

# ENVIRONMENT, WELL-BEING, AND BEHAVIOR

## Effects of Alfalfa and Fructooligosaccharide on Molting Parameters and Bone Qualities Using Dual Energy X-Ray Absorptiometry and Conventional Bone Assays

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**ABSTRACT** The aim of this study was to evaluate effects of alfalfa and fructooligosaccharides (FOS) on molting performance and bone parameters compared with the conventional feed withdrawal molting procedure. A total of 36 Single Comb White Leghorn hens (84 wk of age) were used for this experiment. The hens were divided into 6 treatment groups with 6 birds per treatment: pre-trial control (PC), full fed (FF), feed withdrawal (FW), 100% alfalfa (A100), A100 + 0.375% FOS (A100L), and A100 + 0.75% FOS (A100H). At the end of the 9-d molt period, hens were euthanized, and tibia and femurs were collected to evaluate bone qualities using dual-energy x-ray absorptiometry (DXA), Instron (Model 1011 Instron Universal Testing Machine, Instron Corp., Canton, MA), and conventional bone assays. Egg production was recorded during the molting period to evaluate first day

out of production, and ovary was also collected to measure ovary weight. Alfalfa molting diets had comparable molting parameters, such as percentage of BW loss, ovary weight, and first day out of egg production, to the conventional feed withdrawal molting procedure, and FOS supplementation did not have any detrimental effects on molting performance. Conventional bone assay and DXA results suggest that hens lose a considerable amount of bone minerals during a molting period. The tibia and femur bone strengths of the FF, FW, A100, and A100L hens were significantly lower than the PC hens, whereas hens fed A100H had similar tibia bone breaking strength to that of the PC hens. The bone parameters measured by conventional assays, bone breaking strength measured by Instron, and bone density and mineral content measured by DXA were highly correlated to each other.

**Key words:** skeletal quality, bone density, alfalfa, dual-energy X-ray absorptiometry, bone breaking strength

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### INTRODUCTION

Feed withdrawal is the primary procedure used by the US poultry industry to induce molt and stimulate multiple egg-laying cycles in hens (Brake, 1993; Holt, 1995). The complete removal of feed for 10 to 14 d combined with a reduction in photoperiod from 16 to 8 h remains the method of choice (Bell, 2003; Brake, 1993) because the birds are out of production for a relatively short time (Brake, 1993). However, feed withdrawal molting methods have received increasing levels of scrutiny relative to animal welfare and food safety issues in recent years (Bar et al., 2003; Gast and Ricke, 2003; Webster, 2003; Park et al., 2004).

Because of the Ca demands of high levels of shell egg production after the molt period, structural bone loss in laying hens undergoing molt may also be an important

animal welfare issue for the poultry industry (Aziz-Abdul, 1998; Park et al., 2004). An induced molt using feed withdrawal is a potential factor for increasing structural bone loss and incidence of osteoporosis in laying hens (Park et al., 2004). Molt osteoporosis has been identified for several avian species (Kuenzel, 2003). Structural bone loss in laying hens can enhance fragility and susceptibility to fracture, causing severe pain (Whitehead and Fleming, 2000). Garlich et al. (1984), Newman and Leeson (1999), and Mazzuco et al. (2003) reported that bones from molted hens using feed removal had severely reduced bone mineral densities and bone breaking force compared with laying hens. Therefore, reducing bone losses during molting is important to prevent bone weakness problems in laying hens.

We hypothesized that alfalfa molting diets supplemented with fructooligosaccharides (FOS) would provide balanced nutrients and increase Ca absorption, reducing bone losses during molting compared with feed withdrawal molting method. Alfalfa is high-protein, high-Ca, and high-fiber feedstuff (NRC, 1994). Alfalfa molting diets have shown comparable molting parame-

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ters and post-molting performance to feed withdrawal (Landers et al., 2005a, b). Hens molted with 100% alfalfa compared with birds molted via feed withdrawal exhibited equivalent ovary weight regression, but alfalfa-induced, molted birds exhibited earlier re-entry into egg production, more eggs produced, and less BW loss (Landers et al., 2001b). Landers et al. (2005a) reported that post-molt egg production and egg quality of hens molted with alfalfa were comparable to those of hens molted by feed withdrawal.

Fructooligosaccharides, low molecular weight indigestible sugars, have been shown to stimulate Ca and Mg absorption in the intestine and increase bone mineral concentrations in humans and rats (Ohta et al., 1997; Morohashi et al., 1998; Takahara et al., 2000; Younes et al., 2001; Zafar et al., 2004). Dietary short-chain FOS increase calbindin-D9k levels in rats (Takasaki et al., 2000). Mineo et al. (2001) reported that FOS stimulated net Ca transport from the epithelium of the small and large intestine of rats *in vitro*. Thus, objectives of this study were 1) to evaluate effects of an alfalfa molting diet and FOS supplementation on molting performance and bone parameters during a 9-d molting period and 2) to evaluate correlations of tibia mineral content and density measured by dual-energy x-ray absorptiometry (DXA), bone breaking strength measured by Instron (Model 1011 Instron Universal Testing Machine, Instron Corp., Canton, MA), and bone parameters measured by conventional bone assays.

## MATERIALS AND METHODS

A total of 36 Single Comb White Leghorn hens (84 wk of age) were used for this experiment. Hens were housed 2 per cage at the Texas A&M University (TAMU) Poultry Science Research Center in College Station and were allowed time for acclimation. The birds were fed a complete layer ration *ad libitum* and were allowed full access to water for a period of 6 wk prior to the initiation of the molt experiment. Egg production was monitored to ensure that all hens were healthy and actively producing. The hens were divided into 6 treatment groups with 6 birds per treatment: pretrial control (PC), full fed (FF), feed withdrawal (FW), 100% alfalfa (A100), A100 + 0.375% FOS (A100L), and A100 + 0.75% FOS (A100H). Birds on all treatments were allowed *ad libitum* access to water and their respective diets. Hens were placed on an artificial lighting program of 8-h light:16-h dark for 1 wk prior to molt. Treatments were randomly assigned to cages throughout the house to ensure that there was no variability in egg production or reproductive tract regression caused by light stimulation. Molting lasted for 9 d. For the PC group, hens were euthanized by CO<sub>2</sub> gas, and bones were collected before the molt started. At the end of the 9-d molt period, hens were euthanized, and tibia and femurs were collected to evaluate bone qualities using DXA, Instron, and conventional bone assays. Egg production was recorded during the molting period to evaluate first day of out of production, and ovaries were also collected to measure ovary weight. All procedures

were carried out in accordance with the TAMU Lab Animal Care Committee animal use protocols.

## Bone Parameters

Bone parameters were measured according to the methods described by Zhang and Coon (1997). After tibia and femurs were obtained from each hen, the bones were cleaned of attached tissue. Bones from the right leg were subjected to DXA and then conventional bone assays (see subsequent); bones from the left leg were used for bone breaking strength by Instron.

**DXA.** Bones from the right tibia and femurs were subjected to DXA analysis using a Lunar DPX-L densitometer (Brinkman, Luzern, Switzerland). The bones were placed in groups of 15 to 18 bones on a 7 mm thick Lucite tablet. The bones were scanned and analyzed using the Small Animal Total Body software (version 4.6d) in the high resolution-medium scan mode. The bones were further analyzed individually using a manual region of interest to define each separate bone. The results for each bone were reported as bone mineral content (g) and bone mineral density (BMD; g/cm<sup>2</sup>).

**Conventional Bone Assays.** The bones from right tibia and femurs were dried at 100°C for 24 h and weighed again. The bones were subsequently ashed at 600°C overnight, cooled in a desiccator, and weighed.

**Bone Breaking Strength.** Bone breaking strength was measured using an Instron with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min; bone was supported on a 3.00-cm span (Park et al., 2003).

## Statistical Analysis

All data were subjected to one-way analysis of variance as a completely randomized design using the General Linear Models procedure of SAS<sup>®</sup> (SAS Inst., 2001). Significant differences among the means were determined by using Duncan's multiple-range test at  $P < 0.05$ . Correlations of bone parameters were evaluated by Pearson correlation procedures.

## RESULTS

The effects of dietary treatments on molting parameters compared with the full-fed, non-molted control and feed withdrawal procedures are shown in Table 1. The FF group (741.5 g) consumed significantly more feed compared with the other molting treatments ( $P < 0.05$ ), whereas the feed intakes of the A100, A100L, and A100H groups were higher than the FW group ( $P < 0.05$ ). The percentage of BW losses of the FW (27.8%), A100 (27.5%), A100L (37.4%), and A100H (27.2%) groups were significantly higher compared with the full-fed, non-molted control group (0.0%) ( $P < 0.05$ ), whereas there were no differences among the molting treatments. The FW (13.1 g), A100 (12.2 g), A100L (13.9 g), and A100H (13.4 g) molted groups exhibited significantly lower ovary weight than the FF group (46.1 g). The ovary weights of the A100,

**Table 1.** Effects of dietary treatments on feed intake, percentage of BW loss, ovary weight, and first day out of egg production during a 9-d molting period<sup>1</sup>

Parameter	FF	FW	A100	A100L	A100H	Pooled SE
Feed intake (g/9 d)	741.5 <sup>a</sup>	NA <sup>2</sup>	39.3 <sup>b</sup>	48.5 <sup>b</sup>	47.8 <sup>b</sup>	9.02
Percentage of BW loss	0.0 <sup>b</sup>	27.8 <sup>a</sup>	27.5 <sup>a</sup>	37.4 <sup>a</sup>	27.2 <sup>a</sup>	2.63
Ovary weight (g)	46.1 <sup>a</sup>	13.1 <sup>b</sup>	12.2 <sup>b</sup>	13.9 <sup>b</sup>	13.4 <sup>b</sup>	1.44
First day out of egg production (d)	NA <sup>2</sup>	4.25 <sup>ab</sup>	4.54 <sup>a</sup>	4.00 <sup>ab</sup>	3.63 <sup>b</sup>	0.27

<sup>a,b</sup>Means within a row with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>FF = full fed, FW = feed withdrawal, A100 = 100% alfalfa, A100L = 100% alfalfa + 0.375% fructooligosaccharide (FOS), and A100H = 100% alfalfa + 0.75% FOS.

<sup>2</sup>NA = not applicable.

A100L, and A100H hens were no different from that of the FW hens ( $P > 0.05$ ). The A100H group ceased egg production earlier than the A100 group, whereas there were no significant differences among the FW, A100, and A100L. These results indicated that alfalfa molting diets had molting parameters comparable with the conventional feed withdrawal molting procedure, and FOS supplementation did not have any detrimental effects on molting performance.

The effects of dietary treatments on BW and tibia bone parameters during a 9-d molting period are shown in Table 2. The BW of the PC and FF hens were heavier than the BW of the molted hen groups ( $P < 0.05$ ). The tibia dry weight of the PC hens (5.74 g) was significantly higher than that of the FW hens (4.96 g), whereas there were no significant differences among the PC, FF, A100, A100L, and A100H. The PC hens had a higher tibia ash weight compared with the FW, A100, A100L, and A100H hens ( $P < 0.05$ ), whereas there was no significant difference between the PC and FF groups. The femur dry weight of the PC hens was greater than that of the FW, A100, and A100H hens ( $P < 0.05$ ), whereas there were no significant differences among the PC, FF, and A100L hens. The PC hens had higher femur ash weight compared with the other treatments ( $P < 0.05$ ). These results suggest that hens lose a considerable amount of bone mineral during a molting period. Even the FF hens had higher bone mineral losses compared with the PC group. Because the light schedule was changed from 16L:8D to 8L:16D during a week before molting started and during the 9-d molting period, a shorter light schedule might have influenced hens' activities as well as their feed intakes, increasing their bone losses compared with the PC group.

Tibia and femur breaking strengths of the PC, FF, FW, A100, A100L, and A100H groups are also shown in Table 1. The tibia bone strengths of the FF (25.7 kg), FW (25.6 kg), A100 (24.0 kg), and A100L (25.1 kg) hens were significantly lower than the bone strength of the PC hens (35.2 kg). However, hens fed A100H (30.9 kg) had similar tibia bone breaking strength to that of the PC hens. The femur breaking strength results also showed the same trend as the tibia breaking strength results. There were no significant differences in femur breaking strength between the PC (27.6 kg) and A100H (22.7 kg) hens, whereas the FF (20.1 kg), FW (18.3 kg), A100 (18.7 kg), and A100L (19.7 kg) groups had lower femur breaking strengths than the PC group ( $P < 0.05$ ). These results suggest that FOS have the potential to maintain bone strength during molting.

Bone mineral content and BMD measured by DXA are presented in Table 3. The bone densities for the PC group were significantly higher than those for the other treatments. The tibia mineral content of the PC group (3.02 g) was higher than that of the FF (2.35 g) and FW (2.40 g) groups ( $P < 0.05$ ), whereas there were no significant differences among the PC, A100 (2.75 g), A100L (2.63 g), and A100H (2.62 g). The PC hens had greater femur mineral content compared with the FF, FW, A100L, and A100H hens. These results also suggest that bone integrities were substantially reduced during molting.

Correlations of tibia mineral content and density measured by DXA, bone breaking strength measured by Instron, and bone parameters measured by conventional bone assay are presented in Table 4. Tibia density and mineral content were highly correlated with tibia dry weight and ash weight ( $P < 0.0001$ ). Tibia breaking

**Table 2.** Effects of dietary treatments on BW and bone parameters measured by conventional bone assays during a 9-d molting period<sup>1</sup>

Parameter	PC	FF	FW	A100	A100L	A100H	Pooled SE
BW (g)	1,655 <sup>a</sup>	1,567 <sup>a</sup>	1,129 <sup>b</sup>	1,191 <sup>b</sup>	1,015 <sup>b</sup>	1,172 <sup>b</sup>	76.3
Tibia dry weight (g)	5.74 <sup>a</sup>	5.10 <sup>ab</sup>	4.96 <sup>b</sup>	5.05 <sup>ab</sup>	5.11 <sup>ab</sup>	5.15 <sup>ab</sup>	0.16
Tibia ash weight (g)	3.01 <sup>a</sup>	2.55 <sup>ab</sup>	2.48 <sup>b</sup>	2.29 <sup>b</sup>	2.42 <sup>b</sup>	2.48 <sup>b</sup>	0.12
Tibia breaking strength (kg)	35.16 <sup>a</sup>	25.71 <sup>b</sup>	25.63 <sup>b</sup>	24.03 <sup>b</sup>	25.14 <sup>b</sup>	30.94 <sup>ab</sup>	2.63
Femur dry weight (g)	4.18 <sup>a</sup>	3.72 <sup>ab</sup>	3.16 <sup>b</sup>	3.12 <sup>b</sup>	3.50 <sup>ab</sup>	3.36 <sup>b</sup>	0.19
Femur ash weight (g)	2.47 <sup>a</sup>	1.88 <sup>b</sup>	1.51 <sup>b</sup>	1.62 <sup>b</sup>	1.73 <sup>b</sup>	1.73 <sup>b</sup>	0.15
Femur breaking strength (kg)	27.62 <sup>a</sup>	20.10 <sup>b</sup>	18.38 <sup>b</sup>	18.75 <sup>b</sup>	19.78 <sup>b</sup>	22.72 <sup>ab</sup>	2.01

<sup>a,b</sup>Means within a row with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>FF = full fed, FW = feed withdrawal, A100 = 100% alfalfa, A100L = 100% alfalfa + 0.375% fructooligosaccharide (FOS), and A100H = 100% alfalfa + 0.75% FOS.

**Table 3.** Bone mineral content and mineral density measured by dual-energy X-ray absorptiometry (DXA) following 9-d molting period<sup>1</sup>

Parameter	PC	FF	FW	A100	A100L	A100H	Pooled SE
Tibia density (g/cm <sup>2</sup> )	0.23 <sup>a</sup>	0.18 <sup>b</sup>	0.18 <sup>b</sup>	0.20 <sup>b</sup>	0.20 <sup>b</sup>	0.20 <sup>b</sup>	0.007
Tibia mineral content (g)	3.02 <sup>a</sup>	2.35 <sup>b</sup>	2.40 <sup>b</sup>	2.75 <sup>ab</sup>	2.63 <sup>ab</sup>	2.62 <sup>ab</sup>	0.12
Femur density (g/cm <sup>2</sup> )	0.27 <sup>a</sup>	0.18 <sup>b</sup>	0.18 <sup>b</sup>	0.22 <sup>b</sup>	0.20 <sup>b</sup>	0.20 <sup>b</sup>	0.008
Femur mineral content (g)	2.55 <sup>a</sup>	1.63 <sup>b</sup>	1.57 <sup>b</sup>	2.07 <sup>ab</sup>	1.85 <sup>b</sup>	1.93 <sup>b</sup>	0.11

<sup>a,b</sup>Means within a row with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>FF = full fed, FW = feed withdrawal, A100 = 100% alfalfa, A100L = 100% alfalfa + 0.375% fructooligosaccharide (FOS), and A100H = 100% alfalfa + 0.75% FOS.

strength was also greatly correlated with dry weight (0.692;  $P < 0.0001$ ), ash weight (0.724;  $P < 0.0001$ ), tibia density (0.657;  $P < 0.0001$ ), and tibia mineral content (0.615;  $P < 0.0001$ ). The correlation between tibia density and mineral content showed the highest correlation coefficient (0.958;  $P < 0.0001$ ).

## DISCUSSION

In the present study, hens molted by alfalfa molting diets exhibited effective ovary regression and rapid cessation of egg production. Alfalfa is a high-protein, high-Ca, low-energy feedstuff with readily digestible amino acids such as arginine (82%), methionine (70%), threonine (71%), valine (75%), lysine (59%), and cysteine (40%) (NRC, 1994; Lewis and Bayley, 1995). Because alfalfa has a slow passage rate in the chicken gastrointestinal tract (Sibbald, 1979, 1980), it is beneficial to allow for a better digestion of feed and microbial fermentation, maintaining balanced microflora to limit pathogenic microorganisms in the gastrointestinal tract (Ricke et al., 1982; Vispo and Karasov, 1997; Donalson et al., 2004a,b). Landers et al. (2005a, b) also indicated that alfalfa molting diets were equally effective as conventional feed withdrawal molting methods in ovary regression and post-molting egg production.

The results of the present study also demonstrated that hens lost a substantial amount of bone mineral during molting. These results were in agreement with Garlich et al. (1984). Those researchers reported that hens molted by feed withdrawal had a significant reduction in femur bone density during molting. Mazzuco et al. (2003) also indicated that hens molted by feed withdrawal for 10 d exhibited a precipitous decrease in BMD compared with non-molted, control hens. Moreover, reduced bone qualities during feed withdrawal did not recover after refeed-

ing normal layer rations (Newman and Leeson, 1999). Recently, Kim et al. (2005) evaluated cortical, cancellous, and medullary bone densities of molted hens using peripheral quantitative computed tomography. The results of this study indicated that medullary and cancellous bones are susceptible bone components for bone resorption during molting, reducing overall bone qualities.

Results showed that molted hens fed an alfalfa molt diet supplemented with 0.75% FOS (A100H) had no significant difference in bone breaking strength compared with PC hens, and the other treatment groups (FF, FW, A100, and A100L) had significantly lower bone breaking strength compared with the PC hens. These results suggest that FOS have potential to maintain bone strength during molting. Several studies reported that FOS increased mineral absorption and bone qualities. Morohashi et al. (1998) showed that FOS feeding in rats improved true Ca absorption in the intestine. Mineo et al. (2001) also reported that FOS increased net Ca transport from the epithelium of the small and large intestine of rats in vitro. Besides FOS, several osteogenic agents, such as F, Sr, or Vitamin D3, have potential to reduce bone resorption during molting and enhance bone restoration after molting.

In the present study, correlation analysis indicated that the bone parameters measured by conventional assays, bone breaking strength measured by Instron, and bone density and mineral content measured by DXA were highly correlated to each other. Kim et al. (2004) reported that femur and tibia mineral density and mineral content were highly correlated to ash and ash concentration. Schreiweis et al. (2003) also indicated that bone breaking strength and BMD were highly correlated to each other ( $P < 0.001$ ). Traditional methods (ashing, breaking strength, mineral assay, and histomorphometry) for evaluating bone quality in poultry are normally destructive and inva-

**Table 4.** Correlations of tibia mineral content and density measured by DXA, bone breaking strength measured by Instron, and bone parameters measured by conventional bone assay

Bone parameter	Dry weight	Ash weight	Tibia density	Tibia mineral content
Tibia density	0.643****	0.632****		
Tibia mineral content	0.615****	0.604****	0.958****	
Tibia breaking strength	0.692****	0.724****	0.657****	0.615****

\*\*\*\* $P < 0.0001$ .

sive (Hester et al, 2004; Korver et al., 2004). These methods cannot measure changes in bone quality of individual birds over time (Korver et al., 2004). Thus, non-invasive methods, such as DXA, have great advantages over traditional methods. Because laying hens have dynamic Ca metabolisms for bone and eggshell formations, monitoring changes in bone density using DXA can provide valuable information to develop key management and nutritional strategies to prevent osteoporosis in laying hens.

In summary, this study suggests that the alfalfa-FOS feeding regimen retains the potential as a molting diet for effective ovary regression and rapid cessation of egg production exhibited by non-supplemented alfalfa. More importantly, FOS sugar supplementation may help to maintain bone strength during molting as indicated by the bone parameters measured in this study. In addition, it appears that conventional assays, bone breaking strength measured by Instron, and bone density and mineral content measured by DXA are highly correlated to each other. Non-invasive methods such as DXA may have practical utility for bird management.

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