with the addition of granite chick grit. Both rearing treatments also followed identical vaccination and lighting programs. Lights (incandescent) were on for 16 h/d for wk 1 and 2, alternating 4 h on and 2 h off. At 3 wk, lights were on continuously for 14 h/d starting at 05:00 h, and subsequently reduced by 1 h/wk until maintaining 8 h of light from wk 8–15. The lights were set at 40 lux at placement, and reduced by 5 lux every 5 to 7 d until maintaining 10 lux from wk 4–16. In the aviary rearing system, water lines were located in the middle of the enclosure with a total of 74 nipple drinkers located within the system and an additional 40 nipple drinkers over the platforms accessible after 6 wk once the system was opened. A chain feeder (total length 2,255 cm) was located within the system and allowed for access of the feeder on both sides of the trough. Water lines with nipple drinkers and chain feeders were suspended from the ceiling and could be raised vertically in accordance with pullet growth. In the standard cages, water lines with nipple drinkers were located in the middle of the cages (2 nipple drinkers/cage) with the chain feeders (total length 61 cm) running past the front of the cage allowing access to the feed trough on one side.

A subsample of 100 pullets/flock from each rearing system were weighed biweekly and compared to the target weights outlined in the North American Edition of the LSL-Lite Management Guide Layers (Lohmann Tierzucht GmbH, Germany). Pullets from within the aviary rearing system were sampled from various areas on the floor and within the system. For the conventional rearing system, 2 pullets were selected from each cage. In an attempt to achieve equal body weight (**BW**) between the Conv and Avi pullets, the room temperatures were increased slightly (≤2°C) in the conventional cage rearing room to reduce feeding behavior when the biweekly weight of the Conv pullets exceeded that of the Avi pullets by more than 10%. In accordance with the LSL-Lite Management Guide, which recommends adjusting the feeding program based on BW, Aviary-reared pullets from the second flock remained on the starter diet for 1 additional wk in order to bring them up to a BW matching that of the Conv pullets.

Sample Collection and Measurement

At 16 wk of age, a sample of 20 Avi and 20 Conv pullets from each flock (n = 40 Conv pullets; n = 40 Avi pullets) were euthanized by cervical dislocation and frozen at –20°C for later collection of muscle and bone tissues. To prevent bone breakage due to wing-flapping during convulsions, all birds were restrained using a handmade wrap comprised of cotton cloth and Velcro to contain wing-flapping. Pullets from within the aviary rearing system were sampled from various locations on the floor and within the system. For the conventional rearing system, 2 pullets were selected from each of 10 cages.

At the time of sample collection, the carcasses were thawed and prior to dissection, the distance between the caudal-most tip of the keel and the left pubic bone, and the distance between the pubic bones themselves were measured to estimate the proximity to initiation of egg production as the gap between pubic bones increases and the keel bone gradually tilts ventrally, away from the pubic bones, at the onset of lay (Chapman, 1943).

The protocol for the collection of muscles was designed with the assistance of a veterinary avian pathologist (Dr. Emily Martin) to ensure consistent muscle specimen collection. The bicep brachii, 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 left side of each pullet. The bicep brachii was detached from the bone by severing the muscle at the site of tendon origin (proximal head of the humerus) and insertion (proximal anterior surface of the radius) to include only muscle tissue in the measurements and ensure a consistent visual identification of the site of removal. The pectoralis muscles were removed by severing the attachment at the origin (Carina sternum, furcula and sternal ribs) and insertion of the major (proximal ventral surface of the humerus) and insertion of the minor (proximal dorsal surface of the humerus) and gently freeing the muscles from any additional fascia tissue attachment to the sternum and rib cage. Due to the presence of multiple tendon and ligament bundles, the individual leg muscles could not be easily separated. To prevent inconsistent collection, the entire group of leg muscles, tendons, and ligaments were removed as a group and all included in the measurement. The leg muscle group was detached from the axial skeleton at the distal end of the tibia, severing the Achilles tendon between the tibial condyles and the proximal tarsometatarsus, followed by severing the fascia attachment of the thigh muscle group over the synsacrum at the midline of the ilium. All muscles were weighed immediately upon removal.

Following muscle collection, the right and left radius, humerus, tibia, and keel bone were removed. The bones from the right side were subsequently used for Quantitative Computed Tomography (**QCT**) analysis and the bones from the left side were used to test bone breaking strength (**BBS**). Only 1 freeze and thaw cycle was allowed for all bones. Immediately after the bones were extracted, the bones from the right side were placed into 10% formalin for >7 d for QCT analysis. All the bones from the left side were placed in a moving air fume hood to air dry for >7 d for analysis of BBS (Newman and Leeson, 1998). If the bone on the left side was fractured, the right bone was used instead.

Measurements of the keel were taken immediately following dissection and weighing of muscle tissues. The total length (mm) of the keel metasternum, as well as the length of the cartilaginous caudal tip (mm) of the metasternum were measured with Fisher Science Education™ Traceable™ Digital Carbon Fiber Calipers (Fisher Scientific, Toronto, Ontario, Canada). The total length of the metasternum was 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. The cartilaginous tip was 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. The percentage of cartilage was calculated using the total length of the metasternum and the length of the cartilaginous region of the keel. The height of the keel was measured on the cranial region, from the ventral base of the metasternum to the Carina apex. The keel area was estimated using the equation for the area of a right triangle using the measurements of the total length of the metasternum and the height of the keel.

Quantitative Computed Tomography

A Stratec XCT^3 scanner (Model 922010; Norland Medical Systems Inc., Fort Atkinson, WI) with XMENU software version 5.40C was used for analysis of bone density (mg/cm^3) and area (mm^2) of the total bone, cortical bone, and trabecular bone (Korver et al., 2004). A longitudinal scan of the bone was used to set the mid-points of the bone, and a 1-mm bone cross-section at a view of 30% from the distal end of each bone was used for analysis. Threshold density values of 400 and 500 mg/cm^3 were used for trabecular and cortical bone separation, respectively (Korver et al., 2004). The density and area measures were used to calculate the total bone mineral content (mg/mm) of the total and cortical bone, and bone in the trabecular space within the 1 mm length of bone included in the scan.

Three-Point Bone Breaking Strength

An Instron Dynamic and Static Materials Test System (Model # 4204; Instron Corp., Canton, MA) with Automated Materials Test System software was used to assess BBS. A cradle support with posts 5 cm apart was used to support each bone. A 1 kN static load cell and speed of 100 mm/min was used to apply a shear plate (8 cm long by 3 mm wide) to the mid-point of the bone shaft. All bones were placed in the same orientation on the support cradle. The maximum voltage required to break the bone was recorded, which was then converted to kilograms (kg) using a slope equation of a calibration curve generated by calibrating the machine with 10, 20, 50, 100, and 500 g weights. All BBS

Table 1. Comparison of muscle weights between aviary-reared (Avi) and conventionally reared (Conv) pullets at 16 wk of age.

		Muscle Weight, g/kg[1] (±SE)			
		Bicep brachii	Pectoralis major	Pectoralis minor	Leg Muscle Group[2]
Rearing					
Conv		2.1 (0.05)	44.8 (0.69)	16.8 (0.20)	83.9 (1.28)
Avi		2.3 (0.05)	54.7 (0.68)	17.8 (0.19)	81.9 (1.27)
	DF	75	75	75	75
	F-Value	27.58	157.92	13.02	5.17
	P-Value	<0.001	<0.001	<0.001	0.026

[1]All muscles 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).

Table 2. Comparison of keel bone growth between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wk of age.

		Keel Skeletal Characteristics[1] (±SE)						
		Metasternum[2] Length (mm/kg)	Height[3] (mm/kg)	Area[4] (mm^2/kg)	Cartilage[5] Length (mm/kg)	Cartilage[6] Percentage (%/kg)	Keel to Pubic[7] (mm/kg)	Pubic Gap[8] (mm/kg)
Rearing								
Conv		72.3 (1.30)	25.3 (0.35)	1097.2 (18.64)	12.6 (0.92)	14.4 (1.01)	33.2 (0.93)	15.7 (0.65)
Avi		75.5 (1.29)	25.1 (0.35)	1138.3 (18.41)	16.3 (0.90)	18.0 (0.91)	27.5 (0.90)	15.5 (0.64)
	DF	75	75	75	75	75	75	75
	F-Value	9.70	0.36	5.18	24.01	18.98	19.65	0.13
	P-Value	0.003	0.552	0.026	<0.001	<0.001	<0.001	0.717

[1]All keel bone skeletal characteristics were adjusted for BW.
[2]Length measured on the dorsal metatsternum surface parallel to the cranial region of the sternal notch ending at the caudal border of the keel metasternum tip.
[3]Height as measured from the ventral surface of the metasternum to the peak of the Carina apex.
[4]Area of the keel estimated using the formula for area of a right triangle: Area = (metasternum length × height) × $^1/_2$.
[5]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.
[6]Percentage cartilage = (cartilage length/metasternum length) × 100.
[7]Distance between the caudal tip of the keel metasternum to the tip of the left pubic bone.
[8]Distance between tips of pubic bones.

results are presented as kg of force required to break each bone.

Statistical Analysis

All statistical analyses were completed using SAS statistical software version 9.4 (SAS Institute, Cary, NC). The level for assessment of statistical significance of differences between means was set at $P < 0.05$.

Pullet Muscle and Bone Analyses

To assess the effect of rearing system on the muscle and keel characteristics, a general linear mixed model analysis (PROC MIXED command) was performed with rearing system (Avi, Conv) as a fixed effect and Flock as a random factor to account for any variation due to flock differences. All muscle weights were expressed relative to the BW of the pullet (g/kg). Keel dimensions were also adjusted for pullet BW (mm/kg). To assess the effect of rearing system on the long bones of the pullets (QCT and BBS outcome measures), a general linear mixed model analysis (PROC MIXED command) was performed with rearing system (Avi, Conv) as a fixed effect and rearing BW as a covariate. Flock was included as a random factor.

All data were tested for normality and normality of residuals using PROC UNIVARITE command and no data required transformation. Due to the lack of medullary bone content prior to the onset of lay (Whitehead, 2004), density and bone mineral content of bone in the trabecular space was not assessed for pullets.

RESULTS

Muscle and Keel Bone Characteristics

There was no difference in mean BW ($P = 0.875$) between Avi (1,204.6 g ± 18.1 SE) and Conv (1,202.1 g ± 18.2 SE) pullets at 16 wk.

Rearing system affected all muscle and keel characteristics, except for keel height (Tables 1 and 2). The muscle weight of the bicep brachii, pectoralis major, and pectoralis minor was greater in the Avi pullets compared to Conv pullets ($P < 0.001$); however, the weight of the leg muscle group was greater in the Conv pullets compared to Avi pullets ($P = 0.026$: Table 1). The length of the metasternum was greater

Table 3. Comparison of Quantitative Computed Tomography (QCT) bone measures between aviary-reared (Avi) and conventionally-reared (Conv) pullets at 16 wk of age.

		Density mg/cm^3 (±SE)		Cross-sectional Area mm^2 (±SE)			Bone Mineral Content mg/mm (±SE)	
Bone & Housing		Total	Cortical	Total	Cortical	Trabecular[1]	Total	Cortical
Radius								
Conv		523.4 (9.65)	898.5 (9.36)	5.0 (0.08)	2.9 (0.05)	2.2 (0.06)	2.6 (0.06)	2.7 (0.07)
Avi		615.5 (9.59)	972.3 (9.33)	7.3 (0.07)	4.7 (0.04)	2.9 (0.06)	4.5 (0.06)	4.5 (0.07)
	DF	75	75	75	75	75	75	75
	F-Value	87.91	122.08	437.21	724.00	51.71	927.89	961.70
	P-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Rearing BW[2]	*P*-Value	0.114	0.745	<0.001	<0.001	<0.001	<0.001	<0.001
Humerus								
Conv		71.9 (3.96)	887.1 (5.67)	53.3 (0.70)	9.8 (0.13)	41.9 (0.52)	3.8 (0.21)	8.7 (0.12)
Avi		126.6 (3.90)	928.7 (5.65)	65.9 (0.69)	14.6 (0.13)	50.2 (0.51)	8.3 (0.20)	13.5 (0.12)
	DF	75	75	75	75	75	75	75
	F-Value	96.49	102.13	233.01	745.98	128.67	237.69	798.69
	P-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Rearing BW[2]	*P*-Value	0.052	0.078	<0.001	<0.001	<0.001	0.799	0.005
Tibia								
Conv		429.9 (5.36)	1016.3 (3.04)	37.4 (0.35)	15.9 (0.14)	21.6 (0.32)	16.0 (0.18)	16.2 (0.14)
Avi		456.8 (5.30)	1016.2 (3.00)	38.8 (0.34)	17.2 (0.13)	21.5 (0.31)	17.6 (0.17)	17.5 (0.13)
	DF	75	75	75	75	75	75	75
	F-Value	13.17	0.00	7.03	46.33	0.05	40.86	51.24
	P-Value	<0.001	0.989	0.009	<0.001	0.823	<0.001	<0.001
Rearing BW[2]	*P*-Value	0.052	0.717	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Refers to the bone in the trabecular space.
[2]Rearing BW was a covariate in the statistical model.

in the Avi pullets compared to the Conv pullets ($P = 0.003$). The length of the cartilage on the keel bone ($P < 0.001$) and percentage of the keel comprised of cartilage ($P < 0.001$) were greater in the Avi pullets compared to the Conv (Table 2). Keel area was greater in the Avi pullets compared to the Conv pullets ($P = 0.026$). The distance between the keel and pubic bone ($P < 0.001$) was greater in the Conv pullets compared to the Avi pullets; however, there was no difference in the distance between the pubic bones ($P = 0.717$) for the Avi and Conv pullets.

QCT Bone Composition and Bone Breaking Strength

Rearing system affected each of the QCT characteristics for the radii and humeri, and the majority of the QCT characteristics of the tibiae (Table 3). The total and cortical densities of the radius and humerus of the Avi pullets were greater than that of the Conv pullets ($P < 0.001$). For the tibia, the Avi pullets had a greater total density than the Conv pullets ($P < 0.001$), yet the cortical density was not different ($P = 0.989$). The total cross-sectional area, cortical cross-sectional area, and trabecular cross-sectional area of the radius and humerus were greater in the Avi pullets compared to the Conv pullets ($P < 0.001$). For the tibia, the total cross-sectional area was greater in the Avi pullets compared to the Conv pullets ($P = 0.009$) and the same was true for the cortical cross-sectional area ($P < 0.001$); however, trabecular cross-sectional area of the tibia was not different between the rearing system groups ($P = 0.823$). The total bone mineral content and cortical bone mineral content was greater in the Avi pullets compared to the Conv pullets for the radius, humerus, and tibia ($P < 0.001$). BW did not have an effect or a marginal effect on the total or cortical bone density for any of the bones; however, rearing BW had a positive linear effect on the majority of QCT measurements for cross-sectional area and bone mineral content for all 3 bones. The *P*-values for effects of BW on bone characteristics are reported in Table 3.

The BBS of the humerus, radius, and tibia were greater in the Avi pullets compared to the Conv pullets ($P = 0.014$ or less; Table 4). BW did not have an effect on the BBS of the humerus ($P = 0.568$); however, BW did have a positive linear effect on the BBS of the radius ($P < 0.001$) and the tibia ($P = 0.039$; Table 4).

DISCUSSION

The consistent treatment differences in muscle and bone characteristics of pullets at 16 wk indicates that rearing in an aviary system that provides regular opportunities for varied load-bearing exercise starting at 1 d of age has substantial effects on the musculoskeletal characteristics of pullets. This is the first study to clearly demonstrate that the musculoskeletal frame of growing pullets differs between aviary-reared and conventionally-reared pullets, even when BW is accounted for and genetics, diet, and lighting are held constant between the groups.

Table 4. Comparison of bone breaking strength between aviary-reared (Avi) and conventionally reared (Conv) pullets at 16 wk of age.

		Maximum Bone Breaking Strength (kg)		
		Humerus (±SE)	Radius (±SE)	Tibia (±SE)
Rearing				
Conv		9.8 (0.38)	3.9 (0.09)	15.5 (0.36)
Avi		17.5 (0.37)	7.2 (0.09)	15.7 (0.36)
	DF	72	72	72
	F-Value	206.50	617.32	6.46
	P-Value	<0.001	<0.001	0.014
Rearing BW[1]	*P*-Value	0.568	<0.001	0.039

[1]Rearing BW was a covariate in the statistical model.

Rearing System Effects on Muscle Growth and Keel Bone Characteristics

The larger muscle weights and bone cross-sectional areas of Avi pullets indicates that the musculoskeletal growth of the Avi pullets surpassed that of Conv pullets in the majority of growth characteristics. This suggests that opportunities for diverse load-bearing exercise at an early age stimulated muscle deposition and osteogenesis. Hester et al. (2013) demonstrated that allocation of perches in conventional cages during the pullet rearing phase compared to rearing in conventional cages without perches had a positive effect on muscle growth in adult hens; however, when comparing pullet muscle weights averaged between 3, 6, and 12 wk, there was only a difference in thigh muscle weight, not breast muscle (Enneking et al., 2012). The results reported here clearly show a difference in muscle weights at 16 wk of age between the Avi and Conv rearing systems for wing, breast, and leg muscles. Wing and breast muscle weights of the Avi pullets were greater than the Conv pullets which is likely due to the allowance of flight, wing-flapping, and wing-assisted running in the aviary-rearing system compared to the relative confinement of the conventional-rearing cages. The leg muscle weights were greater in the Conv pullets compared to the Avi pullets. The opposite effect of rearing system on leg muscle weights compared to wing and breast muscles suggests that the constant standing in conventional cages also stimulates muscle growth, with perhaps more lean muscles developing in the Avi pullets due to more diverse, extensive exercise. Alternatively, our method of weighing the leg muscles as a group may not have been sensitive enough as some fatty tissues and ligaments were included in the leg muscle group weights, and perhaps assessing individual leg muscles might yield a different result.

The longer metasternum and larger cartilage portion of the keel of Avi pullets at 16 wk compared to the Conv pullets potentially indicates that ossification of the keel was slower in the Avi pullets. This result suggests that the overall skeletal development of the Avi pullets was slower than that of the Conv pullets; although this hypothesis requires further testing. Buckner et al. (1949) described the process of calcification of the keel as a slow progression of ossification from the cranial portion of the keel to the caudal tip of the metasternum, noting that calcification of the caudal tip was not complete until 28 to 40 wk of age, long after structural growth of the long bones is complete. The percentage of cartilage present in the current study at 16 wk of age was similar for both rearing treatments to the cartilage percentage reported by Buckner et al. (1949) for wk 22 to 26, although direct comparison is difficult due to the lack of thoroughly described anatomical marker measurement details within that study.

Alternatively, the greater proportion of cartilage found in the Avi pullets might be related to the larger area and greater length of the keels of the Avi pullets compared to the keels of the Conv pullets, suggesting that increased wing-loading exercise is stimulating the growth of the keel to produce a larger keel bone overall. This may also explain the differences in distance between the keel and pubic bone, as a larger keel likely leads to a shorter distance to the pubic bone. The longer distance between the keel and pubic bone also could indicate that the Conv pullets were closer to sexual maturity, as the distance between the keel and pubic bones has been used as a measure of proximity to lay (Chapman, 1943); however, both flocks achieved 50% production by 19 wk. The lack of difference in the distance of the gap between the pubic bones at 16 wk of age indicates that neither group had entered the laying phase (Satterlee and Marin, 2004).

Understanding the effect of opportunities for exercise during rearing, and its influence on muscle growth and keel bone characteristics can provide insight into the overall growth rate or metabolic differences initiated by the housing system provided during the rearing phase. Determining when ossification of the keel is complete in modern lines of laying hens, especially in relation to rearing environment and the onset of lay, is an area that requires further study. Perhaps adjusting the timing of photo-stimulation depending on rearing system to ensure optimal skeletal growth or selecting for specific keel bone growth characteristics, can add to the discussion on approaches to reducing keel bone damage in adult laying hens. Longitudinal assessment of keel measures in adult hens is warranted, as certain keel bone characteristics, such as length, area, or degree of ossification at the onset of lay might be precursors to keel bone damage, a prominent welfare concern for laying hens.

Pullet QCT Bone Composition and Bone Breaking Strength

In addition to the differences in the musculature and keel bone characteristics between Avi and Conv pullets, the long bones of the pullets in both the wing and leg were also affected by the opportunity for exercise during the rearing phase. The larger total cross-sectional area of the Avi pullet bones indicates greater bone width, which is a characteristic of increased bone growth in human adolescents (Rauch, 2007). Increased area of aviary-reared pullet bones was also reported by Regmi et al. (2015) for the humerus and tibia. A larger cortical area coupled with a larger trabecular area of the radii, humeri, and tibiae of Avi pullets (for all but the bone in the trabecular space of the tibiae) indicates that bone apposition occurred on the periosteal surfaces, which is also typically observed in human studies in which exercise programs target pre-pubertal and adolescent stages (Linden et al., 2007; Specker et al., 2015). In agreement with the current results, evidence of periosteal growth during the pullet phase was also reported by Biewener and Bertham (1994) and Judex and Zurnicke (2000), whereas Regmi et al. (2015) reported periosteal growth of the humerus but endosteal growth of the tibia. With the exception of the cortical bone density of the tibia, the consistently higher values for bone density and bone mineral content in the Avi pullets reported here indicate that not only were the Avi pullet bones wider than Conv pullet bones, but the Avi bones were also undergoing greater mineral deposition. The bone mineral content of the humerus in aviary-reared pullets, as measured by bone ash, has also been reported as being greater than that of conventionally reared pullets, whereas no differences were observed in the tibia (Regmi et al., 2015).

The result of greater values reported for the Avi pullets for QCT measures compared to the Conv pullets was mirrored by the BBS results with the Avi pullets developing stronger bones as evident by greater BBS values for all 3 bones compared to the Conv pullets. Mild to moderate correlations between QCT and BBS values have been previously reported (Saunders-Blades et al., 2003), and perhaps measuring BBS at 16 wk of age, without the presence of medullary bone complicating the BBS measurement, has the potential to increase the correlation between QCT and BBS. Although correlations between the measurements were not measured in this study, both QCT and BBS measures indicate that rearing in an aviary system alters bone composition in a way that improves the overall strength of the bone increasing the amount of force required for fracture.

Overall, the greater total and cortical bone area and breaking strength observed in the Avi pullets at 16 wk of age indicates that opportunities for diverse, loading exercise during the rearing phase substantially alters the geometry of growing pullet bones. This increase in size of the Avi pullet skeleton potentially affords greater space for bone mineralization and medullary bone deposition during the laying phase of adult hens. Perhaps other avenues of research targeting methods to increase the rate of calcium absorption or medullary bone deposition can be used in conjunction with this increased skeletal growth to capitalize on the newly available skeletal framework.

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