ABSTRACT Osteoporosis, a progressive decrease in mineralized structural bone, causes 20 to 35% of all mortalities in caged White Leghorn hens. Previous research has focused on manipulating the egg laying environment to improve skeletal health, with little research on the pullet. The objective of the current study was to determine the effect of perch access on pullet health, bone mineralization, muscle deposition, and stress in caged White Leghorns. From 0 to 17 wk of age, half of the birds were placed in cages with 2 round metal perches, while the other half did not have perches (controls). Bone mineralization and bone size traits were determined in the tibia, femur, sternum, humerus, ulna, radius, and phalange (III carpometacarpal) using dual energy x-ray absorptiometry. Muscle weights were obtained for the breast and left leg (drum and thigh). A sample of pullets from each cage was evaluated for foot health, BW, right adrenal weight, and packed cell volume. Most measurements were taken at 3, 6, and 12 wk of age. Access to perches did not affect breast muscle weight, percentage breast muscle, percentage leg muscle, bone mineral density, bone length, bone width, adrenal weight, packed cell volume, and hyperkeratosis of the foot-pad and toes. There were no differences in BW, bone mineral content, and leg muscle weight at 3 and 6 wk of age. However, at 12 wk of age, BW ($P = 0.025$), bone mineral content of the tibia, sternum, and humerus ($P = 0.015$), and the left leg muscle weight ($P = 0.006$) increased in pullets with access to perches as compared with controls. These results suggest that perch access has beneficial effects on pullet health by stimulating leg muscle deposition and increasing the mineral content of certain bones without causing a concomitant decrease in bone mineral density.

Key words: perch, pullet, White Leghorn, bone mineralization, muscle deposition

INTRODUCTION

Osteoporosis is a noninfectious disease caused by a progressive decrease in the amount of mineralized structural bone leading to skeletal fragility and susceptibility to fracture (Whitehead, 2004). At sexual maturity, the estrogens stimulate the formation of medullary bone, used as a labile source of calcium during eggshell formation. While medullary bone deposition increases with age, structural bone mineralization gradually deteriorates (Whitehead and Wilson, 1992; Hudson et al., 1993). Age-related loss of structural bone, which is generalized throughout the skeleton, eventually leads to osteoporosis (Whitehead and Wilson, 1992).

The effect of pullet rearing on skeletal quality during egg laying has not been extensively studied. Previous research has focused on manipulating the egg laying environment to improve skeletal health which may be too late. In humans, participation in prepubertal exercise had reduced risk of osteoporotic fractures as adults (Bass et al., 1998), thus childhood exercise has a greater influence on adult skeletal health than any intervention plan in adulthood (American Academy of Orthopaedic Surgeons, 2007). Therefore, it is reasonable to assume that chickens may also need early exposure to exercise to avoid fractures.

The addition of perches to a housing system can have both positive and negative consequences. Installation of perches in laying cages improved bone quality measures (Hughes and Appleby, 1989; Duncan et al., 1992; Abrahamsson and Tauson, 1993, 1997; Hughes et al., 1993; Tauson and Abrahamsson, 1994b; Barnett et al., 1997; Jendral et al., 2008; Tactacan et al., 2009), but failure in jumping gaps between perches in extensive systems contributed to bone breaks which occurred earlier in the production cycle (referred to as old breaks). High use of perches can also lead to foot problems and keel bone deviations (Abrahamsson and Tauson, 1993;
Tauson and Abrahamsson, 1994a; Abrahamsson et al., 1996; Tauson, 1998; Vits et al., 2005; Struelens and Tuyttens, 2009). With an increased interest by the egg industry for housing laying hens in enriched cages, consideration should also be given to enriching the cage environment of the pullet. Furthermore, early exposure to perches during rearing encouraged adult perching behavior (Faure and Jones, 1982). However, it is unknown if use of perches during the growing phase has a long-term benefit in improving skeletal health during the laying phase which is a goal of an ongoing study. Nevertheless, it is known that long-term use of perches by hens during the laying cycle (18 to 72 wk of age) increases trabecular bone volume as compared with that of nonperching controls (Wilson et al., 1993).

Perch use may contribute to increased muscle deposition through increased exercise. Weight loading builds muscles in animals. For example, the loading of the anterior latissimus dorsi of chickens culminated in a massive rate of muscle growth due to hyperplasia and hypertrophy of muscle fibers (Sola et al., 1973). Perch-induced exercise may also alleviate stress. Adrenal hypertrophy is an indicator of stress (Siegel, 1971, 1995), thus it is hypothesized that pullets with access to perches would have lower adrenal weights. Packed cell volume was measured as a health parameter as hemococoncentration is an indicator of dehydration (Hester et al., 1996). Therefore, the objective of the current study was to determine the effect of perch access on pullet health, bone mineralization, muscle deposition, and stress in caged White Leghorns.

**MATERIALS AND METHODS**

Chickens were raised at the Purdue University Poultry Research Farm using standard management and vaccination practices and under the guidelines approved by the Purdue University Animal Care and Use Committee. Infrared beak trimming was performed at the hatchery. Two drip nipple drinkers/cage provided a source of water. Typical starter (0 to 3.9 wk of age, CP = 20%, Ca = 1.00%, and nonphytate P = 0.45%) and grower (4 to 12 wk of age, CP = 18.6%, Ca = 1.00%, and nonphytate P = 0.40%) diets were fed using the recommendations for nutrients by Hy-Line International (2009–2011) or the National Research Council (1994). Feed and water were provided for ad libitum consumption. Feed was also provided on paper lining the cage floor for the first few days following hatch. White Leghorn females of the Hy-Line W36 strain (n = 1,064) were banded in the right wing and housed in 14 cages each with and without perches for a total of 28 cages beginning at day of age. Cages in 2 of 3 decks (top and middle) arranged in a double row of one room of the Grower Research Unit were used. The assignment of perch treatment to cages used a restricted randomization scheme that ensured equal number of perch treatments on each deck level. Chicks were assigned randomly to cages with group BW determined for each cage before placement of chicks in the cages. Information on floor, perch, and feeder space per bird can be found in Table 1. Stacking density, feeder space, and the number of drinkers/cage were identical between treatment groups. Big Dutchman metal round perches with a smooth surface, 32 mm in diameter, were used. Arrangement of perches within pullet cages is depicted in Figure 1. There was a distance of 20 cm (8 in) between the 2 perches and 18 cm (7 in) between the front perch and the feed trough and between the rear perch and the back of the cage.

Chickens were removed from each cage beginning at 3, 3.4 (24 d of age), 4.4 (31 d of age), 6, and 12 wk of age (Table 1) for sampling purposes and also to provide each chicken with more space. They were euthanized using sodium pentobarbital followed by cervical dislocation and were weighed. At 3, 6, and 12 wk of age, the

<table>
<thead>
<tr>
<th>Age of bird (wk)</th>
<th>Birds/cage</th>
<th>Floor space [cm²/bird (in²/bird)]</th>
<th>Perch space/bird [cm (in)]</th>
<th>Feeder space/bird [cm (in)]</th>
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<tbody>
<tr>
<td>0</td>
<td>38</td>
<td>98 (15)</td>
<td>3.2 (1.3)</td>
<td>1.6 (0.6)</td>
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<tr>
<td>3</td>
<td>28</td>
<td>133 (21)</td>
<td>4.4 (1.7)</td>
<td>2.2 (0.9)</td>
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<tr>
<td>3.4</td>
<td>27</td>
<td>138 (21)</td>
<td>4.5 (1.8)</td>
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<tr>
<td>4.4</td>
<td>24</td>
<td>155 (24)</td>
<td>5.1 (2.0)</td>
<td>2.5 (1.0)</td>
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<tr>
<td>6</td>
<td>16</td>
<td>233 (36)</td>
<td>7.6 (3.0)</td>
<td>3.8 (1.5)</td>
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<td>12</td>
<td>12</td>
<td>310 (48)</td>
<td>10.2 (4.0)</td>
<td>5.1 (2.0)</td>
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1Pullets were housed in grower cages from 0 to 17 wk of age.
foot-pad and toes were examined for hyperkeratosis. Severity of hyperkeratosis for the foot-pad and toes was evaluated using a scoring system of values ranging from 1 to 4 points, with 1 being the worst condition and 4 being representative of the healthy control (Tauson et al., 1984). The whole breast and the left wing, thigh, and drum were retrieved, labeled, and frozen for later analysis. The carcass parts were thawed. The sternum and keel were dissected from breast muscle. The breast muscle, which included the pectoralis major and minor with respective tendons, was weighed following dissection from bones.

Bone mineral density (BMD) and bone mineral content (BMC) were accessed using dual energy x-ray absorptiometry (DEXA; Norland Medical Systems Inc., Fort Atkinson, WI). As a brief overview on validation of DEXA’s technology, BMD and BMC of live hens computed from DEXA scans were positively correlated with bone ash weights and bone breaking strength (Schreiweis et al., 2003; Hester et al., 2004). Scans conducted on live hens followed by scans of their excised bones cleaned of muscle and tendons were highly correlated (Schreiweis et al., 2005). In addition, as the incidence of broken bones increased in aging White Leghorn hens, the tibial BMD and BMC decreased (Mazzuco and Hester, 2005).

The sternum including its keel (but minus its muscle) and the carcass parts (left wing, thigh, and drum) were scanned using DEXA. The sternum was cleaned of muscle so as to allow the bone to be oriented laterally in a similar manner between samples, culminating in more consistent and reliable scans. The BMD, BMC, and bone size traits of the tibia/fibula, femur, sternum, humerus, ulna, radius, and phalange (III carpometacarpal) were determined from the scans. The wing phalange was selected because it might be the least weight bearing of all bones measured, thus the hypothesis that there would be no response with this bone with regard to mineralization as a result of perching.

After scanning, the skeletal muscle and tendons of the drum and thigh were excised from the bone and weighed. Muscle weight was expressed relative to BW. The sternum with respective keel and the left tibia/fibula, femur, and phalange were examined visually to confirm the presence or absence of a fracture. The keel bone was examined for deformations using the same scoring system as described for hyperkeratosis (Tauson et al., 1984).

The right adrenal gland was retrieved from chickens at 3, 6, and 12 wk of age, weighed, and expressed relative to BW. Duplicate packed cell volumes were determined from each chicken at 4.4, 6, and 12 wk of age after spinning the microhematocrits for 15 min.

A one-way ANOVA was performed on hatchling BW. Bone mineralization, bone size traits, and gross muscle weights were analyzed as an analysis of covariance with BW as the covariate (Steel et al., 1997). For bone mineralization and size traits, type of bone (for example, tibia/fibula, femur, sternum, humerus, ulna, radius, and phalange) was used as a subplot. A 2-way ANOVA was used on all remaining traits. Fixed effects included perch treatment, age of the chicken, and for the subplot analysis, type of bone. Percentage data were transformed to arcsine square root and reanalyzed. Because statistical trends were similar for both transformed and untransformed data, the untransformed results will be presented. The variability of least squares means was reported as the SEM. The mixed model procedure of SAS Institute (2008) was used. The Tukey-Kramer test was used to partition differences among means (Oehlert, 2000) with the exception for the 3-way interaction for BMC where the SLICE option was used (Winer, 1971).

RESULTS

Hatchling BW were similar between chicks assigned to the perch (39.0 ± 0.1 g/chick) and control (39.2 ± 0.1 g/chick) treatments (P = 0.41). There were no differences in BW, BMC, and leg muscle weight at 3 and 6 wk of age. However, at 12 wk of age, the BW (perch = 974 ± 6 g vs. controls = 948 ± 6 g, P = 0.025), the BMC of the tibia, sternum, and humerus (P = 0.015; Figure 2) and the left leg muscle weight (perch = 27.5 ± 1.5 g vs. controls = 26.0 ± 1.4 g, P = 0.006) increased in pullets with access to perches as compared with controls. Breast muscle weight, percentage breast muscle, percentage leg muscle, BMD, bone length, and bone width did not differ between treatments (Table 2). Bone area was greater for chickens with access to perches as compared with controls (P = 0.03; Table 2). In addition, all interactions of treatment with bone were not significant for BMD, length, width, and area. There were no keel bone deformations or bone fractures observed in any of the examined samples.

The gross right adrenal weight was not affected by the perch treatment, but the relative right adrenal weighed less (P = 0.06) for pullets given access to perches as compared with controls. Packed cell volume and the score for hyperkeratosis of the foot-pad and toes were not affected by the perch treatment (Table 3).

DISCUSSION

Past studies evaluating intervention strategies for improving bone integrity in laying hens have done so during the egg production cycle when the adult birds may already be experiencing osteoporosis. At this point, nutritional manipulation and changes in management practices such as increased exercise may not have as large an effect in alleviating bone fractures of hens as would earlier intervention with pullets. Early intervention to improve skeletal health in egg laying strains of chickens has received little attention. The results of the current study suggest that perch access has beneficial effects on pullet health by stimulating leg muscle deposition and increasing the BMC of certain bones without causing a concomitant decrease in BMD. Not all bones showed an increase in BMC at 12 wk of age.
as a result of perching, in particular the femur, ulna, radius, and phalange (Figure 2). It is unknown why the BMC of the femur did not respond to perching as it was hypothesized that its response would be similar to the tibia where an increase in BMC was observed. Wing flapping (data not collected) during perching would encourage movement of wing bones, but only the humerus responded with an increase in BMC. The BMC of the radius, ulna, and phalange were not affected, perhaps indicating less mobility or improved bone strength through loading exercise of these bones during perching.

Perch use by a few chicks of the current study was noted infrequently as early as 2 wk of age (Enneking et al., 2011). Perch use increased as chicks aged (Enneking et al., 2012). For example, nighttime behavioral observations of pullets showed little perching activity before 6 wk of age, but use of the front perch increased to 8, 23, and 31% by 8, 10, and 12 wk of age, respectively, after which no further increases in front perch use were noted to 16 wk of age (P < 0.0001, SEM = 1.7, Enneking et al., 2012). Caged pullets were also using the back perch at night, but the actual numbers could not be accessed accurately because the pullets of the front perch were blocking the camera view of the pullets on the back perch. The low perch use at 3 and 6 wk of age and the much greater perching activity at 12 wk of age perhaps explains why an increase in BW, leg muscle deposition, and BMC of some bones of pullets with access to perches was not noted until 12 wk of age.

The results of the current study suggest a chicken’s availability to activity is important early in life. After genetics, exercise has the next biggest effect on skeletal quality (Fleming et al., 2006). The mechanism of action is that more active birds with opportunities for exercise have fewer osteoclasts breaking down the endosteal bone surface than nonactive birds. Unfortunately, as hens age, the differences in osteoclast numbers dissipate in hens experiencing different levels of activity. Exercise fails to maintain the suppression of osteoclastic resorption, leading to bone loss in older hens. Downregulation of the osteoblastic estrogen receptor as animals age may contribute to exercise having less effect on improving skeletal quality (Fleming et al., 2006).

Additional information gleaned from this study was to determine when bone fractures occur during the pullet phase, which is important for assessment of chronic pain. Old bone breaks, in particular, are of great concern from a welfare point of view because of fracture and its associated inflammation, which activate nociceptors, causing chronic pain (Carstens and Moberg, 2000; Underwood, 2002; Kuenzel, 2007). Though bone strength of caged hens is poorer as compared with hens of extensive housing (McLean et al., 1986; Abrahamsson and Tauson, 1995; Tauson et al., 1999; Wilkins et al., 2004; DEFRA, 2006; Fleming et al., 2006), the incidence of old fractures is much lower at around 5% (Gregory et al., 1990). For hens in noncage systems, the incidence of old keel breaks can be as high as 52 to 73% (Freire et al., 2003; Nicol et al., 2006) and is likely due to the increased mobility and bumps of the keel bone.
when hens move from litter to raised slats or perches or access the nest boxes (DEFRA, 2006). Thus, perches provide benefits of improving bone strength thorough loading exercise and allowing for the expression of natural perching behavior, but the disadvantage, particularly in extensive housing with aerial perches, is the increased incidence of bone fractures (Sandilands et al., 2009). Our results show that bone fracture did not occur in caged pullet flocks before 12 wk of age regardless of whether pullets had access to a perch or not. Likewise, Wilkins et al. (2005) reported no problem with bone fractures in pullet flocks. Perch access in the current study provided benefits to pullets as compared with those chickens without access. Perches encouraged pullet activity, leading to larger pullets with perhaps larger skeletal frame and with greater BMC. These changes in pullet development as a result of perching could ultimately lead to long-term health benefits during adulthood and particularly at end of lay when osteoporosis is especially problematic. In addition, perches allow for natural roosting, a behavior greatly sought out by chickens as shown through a motivational study (Olsson and Keeling, 2000).

Keel bone deformation is perhaps caused by continued pressure exerted on the keel when chickens sit on the perch (Sandilands et al., 2009). Callus formation of the keel, as a result of bone fracture, occurs in hens with moderate to severe keel deformities (Fleming et al., 2004; Käppeli et al., 2011). Pullets of the current study showed no keel bone deformities regardless of whether or not they had access to the perch, similar to the results of Käppeli et al. (2011), who reported few deformities of the keel during rearing for pullets given access to perches as compared with controls is an indicator that pullets with perches were less stressed (Siegel, 1971, 1995). Circulating glucocorticoids from the adrenal increase during stress and inhibits calcification of the skeleton in growing birds and induces osteoporosis in adult birds (Urist and Deutsch, 1960; Siegel and Latimer, 1970). The presence of perches in the cages did not appear to interfere with pullet access to drinkers as there was no evidence of hemococoncentration as indicated by packed cell volume (Table 3).

Perches used in the current study have been field-tested by Big Dutchman, mainly being used by the European egg industry in aviary systems. Material cost for perches is estimated at $4.92/m ($1.50/ft). Assuming a labor cost of $12/h and 10 to 15 min to install, the estimated labor cost of the installation of metal perches is $2 to $3/cage. (K. Krogman and T. Pollard, Big Dutchman, Holland, MI, personal communication).

In conclusion, these results suggest that perch access has beneficial effects on pullet health by stimulating leg muscle deposition and increasing the mineral content of certain bones without causing a concomitant decrease in BMD.

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REFERENCES


Description.


