The Effect of an Induced Molt Using a Nonfasting Program on Bone Mineralization of White Leghorns

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ABSTRACT To determine changes in bone mineralization during molt, 66-wk-old White Leghorns were assigned to either a fasted molting regimen using feed removal for 10 d, followed by the ad libitum consumption of cracked corn for 7 d and a pullet developer diet for 10 d or a nonfasted molting regimen lasting 27 d that included the ad libitum consumption of a diet containing 71% wheat middlings and 23% corn. Both molting regimens restricted light to 8 h/d, and water was provided ad libitum. At 28 d postmolt, hens from both molting treatments were returned to a regular egg-laying diet and 16 h/d of photoperiod. Control hens consumed a regular egg laying diet and were kept on 16 h/d of light throughout the study. Using dual-energy x-ray absorptiometry, bone mineral density (BMD) and bone mineral content (BMC) of the left tibia were measured in 7 live hens per treatment immediately prior to, during, and following the molt. Results showed that by 28 d postmolt, BW loss was 22 and 18% in the fasted and nonfasted molting regimens, respectively (P ≤ 0.0001). Compared with premolt values, tibial BMD at 28 d postmolt decreased 35 and 18% in the fasted and nonfasted molt groups, respectively (treatment × age interaction, P ≤ 0.0001). Similarly, tibial BMC values decreased 39 and 27% in the fasted and nonfasted molt groups, respectively (treatment by age interaction, P ≤ 0.01). The tibial BMD and BMC of controls at 28 d postmolt were similar to premolt values. Recovery in tibial BMD and BMC of fasted and nonfasted hens occurred by 126 d postmolt with values similar to controls. These results suggest that a nonfasted molting regimen is less deleterious to tibial BMD and BMC than a fasted molting regimen.

(Key words: bone mineral density, bone mineral content, fasted molt, nonfasted molt, laying hen)

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INTRODUCTION

In laying hens, in order to minimize the age-related declines in shell quality and egg production, cessation of egg laying can be induced through molt, which involves feed and light restriction. With this management system, the rejuvenation of the laying flock for a second cycle of production is acquired with the enhancement of shell quality (Bell, 2003). Economic and welfare benefits of an induced molt include the use of approximately 50% fewer hens in commercial egg flocks of the United States with fewer male chicks hatched (Bell et al., 2004). However, in recent years, the induction of molt by temporary feed withdrawal has become more of a concern to the poultry industry due to welfare and food safety reasons (Bell, 2003; Gast and Ricke, 2003). Therefore, alternative molting techniques that would reduce or eliminate fasting are being evaluated (Keshavarz and Quimby, 2002; Biggs et al., 2003, 2004; Park et al., 2004).

Alternative molting procedures rely on nutrient restriction by limiting daily feed intake, by reducing nutrient density, or changing nutrient balance of feed offered ad libitum (Webster, 2003). Induction of molt through dietary imbalances of certain minerals such as Cu, Zn (Stevenson and Jackson, 1984), Na, Cl (Whitehead and Shannon, 1974; Harms, 1991), or Al (Hussein et al., 1989) were compared with a fasting molting regimen, but these methods have not been used substantially in commercial programs because of their expense and inconsistent results (Ruszler, 1998; Biggs et al., 2003). Full feeding of a 15% guar meal diet caused a slow cessation and reinitiation of lay and was considered a feasible alternative molt method (Zimmerman et al., 1987). The use of a grape pomace diet plus thyroxine resulted in similar hen performance as a conventional fasting molt (Keshavarz and Quimby, 2002).

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Abbreviation Key: BMD = bone mineral density; BMC = bone mineral content; DEXA = dual energy X-ray absorptiometry; pQCT = peripheral quantitative computed tomography; SE = Salmonella enteritidis.
Feeding molting diets consisting of 95% corn or wheat middlings for 28 d resulted in effective alternative programs providing comparable postmolt performance to 10-d feed removal (Biggs et al., 2003). The use of diets containing wheat middlings, corn, corn gluten feed, or a combination of wheat middlings and corn were considered effective nonfeed removal methods for molting laying hens (Biggs et al., 2004). Recently, the ad libitum feeding of alfalfa for 9 d to White Leghorns over 50 wk of age was identified as an alternative method for inducing molt (Woodward et al., 2005). Endocrine changes observed in fasted hens include a large and temporary increase in circulating levels of corticosterone (Etches et al., 1983). Because corticosterone affects immunological defenses against infection and disease, feed withdrawal reduces the hen’s cell-mediated immune response. Fasting also reduces the ability of the hen to resist microbial colonization of the gut (Webster, 2003). Flocks may be vulnerable to infection by a number of organisms, particularly, Salmonella enteritidis (SE), which was excreted significantly in the feces and present in higher numbers in internal organs during the feed withdrawal phase (Holt, 2003). Interestingly, a SE challenge of hens subjected to a nonfasted molt, which involved the ad libitum consumption of wheat middlings, exhibited significantly less SE shedding and internal organ contamination compared with hens molted via feed withdrawal (Seo et al., 2001). Ad libitum feeding of White Leghorns (over 50 wk old) for 9 d with a molting diet containing wheat middlings, corn, corn gluten feed, or a combination of wheat middlings and corn was employed as an alternative method for molting, without increasing the incidence of SE in eggs and internal organs (Woodward et al., 2005). In addition to reduced resistance to pathogens, induced molting issues also reside in the fact that mineralization of the medullary tibia and the humerus (more representative of structural bone) declined during a 10-d fasted molt with gradual recovery during the postmolt period (Mazzuco and Hester, 2005). Little information is available in the scientific literature on the effects of a nonfasting molt regimen on hen skeletal health. Therefore, the objective of the current study was to determine the effect of an induced molt using nonwithdrawal of food (nonfasted molt) on the bone mineralization of White Leghorn hens.

MATERIALS AND METHODS

A 19-wk trial was conducted from 66 to 85 wk of age using 202 White Leghorns nearing the end of their first cycle of lay (60 wk of age). Hens were housed randomly in 6 rows of cages with one bird placed in a cage resulting in 1,084 cm² of floor space per hen. Birds were weighed individually at 64 wk of age for purposes of eliminating potential culls prior to molt and to ensure similar BW means among treatments prior to the initiation of the experiment. There were no culls, so all hens were assigned to one of the following 3 treatments with 2 rows of cages per treatment: a fasted molt (67 hens), a nonfasted molt (68 hens), and controls (67 hens). Molt was initiated at 66 wk of age. The fasted molt included feed removal for 10 d followed by the ad libitum consumption of cracked corn for 7 d and a pullet developer diet (13.07% CP, 2,843 kcal/kg of ME, 0.72% Ca, 0.38% nonphytate P) for 10 d (see Schreiweis et al., 2004, for composition of the developer diet). The nonfasted molt included the ad libitum consumption of a diet containing 71% wheat middlings and 23% corn for 27 d (Biggs et al., 2004; Table 1). Both molting regimens provided water ad libitum consumption and restricted light to 8 h/d. At 28 d postmolt, hens from both molting treatments were returned to a photoperiod of 16 h with a light intensity of 5 lx and provided with an egg-laying diet (Table 1). Hens were kept on the egg-laying diet until the end of the experiment. The nonmolted control hens consumed the egg laying diet and water ad libitum throughout the experiment and were exposed to an unchanging daily photoperiod of 16 h.

To monitor bone mineralization, 21 hens selected out of 202 birds, based on the average of their BW closest to the mean of the entire flock, were repeatedly scanned in vivo using dual-energy x-ray absorptiometry (DEXA). Briefly, DEXA technology consists of a moving x-ray generator that produces photons at 2 energy levels. A collinear...

### Table 1. Composition of the wheat-middlings molt and egg laying diets

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Molt diet</th>
<th>Egg laying diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn (6.12% CP)</td>
<td>23.00</td>
<td>65.43</td>
</tr>
<tr>
<td>Dehulled soybean meal (47.5% CP)</td>
<td>—</td>
<td>21.35</td>
</tr>
<tr>
<td>Standard midds</td>
<td>71.39</td>
<td>—</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>—</td>
<td>1.75</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>—</td>
<td>5.40</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.10</td>
<td>1.50</td>
</tr>
<tr>
<td>Limestone, ground</td>
<td>4.96</td>
<td>—</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>—</td>
<td>2.75</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.30</td>
<td>0.45</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mold inhibitor</td>
<td>—</td>
<td>0.05</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

2. Provided per kilogram of diet: vitamin A, 6,600 IU; vitamin D₃, 2,695 IU; vitamin E, 33 IU; vitamin K, 1.2 mg; riboflavin, 4.4 mg; pantothenic acid, 6.6 mg; niacin 21 mg; choline, 358 mg; vitamin B₁₂, 0.006 mg; manganese, 63 mg; zinc, 61 mg; iron, 32 mg; copper, 3.9 mg; iodine, 1.1 mg; selenium, 0.256 mg.
3. Myco curb Dry, Kemin Industries, Inc., Des Moines, IA.
4. Ethoxyquin (66%), Prime Quality Feeds, North Little Rock, AR.
5. Based on NRC (1994) feed composition tables.
mated scintillation detector moves simultaneously on the opposite side of the bone measuring flux. As the beam passes through the limb or bone, photon output is filtered to produce 2 distinct peaks that distinguish soft tissue from bone, generating bone density values (Hester et al., 2004). Bone mineral content (BMC) is the amount of mineral in the specific site scanned and, when divided by the area measured, is used to derive a value for bone mineral density (BMD; Kanis, 2002). The BMD (g/cm²) and BMC (g) of the left leg (tibia and fibula) of 7 live, unanesthetized hens per treatment was determined repeatedly at weekly intervals from 66 to 72 wk of age and then from 76 to 84 wk of age at 4 wk intervals. During scans, birds were restrained on their backs in a foam holding device and secured with straps (Schreiweis et al., 2003). Scanning began at the proximal end of the tibia and took approximately 10 min. Individual BW was recorded following each scan.

Mortalities and eggs laid by each hen were recorded daily throughout the entire experimental period. Egg production for the flock (n = 202) was calculated as hen-day production similar to the controls beginning at 76 wk of age. Egg production of nonmolted control hens averaged 81% during the weeks of the molt (66 to 70 wk of age), with a decline during second cycle of lay when compared with molted hens (e.g., 83 wk of age). Hen livability was not different among treatments or ages (P ≥ 0.05) and averaged 96% for the entire experimental period (data not presented).

Fasted and nonfasted hens experienced a 35 and 18% loss, respectively, in their tibial BMD by d 28 of molt (treatment × age interaction, P ≤ 0.0001, Figure 2). Fasted and nonfasted hens experienced 39 and 27% loss, respectively, in their tibial BMC by d 28 of molt (treatment × age interaction, P ≤ 0.01, Figure 4). The BMD and BMC of the tibia of control hens remained relatively stable during the study. A gradual and slow recovery in BMD and BMC occurred in both molted groups upon the return of the higher calcium egg-laying diet on d 28 of molt. By 126 d postmolt, there were no significant differences among treatments in tibial BMD and BMC.

Shell thickness (Figure 5) and percentage of shell (Figure 6) of eggs from hens of both molting regimens improved after molt at 76 and 80 wk of age compared with controls. Shell thickness and percentage of shell were sustained through 84 wk of age for hens subjected to the nonfasted molt, whereas the hens of the fasted molt had shell thickness and percentage of shell similar to controls by 84 wk of age (treatment × age interaction, P ≤ 0.05). Shell thickness and percentage of shell did not differ between fasted and nonfasted molting hens at 84 wk of age. Egg weight was unaffected by molting treatment (data not presented).

**Statistical Analysis**

An ANOVA with repeated measurements was conducted on all variables with treatment (fasted molt vs. nonfasted molt vs. control) and age as fixed effects. The individual hen was the experimental unit with the exception of egg production in which a row of birds was the experimental unit. Differences of least squares means were used to partition means for significant interactions (Oehlert, 2000). The mixed model procedure of the SAS system was used to conduct the statistical analysis (SAS Institute, 1988).

**RESULTS**

Fasted and nonfasted hens lost 22 and 18%, respectively, of their BW by 28 d postmolt. At 28 d postmolt, BW was similar between birds of the fasted and nonfasted molting regimens but at 7 and 35 d postmolt, fasted hens had lower BW than nonfasted hens. A gradual recovery in BW of molted hens to premolt values occurred by 70 d postmolt. Control hens did not lose weight during the trial (treatment × age interaction, P ≤ 0.0001, Figure 1). Fasted hens ceased laying eggs by 67 wk of age. Nonfasted hens never went completely out of production but decreased their egg production to 3.8% on the 7th day of molt (treatment × age interaction, P ≤ 0.0001, Figure 2). Both molted groups returned to normal egg production by 74 wk of age, but hens of the nonfasted molt regimen experienced a dip in egg production with levels of production similar to the controls beginning at 76 wk of age.

**DISCUSSION**

Previous research has shown that use of feed withdrawal to induce a molt adversely affects bone mineralization and biochemical properties of bone during molt (Newman and Leeson, 1999; Mazzuco and Hester, 2005). Garlich et al. (1984) reported that the femur density of White Leghorn hens molted at 67 wk of age decreased during molt. The bone-breaking force, bone stress, and ash of the tibia of hens (68 wk old) subjected to 8 d of feed deprivation were reduced immediately following feed withdrawal (Newman and Leeson, 1999). Yosefi et al. (2003) also observed that the tibial ash of fasted hens decreased during an induced molt (feed withdrawal for 8 or 11 d at 67 wk of age). The results of the current study also showed reduced bone mineralization during a fasted molt (Figures 3 and 4). However, a nonfasted molt regimen was less deleterious to bone mineralization as compared to a fasted molt. Tibial BMD values of hens on the nonfeed removal molt regimen were intermediate be-
FIGURE 1. Body weight of White Leghorns submitted to either a fasted (▲) or a nonfasted molt (■) at 66 wk of age (d 1) as compared to nonmolted controls (○). Within age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, \( P \leq 0.0001 \)). Each value represents the average of 7 hens.

tween the fasted molt hens and controls at 35 d postmolt (Figure 3). Fasted hens experienced a 35% loss in BMD by 28 d postmolt compared with an 18% loss in BMD of molted hens consuming a wheat middlings molt diet. Likewise, using peripheral quantitative computerized tomography (pQCT) to assess bone mineralization, a nonfasted alfalfa-based molt diet resulted in less medullary and cancellous femur resorption than a fasted molt (Kim et al., 2005). These results from two different laboratories using two different technologies to assess bone qualities (DEXA and pQCT) suggest that a fasted molting regimen compromises bone mineralization more severely than a nonfasted molt regimen. Because DEXA cannot distinguish medullary bone from structural bone (cortical, cancellous, and trabecular), the possibility exists that the decline in tibial mineralization during molt could be representative of medullary rather than structural bone loss. However, since the humerus, which is more representative of structural bone, also showed a decrease in BMD during a fasted molt (Mazzuco and Hester, 2005), it is suspected that the molt-induced decline in tibial mineralization was due to both the medullary as well as the structural components. Even if medullary bone was the major source of tibial mineralization loss, skeletal integrity during molt is compromised because the medullary component of bone contributes to bone strength (Fleming

FIGURE 2. Weekly hen-day egg production of 202 hens before, during, and after the molt period (▲ = fasted, ■ = nonfasted molt) compared with controls (○). Within age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, \( P \leq 0.0001 \)). Each value represents the average of 2 rows of hens with 26 to 37 hens/row.
et al., 1996). Our previous work has shown that the BMD from DEXA scans is positively correlated with bone breaking force (0.58, \( P \leq 0.0001 \)) and as tibial BMD decreases, the incidence of bone breakage increases (r = −0.54, \( P \leq 0.05 \), Mazzuco and Hester, 2005). Other researchers have shown that the tibia ash of hens on 9 d of feed withdrawal was lower than those of hens on a high-Zn molt diet (Park et al., 2004). Feeding low-Ca diets showed similar effects on bone mineralization, indicating that a rapid depletion of Ca reserves from bone caused a decrease in tibial mineralization during molt. Specifically, a decrease in bone strength and ash of 120-wk-old hens fed a 0.08% Ca diet for 28 d compared with hens receiving 3.75% Ca diet suggested depletion of skeletal mineral reserves (Elaroussi et al., 1994). Using DEXA, bones of birds fed a hypocalcemic diet (1.8% dietary Ca) compared with a control diet (3.9% Ca) showed a detrimental effect on bone mineralization (Schreiweis et al., 2003).

A recovery in BMD and BMC was observed in hens of both molting treatments by 84 wk of age or 126 d after molt (Figures 3 and 4, respectively). Likewise, hens fed a Ca depletion diet (0.08% dietary Ca) for 28 d followed by a repletion diet (3.75% Ca) for an additional 28 d recovered their bone mineralization as indicated by tibia breaking strength and bone ash values similar to controls (Elaroussi et al., 1994). A recovery in bone ash was noted 32 d postmolt, but not in the other bone mechanical variables (bone breaking force and bone stress) measured in hens submitted previously to 8 d feed deprivation.

FIGURE 3. Tibial mineral density (g/cm²) measured in live White Leghorns submitted to either a fasted (▲) or a nonfasted molt (■) between 66 and 70 wk of age compared with nonmolted control hens (○). \(^{ab}\)Within age, least squares means ± SEM with no common letter are significantly different (treatment \( \times \) age interaction, \( P \leq 0.0001 \)). Means represents 7 hens per age.

FIGURE 4. Tibial mineral content (g) measured in live White Leghorns submitted to either a fasted (▲) or a nonfasted molt (■) between 66 and 70 wk of age compared with nonmolted control hens (○). \(^{ab}\)Within age, least squares means ± SEM with no common letter are significantly different (treatment \( \times \) age interaction, \( P \leq 0.01 \)). Means represents 7 hens per age.
FIGURE 5. Egg shell thickness (mm) of White Leghorns submitted to either a fasted (▲) or a nonfasted molt (■) between 66 and 70 wk of age compared with nonmolted control hens (○). *ab*Within age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P \leq 0.05$). Means represent 2 eggs collected from each hen for a total of 7 hens per treatment per age.

FIGURE 6. Percentage of shell of White Leghorns submitted to either a fasted molt (▲) or nonfasted molt (■) between 66 and 70 wk of age compared with nonmolted control hens (○). *ab*Within age, least squares means ± SEM with no common letter are significantly different (treatment × age interaction, $P \leq 0.05$). Means represent 2 eggs collected from each hen for a total of 7 hens per treatment per age.

(Newman and Leeson, 1999). Yosefi et al. (2003) indicated that bone ash of hens previously induced to molt by feed withdrawal for 8 or 11 d showed a gradual increase during postmolt and increased markedly at the onset of the egg production. A gradual recovery by 67 d postmolt in BMD was reported in a pedigree line of White Leghorn hens fasted for 10 d (Mazzuco and Hester, 2005). Molt improved shell quality at the beginning of the second cycle of lay in hens of both fasted and nonfasted molting regimens. However, hens subjected to the nonfasted molt regimen demonstrated better shell quality (Figures 5 and 6), most likely because they were laying fewer eggs (75 and 76 wk old, Figure 2). Other studies have shown that shell quality postmolt did not differ between fasted and nonfasted molting regimens, but this is perhaps due to similar egg production during the second cycle of lay. For example, egg production and shell thickness of hens subjected to feed withdrawal for 8 d
and supplemented daily with 6 g of Ca/bird or a high-Zn diet (2.5% Zn oxide for 10 d) were similarly improved postmolt when compared to nonmolted control hens (Bar et al., 2003). Likewise, Biggs et al. (2004) indicated no differences between feed removal vs. nonfeed removal molting treatments on egg production, egg specific gravity, and egg weight. Park et al. (2004) also demonstrated that there were no differences in egg production and the interior and exterior egg quality of eggs laid by hens subjected to alternative molting regimens using Zn acetate or Zn propionate and hens on a 9-d fasting molting regimen.

Notably, during the time tibial mineralization was still low (from 76 to 80 wk of age, Figures 3 and 4) in both molted groups, shell thickness and percentage of shell were improved compared with nonmolted controls (Figures 5 and 6), suggesting that Ca was perhaps being mobilized from medullary bone for use in shell formation. This adaptive response to previous Ca restriction during molt is most likely parathyroid hormone-dependent, utilizing bone reservoirs for shell calcification (Bar et al., 1999).

The BW loss experienced by molting hens of the current study was similar to previous reports (Biggs et al., 2003; Kubena et al., 2005), with most of the BW loss likely due to the involution of the reproductive organs (Sherry et al., 1980; Ruszler, 1998). The BW recovery for the second cycle of lay (Figure 1) occurred in both molted groups at the same time when egg production (Figure 2) was increasing and improvements in shell quality were noted (Figures 5 and 6). The resumption of lay induced by the return to an egg laying diet and the improvement in egg shell quality postmolt could be linked to an improvement of intestinal Ca transport. Higher Ca uptake by the gut of hens after molt can be explained by the improved ability of duodenal cells to transport Ca due to an increase in Ca-regulating proteins (e.g., calbindin) in response to estrogen (Beck and Hansen, 2004). This protein appeared in the uterine mucosa during the formation of the first egg at the start of lay, decreased when egg production ceased, and recovered upon reintroduction of feed after 22 d of a nonfeed removal molting regimen (Balnave et al., 1992). An induction of molt of 63-wk-old hens by feed deprivation for 2 wk or 30% BW loss caused duodenal Ca uptake to increase after molt with a concurrent increase in percent shell and eggshell thickness (Al-Batshan et al., 1994).

In summary, the data from this experiment confirm a previous report (Mazzuco and Hester, 2005) that feed withdrawal used to induce a molt adversely affects bone mineralization during molt. We further demonstrated that a nonfeed-removal molt diet using wheat middlings as a major feed ingredient was less deleterious to bone mineralization during molt compared with a fastered molting program. Additional research using nonfeed removal regimens for molting birds should be done in order to confirm these findings.

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