Influence of Production System and Level of Dietary Soy on Bone Composition and Bone Strength in Laying Hens

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Submission: February 02, 2018; Published: March 09, 2018

Abstract

Common poultry feed ingredients such as soybean meal and its subsequent isoflavones and metabolites (daidzein & genistein) have been found to have positive effects on bone health in estrogen deficient animals; however, the exact role these compounds play has not been well established. A field study was conducted to determine the effects of varying levels of dietary soy (high, standard or no soy) on bone characteristics in Free-range (FR) and Battery caged (BC) laying hens. At the end of an 8-week feeding trial, hens were euthanized and both femurs were removed to determine physical (weight, width, length, volume, density and breaking strength) and chemical characteristics (bone mineral content or BMC). Bird weight and feed consumption was similar in hens living in the FR system and BC system with the exception of hens in the BC system fed a soy-free diet. These hen weights were significantly lower than all other feed treatments in both housing systems, consistent with feed consumption data. Under the conditions of the present study, dietary soy supplementation in FR hens resulted in higher levels of phosphorus, calcium, and magnesium levels in femurs as compared to BC hens on a high soy diet. Within the BC production system, hens receiving soy supplementation had longer femurs that were similar in length to femurs in FR hens. Within the constraints of this study, bones in young laying hens showed minimal signs of fragility, some evidence of demineralization, and the ability to be altered with the combination of exercise and dietary soy supplementation.

Keywords: Laying hen production; Free-range chickens; Bone strength; Bone composition

Introduction

Bone fragility and osteoporosis have become major problems in the poultry industry leading to economic losses and animal welfare concerns. Osteoporosis in laying hens is characterized by the decrease in mineralized structural bone over time. The condition eventually leads to bone fragility and an increased likelihood of fractures during either the production period or depopulation [1]. While laying hens are predisposed to osteoporosis as a result of prolonged periods of egg production, battery cage systems have exacerbated the disease, causing increased incidence of fractures [2]. In advanced stages, osteoporosis can manifest itself in the form of cage-layer fatigue characterized by spontaneous bone fractures, vertebral weakness and in extreme cases even paralysis [3]. Osteoporosis in laying hens may be the result of several nutritional, environmental and genetic factors; however, physical activity and endogenous estrogen levels are two notable factors that influence the incidence of osteoporosis in commercial hens [1, 4,5].

Cage layer fatigue occurs almost exclusively in hens housed in battery cages [6-8]. These hens have considerably weaker bones than hens grown in high-level aviaries or free-range production systems where they have more opportunities for flight, flapping and perching [6]. A study conducted by Gregory and Wilkins [2] examined hens over the course of their entire production period and found that 29% of the hens housed in battery cages had at least one bone fracture during their lifetime. A subsequent survey found that 24% of hens from battery cages had freshly broken bones compared to broken bones in 10% of the hens in an aviary system [9]. Confinement of birds in cages where they have limited mobility and negligible physical activity contributes to the manifestation of disuse atrophy and osteoporosis [1].

Another factor that affects the incidence of osteoporosis in laying hens is endogenous estrogen which largely controls the formation of medullary bone [4]. Medullary bone (MB), common in long-bones, is a non-structural type of woven bone exclusive to female egg-laying birds that can be resorbed 10-15 times faster than structural bone and provides the necessary calcium (Ca) for egg shell formation [10]. Shortly before hens enter lay, circulating plasma 17-β estradiol increases triggering follicular maturation and medullary bone formation [1]. During the laying period, structural bone formation ceases and is continuously resorbed to supply calcium for medullary bone synthesis causing skeleton...
fragility and increasing the chance of fractures [6]. Decreased 17-β estradiol circulation signals a decline in egg production and causes medullary bone formation to cease [1]. As estradiol levels decline, formation of structural bone recommences, and is evident by the appearance of a layer of structural bone deposited on top of the existing medullary bone [11]. This homeostatic mechanism is normal in all laying hens enabling them to lay in clutches with the follow-up incubation giving them time to regenerate structural bone [1]. However, commercial laying hens are typically selected for production capacity, and prolonged lay leads to bone fragility and eventual osteoporosis [4].

Recent research has begun to investigate the effects of soybean isoflavones on avian performance, hatchability, egg quality, blood parameters and organ development [12-16]. Structurally similar to estrogen, isoflavones and their subsequent metabolites (daidzein and genistein) have been found to exhibit estrogenic behavior by binding to estrogen receptors (ER's) [17]. Based on levels of endogenous estrogen, the effects of isoflavones can be classified as positive (amplifying the effects of estrogen), or negative (suppressing and down-regulating estrogen) [18]. Reports have suggested that soybean isoflavones may reduce the risk of osteoporosis [12]. In avian species, few studies have examined the impact of high-doses of soybean isoflavones on bone development and maintenance. Thus, the purpose of this field trial was to examine the effects of dietary isoflavone supplementation (in the form of soy germ) on the physical and chemical properties of femurs harvested from laying hens that were reared in either battery cages (BC) or free range (FR) production systems.

Materials and Methods

This research project was reviewed and approved by the Clemson University Institutional Animal Care and Use Committee (IACUC) prior to the procurement of chicks (Institutional Animal Care and Use Number IAP 2011-008). Researchers completed the Animal Care and Use Series–Live Vertebrate Animals, through the Clemson University IACUC training program. The series meets USDA and Office of Laboratory Animal Welfare requirements for training in the humane care and use of animals. It covers general principles of the ethical care and use of animals in research. These institutional and national guidelines were followed for the care and use of the laying hens used in this study.

Housing

Eighty-four, 2 day old, Bovan Brown chicks from a commercial hatchery were transferred to the University Poultry Production Center. Chicks were maintained in 6 indoor floor pens under brooders and on pine shavings in groups of 14 chickens per pen and given ad libitum access to feed and water. At 16 weeks of age, pullets from three of the pens were moved to a FR housing system and pullets in the remaining three pens were moved into the BC housing system.

Caged layers were housed in two banks of BC in an indoor poultry house. Banks were 3 cages high, 4 cages per level, and 2 cages wide. Each BC had 61 X 61 X 41 cm (0.15m3) of space. Sets of 3 or 4 pullets from a single floor pen were placed into individual BC along a single bank row. A total of three rows, one row from each floor pen, were staggered among the 2 banks to avoid cross-over of feed or feces from one group to another during the study. Cages had wire mesh flooring and were equipped with feed troughs and nipple drinkers. Egg collection troughs with wire dividers were attached to the front of the cages to preclude eggs from one cage mixing with eggs from another cage.

The FR house was partitioned into three 1.5 x 3m (4.5m2) indoor pens, each with access to a separate 7.6x13.7m (104.5m2) outdoor range. Chain link fencing, an outside electric wire and overhead nylon netting were used to prevent entrance by predators. A light-weight shading tarp was spread over 1/3 of the overhead netting to supply shade for each range area. Grass was mowed short in each range and surrounding areas prior to introducing the pullets to the FR system. The three indoor pens of the range house were separated by fencing and were equipped with 9 nest boxes each. Nest boxes contained pine shavings and were accompanied by wooden perches. Each pen contained a separate waterer and feeder and the floor was covered with pine shavings bedding.

Diet

All diets met the National Research Council nutrient requirements for poultry [19]. During the grow-out period (2 days to 20 weeks of age), parallel diets for FR and BC hens of standard 25% soy (SS) formulations were used. FR diets were plant-based while the BC diets included animal sources of fat and protein to mimic typical commercial diets in these production systems. Birds received starter feed crumbles consistent with their pen’s housing system assignments until 18 weeks of age when starter feeds were substituted with layer feeds. Standard soy layer feeds were fed from 18-20 weeks of age. When hens turned 20 weeks of age, all hens were fed a washout diet of either the BC or FR soy free diets. At 21 weeks, hens began 3 weeks of their assigned treatment diets.

Table 1: Diet fed to laying hens after 20 wk-of-age.

| Ingredient (% by volume) | Battery Cage (BC) | Free-Range (FR) |
|-------------------------|-------------------|-----------------|
|                         | Standard Soy1 BCSS| Soy-Free2 BCSF  | Standard Soy1 FRSS| Soy-Free1 FRSF |
| Corn %                  | 56.4              | 58.8            | 56.4              | 18.2            |
| Soybean meal %          | 25                | 0               | 25                | 0               |
| Poultry meal %          | 0                 | 19.67           | 0                 | 0               |
| Poultry fat %           | 4.75              | 0               | 4.75              | 0               |
| Whole soybeans %        | 0                 | 0               | 0                 | 0               |
Layer feeds (Table 1) included: 1) FR standard soy feed (FRSS) which contained 18% crude protein with 2,750kcal/kg ME; 2) FR soy free feed (FRSF) containing 19% crude protein with 2,684kcal/kg ME; 3) BC standard soy feed (BCSS) containing 19% crude protein with 2,750kcal/kg ME and 4) BC soy free feed (BCSF) which contained 18% crude protein with 2,860kcal/kg ME. Two soy enhanced feeds were formulated - one for the FR hens and one for the BC hens. These were created by adding 5 grams of soy-germ/100 grams of feed to the 25% standard FR feed and BC feed. Soy germ was procured in a bulk shipment and mixed with the SS feeds in the feed mixing room at the University of Georgia’s (UGA) Poultry Research Farm facility in Athens, Georgia. All feeds were mixed on the same day and in the total amount needed for the duplicate trials.

Table 2: Proximate composition and mineral analyses of diets prior to feeding to laying hens and reported on a dry weight basis.

| Hen Diets | BCSS | BCSE | BCSF | FRSS | FRSE | FRSF |
|-----------|------|------|------|------|------|------|
| Crude Protein (%) | 18.8 | 19.7 | 18.4 | 18.6 | 19  | 18.1 |
| Fat (%) | 2.5 | 2 | 7.7 | 3.3 | 3.2 | 3 |
| Moisture (%) | 10.7 | 10.2 | 10.1 | 10.3 | 9.7 | 11.2 |
| Dry matter (%) | 89.3 | 89.8 | 89.9 | 89.7 | 90.3 | 88.8 |
| P (%) | 0.72 | 0.77 | 1.49 | 0.79 | 0.83 | 1.09 |
| Ca (%) | 3.3 | 3.2 | 6.44 | 4.25 | 3.33 | 4.9 |
| Ca/P | 4.61 | 4.16 | 4.32 | 5.37 | 4.01 | 4.48 |
| Mg (%) | 0.19 | 0.2 | 0.22 | 0.35 | 0.34 | 0.24 |
| K (%) | 0.9 | 0.9 | 0.54 | 1.14 | 1.13 | 0.71 |
| S (%) | 0.24 | 0.23 | 0.33 | 0.28 | 0.27 | 0.26 |
| Zn (ppm) | 180 | 138 | 136 | 172 | 134 | 194 |
### Results and Discussion

#### Bone parameters

At the end of the feeding study, 18 hens (9 from each production system) were euthanized by cervical dislocation. Hens were chosen at random within the two production systems to include 3 hens from each soy treatment (SF, SS and SE).

Both femurs were removed from each carcass, cleansed of adhering tissue and stored at 4.4°C for less than 24h. Femur length was measured from medial condyle to the greater trochanter and stored at 4.4°C for less than 24h. Femur length was measured from medial condyle to the greater trochanter using digital calipers and recorded to the nearest 100th decimal place. Similarly, width measurements were taken at the calculated midpoint or 50% of length [21-23]. Bone volume was determined by utilizing the water displacement method described by Cheng and Coon [24]. Briefly, this method measures bone volume by placing femurs in a 100mL graduated cylinder filled with 70mL of distilled water. Femur volume can be determined from the change in volume after the femur is fully submerged. Wet densities of the femurs were calculated by dividing the mass in g by the volume (mL) of the bone [24].

After the physical properties were measured, bone strength was determined using a TA-TX Plus Texture Analyzer equipped with a 50kg load cell and programmed to conduct a 3-point bend test using a 1mm blade. Fulcrum points on femurs were set 62.33mm apart and the femur was subjected to shear test to failure at a constant loading rate of 20mm/s.

Broken femurs were then crushed and pulverized in a Waring® blender. The pulverized particles were placed in labeled Whirl-Pak bags and sent to the Agricultural Service Laboratory for standard mineral analysis to determine phosphorous (P), potassium (K), calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu), Manganese (Mn), Iron (Fe), Sulfur (S) and Ca/P amounts. Mineral analysis was performed using the inductively coupled plasma-mass spectrometry method recommended by the US Environmental Protection Agency with detection limits between 1 and 100 ng/L [20].

#### Statistical Analysis

Data were analyzed by the General Linear Model procedure of the SAS/STAT program [25] using feed formulation, production system (FR or BC) and their interactions as the main effects for the model. All first order interactions were tested for statistical significance (P < 0.05) using the residual error mean squares. Means were separated using the least-squares means option and reported along with the standard error [25].

### Table 3: Laying hen body weight (Kg) and feed consumption (Kg) for Battery Cage and Free Range production systems and diets[^1^]

| Age 23 (Treatment Week 3, Cohort 1) | Age 27 (treatment week 3, cohort 2) |
|----------------------------------|----------------------------------|
| **FR Diets[^2^]**                | **BC Diets[^2^]**                |
| FRSS                            | BCSS                            |
| FRSE                            | BCSE                            |
| FRSF                            | BCSF                            |
| **Weight[^3^]** (Kg)             | **Weight[^3^]** (Kg)             |
| 1.74a                           | 1.77a                           |
| 1.76a                           | 1.72a                           |
| 1.77a                           | 1.56a                           |
| **Weekly Feed Consumption[^4^]** (Kg) | **Weekly Feed Consumption[^4^]** (Kg) |
| 1.45a                           | 1.11a                           |
| 1.45a                           | 0.92a                           |
| 1.11a                           | 0.83a                           |

[^1^]: Standard mineral analyses includes: phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe).

[^2^]: FRSS=Free-Range Standard Soy Feed; FRSE=Free-Range Soy Enhanced Feed; FRSF=Free-Range Soy Free Feed; BCSS=Battery Cage Standard Soy Feed; BCSE=Battery Cage Soy Enhanced Feed; BCSF=Battery Cage Soy Free Feed.

[^3^]: Means were separated using the least-squares means option and reported along with the standard error [25].
Table 4: Effect of production system and diet on the physical properties of laying hen femurs post-slaughter<sup>1,2</sup>

| Production System | Diet<sup>3</sup> | Weight (g) | Width (mm) | Length (mm) | Volume (cm<sup>3</sup>) | Density (g/cm<sup>3</sup>) |
|-------------------|-----------------|------------|------------|-------------|-------------------------|---------------------------|
|                   |                 |            |            |             |                         |                           |
| Battery Cage      | SE              | 10.6±0.3   | 8.14±0.04  | 86.6±0.4    | 8.1±0.2                 | 1.31±0.02                 |
|                   | SS              | 9.92±0.4   | 8.1±0.08   | 84.4±1.2    | 7.3±0.3                 | 1.35±0.01                 |
|                   | SE              | 11.6±0.4   | 8.1±0.14   | 89.9±1.2    | 8.2±0.3                 | 1.42±0.01                 |
|                   | SS              | 10.2±0.1   | 8.1±0.16   | 87.1±0.7    | 7.7±0.2                 | 1.34±0.03                 |
|                   | SF              | 10.1±0.2   | 8.2±0.1    | 86.3±0.8    | 7.5±0.3                 | 1.35±0.03                 |

<sup>1</sup>Means±standard error in a column without common superscripts are significantly different at P ≤ 0.05.

<sup>2</sup>N =14 laying hens.

<sup>3</sup>SE represents standard soy diet (25%) with added soy germ in the amount of 5 g soy-germ/100 grams diet; SS indicates standard soy diets with 25% soy content; SF represents a diet that did not contain soy.

Bone strength and bone mineralization were measured on femurs to determine if there was any evidence of osteoporosis in laying hens. Femur bone strength as measured by force-to-fracture varied widely (217.4 N to 304.7 N) and showed no distinct difference due to hen diet or production system (data not shown). Indiscernible patterns in bone strength data may stem from the young age of the hens and the genetics of the strain used (Bovan Brown), a typical free-range bird. Previous research has...
demonstrated that bone strength of femurs harvested from hens housed in conventional battery cages was significantly lower (21.92kgf or 215 N) than bone strength of femurs harvested from hens in colony cages modified to allow increased movement (approximately 27-30kg for 265-290 N) [3]. Values in the present study are similar to those previously reported, but wide variations in the data suggest that more information is need to draw a similar conclusion.

Table 5: Effect of production system and diet on bone mineral content (BMC) of femurs from laying hens post-slaughter1,2

| Production System | Diet3 | P (%) | Ca (%) | Mg (%) | Ca/P (%) |
|-------------------|-------|-------|--------|--------|----------|
|                   | SE    | 7.17±(0.45) | 15.3±(0.98) | 0.227±(0.016) | 2.14±(0.006) |
|                   | SS    | 7.97±(0.17) | 16.9±(0.38) | 0.263±(0.006) | 2.12±(0.006) |
| Battery Cage      | SF    | 7.87±(0.22) | 16.4±(0.50) | 0.272±(0.008) | 2.09±(0.015) |
|                   | SE    | 8.12±(0.14) | 18.0±(0.35) | 0.313±(0.007) | 2.22±(0.020) |
|                   | SS    | 7.56±(0.19) | 17.2±(0.41) | 0.283±(0.009) | 2.27±(0.014) |
| Free Range        | SF    | 7.40±(0.18) | 17.0±(0.42) | 0.256±(0.009) | 2.30±(0.009) |

1,2 Means with standard error in parenthesis in a column without common superscripts are significantly different at P < 0.05.

Acknowledgements

The authors would like to acknowledge the help and poultry expertise of University of Georgia poultry nutritionist, Nicholas M. Dale, and the Poultry Research Center Supervisor, Christopher A. McKenzie. Additionally, the authors gratefully acknowledge expert technical assistance by Randy Koch of Texture Technologies and Carol Foster-Mosley and Karen Tankersley of Clemson University Morgan Poultry Farm.

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