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Effects of a premolt calcium and low-energy molt program on laying hen behavior and heterophil-to-lymphocyte ratios

Emily R. Dickey
Iowa State University

Kristjan Bregendahl
Iowa State University

Kenneth J. Stalder
Iowa State University, stalder@iastate.edu

Robert F. Fitzgerald
Iowa State University

Anna Johnson
Iowa State University, johnsona@iastate.edu

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Abstract
The objectives of this study were to compare the behaviors, postures, and heterophil-to-lymphocyte ratios (H:L) of laying hens housed in a cage system when offered a Ca premolt treatment and low-energy molt diets vs. a traditional feed withdrawal (FW) treatment during and after molt. A total of 144 Hy-Line W-36 hens (85 wk of age), housed 3 hens/cage (413 cm2/hen), were used. Hens were allotted to treatments according to a randomized complete block design, with the cage location and initial BW as the blocking criteria. Six treatments were compared in a 2 × 3 factorial arrangement with 2 Ca premolt treatments (fine or coarse) and 3 low-energy molt diets (FW, soybean hulls, or wheat middlings). The 2 Ca premolt treatments differed only in Ca particle size (fine was 0.14 mm and coarse was 2.27 mm mean diameter). Two postures and 5 behaviors were recorded and H:L was measured. Data were analyzed using the MIXED procedure of SAS, with $P < 0.05$ considered significant. There were no differences in behaviors, postures, or H:L during the premolt baseline period. The Ca premolt treatment had no carryover effects during or after molt for behaviors or postures. During molt, hens in the FW treatment were more active, and they ate and drank less compared with hens fed soybean hulls or wheat middlings, but there were no differences in aggression, nonnutritive pecking, or sitting. Drinking and aggression during and after molt were not different, but hens postmolt engaged in more sitting and feeding and less activity, nonnutritive pecking, and preening compared with during molt. There were no differences in H:L during or after molt. In conclusion, a Ca premolt treatment did not affect the behavior of the laying hen. The low-energy molt diets did not adversely affect behavior compared with FW and did not increase H:L; therefore, they could be useful alternatives for inducing molt in laying hens.

Keywords
behavior, feed withdrawal, laying hen, low-energy molt diet, well-being

Disciplines
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Effects of a Pre-molt Calcium and Low-Energy Molt Program on Laying Hen Behavior and Heterophil to Lymphocyte Ratios

E. R. Dickey,* K. Bregendahl,*,# K. Stalder,* R. Fitzgerald,* and A. K. Johnson*,[1]

*Department of Animal Science, Iowa State University, Ames, IA

#Present address: Hy-Line International, Dallas Center, IA

[1]Corresponding author: 2356F Kildee Hall, Ames, IA; johnsona@iastate.edu

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ABSTRACT: The objectives of this study were to compare the behaviors, postures, and heterophil to lymphocyte ratios (H:L) from laying hens housed in a cage system when offered a Ca pre-molt treatment and low-energy molt diets versus a traditional feed withdraw (FW) during and post-molt. A total of 144 Hy-Line W-36 hens (85 wk of age), housed 3 hens per cage (413 cm^2/hen), were used. Hens were allotted to treatments according to a randomized complete block design with the cage location and initial body weight as the blocking criteria. Six treatments were compared in a 2 × 3 factorial arrangement with 2 Ca pre-molt treatments (fine or coarse) and 3 low-energy molt diets (FW, soybean hulls [SH] or wheat middlings [WM]). The 2 Ca pre-molt treatments differed only in Ca particle size (fine 0.14 and coarse 2.27 mm mean diameter). Two postures and 5 behaviors were recorded and H:L was measured. Data were analyzed using PROC MIXED procedure of SAS with $P < 0.05$ significant. There were no differences in behaviors, postures, or H:L during the pre-molt baseline period. The Ca pre-molt treatment had no carryover effects during or post-molt for behaviors or postures. During molt, FW hens were more active, fed and drank less compared to hens fed SH or WM, but there were no differences in aggression, non-nutritive pecking, or sitting. Drinking and aggression during and post-molt were not different, but hens post-molt engaged in more sitting and feeding and less activity, non-nutritive pecking, and preening compared to during molt. There were no differences in H:L during or post-molt. In conclusion, a Ca pre-molt treatment did not affect the behavior of the laying hen. The low-energy molt diets did not adversely affect behavior compared to FW and did not increase H:L and could therefore be useful alternatives for inducing molt in laying hens.

Key Words: Behavior, Feed withdrawal, Laying hen, Low-energy molt diet, Well-being

INTRODUCTION

In the egg laying industry, hens may be exposed to an induced molt to extend their productive life which allows for a second, more productive egg laying cycle. During molt, the reproductive tract regresses and egg production ceases (Webster, 2003). Traditionally, molt has been induced by feed withdrawal (FW) ranging from 4 to 14 d accompanied by light restriction or total removal of water for up to 3 d (Cunningham and Mauldin, 1996; Berry, 2003). However, this practice has raised societal concerns about its possible effects on the overall well-being of the laying hen (Holt, 1992; Webster, 2003; McCowan et al., 2006). In the United States, industry groups have recommended that producers implement only non-fasting molt programs after January 1, 2006, which has been defined as having available water and a feed source suitable for non-producing hens (AVMA, 2005; United Egg Producers, 2008) and some fast-food chains (McDonald's, Wendy's, and Burger King) have stated that their companies will no longer purchase eggs that are produced from a laying operation that uses a FW molting program (Anonymous, 2000).

In the United States, molt programs are typically induced for laying hens between 65-75 wk of age based on economics. However, there is no reason to expect a molting age by treatment effect on behavioral traits. A number of studies have compared the effectiveness of feeding low-energy feeds, such as wheat middlings (WM) or soybean hulls (SH), as an alternative to FW for inducing molt (Biggs et al., 2003, 2004; Koelkebeck et al., 2006; Koelkebeck and Anderson, 2007). Although FW resulted in a more complete and better post-molt performance, Biggs et al. (2003, 2004), Koelkebeck et al. (2006), and Mejia et al. (2010) concluded that the low-energy feeds were alternatives for inducing a molt with regard to better post-molt performance. In addition to behavioral changes when undergoing a molt, the laying hen may have altered her physiology when they experience different types of stress. Biggs et al. (2004) has reported that the heterophil to lymphocyte ratio (H:L) is a good predictor for laying hen stress, with an increased ratio indicating elevated levels of stress. Biggs et al. (2004) found no differences in social behaviors or H:L between hens subjected to a 10 d FW and hens that were fed a WM molt diet, but alternatively, McCowan et al. (2006) reported increased aggression in both fasted and non-fasted hens during molt compared to their behaviors prior to molting. Therefore, the non-fasting molt programs have not conclusively shown what effects they may have on laying hen behavior or physiology.

An additional consideration is that feeding a Ca-deficient diet can inhibit ovulation and induce molt (Douglas et al., 1972; Hurwitz et al., 1975). Although supplying sufficient calcium to meet the recommended consumption, it may be possible that a fine Ca pre-molt treatment, compared to coarse Ca that is typically used in laying hen feed, will not allow the hen to mobilize sufficient Ca from bone to meet the needs for eggshell formation and the generation of the luteinizing-hormone surge needed for ovulation (Luck and Scanes, 1979, 1980; Johnson, 2000). Furthermore, fine Ca may be absorbed faster than coarse Ca, resulting in little or no Ca present in the crop and intestines at night and therefore less Ca available for egg shell formation. However, the effect of Ca pre-molt supplementation on laying behavior and physiology is unknown.

The objectives of this study were to compare the behaviors, postures, and heterophil to lymphocyte ratios (**H:L**) of the laying hen housed in a cage system when offered a Ca pre-molt treatment and low-energy molt diets versus a traditional feed withdraw (**FW**) during and post-molt.

MATERIALS AND METHODS

Housing and Husbandry

The project was approved by the Iowa State University Institutional Animal Care and Use Committee. The research was conducted over a 29 wk period (July 2007 to February 2008) at the Iowa State University Poultry Research Center in Ames, IA. A total of 333 Hy-Line W-36 laying hens (85 wk of age) weighing 1.7 ± 0.2 kg were used (144 hens for behavior and 189 hens for H:L). Hens were obtained from a single source and were considered to have a healthy reproductive status. Hen beaks were trimmed at the hatchery immediately post-hatch according to recommendations from the Hy-Line W-36 commercial management guide. All cages were located in 2 identical, light-controlled mechanically-ventilated rooms. Hens were housed 3 per cage (30.5 cm wide × 40.6 cm deep × 44.5 cm high), providing 413 cm^2/hen. Wire flooring was used in all cages (Chore-Time, Milford, IN) and each cage was equipped with a plastic self-feeder and a nipple drinker. In room 1, the feeders were 29.2 cm in length, whereas in room 2 the feeders were 20.3 cm in length. Hens were able to see neighboring feed troughs, but were unable to reach them due to vertical plastic barriers between troughs.

Treatments and Experimental Design

Forty-eight cages were used during the behavioral trial and 63 cages were used for the H:L trial. Hens were weighed and assigned to cages (n = 3 hens / cage) so that cage BW weight was even across treatments. The experimental design was a randomized complete block design with treatments in a 2 × 3 factorial arrangement with 2 Ca pre-molt treatments and 3 low-energy molt diets (Figure 1). The behavioral trial had a total of 8 blocks, within each block there were 6 individual laying hen cages representing the 6 dietary treatment combinations. The experimental unit was the cage containing 3 hens (n = 48) for the behavioral data, whereas the experimental unit for the H:L was the individual hen (n = 189).

Baseline Period. Hens were exposed to a 16-h light photoperiod. Hens were 85 wk of age at the start of the 2-wk baseline period which was defined as the period before any experimental diets were applied. The hens had free access to water and a laying hen diet formulated to meet or exceed recommendations from the Hy-Line W-36 commercial management guide (Table 1).

Calcium Pre-molt Treatment. Hens were exposed to a 24-h light photoperiod for a 1 wk period (87 to 88 wk of age; Anderson and Havenstein. 2007). Following the baseline period, hens (87 wk of age) received either a combination (50:50) of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$, or all-fine $CaCO_3$, mixed into a laying hen diet that was fed to the hens for a 1 wk period (Table 1). Both diets contained 4.61% Ca, such that only the particle size of the Ca supplement differed between the 2 treatments (which both supplied the recommended amounts of calcium). Hens had free access to water.

During Molt. Hens were exposed to an 8-h light to 16-h dark photoperiod for the first 3 wk and then light was increased to 12 h at the start of the last week of molt. The 3 low-energy molt diets (feed withdrawal [FW], soybean hulls [SH], or wheat middlings [WM]) were applied for a total of 28 d (from 88 to 92 wk of age). Hens fed the FW molt diet (Table 1) were restricted from feed consumption for 7 d with free access to water, followed by 21 d of skip-a-day feeding restricted to 60 g of feed per feeding day per hen. This feeding regime allowed hens assigned to the FW group to engage in feeding related behaviors during molt. The hens fed the WM and SH low-energy molt diets (Table 1) were provided free access to feed and water during the entire 28-d molt period. Vitamins and minerals were added to the WM and SH molt diets to make acceptable diets for non-producing hens, with the exception of energy content, according to recommendations from the Hy-Line W-36 commercial management guide. Ground corn grain was added to the molt diets to improve flowability (75:25 WM:corn and 50:50 SH:corn; Koelkebeck et al., 2006).

Post-molt. Hens were exposed to an incremental 1-h increase in light each week until a 16 h photoperiod was reached. Following the 4 wk of molt diets, all hens were fed a commercial-type laying hen diet for egg-producing hens (Table 1) for 22 wk (from 92 to 114 wk of age). This period was divided into the first 2 wk post-molt and the next 20 wk according to diet recommendations from the Hy-Line W-36 commercial management guide. Hens were given free access to water.

Heterophil to Lymphocyte Ratios

Blood was collected from a total of 189 additional laying hens at 5 time points over the course of the trial. The first time point was at the end of *baseline* (n = 9 laying hens aged 86 wk), second, at the end of the *Ca pre-molt treatment* (n = 9 laying hens aged 87 wk / trt for a total of 18 hens), third *middle of the molt period* (n = 9 laying hens aged 89 wk / trt for a total of 54 hens) fourth *end of the molt period* (n = 9 laying hens aged 91 wk / trt for a total of 54 hens) and finally at the *end of the post-molt period* (n = 9 laying hens aged 113 wk / trt for a total of 54 hens). Blood could not be collected from the same bird more than once and hens were randomly selected for a representation of the current treatments. Approximately 9 -mL of blood was collected from the brachial vein into heparinized 15-mL centrifuge tubes. The tubes were stored on ice until analysis within 1 hr of collection. Blood smears were made on a glass slide and were stained using materials from a commercially available kit (Camco Quik Stain II, Cambridge Diagnostics Products, Inc., Fort Lauderdale, FL). A total of 50 heterophils and lymphocytes were counted by a trained person and the number of heterophils was divided by the number of lymphocytes for the H:L.

Behavioral Equipment and Collection

Twelve cameras (12 V color closed circuit television camera; Model WV-CP484, Panasonic Matsushita Co. Ltd., Kadoma, Japan), each filming 4 cages, were mounted on the ceiling (1.5 m above the cages) to record hen behaviors and postures at baseline, during, and post-molt onto a digital video recorder (RECO-204, Darim Vision, Pleasanton, CA) at a rate of 30 frames*s^{-1}. Behaviors and postures were recorded (Figure 1) on d 7 (baseline), on d 23 and 49 (during molt), and on d 51 and 66 (post-molt) for 2 h once the lights were turned on in the morning and for 2 h before lights went out at night (dependent on the photoperiod). This resulted in a total of 960 h of behavioral recordings. The collection of laying hen behaviors and postures were collected by 2 experienced observers who viewed the DVD at 30 frame/s using a 1 min scan sampling technique on the Observer software (The Observer, Ver. 5.0.25 Noldus Information Technology, Wageningen, The Netherlands).

Behaviors and Postures

A total of 48 cages (containing 3 hens per cage [144 hens total]) were used to collect two postures (sitting and active) and 5 behaviors (feeding, drinking, non-nutritive pecking, preening, and aggression), adapted from Webster (2000). *Sitting* was defined as a crouched posture with shanks or breast in contact with the cage floor. *Active* included standing with an erect posture, standing on top of another cage mate, or engaging in a comfort movement to relieve muscular tension (wing flap, wing shake, and body stretch). *Feeding* was defined as pecking behavior directed toward the feed trough or toward a neighboring feed trough. *Drinking* was defined as the appearance of ingesting water from the nipple drinker at the rear of the cage. *Non-nutritive pecking* was defined as non-aggressive pecking at anything other than feed, which included cage pecking, feather pecking, bill pecking, and air pecking. *Preening* behavior involved the manipulation of the plumage with the beak. *Aggression* was observed as a forceful peck directed toward the head of another hen that either made contact or caused an avoidance response in the target hen. Aggression was the sum of pecks that occurred within a cage or between neighboring cages.

Statistical Analysis

The experimental design was a randomized complete block design with treatments in a 2 × 3 factorial arrangement with 2 Ca pre-molt treatments and 3 low-energy molt diets. The experimental unit was the cage containing 3 hens (n = 48) for the behavioral data, whereas the experimental unit for the H:L was the individual hen (n = 189). The behavioral data for each observational day for the 3 hens in a cage were averaged. Behavioral data were expressed as a percentage of total recorded time and were subjected to a square root arcsine transformation process to achieve a normal distribution, which was evaluated using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed using the MIXED procedure of SAS for parametric data on a cage basis.

For the baseline behavioral data that was collected on d 7, the statistical model included the fixed effects of treatment (2 Ca pre-molt treatments by 3 molt diets) and room (1 [larger feeder] or 2 [smaller feeder]), and the subplot included the 2-way interaction of treatment by room. The location of cage within a room (north or south half of room1 or 2) was included in the initial behavioral model, but was not significant and was removed for the final analyses.

The data collected on d 23 and 49 during molt and on d 51 and 66 post-molt were first analyzed as 4 separate days. However, there were no differences within each period when the days were analyzed separately compared to when the days were averaged together. Therefore, the data collected on the 2 d during molt and the data collected on the 2 d post-molt were averaged for the final behavioral analysis. Calcium treatment and the interaction of Ca treatment by molt diet were included in the initial behavioral model, but Ca pre-molt treatment effects were not significant ($P > 0.05$) and were not included in the final analyses. The final statistical model included the main effects of period (during or post-molt), room (1 or 2) and, low-energy molt diet (FW, WH or SH), and the 2-way interactions between period and low-energy molt diet and room and low-energy molt diet. Cage was included as a random effect. The 3-way interaction between period, room and low-energy molt diet was evaluated, but it was not significant and therefore was removed from the final model before analysis. Room was significant ($P < 0.05$) at baseline for activity, feeding and drinking and for room x molt diet interaction for activity and feeding (*data not presented*). The H:L were analyzed with ANOVA using JMP (version 6.0.3; SAS Institute, Cary, NC). During the molt and post-molt periods, molt diet and Ca pre-molt treatment were included in the model as fixed effects. The 2-way interaction of molt diet by Ca pre-molt treatment was included in the subplot and cage was included as a random effect. Means of the 3 molt diets were assessed by Fisher's least significant difference. The mean H:L values from the molt and post-molt periods were compared to baseline values using Dunnett's t-test. A *P*-value less than 0.05 were considered significant in all comparisons.

RESULTS

Heterophil to Lymphocyte Ratios

The mean H:L during the baseline period was 40% (SD 11%). There was no ($P = 0.59$) effect on H:L between coarse or fine Ca during the Ca pre-molt treatment. The Ca pre-molt treatment had no effect on H:L during molt and they did not differ from baseline values

($P > 0.05$). The fine-Ca pre-molt treatment resulted in lower H:L compared to the coarse-Ca pre-molt treatment during the post-molt period ($P = 0.01$), but neither treatment differed from baseline values ($P > 0.05$). There were no differences ($P > 0.05$) among the 3 molt diets for H:L during or post-molt and the values did not differ from baseline values, respectively (Table 2).

Baseline and Ca Pre-Molt Periods: Laying Hen Postures and Behaviors

There was no difference ($P > 0.05$) among treatments (defined as the interaction of the 2 Ca pre-molt treatments and the 3 low-energy molt diets) during the baseline period (Table 3). The Ca pre-molt treatment had no carryover effect on the behaviors or postures of the laying hens during or post-molt ($P > 0.05$; *data not shown*).

During and Post Molt (period) by Low Energy molt (FW, SH and WM) Diet Interaction: Laying Hen Postures and Behaviors

Hens provided the FW treatment were more ($P < 0.001$) active than hens fed SH and WM low-energy molt diets during molt. Post-molt, there were no ($P > 0.05$) differences among low-energy molt diets for activity levels. When comparing during and post-molt hens provided the FW treatment decreased their activity, but SH and WM hens activity levels increased (Figure 2).

Hens provided the FW treatment engaged in less ($P < 0.001$) feeding behavior than hens fed SH and WM low-energy molt diets during molt. Post-molt, there were no (P > 0.05) differences among low-energy molt diets for feeding behavior. When comparing during and post-molt, hens provided the FW treatment increased ($P < 0.001$) their feeding activity but SH and WM hens decreased ($P < 0.001$; Figure 3).

Hens provided the FW treatment engaged in less ($P < 0.001$) drinking behavior than hens fed SH and WM low-energy molt diets during molt. Post-molt, there were no ($P > 0.05$) differences among low-energy molt diets for drinking behavior. When compared during and post-molt for hens provided the FW treatment drinking activity increased ($P = 0.003$), but there was no change for SH and WM hens ($P = 0.29$ and 0.71, respectively; Figure 4).

Feed withdrawal and WM hens engaged in more ($P < 0.001$) preening behavior than SH hens during molt. Post-molt, WM hens engaged in more preening related behaviors than FW and SH hens ($P = 0.002$ and 0.001, respectively). When comparing during and post-molt, preening behavior decreased ($P < 0.001$) for FW hens, but did not change for SH and WM hens ($P = 0.86$ and 0.44, respectively; Figure 5). Finally, there were no differences between low-energy molt diets during or post-molt for sitting ($P = 0.37$; Figure 6), non-nutritive pecking ($P = 0.66$; Figure 7) or aggressive behaviors ($P = 0.48$; Figure 8).

During and post molt: Laying hen Postures and Behaviors

During molt, hens were more ($P < 0.001$) active and engaged in higher levels ($P < 0.001$) of non-nutritive pecking and preening related behaviors ($P < 0.001$) compared to post-molt. During molt, laying hens spent less time sitting and feeding ($P < 0.001$) compared to post-molt. There were no differences in the levels of drinking ($P = 0.35$) and aggression ($P = 0.30$) between during and post-molt, respectively (Table 4).

DISCUSSION

Baseline Period

During the baseline period, hens assigned to the different Ca pre-molt treatments and low-energy molt diets did not display differences in the postures and behaviors collected in this trial. The time utilized by these hens in each respective posture and behavior is in agreement with previous laying hen behavioral research (Webster, 2000; Anderson et al., 2004). Therefore, any differences reported in the subsequent molt and post-molt periods could be attributed to the low-energy molt diets. The mean H:L during the baseline period was also in agreement with previously published literature for laying hens (Biggs et al., 2004).

Calcium Pre-molt Treatments

The Ca pre-molt treatment had not been previously investigated for its possible effects on laying hen behavior or H:L during or post-molt. Hypothetically, fine-$CaCO_3$ may be absorbed faster than the coarse $CaCO_3$, resulting in little or no $CaCO_3$ present in the crop and intestines at night. In turn, there would be less Ca available for egg shell formation at night than in hens fed the coarse $CaCO_3$. Additionally, restricting Ca may inhibit ovulation by negatively affecting the surge of luteinizing hormone. Overall, decreasing Ca in the laying hen diet may produce a more efficient molt by assisting in the cessation of egg production. However, the Ca treatment did not have a carryover effect on the laying hens' behaviors and postures during or post-molt and had no effect on the H:L during the Ca pre-molt treatment period. Production data from the current study found that the fine-Ca pre-molt treatment was beneficial to egg production in the next laying cycle and overall laying hen performance (Dickey, 2008).

During vs. Post-molt

There were no differences before and post-molt for time engaged in drinking in the present study. During molt there was a difference; FW hens decreased drinking and feeding behavior. Furthermore, hens during and post-molt did not engage in increased aggression. Although aggression can increase due to a disturbance such as changing or withdrawing feed, aggressive activity is rare in small groups of caged hens because the hens are able to develop a dominance relationship (Appleby et al., 2004). It may be possible that in larger commercial systems with more hens housed per cage or higher cage density, time spent engaged in aggressive behaviors may change. Preening, activity, and non-nutritive pecking increased during molt compared to post-molt. The increased preening may be due to sensitivity from the loss of feathers (Webster, 2000) or displacement behavior (an unrelated behavioral response to anxiety) as a result of frustration (Duncan and Wood-Gush, 1972). The increase in non-nutritive pecking agrees with research by McCowan et al. (2006) who reported an increase in cage pecking for hens assigned to a FW and non-FW molt during the molt period compared to the pre-molt period. This increase may be due to hunger or a redirection of foraging behaviors (Webster, 2003). However, once hens were fed a diet that met or exceeded their physiological requirements post-molt, feeding and sitting behaviors were higher than during molt. This increase may be due to the hens being provided a more palatable non-molt diet and no longer needing to search for food as low-energy feeds increase hunger in a molted hen at least as much as a FW molt (Koch et al., 2007).

Low-Energy Molt Diets During and Post-molt

Concern over individual hen well-being has been expressed by numerous groups who oppose the traditional methodology of withdrawing feed to induce molt in the laying hen (Cunningham, 1996; Ruszler, 1998). Previous research addressing the behavior of the laying hen during a traditional FW molt is conflicting. Webster (2000) reported that aggressive pecking within a cage (housed 3 per cage with 344 cm^2/hen) was reported in 14% of hens during the first day of FW compared to 0% of fed control hens, but was not seen at all on d 2 and 3 of the FW molt. Anderson et al. (2004) reported that feather pecking increased during a 2 wk FW for hens kept in a cage system (6 hens/cage at 361 or 482 cm^2/hen), but the frequency of aggression and submissive acts were lower during the same time period. Webster (2000) and Biggs et al. (2004; housed 3/cage at 460 cm^2/hen) reported no differences in aggressive pecking behaviors when comparing WM and FW molt treatments, but McCowan et al. (2006) noted that cage pecking increased in hens (housed 3/cage at 417 cm^2/hen) assigned to a FW molt and aggression increased in hens assigned to a FW and a non-FW molt during the molt period. In agreement with Webster (2000), Anderson et al. (2004), and Biggs et al. (2004) reported that across studies FW birds did not show an increase in aggression, non-nutritive pecking, or sitting when compared to birds that still had access to a low-energy feed during molt. The 2 d of observations during molt were averaged prior to final analysis because no difference was observed between observation days for each behavioral trait. This result suggests that the hens did not show increased aggression on the first day of molt. Group selection has been reported to result in relatively low aggressive behavior birds that remain productive (Muir, 1995; Weary and Fraser, 2004). Therefore, the differences observed in aggressive behaviors may be due to the type of bird used in any given experiment and can make comparisons and general assumptions about bird behavior challenging. Biggs et al. (2004) and McCowan et al. (2006) used Single Comb White Leghorn hens of the Dekalb White strain, whereas Webster (2000) and Anderson et al. (2004) used Hy-Line W-36 hens, the same line used in the present study.

Laying hens in non-cage systems spend 5 to 25% of their time engaged in foraging behaviors (Appleby and Duncan, 1989), therefore caged hens that are unable to forage spend more time feeding and manipulating their feed (Appleby et al., 2004). However, when presented with a new kind of feed, hens may decrease their feed intake due to novelty or palatability (Appleby et al., 2004). In the present study, both feeding and drinking behaviors during molt occurred less frequently in FW hens compared to the SH and WM hens. Leeson and Summers (2005) have reported that laying hens are prandial drinkers and that there is a clear relationship between feeding and drinking. Therefore, with the FW hens engaged in less feeding related activities due to withdrawal of feed followed by restricted skip-a-day feeding, it would be expected that the time engaged in drinking would also decrease. Once the FW hens were allowed free access to feed, no differences were seen among the treatments. Hens assigned to the FW molt diet spent more time preening during molt compared to post-molt, whereas hens fed the SH and WM molt diets did not alter their time engaged in preening between the 2 periods. Preening is a maintenance behavior that is important for keeping feathers in good condition and birds will often preen when they do not have access to feed (Duncan, 1970; Appleby et al., 2004).

The hens assigned to the FW molt diet spent more time engaged in active postures compared to hens fed the SH and WM molt diets. Activity may have been increased in the hens assigned to the FW molt diet because they were without feed for 7 d and shifted time that would have normally been spent in feeding related behaviors to other postures. Webster (2000) reported that activity increased during a FW molt and hypothesized that the increase in activity would improve the likelihood of a hen finding food. Anderson et al. (2004) used a 14 d FW molt and reported that standing behavior was highest during molt compared to any other period. Aggrey et al. (1990) associated an increase in locomotion during a FW molt to hunger and the search for food. Further, the hens returned to normal activity levels post-molt, once they were being fed a diet that met or exceeded their physiological requirements.

The H:L were not affected by the low-energy molt diets during or post-molt and they did not differ from baseline values, which suggests that hens were not under additional stress and the molt diets were comparable in their effects on stress. During the post-molt period, the fine-Ca pre-molt treatment resulted in lower H:L compared to the coarse-Ca pre-molt treatment. The Ca result suggests hens fed the fine-Ca pre-molt treatment were under less stress than hens fed the coarse-Ca pre-molt treatment; however, this treatment was applied 28 wk before this measurement and would not be expected to have a long-term effect (Appleby et al., 2004). Additionally, the H:L values did not differ from baseline values.

In conclusion, a Ca pre-molt treatment did not have a carryover effect on the behaviors and postures of the laying hen during or post-molt. Low-energy diets consisting mainly of SH or WM did not result in increased aggression or non-nutritive pecking compared to the FW treatment during or after an induced molt. Hens fed the WM or SH molt diets were less active during molt compared to hens fed the FW molt diet. Additionally, the treatments had no effect on the H:L of the laying hens suggesting minimal effects on stress. Therefore, with regard to laying hen behavior and physiological stress, low-energy diets containing SH or WM may be considered for use by the laying hen industry as dietary alternatives to FW during induced molt.

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Table 1. Experimental diets for laying hens before, during, and post-molt[1]

Measure

[1]Calculated values. Diets contained corn, soybean meal, vitamins, trace minerals, dicalcium phosphate, and calcium carbonate.
[2]Fine Ca was 100% fine $CaCO_3$ and coarse Ca was 50% fine:50% coarse $CaCO_3$ added to the diet for a 1-wk pre-molt treatment.
[3]The 7 d feed withdrawal was followed by restricted skip-a-day feeding (60 g/hen).

Table 2. Heterophil to lymphocyte percentages for laying hens before, during, and post-molt when assigned to a fine- or coarse-Ca pre-molt treatment and feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low-energy molt diet [1]

	Treatments		
	Ca pre-molt[2]	**Molt**	***P*-values**[3]

[1]Values are least squares means ± SEM of 9 observations per treatment.
[2]Calcium was supplied as either a 50:50 combination of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]*P* values less than 0.05 significant.

Table 3. Postures and behaviors of the laying hen during the baseline period (from 85 to 87 wk of age)[1]

Measure	**Treatment**[2]	***P*-value**[3]
Number of cages	48	.
Postures, %		
Sitting	5.4 ± 6.0	0.97
Active[4]	57.2 ± 7.4	0.34
Behaviors, %		
Feeding[5]	22.1 ± 6.7	0.21
Drinking	3.7 ± 1.8	0.36
Non-nutritive pecking	0.2 ± 0.4	0.79
Preening	11.1 ± 3.4	0.25
Aggression	0.2 ± 0.3	0.71

[1]All hens were observed using a 1-min scan sample.
[2]Treatment is defined as the combination of 2 Ca pre-molt treatments and 3 molt diets. Mean ± SD.
[3]*P*-values less than 0.05 significant.
[4]Active postures included standing, standing on top of another cage mate, and comfort movements.
[5]Feeding behaviors included feeding from own feed trough and attempting to feed from a neighboring feed trough.

Table 4. Postures and behaviors of the laying hen during and post-molt[1]

	Period		
Measures, %	**During molt**	**Post-molt**	***P*-value[2]**
Postures			
Sitting	4.1 ± 0.01	6.0 ± 0.01	<0.0001
Active[3]	59.8 ± 0.01	56.3 ± 0.01	<0.0001
Behaviors			
Feeding[4]	21.5 ± 0.01	24.6 ± 0.01	<0.0001
Drinking	4.6 ± 0.01	4.8 ± 0.01	0.35
Non-nutritive pecking	0.2 ± 0.01	0.0 ± 0.01	<0.0001
Preening	9.7 ± 0.01	8.2 ± 0.01	0.0002
Aggression	0.1 ± 0.01	0.1 ± 0.01	0.30

[1]Values are least squares means ± SEM of 8 observations per treatment. All hens were observed using a 1-min scan sample.
[2]*P*-values less than 0.05 significant.
[3]Active postures included standing, standing on a cage mate, and comfort movements.
[4]Feeding behaviors included feeding from own feed trough and attempting to feed from a neighboring feed trough.

Figure 1. Project timeline

Figure 2. Active posture of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low-energy molt diet[1]

90
80
70
60
50
40
30
20
10
0

FW
SH
WM

During molt **Post-molt**

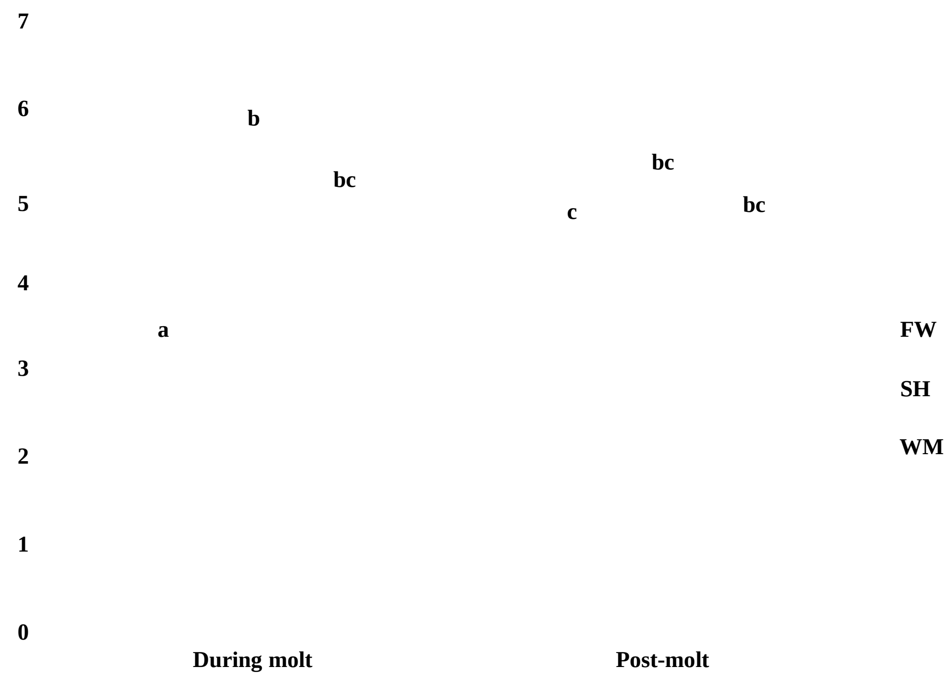
[1]Values are least squares means ± SEM (0.01) with 8 observations per treatment.
[a-c]Superscripts above columns differ at $P < 0.0001$.

Figure 3. Feeding behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low-energy molt diet[1]

35
b
b
30
c c c
25
20
FW
15
SH
10
WM
5
a
0
During molt **Post-molt**

[1]Values are least squares means ± SEM (0.01) with 8 observations per treatment.
[a-c]Superscripts above columns differ at $P < 0.0001$.

Figure 4. Drinking behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low-energy molt diet[1]



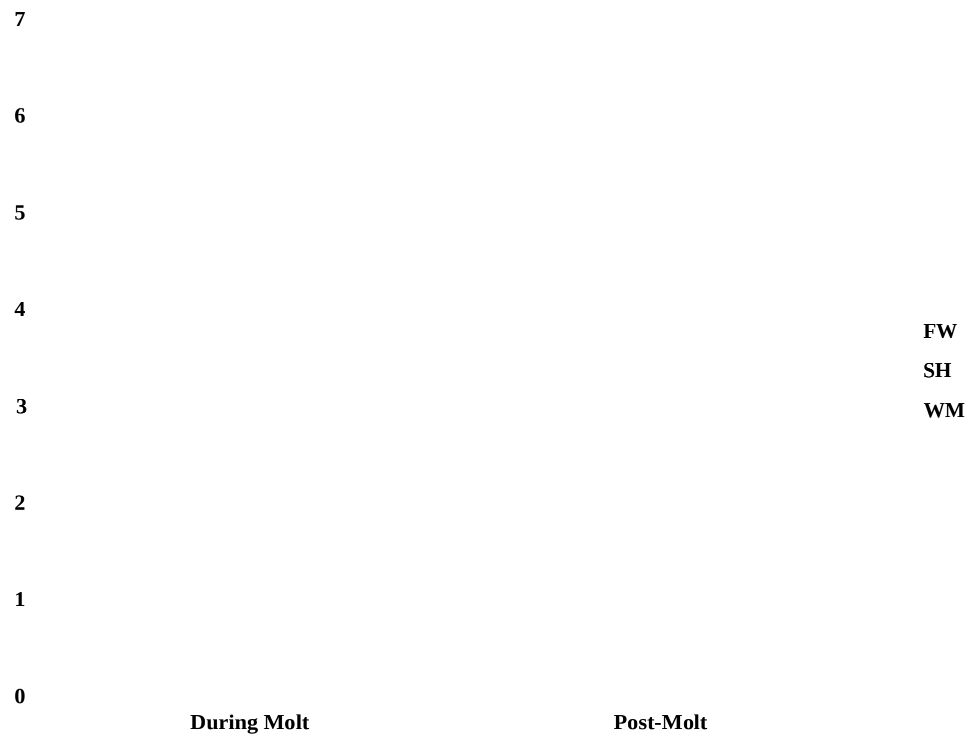
[1]Values are least squares means ± SEM (0.003) with 8 observations per treatment.
[a-c]Superscripts above columns differ at $P = 0.004$.

Figure 5. Preening behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low-energy molt diet[1]

12
a
ac
10
c
8
b
b
b
FW
6
SH
4
WM
2
0
During molt
Post-molt

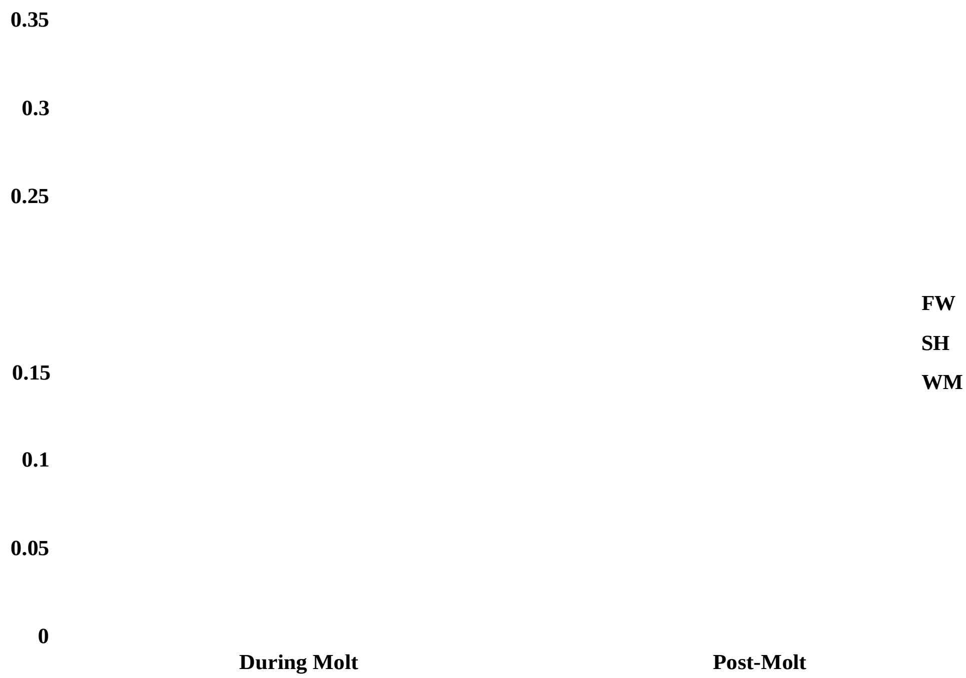
[1]Values are least squares means ± SEM (0.005) with 8 observations per treatment.
[a-c]Superscripts above columns differ at $P = 0.0003$.

Figure 6. Sitting behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low energy molt diet[1]



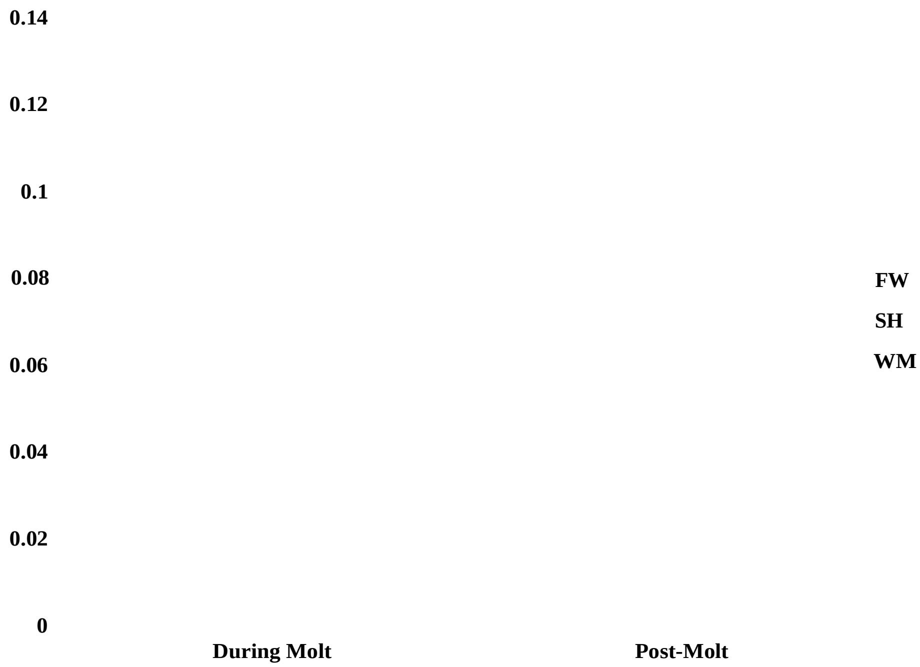
[1]Values are least squares means ± SEM with 8 observations per treatment. There were no statistical differences with $P = 0.37$.

Figure 7. Non-nutritive pecking behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low energy molt diet[1]



[1]Values are least squares means ± SEM with 8 observations per treatment. There were no statistical differences with $P = 0.66$.

Figure 8. Aggressive behavior of the laying hen during and post-molt when assigned to either a feed withdrawal (FW), soybean hulls (SH), or wheat middlings (WM) low energy molt diet[1]



[1]Values are least squares means ± SEM with 8 observations per treatment. There were no statistical differences with $P = 0.48$.