Comparisons of Molting Diets on Skeletal Quality and Eggshell Parameters in Hens at the End of the Second Egg-Laying Cycle

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ABSTRACT A study was conducted to evaluate skeletal quality and eggshell parameters of molted hens at the end of the second laying cycle. Sixty Single Comb White Leghorn hens were used for this study. There were 2 controls and 4 molting treatments: full-fed control 1 (82 wk old; FF1), full-fed control 2 (122 wk old; FF2), feed withdrawal (FW), 100% alfalfa (A100), 90% alfalfa/10% layer ration (A90), and 70% alfalfa/30% layer ration (A70). At the end of the second laying cycle (approximately 122 wk of age), hens were euthanized by CO_2. Tibia and femur were collected. There were no differences in bone parameters between FF1 and FF2 ($P > 0.05$) hens. There were no differences in bone parameters among the different molting dietary treatments ($P > 0.05$). In the eggshell parameters, the FF2 hens exhibited heavier egg weights than the FF1 ($P < 0.05$), whereas the percentage shell and egg production of the FF1 birds were significantly higher than those of the FF2 birds. Shell weights of the FW and A90 birds were significantly heavier than that of the A100. The correlation analysis showed that overall bone parameters were negatively correlated with eggshell parameters. Bone parameters were highly correlated with each other. Shell weight, percentage shell, and shell thickness were positively correlated with each other, whereas egg weight was negatively correlated with percentage shell. These results suggest that age of hens and molting dietary treatments influence egg parameters, and eggshell formation is closely related to bone metabolism in laying hens.

(*Key words*: skeletal quality, eggshell, molting, alfalfa, bone-breaking strength)

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INTRODUCTION

The incidence of bone fractures and weakness in older laying hens at the end of lay is a concern in the poultry industry (Riczu et al., 2004). The structural bone loss related to osteoporosis can enhance the skeletal fragility and contribute to the high fracture incidence at the end of the laying cycle in older laying hens (Gregory and Wilkins, 1989). Osteoporosis in laying hens is defined as a condition that involves progressive structural bone loss (Whitehead and Fleming, 2000), and is one of the main causes of subsequent fractures in older laying hens (Randall and Duff, 1988). The pain associated with such extreme physical damage can be severe (Knowles and Wilkins, 1998). Gregory et al. (1990) reported that 24% of laying hens from battery cages have freshly broken bones. Gregory and Wilkins (1989) reported that approximately 30% of hens housed in batteries suffer at least one broken bone during their lifetime. Approximately one-third of these occur while hens are housed in cages, with the remaining breakage occurring during depopulation, transport, and processing (Gregory and Wilkins, 1989). The presence of old healed bone breaks at slaughter can be considered indicators of poor welfare, and it can be assumed that the pain associated with an old break will have been felt over a prolonged period (Knowles and Wilkins, 1998). Knowles and Wilkins (1998) emphasized that the numerous bone breaks can be reduced by increasing bone strength. In addition, skeletal problems related to structural bone loss, such as cage layer fatigue or osteoporosis, clearly influence egg production and shell quality in laying hens (Parkinson and Cransberg, 2002), because bone of the laying hen contributes approximately 35% of the calcium in each eggshell (Mueller et al., 1964).

Induced molt using feed withdrawal is a potential factor exacerbating structural bone loss in older laying hens. Feed withdrawal is the most common molting practice to stimulate multiple egg-laying cycles in laying hens (Breeding et al., 1992). Typically, feed is completely removed for 10 to 14 d combined with a reduction in photoperiod from 16 to 8 h (Brake, 1993). After resting periods (0 to 21 d) following feed withdrawal, molted hens are

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Abbreviation Key: A100 = 100% alfalfa; A90 = 90% alfalfa/10% layer ration; A70 = 70% alfalfa/30% layer ration, FF1 = full-fed control 1, 82 wk old; FF2 = full-fed control 2, 122 wk old; FW = feed withdrawal.

fed layer rations to resume egg production. Garlich et al. (1984) indicated that induced molt using feed removal decreased femur weight and density in laying hens. Mazzuco et al. (2003) reported that molted hens showed a precipitous decrease in bone mineral densities compared with nonmolted control hens. Medvedev et al. (2001a,b) evaluated alfalfa as an alternative molting diet and demonstrated that it yielded second egg production responses that were equivalent to feed-withdrawal hens. Recently, Donalson et al. (2004) evaluated the effects of the mixtures of alfalfa and layer ration (100% alfalfa, 90% alfalfa/10% layer ration, 80% alfalfa/20% layer ration, and 70% alfalfa/30% layer ration) on molting parameters and postmolting performance. The results showed that the alternative molting diets had comparable molting parameters and postmolting performances to feed withdrawal. However, in a long-term postmolting egg production study, a 70% alfalfa/30% layer ration molting diet showed lower egg production and egg qualities compared with the other treatments, whereas 100% alfalfa and 90% alfalfa/10% layer ration had comparable egg production and egg quality responses to feed withdrawal. There are questions regarding bone and eggshell quality that need to be addressed with alternative molting diets, namely: 1) the effects of alternative molting diets on skeletal integrity and eggshell quality at the end of the second laying cycle, and 2) whether reduced bone qualities during molting influence eggshell and bone parameters of laying hens at the end of the second laying cycle. Therefore, objectives of this study were to evaluate the effects of different molting dietary treatments on bone and eggshell qualities at the end of the second laying cycle, and to evaluate the correlation among the bone and eggshell parameters.

MATERIALS AND METHODS

Samples

The 60 Single Comb White Leghorn hens used for this study were part of an overall study to examine alfalfa molt induction and egg production (Donalson et al., 2004). Ten hens were euthanized using CO_2 gas at 82 wk of age without molting for the full-fed control group 1 (FF1). Left tibia and femur were obtained from each hen to evaluate bone parameters compared with those of older molted hens. Fifty 82-wk-old hens were then assigned to 1 nonmolted control and 4 molt induction treatment groups with 10 birds per treatment, as part of an earlier experiment (Donalson et al, 2004): full-fed control 2 (FF2), feed withdrawal (FW), 100% alfalfa (A100), 90% alfalfa/10% layer ration (A90), and 70% alfalfa/30% layer ration (A70). All hens 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 before molt. Treatments 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. Hens were molted for 9 d. After a 9-d molting period, hens were fed a Texas A&M University layer ration (Park et al., 2004), and the lighting program was changed to 16L:8D to stimulate egg production. At the end of the second laying cycle (122 wk of age), eggs were collected, and hens were euthanized by CO_2 to collect left tibia and femur. The bones were cleaned of attached tissue.

Bone parameters were measured according to the methods described by Zhang and Coon (1997) and Park et al. (2003). All bones were first weighed in the air, then reweighed while suspended in water at room temperature. Bone volume was calculated assuming that the specific gravity of water is 1 g/cm^3 at room temperature. For the fresh bone preparation, bone-breaking strength was initially measured, and bones were subsequently dried at 100°C for 24 h and weighed again. The bones were then ashed at 600°C for 24 h, cooled in a desiccator, and weighed. For the dry bone preparation, the bones were initially dried at 100°C for 24 h and weighed again, and bone-breaking strength was measured. After bone-breaking strength measurements were taken, the bones were ashed at 600°C for 24 h, cooled in a desiccator, and weighed. For the fat-free dry preparation, the bones were dried at 100°C for 24 h. In order to determine their fat-free dry matter, the dried bones were refluxed in a Soxhlet apparatus for 48 h in 333 mL of 95% ethanol and 667 mL of benzene at 70°C. The fat-free bones 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. Bone ash concentrations were calculated by dividing the ash weight of each bone by its volume. This measurement has been reported to best reflect the bone status of laying hens (Zhang and Coon, 1997). Percentage ash was calculated by dividing the ash weight of each bone by its fat-free dry matter. Bone-breaking strength was measured using an Instron[2] with 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min with bone supported on a 3.00-cm span (Park et al., 2003). Animal care procedures described herein were approved by Texas A & M University Institutional Animal Care and Use Committee.

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 using Duncan's multiple-range test at $P \leq 0.05$. Correlations of bone parameters were evaluated by Pearson correlation procedures.

RESULTS AND DISCUSSION

The effects of age and different molting dietary treatments on various bone parameters of the tibia at the end of the second laying cycle are presented in Table 1.

[2]Model 1011 Instron Universal Testing Machine, Instron Corp., Canton, MA.

TABLE 1. Effects of age and different molting dietary treatments on various bone parameters of tibia at the end of the the second laying cycle

	Bone parameter							
Treatment[1]	Bone volume (cm^3)	Fresh weight (g)	Dry weight (g)	Fat-free dry weight (g)	Ash weight	Percentage ash (%)	Ash concentration (g/cm^3)	Bone-breaking strength (kg)
FF1	3.52	4.90	3.28	2.75	1.70	39.1	0.304	4.68
FF2	3.58	4.94	3.29	2.69	1.69	35.9	0.270	5.22
Pooled SE	0.11	0.17	0.12	0.10	0.07	1.61	0.014	0.58
FF2	3.58	4.94	3.29	2.69	1.69	35.9	0.270	5.22
FW	3.81	5.12	3.35	2.68	1.65	35.4	0.249	3.91
A100	3.77	5.15	3.45	2.73	1.72	36.2	0.262	4.42
A90	3.53	4.71	3.16	2.43	1.51	33.7	0.233	3.78
A70	3.70	5.03	3.37	2.69	1.69	37.0	0.270	4.03
Pooled SE	0.11	0.14	0.11	0.10	0.07	1.05	0.011	0.46

[1]FF1 = full-fed control (82 wk old); FF2 = full-fed control (122 wk old); FW = feed withdrawal; A100 = 100% alfalfa; A90 = 90% alfalfa/10% layer ration; A70 = 70% alfalfa/30% layer ration.

Although the FF1 hens (82 wk old) had numerically higher fat-free dry weight, ash weight, percentage ash, and ash concentration compared with the FF2 group (122 wk old), there were no statistical differences between the FF1 and FF2 ($P > 0.05$). There were no differences in bone parameters among the FF2, FW, A100, A90, and A70 hens ($P > 0.05$). Femur bone parameters showed similar trends to the tibia bone parameters (Table 2). The dry weight, percentage ash, ash concentration, and bone-breaking strength of the FF1 were numerically higher but not significantly. There were no differences in bone parameters among the different molting treatments ($P > 0.05$). This result indicates that bone qualities at the end of the second egg-laying cycle are not influenced by different molting dietary treatments. Garlich et al. (1984) reported that feed withdrawal molt reduced bone weight and density. Mazzuco et al. (2003) indicated that induced molt using feed withdrawal reduced bone mineral densities compared with nonmolted hens. After molted hens were fed standard layer rations to stimulate egg production, bone qualities were slowly restored (Garlich et al., 1984; Mazzuco et al., 2003). However, bone strength did not return to prefast levels although bone strength increased when fasted hens were fed a standard layer ration (Newman and Leeson, 1999). In the present study, however, there were no significant differences in bone qualities among the full-fed and molting dietary treatments at the end of the second egg-laying cycle.

Effects of age and different molting dietary treatments on egg weight, shell weight, percentage shell, and shell thickness at the end of the second laying cycle are shown in Table 3. The FF2 birds exhibited heavier egg weights (69.4 g) compared with the FF1 (64.9 g) ($P < 0.05$), whereas the percentage shell (8.82%) of the FF1 birds was significantly higher than the FF2 (7.85%) birds. There were no significant differences in shell weight and shell thickness between the younger (FF1) and older (FF2) hens. Among the different molting dietary treatments, shell weights of the FW (5.72 g) and A90 (5.88 g) birds were significantly heavier than those of A100 (4.90 g) birds at the end of the second laying cycle. However, there were no significant differences in egg weight, percentage shell, and shell thickness among the different molting dietary treatments at the end of the second laying cycle. These results indicated that molted hens produced heavier eggs than younger nonmolted hens. Bell (2003) indicated that the most substantial difference in performance between laying cycles is egg weight responses.

The correlations between BW and tibia bone parameters are shown in Table 4. Body weight was positively correlated with fresh bone weight (0.559; $P < 0.0001$), bone volume (0.533; $P < 0.0001$), dry bone weight (0.455; $P <$

TABLE 2. Effects of age and different molting dietary treatments on various bone parameters of femur at the end of the second laying cycle

	Bone parameter							
Treatment[1]	Bone volume (cm^3)	Fresh weight (g)	Dry weight (g)	Fat-free dry weight (g)	Ash eight	Percentage ash (%)	Ash concentration (g/cm^3)	Bone-breaking strength (kg)
FF1	3.10	4.30	2.61	2.32	1.37	37.3	0.279	4.18
FF2	3.15	4.32	2.50	2.30	1.39	34.6	0.255	3.40
Pooled SE	0.10	0.15	0.15	0.11	0.08	1.83	0.018	0.52
FF2	3.15	4.32	2.50	2.30	1.39	34.6	0.255	3.40
FW	3.35	4.46	2.50	2.32	1.37	33.2	0.232	2.74
A100	3.30	4.54	2.63	2.39	1.43	34.2	0.249	3.19
A90	3.05	4.14	2.37	2.15	1.28	32.4	0.232	3.00
A70	3.28	4.52	2.62	2.43	1.48	34.7	0.259	3.01
Pooled SE	0.10	0.14	0.11	0.11	0.08	1.15	0.016	0.49

[1]FF1 = full-fed control (82 wk old); FF2 = full-fed control (122 wk old); FW = feed withdrawal; A100 = 100% alfalfa; A90 = 90% alfalfa/10% layer ration; A70 = 70% alfalfa/30% layer ration.

TABLE 3. Effects of age and different molting dietary treatments on egg weight, shell weight, percentage shell, and shell thickness at the end of the second laying cycle

	Egg parameters			
Treatment[1]	Egg weight (g)	Shell weight (g)	Percentage shell (%)	Shell thickness (mm)
FF1	64.9^{b}	5.71	8.82^{a}	3.73
FF2	69.4^{a}	5.46	7.85^{b}	3.48
Pooled SE	1.77	0.20	0.29	0.09
FF2	69.4	5.46^{ab}	7.85	3.48
FW	71.6	5.72^{a}	8.01	3.48
A100	66.9	4.90^{b}	7.35	3.23
A90	70.5	5.88^{a}	8.35	3.55
A70	69.9	5.32^{ab}	7.68	3.25
Pooled SE	1.77	0.20	0.29	0.09

^{a,b}Means within a column with different superscripts differ significantly (P < 0.05).

[1]FF1 = full-fed control (82 wk old); FF2 = full-fed control (122 wk old); FW = feed withdrawal; A100 = 100% alfalfa; A90 = 90% alfalfa/10% layer ration; A70 = 70% alfalfa/30% layer ration.

0.001), fat-free dry bone weight (0.449; P < 0.001), and ash weight (0.394; P < 0.01). Fresh bone weight was highly correlated with bone volume (0.914; P < 0.0001), dry bone weight (0.848; P < 0.0001), fat-free dry bone weight (0.855; P < 0.001), and ash weight (0.795; P < 0.0001). Bone volume was highly correlated with dry bone weight (0.742; P < 0.0001), fat-free dry bone weight (0.584; P < 0.0001), and ash weight (0.497; P < 0.0001). Fat-free dry bone weight and ash weight had the highest correlation (0.978; P < 0.0001) among the bone parameters. Percentage ash had positive correlations with dry bone weight (0.403; P < 0.01), fat-free dry weight (0.448; P < 0.001), and ash weight (P < 0.624), but no significant correlations with BW, fresh bone weight, and bone volume. Ash concentration was positively correlated with fresh bone weight (0.254; P < 0.05), dry bone weight (0.381; P < 0.01), fat-free dry bone weight (0.698; P < 0.0001), ash weight (0.784; P < 0.0001), and percentage ash (0.727; P < 0.0001), but no significant correlations with BW and bone volume. These results indicated that bone parameters are positively correlated with each other and with BW. In a previous study involving production hens, Kim et al. (2004) reported that bone parameters were positively correlated with each other. Schreiweis et al. (2003) indicated that BW was positively correlated with bone parameters including bone mineral density (P < 0.001), mineral content (P < 0.01), bone breaking strength (P < 0.001), and ash (P < 0.01). However, in the present study, it was apparent that percentage ash and ash concentration are not significantly correlated with BW, fresh bone weight, or bone volume. Zhang and Coon (1997) indicated that bone volume showed low correlation coefficiencies with percentage ash (0.231) and ash concentration (0.117). One of the reasons for this is that percentage ash and ash concentration are normalized by dry bone weight and bone volume, respectively. It suggests that percentage ash and ash concentration would be better bone parameters for comparing bone quality between subjects that have substantially different body sizes or weights.

Hen BW had a positive correlation with egg weight (0.308; P < 0.05) but it was not significantly correlated with the other eggshell parameters (Table 5). Egg weight was positively correlated with shell weight (0.322; P < 0.05). However, egg weight was negatively correlated with percentage shell (–0.305; P < 0.05). Shell weight was highly correlated with percentage shell (0.780; P < 0.0001) and shell thickness (0.831; P < 0.0001). Percentage shell and shell thickness had the highest correlation (0.901; P < 0.0001) among the eggshell parameters. These results are also in agreement with Schreiweis et al. (2003). They reported that percentage eggshell was positively correlated with shell thickness (0.770; P < 0.001). When data from the egg production component of the study (Donalson et al., 2004; data not shown) were compared with the eggshell parameters reported in the current study, there were no significant correlations between egg production data from an earlier experiment and other eggshell parameters.

The relationships between eggshell and tibia bone parameters are shown in Table 6. Egg weight was positively correlated with fresh bone weight (0.357; P < 0.01) and negatively correlated with bone percentage ash (–0.402; P < 0.01) and bone ash concentration (–0.307; P < 0.05). Trends of the correlations between eggshell and bone parameters were negative. Shell weight exhibited negative correlations with dry bone weight (–0.388; P < 0.01),

TABLE 4. The correlations among BW and tibia bone parameters

Bone parameters	BW	Fresh bone weight	Bone volume	Dry bone weight	Fat-free dry bone weight	Ash weight	Percentage ash
Fresh bone weight	0.559****						
Bone volume	0.533****	0.914****					
Dry bone weight	0.455***	0.848****	0.742****				
Fat-free dry bone weight	0.449***	0.855***	0.584****	0.810****			
Ash weight	0.394**	0.795****	0.497****	0.799****	0.978****		
Percentage ash	0.004	0.228	–0.029	0.403**	0.448***	0.624****	
Ash concentration	0.056	0.254*	–0.147	0.381**	0.698****	0.784****	0.727****

*P < 0.05.

**P < 0.01.

***P < 0.001.

****P < 0.0001.

TABLE 5. Correlations among BW and eggshell parameters

Shell parameters	BW	Egg weight	Shell weight	Percentage shell
Egg weight	0.308*			
Shell weight	0.185	0.322*		
Percentage shell	–0.035	–0.305*	0.780****	
Shell thickness	–0.009	–0.125	0.831****	0.901****

*P < 0.05.

****P < 0.0001.

ash weight (–0.305; $P < 0.05$), and percentage ash (–0.450; $P < 0.001$). Percentage shell also had negative correlations with fresh bone weight (–0.467; P < 0.001), dry bone weight (–0.539; P < 0.001), fat-free dry weight (–0.354; P < 0.01), ash weight (–0.363; P < 0.01), and percentage ash (–0.450; P < 0.001). Shell thickness was negatively correlated with fresh bone weight (–0.348; P < 0.05), dry bone weight (–0.452; P < 0.001), and ash weight (–0.281; P < 0.05). When data from the egg production component of the study (Donalson et al., 2004; data not shown) were compared with tibia bone parameters, negative correlations were seen with fat-free dry weight (–0.400; P < 0.01), ash weight (–0.414; P < 0.01), and ash concentration (–0.448; P < 0.01). These results showed that bone and eggshell parameters were negatively correlated with each other. Because the laying hen bone contributes substantial calcium to each eggshell (Mueller et al., 1964), bone qualities of laying hens would likely exhibit a negative correlation with eggshell qualities. Bishop et al. (2000) characterized hens selected for resistance or susceptibility to osteoporosis based on a bone index. The hens susceptible to osteoporosis had significantly higher shell thickness and better shell quality but lower bone quality compared with hens selected for resistance to osteoporosis. This result suggests that the hens susceptible to osteoporosis have more active bone resorption to supply eggshell calcium.

The medullary bone of laying hens plays an important role in calcium metabolism and eggshell formation because it functions as a calcium store for eggshell formation in laying hens (Wilson and Duff, 1990). Medullary bone is a type of woven bone and is formed immediately before the onset of egg laying influenced by estrogen (Dacke et al., 1993; Wilson and Thorp, 1998). The quantities of medullary bone and structural bone have a reciprocal relationship because the amount of medullary bone increases in the replacement of structural bone during the egg-laying cycle (Wilson and Thorp, 1998; Whitehead and Fleming, 2000; Cransberg et al., 2001). Such a replacement of structural bone with medullary bone during the egg-laying cycle may weaken the bone strength of laying hens and increase the risk of bone fracture (Whitehead, 2004). Therefore, maximizing bone mineral deposition before onset of egg production and after molting is critical to prevent bone weakness problems and improve eggshell qualities in older laying hens (Park et al., 2004).

TABLE 6. The correlations between eggshell and tibia bone parameters

	Eggshell parameters			
Bone parameters	Egg weight	Shell weight	Percentage shell	Shell thickness
Fresh weight	0.357**	–0.212	–0.467***	–0.348*
Dry weight	0.213	–0.388**	–0.539***	–0.452***
Fat-free dry weight	0.188	–0.218	–0.354**	–0.252
Ash weight	0.071	–0.305*	–0.363**	–0.281*
Percentage ash	–0.402**	–0.450***	–0.450***	–0.221
Ash concentration	–0.307*	–0.204	–0.004	–0.005
Bone-breaking strength	0.028	–0.051	–0.081	0.002

*P < 0.05.

**P < 0.01.

***P <0.001.

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