Molt Performance and Bone Density of Cortical, Medullary, and Cancellous Bone in Laying Hens During Feed Restriction or Alfalfa-Based Feed Molt

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ABSTRACT A study was conducted to evaluate the effects of alfalfa-based molt diets on molting performance and bone qualities. A total of 36 Single Comb White Leghorn hens were used for the study. There were 6 treatments: pretrial control (PC), fully fed (FF), feed withdrawal (FW), 90% alfalfa:10% layer ration (A90), 80% alfalfa:20% layer ration (A80), and 70% alfalfa:30% layer ration (A70). For the PC treatment, hens were euthanized by CO_2 gas, and bones were collected before molt was initiated. At the end of the 9-d molt period, hens were euthanized, and femurs and tibias were collected to evaluate bone qualities by peripheral quantitative computed tomography, mechanical testing, and conventional ash weights. The hens fed alfalfa-based molt diets and FW stopped laying eggs within 5 d after molt started, and all hens in these groups had reduced ovary weights compared with those of the FF hens. In the FW and A90 groups, total femur volumetric bone mineral densities (vBMD) at the midshaft were significantly lower, but those of the A80 and A70 groups were not significantly different from the values for the PC and FF hens. In cortical bone density, the midshaft tibial vBMD were significantly higher for FF and A70 hens than for PC hens. The medullary bone densities at the midshaft femur or tibia of the FW, A90, A80, and A70 hens were reduced compared with those of the PC hens. Femur cancellous densities at the distal femur for the FW and A90 hens were significantly reduced compared with those of the PC and FF hens. The FW, A80, and A70 hens yielded significantly higher elastic moduli, and the A80 hens had higher ultimate stress compared with the PC hens, suggesting that the mechanical integrity of the midshaft bone was maintained even though the medullary vBMD was reduced. These results suggest that alfalfa-based molt diets exhibit molt performance similar to FW, that medullary and cancellous bones are labile bone compartments during molting, and that alfalfa-based molt diets may be beneficial to maintain the mechanical properties of bones during molt.

Key words: molt, peripheral quantitative computed tomography, alfalfa, cortical bone, medullary bone

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INTRODUCTION

Molting is a viable management strategy in the egg industry to stimulate multiple egg-laying cycles in commercial hens (Brake, 1993; Park et al., 2004). The primary method used in the egg industry is feed withdrawal (**FW**) because of the management and economic benefits associated with multiple laying cycles and higher quality egg production (Holt et al., 1995; Bell, 2003; Park et al., 2004). A typical induced molting method is the complete removal of feed for 10 to 14 d, along with a reduction in the photoperiod from 16 to 8 h (Bell, 1987; Brake, 1993). After resting periods (0 to 21 d) following FW, molted hens are fed regular layer rations to start the second egg-laying cycle. However, FW methods have received tremendous negative attention related to the animal welfare issue in recent years because they cause marked stress to the hens, decrease the cell-mediated immune response, and increase susceptibility to pathogenic infections (Holt et al., 1995; Davis et al., 2000; Bar et al., 2003; Gast and Ricke, 2003; Holt, 2003; Ricke, 2003; Webster, 2003). Furthermore, inducing molt by FW is detrimental to skeletal integrity (Garlich et al., 1984; Mazzuco and Hester, 2005; Kim et al., 2006). The most common techniques for measuring skeletal integrity in molted hens are conventional bone assays (Garlich et al., 1982; Cheng and Coon, 1990),

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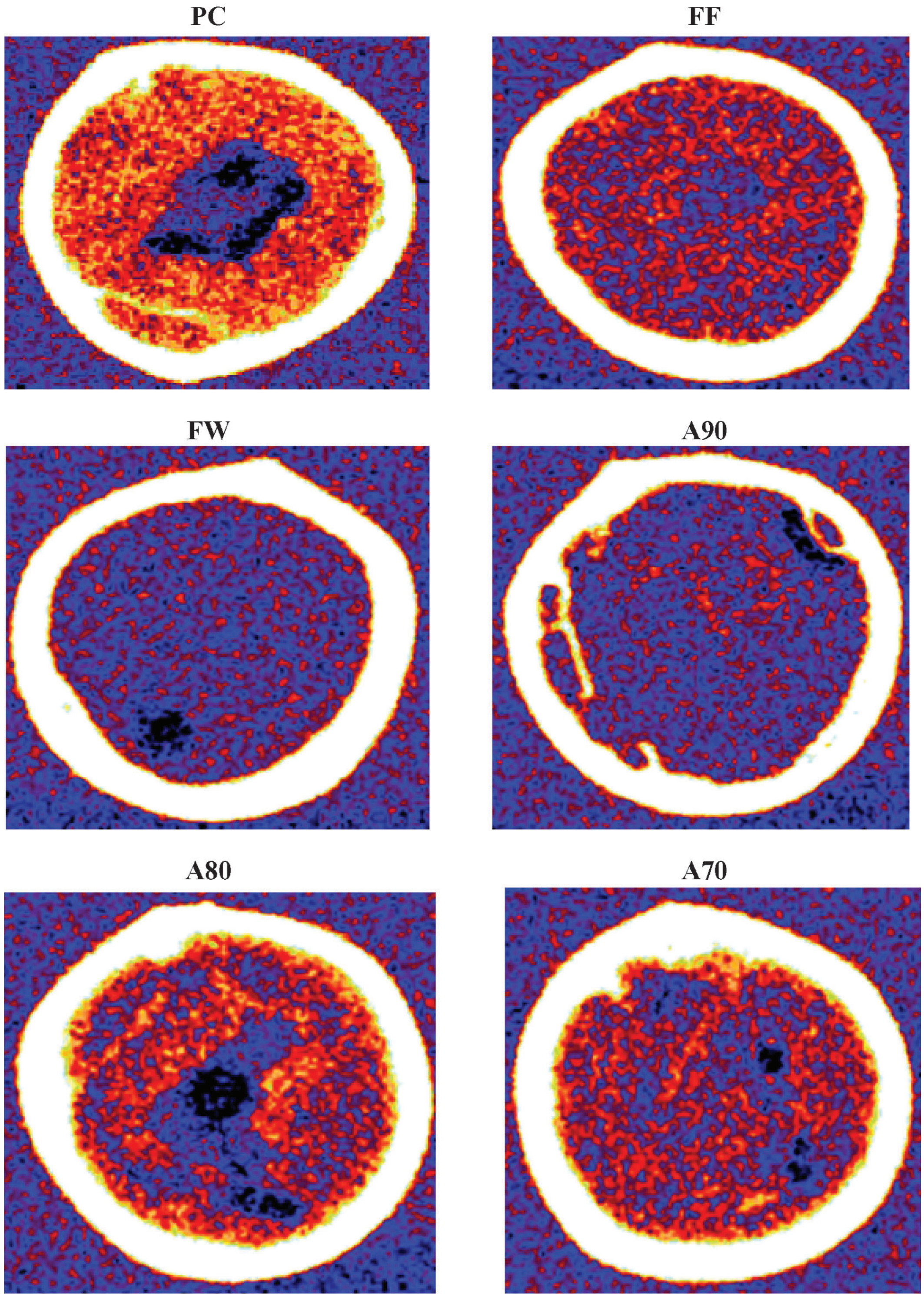

Figure 1. Peripheral quantitative computed tomographic image of midshaft femur bone of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period. PC = pretrial control; FF = fully fed 100% layer ration; FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; and A70 = 70% alfalfa:30% layer ration.

such as ash weight, ash percentage, and ash concentration or bone mineral density, by using dual-energy x-ray absorptiometry (**DEXA**; Watkins and Southern, 1992; Schreiweis et al., 2003; Kim et al., 2004). Although it may be important to evaluate changes in bone mineral distribution and bone density in different bone compartments during molting, none of these techniques can provide such information.

Skeletal problems in laying hens are an important welfare, health, and economic issue for the poultry industry. One of the major skeletal problems in laying hens is structural bone loss related to osteoporosis (Gregory and Wil-

Table 1. Feed, energy, total protein, calcium, and phosphorous intakes of hens fed different diets during a 9-d molt period

Treatment[1]	Feed (g/bird)	Energy (kcal/bird)	Protein (g/bird)	Ca (g/bird)	P (g/bird)
FF	997.0[a]	2863.4[a]	149.6[a]	32.4[a]	13.46[a]
FW	—	—	—	—	—
A90	136.5[c]	186.6[b]	22.9[c]	2.2[b]	0.46[b]
A80	121.5[c]	186.4[b]	20.2[c]	2.2[b]	0.50[b]
A70	263.2[b]	447.9[b]	43.2[b]	5.2[b]	1.32[b]
Pooled SE	30.1	66.2	4.7	0.7	0.26

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]FF = fully fed (100% layer ration); FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration.

kins, 1989; Whitehead, 2004; Kim et al., 2005). Widespread structural bone loss related to osteoporosis in laying hens can cause a high incidence of fractures at various sites of the skeleton, with an average of 34% of processed birds exhibiting freshly broken bones (Gregory and Wilkins, 1989; Fleming et al., 1998; Whitehead and Fleming, 2000). Inducing molt by feed removal is a factor that potentially increases structural bone loss in old laying hens (Park et al., 2004). Garlich et al. (1984) showed that feed-removal molt decreased femur weight and density in laying hens. Mazzuco and Hester (2005) also indicated that feed removal greatly reduced bone mineral density as assessed by DEXA. Reduced bone mineral density was associated with an increased incidence of broken bones at 126 wk of age.

Structural bone loss and the development of osteoporosis in laying hens might be related to the modeling and remodeling of the medullary bone, which serves as a primary calcium source for eggshell formation (Wilson and Thorp, 1998; Wilson et al., 1998; Cransberg et al., 2001). The formation of medullary bone occurs at sexual maturity when the estrogen level increases (Whitehead and Fleming, 2000). During the period of egg production, the content of medullary bone increases at the expense of cortical bone, leading to progressive structural bone loss (Wilson and Thorp, 1998; Whitehead and Fleming, 2000). Rapid changes in bone resorption and formation in the medullary compartment are synchronized with a daily egg-laying cycle (Cransberg et al., 2001; Kim et al., 2003). Although formation of the medullary bone is enhanced throughout the laying period, formation of new cortical bone profoundly decreases, causing an imbalance of skeletal metabolism between these 2 bone compartments (Hudson et al., 1993; Whitehead and Fleming, 2000). Therefore, quantifying the medullary bone status in laying hens during the molting period would provide valuable information on bone metabolism, interactions with structural bone, and overall skeletal integrity. However, little information exists on the medullary bone status of laying hens during molting. In the present study, peripheral quantitative computed tomography (**pQCT**) was used to measure volumetric bone mineral density (**vBMD**) at 4 different bone sites in the femur and tibia. This 3-dimensional methodology also allows quantification of separate bone compartments, that is, cortical vs. medullary or cancellous bone, which can yield more precise information on the bone status of laying hens during the molting period. The objective of this study was to evaluate molt performance and the resulting skeletal integrity of cortical, medullary, and cancellous bone compartments in laying hens during feed restriction or alfalfa-based feed molt.

MATERIALS AND METHODS

Molting Procedure

A total of 36 Single Comb White Leghorn hens (80 wk of age) were obtained from a commercial laying hen facility for this experiment. Hens were housed 2 per cage

Table 2. First day out of egg production, ovary weight, and BW loss of hens fed different diets during a 9-d molt period

Treatment[1]	First day out of egg production (d)	Ovary weight (g)	Ovary weight (% as % of BW)	BW loss (g)	BW loss (%)
FF	—	40.1[a]	2.64[a]	45.3[c]	2.9[c]
FW	4.36	11.9[b]	1.09[b]	434.5[a]	28.2[a]
A90	4.00	9.7[b]	0.90[b]	367.8[ab]	25.4[ab]
A80	4.90	9.4[b]	0.83[b]	294.2[b]	20.2[ab]
A70	4.25	10.1[b]	0.81[b]	290.4[b]	18.8[b]
Pooled SE	0.32	2.6	0.19	36.3	2.2

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]FF = fully fed (100% layer ration); FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration.

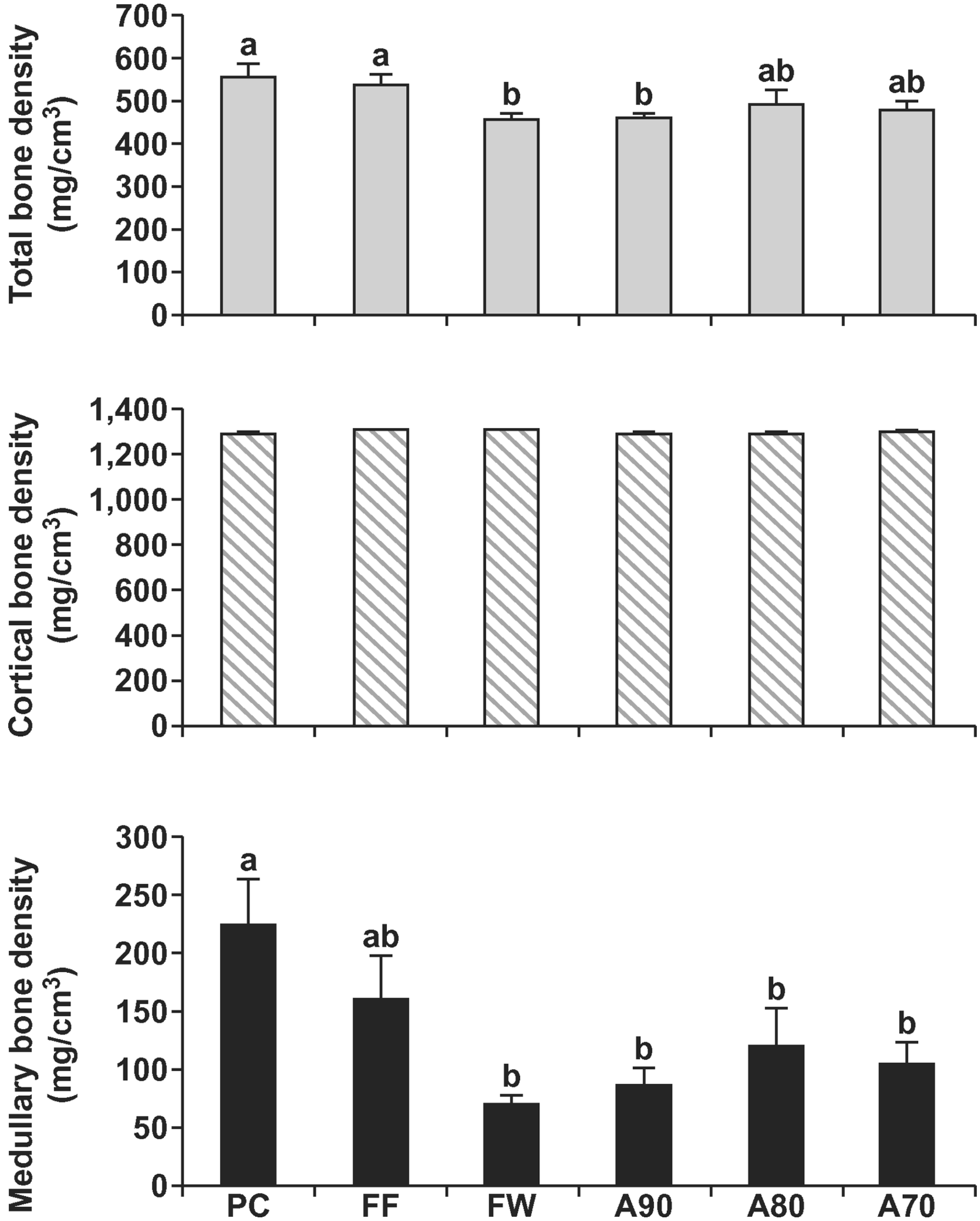

Figure 2. Midshaft femur bone density by peripheral quantitative computed tomography of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period. PC = pretrial control; FF = fully fed 100% layer ration; FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration. [a,b]Means ± SE with different letters differ ($P < 0.05$).

at the Texas A&M University Poultry Science Research Center in College Station, Texas, and allowed time for acclimation. During this time, the birds were fed a complete layer ration ad libitum and allowed full access to water for a period of 6 wk. Egg production was monitored to ensure that all hens were healthy and actively producing eggs. The hens were divided into 6 treatment groups with 6 birds per treatment: pretrial control (**PC**), fully fed (**FF**), FW, 90% alfalfa:10% layer ration (**A90**), 80% alfalfa:20% layer ration (**A80**), and 70% alfalfa:30% layer ration (**A70**; Donalson et al., 2005). All treatment groups were allowed ad libitum access to water and their respective diets. Hens were placed on an artificial lighting program of 8L:16D for 1 wk prior to molt. Treatment groups were randomly assigned to cages throughout the house to ensure there was no variability in egg production or reproductive tract regression due to light stimulation, and the groups molted for 9 d.

In accordance with the Texas A&M University Lab Animal Care Committee animal use protocols, any hen reaching 25% weight loss prior to the end of the trial (d 9) was removed from its diet and immediately placed on full feed. Feed intake was measured by weighing each treatment diet prior to the start of the molt and after the 9-d

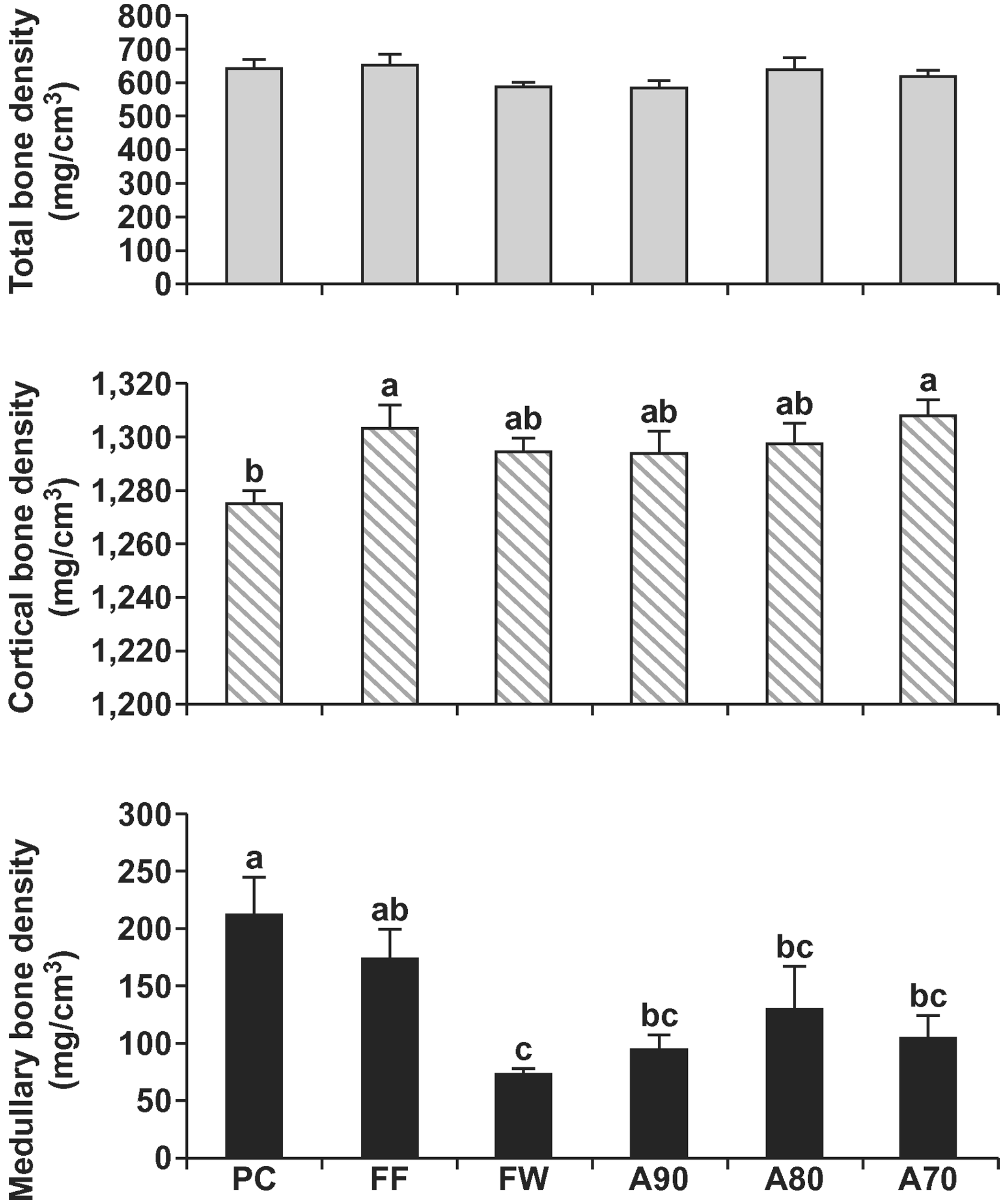

Figure 3. Midshaft tibia bone density by peripheral quantitative computed tomography of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period. PC = pretrial control; FF = fully fed 100% layer ration; FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration. [a–c]Means ± SE with different letters differ ($P < 0.05$).

molt period. Egg production was recorded daily during the molt period.

Sample Collection

Before molt started, PC hens were euthanized by CO_2 gas, BW was measured, and ovaries and bones were collected. At the end of the 9-d molt period, hens were euthanized, and the right tibias and femurs were collected to evaluate bone quality by pQCT, testing of mechanical properties, and conventional ash weight determination. After tibias and femurs were obtained from each hen, the bones were cleaned of attached tissue, wrapped in PBS-soaked gauze for pQCT and mechanical testing, and stored at −20°C.

pQCT. Bone scans of right tibias and femurs were performed with an XCT Research M instrument (Stratec, Norland, Fort Atkinson, WI). This model has a minimum voxel size of 0.07 mm and a scanning beam thickness of 0.50 mm. Scan sites included the tibia and femur middiaphyses (3 slices of each bone located at one-half the total bone length ± 2 mm) for vBMD of the medullary and cortical bone; the proximal tibia and distal femur were scanned to assess metaphyseal cancellous vBMD (3 slices located 8.0, 8.5, and 9.0 mm from the proximal or distal end of the bone). All scans were obtained at a scan speed of 2.5 mm/

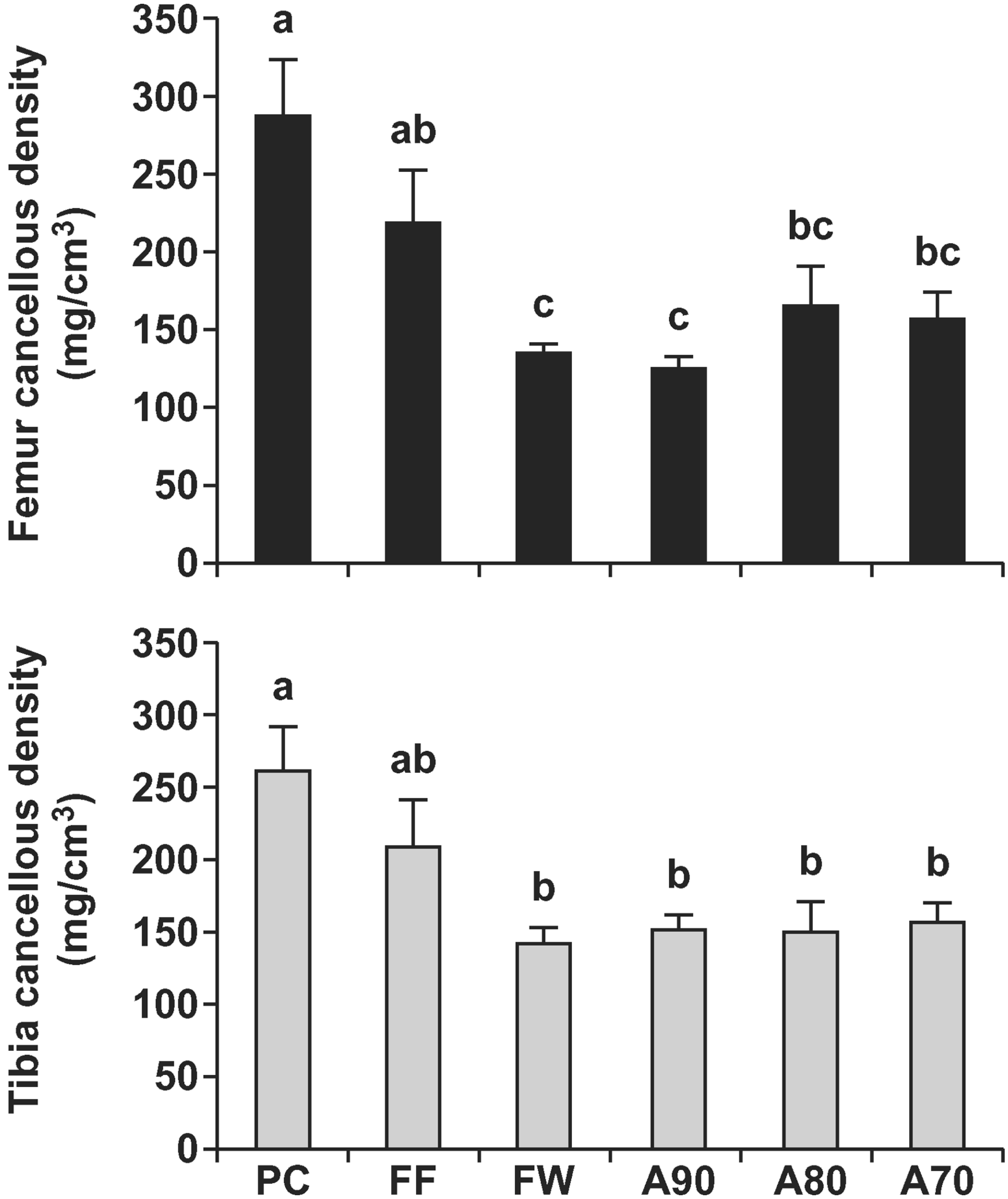

Figure 4. Metaphyseal bone density by peripheral quantitative computed tomography of the distal femur and proximal tibia of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period. PC = pretrial control; FF = fully fed 100% layer ration; FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration. [a–c]Means ± SE with different letters differ ($P < 0.05$).

s, with a voxel resolution of 0.07 × 0.07 × 0.50 mm. In addition, the middiaphyseal cross-sectional moment of inertia (**CSMI**) was obtained with respect to the neutral bending axis of the tibial bone shaft during 3-point bending (mechanical property testing).

Mechanical Testing. After pQCT scans were completed, mechanical properties of the midshaft tibia were determined by 3-point bending to failure with an Instron 1125 servo-controlled testing machine (Instron Corp., Canton, MA) according to previously published procedures (Allen and Bloomfield, 2003). The bones were thawed at room temperature and placed posterior side down on metal pin supports located ±10 mm (femurs) or ±30 mm (tibias) from the middiaphysis testing site. With a 1,000-lb (455 kg) load cell, quasi-static loading (2.5 mm/min) was applied to the anterior surface of both the tibia and femur until fracture. All specimens were sprayed with PBS just before testing to maintain hydration. Displacements were monitored by a linear variable differential transformer interfaced with a personal computer. Raw data, collected at 10 Hz as load vs. displacement curves, were analyzed with Table-Curve 2.0 software (Jandel, San Rafael, CA). Structural variables (ultimate load and stiffness) were obtained directly from the load:displacement curves. The maximum load obtained was defined as the ultimate load (UL, in N), and the slope of the elastic portion of the

Table 3. Dry weight, ash weight, and ash percentage of tibia of hens fed a normal layer ration or an alfalfa-based molt diet[1]

Parameter	PC	FF	FW	A90	A80	A70	Pooled SE
Dry weight (g)	5.18	5.02	4.63	4.68	5.11	5.23	0.19
Ash weight (g)	2.55[a]	2.51[a]	2.19[b]	2.19[b]	2.36[ab]	2.48[ab]	0.08
Ash percentage[2]	49.1	50.2	47.6	46.9	46.4	47.7	1.67

[a,b]Means within a column with different superscripts differ ($P < 0.05$).

[1]PC = pretrial control; FF = fully fed (100% layer ration); FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration.

[2]Ash percentage = ash weight/dry weight × 100.

curve was defined as stiffness (S, in N/mm). Bone tissue material properties were calculated by normalizing structural properties for cross-sectional bone geometry at the site of testing by using CSMI (in mm^4 from pQCT), bone diameter (D, in mm) as measured by calipers, and the appropriate bottom support span distance (L, which was 20 or 60 mm). The appropriate formulas for elastic modulus (E, in GPa) and ultimate stress (US, in MPa) are as follows:

$$E = S \times L^3/(48{,}000 \times \mathrm{CSMI}), \text{ and}$$

$$US = UL \times L \times D/(8 \times \mathrm{CSMI})$$

Bone Ash Measurement

Following bone density and bone breaking strength measurements, tibia samples were dried in an oven at 100°C for 24 h and weighed. The bones were then ashed at 600°C overnight, cooled in a desiccator, and weighed. The percentage ash was calculated by dividing the ash weight of each bone by its DM.

Statistical Analysis

All data were subjected to 1-way ANOVA as a completely randomized design using the GLM procedure of SAS (SAS Institute, 2001). Significant differences among the means were determined by Duncan's multiple-range test at $P < 0.05$. Correlations of bone parameters were evaluated by Pearson correlation procedures.

RESULTS AND DISCUSSION

Feed, energy, total protein, calcium, and phosphorous intakes of hens fed different molt diets during the 9-d molt period are shown in Table 1. The hens fed the 100% layer ration (FF) had significant higher feed, energy, protein, calcium, and phosphorous intakes compared with the other groups ($P < 0.05$). When ratios of layer ration to alfalfa were increased in the alfalfa-based molt diets, feed and nutrient intakes were increased. The hens fed A70 exhibited significant higher feed and protein intakes compared with the hens fed A90 and A80 ($P < 0.05$). Donalson et al. (2005) also reported that feed intakes of hens fed alfalfa molt diets were subsequently lower than those of hens fed a 100% layer ration. The lower feed intake could have been due to the low palatability of alfalfa for hens. A high level of saponins is one of the potential factors suppressing feed intake by hens (Matsushima, 1972). In the present study, increases in the ratios of layer ration to alfalfa in the alfalfa-based molt diets improved feed intake, increasing nutrient intakes.

The first day of egg production, ovary weight, and BW loss during the 9-d molt period are presented in Table 2. The hens fed alfalfa-based molt diets (A90, A80, and A70) and FW hens stopped laying eggs within 5 d after molt started, whereas hens fed the 100% layer ration (FF) laid eggs continuously throughout the 9-d molt period. Ovary weights of the FW, A90, A80, and A70 groups were lower than those of the FF hens ($P < 0.05$), indicating that those diets effectively caused ovary regression during the 9-d molt period. The FW, A90, A80, and A70 groups had significantly lower ovary weights as a percentage of BW compared with the FF group. Body weight loss and the percentage of BW loss of the FW hens were subsequently higher than those of the FF, A80, or A70 groups. Body weight loss of hens was reduced when the ratios of layer ration to alfalfa were increased in the molt diets. Body weight loss appeared to be related to the feed intakes of hens. These results are in agreement with those of Donalson et

Table 4. Mechanical properties of tibia from hens fed a normal layer ration or an alfalfa-based molt diet[1]

Parameter[2]	PC	FF	FW	A90	A80	A70	Pooled SE
Elastic modulus (GPa)	2.34[b]	2.85[ab]	3.15[a]	2.94[ab]	3.36[a]	3.14[a]	0.23
Ultimate stress (MPa)	81.8[b]	98.6[ab]	106.7[ab]	92.9[ab]	116.6[a]	93.4[ab]	9.00
Ultimate load (N)	114.6	129.6	127.4	114.5	144.9	120.7	11.1
Stiffness (N/mm)	38.1	43.0	43.3	41.6	47.0	45.6	3.39

[a,b]Means within a column with different superscripts differ ($P < 0.05$).

[1]PC = pretrial control; FF = fully fed (100% layer ration); FW = feed withdrawal; A90 = 90% alfalfa:10% layer ration; A80 = 80% alfalfa:20% layer ration; A70 = 70% alfalfa:30% layer ration.

[2]Elastic modulus = tendency of a material to be deformed when a force is applied; ultimate stress = bone-bending strength (load per unit area); ultimate load = maximal load at failure points; stiffness = load required to deform bone a given amount (slope of the elastic portion of the load vs. displacement curve).

al. (2005). The authors also indicated that increases in the portion of alfalfa in the molt diets increased BW loss during molting. Landers et al. (2005) reported that hens fed 100% alfalfa diets exhibited a significantly lower percentage of BW loss compared with FW hens during molting, whereas they had ovary weights similar to those of the FW hens. Woodward et al. (2005) also indicated that alfalfa molt diets could be used as an alternative method for feed removal molting. Body weight loss and ovary regression are related to increased postmolt performance, such as higher egg production, better feed efficiency, and better shell quality in laying hens (Lee, 1982; Park et al., 2004). Body weight loss in hens during molt is mainly due to increased muscle and adipose tissue degradation, and decreased organ weights (Brake and Thaxton, 1979; Berry and Brake, 1985; Park et al., 2004).

Peripheral quantitative computed tomography images of midshaft femur bones of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period are presented in Figure 1. The highest bone density in the image is expressed as a white color, whereas the blue-colored areas are background. Cortical bones exhibited the highest bone densities in the image. The bone density differences in medullary bone were distinguished among the hens fed different dietary treatments. The PC hens possessed very dense medullary bone compared with those from the other groups. The FW hens had almost no medullary bone in the marrow, indicating that the medullary bone was completely resorbed during the 9-d molt period. Although the hens fed the A90 molt diet also had detectable medullary bone remaining in the bone marrow, the hens fed the alfalfa molt diets containing higher portions of layer ration (A80 and A70) stored a greater amount of medullary bone when compared with the FW and A90 hens.

Midshaft femur bone density, as measured by pQCT, of hens fed a normal layer ration or an alfalfa-based molt diet for a 9-d molt period is presented in Figure 2. The total femur densities of the FW and A90 groups were significantly lower than those of the PC and FF groups, whereas the A80 and A70 groups exhibited no significant differences when compared with the PC and FF groups. However, there were no significant differences in cortical bone density among the groups. The medullary bone densities of the FW, A90, A80, and A70 hens were subsequently reduced compared with that of the PC hens during the 9-d molt period. Midshaft tibia bone densities are shown in Figure 3. There were no significant differences in total bone density among the treatment groups. However, the FF and A70 groups yielded higher cortical bone density compared with the PC group ($P < 0.05$). Medullary bone densities of the FW group were significantly lower than those of the PC and FF groups. The A90, A80, and A70 groups exhibited more medullary bone resorption than the PC group, whereas there were no significant differences among the FF, A90, A80, and A70 groups. These results indicate that medullary bone is a labile component of hen bones for bone resorption during molt. The major function of medullary bone is as a special eggshell calcium reservoir (Dacke et al., 1993). Medullary bone is a woven-type trabecular bone formed in the marrow cavities, depending on ovarian estrogen (Yamamoto et al., 2001). Medullary bone turnover is synchronized with daily egg cycle and provides 30 to 40% of the eggshell calcium (Mueller et al., 1974; Miller, 1992). Compositions of medullary bone are different from typical cortical bone, causing it to be easily resorbed during molt. Medullary bone has a 3-fold higher mineral to collagen ratio and higher noncollagenous protein than cortical bone (Knott and Bailey, 1999). These noncollagenous proteins in medullary bone are highly glycosylated (Candlish and Holt, 1971; Fisher and Schraer, 1982). The major glycosaminoglycan of avian medullary bone is keratan sulfate, whereas the second predominant glycosaminoglycan is chondroitin sulfate (Fisher and Schraer, 1982). Furthermore, there are large differences in the collagen cross-link profile. Medullary bone contains a very high level of lysine hydroxylation compared with cortical bone (Knott and Bailey, 1999). Medullary bone has a lower concentration of mature collagen cross-links and a higher level of immature collagen cross-links, reflecting a rapid turnover compared with cortical bone (Knott and Bailey, 1999).

Metaphyseal bone density of the proximal femur and proximal tibia of hens fed a normal layer ration or the alfalfa-based molt diets is presented in Figure 4. Femur cancellous densities of the FW and A90 hens were significantly reduced when compared with those of the PC and FF hens during a 9-d molt period. The PC hens had a higher femur cancellous density compared with those of the A80 and A70 hens ($P < 0.05$), whereas there were no significant differences among the FF, A80, and A70 hens. For tibia cancellous density, there were no significant differences among the FF, FW, A90, A80, and A70 hens, whereas the PC hens had a higher tibia cancellous density than the FW, A90, A80, and A70 hens ($P < 0.05$). These results suggest that regions of cancellous bone are also susceptible to bone resorption during molt. Cancellous bone density can be considered as one of the measurements correlated with production traits in laying hens. Nadeau et al. (2003) indicated that cancellous bone mineral density of laying hens at 68 wk was negatively correlated with the total number of soft-shelled eggs. They also reported that feed consumption was highly correlated with cancellous bone mineral density, whereas there was no correlation between feed consumption and cortical bone mineral density. These results suggest that cancellous bone is more sensitive to nutritional and physiological changes than cortical bone.

In the present study, bones were collected postmortem to evaluate several bone parameters. Although bone status and resistance to bone fractures in vivo could be predicted by the ex vivo measurements, the in vivo measurements provided more accurate data for predicting skeletal integrity over time. Dual energy x-ray absorptiometry and pQCT can be useful tools for in vivo measurements in laying hens. Mazzuco and Hester (2005) evaluated the bone density of laying hens during the premolt, molt, and postmolt periods by DEXA in vivo. They also reported that bone density, as measured by DEXA, was highly correlated with conventional bone assays such as bone ash and bone-breaking force. Although the bone density values did not show a high correlation with conventional assay values, such as DEXA bone density values, the values by pQCT were correlated with bone ash or bone-breaking strength (Saunders-Blades et al., 2003). Although DEXA gives information on total bone density, pQCT provides information on the distribution of bone density in specific sites (Korver et al., 2004). In laying hen studies, pQCT may have some advantages over DEXA because pQCT can distinguish medullary bone from cortical bone. As noted previously, medullary bone in laying hens is a labile component, developed at the expense of cortical bone and synchronized with the daily egg cycle. Therefore, bone density measurements of laying hens in different bone types can provide valuable data on the influence of medullary bone compared with cortical bone and on the overall skeletal health of hens throughout the egg production period.

Dry weight, ash weight, and ash percentage of the tibia of hens fed a normal layer ration or an alfalfa-based molt diet are shown in Table 3. Tibia ash weights of the FW and A90 hens were significantly lower than those of the PC and FF hens, whereas there were no significant differences among the PC, FF, A80, and A70 hens. There were no significant differences in dry weight and ash percentage among the treatments. In the present study, hens fed the A80 and A70 molt diets maintained levels of tibia ash weight similar to those of the PC or FF hens, indicating that the A80 and A70 diets could provide proper nutrients for overall tibia mineral deposition. It has been demonstrated that mineral content is positively correlated with bone strength (Crenshaw, 1986; Martin and Boardman, 1993). Crenshaw (1986) reported that ash weight was highly correlated with dietary Ca and P levels, indicating that ash weight could be a reliable predictor of bone mechanical properties. Mechanical properties of tibia from hens fed a normal layer ration or the alfalfa-based molt diets are present in Table 4. The FW, A80, and A70 hens had significantly higher elastic moduli than the PC hens, whereas there were no significant differences among the FF, FW, A90, A80, and A70 hens. The A80 hens exhibited higher ultimate stress compared with the PC hens. There were no significant differences in stiffness among the treatments. Interestingly, molted hens on FW or on the A80 or A70 molt diets improved mechanical integrity, although vBMD of the medullary bone from molted hens was decreased compared with PC hens, suggesting that losing medullary bone does not impair bone structural properties. In particular, the A80 molt diet increased the elastic modulus and ultimate stress compared with the PC diet. It has been demonstrated that elastic modulus is positively correlated with calcium content and overall mineral content (Currey, 1988; Martin and Boardman, 1993; Rath et al., 1999). Improving bone mechanical properties can be beneficial to maintain overall bone strength, because bone strength is highly correlated with its structural and material properties (Zernicke et al., 1995; Wilson and Ruszler, 1998; Rath et al., 1999).

In summary, the present study suggests that the alfalfa-based molt diets exhibited molt performance similar to FW, and that medullary and cancellous bones are labile bone compartments during molting. Losing medullary bone during molting does not impair the mechanical properties of hen bones, indicating that short-term feed restriction causes acute bone resorption in medullary bone, but not in cortical bone, and that medullary bone does not appear to contribute to the mechanical strength of bone. The present study also suggests that alfalfa-based molt diets may be beneficial for reducing overall bone loss and maintaining mechanical properties during molt. In the future, more study is necessary to evaluate the effects of alfalfa-based molt diets on medullary, cancellous, and cortical bone qualities during the second laying cycle.

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