ABSTRACT Several dietary alternatives to feed withdrawal have been proposed to induce a molt in laying hens. This study compared the behavior of laying hens on an alfalfa crumble diet (ALC) to hens that were either on a conventional layer diet (FF) or hens that had feed withdrawn (FW) during a 9-d trial. Each treatment consisted of 24 hens (3 hens per battery cage), and treatment began after a 2-week acclimation period. Video cameras connected to a digital multiplexer recorded the behavior of the hens. The percentages of observations performing nonnutritive pecking, feeder activity, drinking, walking, preening, head movement, and aggression were quantified for two 10-min periods at daily intervals. The FF hens spent significantly more \( P \leq 0.05 \) time drinking than the other treatments, whereas FW hens displayed the most head movements. From d 1 through 7, FW hens walked less than ALC hens except on d 8 when FW hens walked more than ALC and FF hens. On d 4 and 6, the FW hens spent an increased amount of time preening compared with FF hens until the last few days of the molt period. For the most part, FW hens generally displayed more nonnutritive pecking than ALC and FF hens throughout the molt period. However, FW hen visits to the feeders declined as the trial proceeded, whereas ALC and FF hens generally spent more time at the feeder. In summary, the ALC diet showed potential as an alternative to FW for inducing a molt in laying hens based on reduced nonnutritive pecking behavior, head movements, and greater feeding activity.

Key words: behavior, alfalfa crumble, laying hen, molt

INTRODUCTION Commercial laying hens, like most species of wild birds, experience naturally occurring molts (Swanson and Bell, 1975). In the avian species, molting usually involves the periodic shedding and replacement of feathers but is usually incomplete in commercial laying hens, and they continue to lay eggs at low rates for a prolonged period of time (Swanson and Bell, 1975). An incomplete molt means a period of unprofitability due to a reduction of egg production and the end of the useful life of a flock (Berry, 2003). One of the largest incurred expenses in the commercial layer industry is that of replacement pullets (Bell, 2003). Commercial egg farmers in the United States typically extend the productive laying life of their flock from less than 80 wk to 110 or even 140 wk through the use of induced molting (Bell, 2003). After the induced molt, egg production, and egg quality are improved significantly compared with the premolt period (Swanson and Bell, 1975; Bell, 2003; Webster, 2003). Molting may be induced by feed withdrawal for up to 10 d (Christmas et al., 1985), water withdrawal for 2 d (North and Bell, 1990), or both, along with a reduction of day length (Hembree et al., 1980). Feed and water withdrawal is controversial and has been outlawed in Europe, but feed withdrawal has been used for molt induction in the United States (Appleby et al., 2004; Park et al., 2004). Feed withdrawal is also problematic, because it has been shown in experimental models to increase Salmonella Enteritidis colonization and infectivity in the gastrointestinal tract of laying hens (Holt, 2003; Ricke, 2003).

Feed withdrawal has been characterized as a stressful management practice (Beuving and Vonder, 1978) causing a general deterioration of the well-being of animals and usually involving a cascade of physiological adaptive responses (Thaxton and Puvadolpirod, 2000). Duncan and Wood-Gush (1971) stated that feed deprivation was...
frustrating and negatively affected the welfare of the bird due to the initial period of fasting to bring about the cessation of lay. Webster (2000) postulated that behavior might be a direct indicator of the well-being of hens undergoing feed withdrawal; however, studies that quantified aggressive behavior during induced molts have been contradictory. Aggrey et al. (1990) and Haskell et al. (2000) reported increased aggression in feed-deprived hens, whereas Hembree et al. (1980) did not observe this increased aggression. Webster (1995) found no significant differences in aggression between caged hens on a 4-d fast and hens that were not deprived of feed. Although there is no evidence that management programs involving feed withdrawal cause debilitation when properly implemented, there is debate whether or not the hunger involved in feed withdrawal causes suffering (Webster, 2003).

Given the concerns for potential bird stress, various methods of nutrient restriction that would avoid long-term feed withdrawal have been investigated (Berry, 2003; Webster, 2003; Park et al., 2004). Alternative methods for molt induction include feeding low-Ca (Breeding et al., 1992) or low-Na diets and high-dietary Zn (Berry and Brake, 1985) or high-fiber, low-energy diets (Seo et al., 2001; Biggs et al., 2003, 2004; Donalson et al., 2005; Landers et al., 2005a,b; Woodward et al., 2005). Each method is typically employed in combination with a change in the photoperiod and usually leads to BW loss, the cessation of egg production, regression of the reproductive system, and induction of a molt (Shippee et al., 1979; Bell, 2003; Park et al., 2004).

Among the high-fiber, low-energy molt diets, alfalfa molt diets have been shown to effectively regress the reproductive system and bring about a rapid return to egg lay at a rate similar to feed-withdrawal hens (Donalson et al., 2005; Landers et al., 2005a,b). Alfalfa diets also appear to reduce Salmonella Enteritidis colonization in the organs and reduce intestinal shedding of the pathogen during molting when compared with feed-withdrawal hens (McReynolds et al., 2005, 2006; Woodward et al., 2005; Dunkley et al., 2007). However, hen behavior during induced molting using alfalfa molt diets has not been investigated. The objective of this study was to evaluate the behavior of hens and compare these behavioral patterns to that of feed-withdrawal hens over a 9-d induced molt.

**MATERIALS AND METHODS**

Ninety Single Comb White Leghorn hens, commercial hybrid line Lohman (LSL) that were approximately 60 wk old, were obtained from a local commercial layer farm. The hens were divided into 2 groups and placed in floor pens with 5 broiler birds in each pen, for a period of 2 wk. This was done to train the hens to use the nipple waterers in the battery cages. Because the behavior of hens in one treatment could influence the behavior of hens in the other treatment groups, each treatment was housed in a separate identical animal room within the same building. Lighting, temperature, and ventilation were carefully monitored 2 times daily to minimize possible confounding arising from variation between rooms.

The hens were randomly placed on both tiers of 2-tier battery cages with 3 hens per cage and an allowance of 800 cm² per bird. Twenty-four hens were placed in each room for a 2-wk acclimatization period, during which they were fed a balanced unmedicated corn-soybean meal-based mash layer ration that met the NRC requirements for nutrients (NRC, 1994). Water was provided ad libitum. The full-fed (FF) group served as the control group and remained on the acclimatization ration. Alfalfa typically contains 17 to 18% CP and 24 to 25% crude fiber and has a low ME (1,200 kcal/kg) when compared with a layer ration with 2,965 kcal/kg (NRC, 1994). Hens in the ALC treatment were fed alfalfa crumble ad libitum for the 9-d trial. The alfalfa crumble was obtained by passing 2-cm-long alfalfa pellets through a crumber that reduced the pellets to approximately 0.5 to 0.8 cm. Feed was withdrawn (FW) from the third group for the 9-d duration of the trial. One week before the beginning of the trial, the lighting schedule in the 3 rooms was changed from 16L:8D to 8L:16D. On d 1, only the alfalfa diet was offered to the ALC hens, and feed was withdrawn from the FW hens.

**Behavior Parameters**

Eight cages (3 hens in each cage) on both the upper and lower tiers in each room were observed. Two cameras were mounted approximately 1.2 m away and 30° above the cages in each of the 3 rooms, with 1 camera focused on 4 cages. The cameras were attached to a digital multiplexer (Kalatel DVMRe Triplex eZ Digital Video Multiplexer Recorder, Corvallis, OR). Two 10-min intervals were analyzed each day from d 0 to 9 of the trial. Recordings began at 1200 h each day and ended at 1400 h with one 10-min interval starting at 1230 h and a second 10-min observation starting at 1330 h. The times were selected to provide the best comparison of the behavior of the control (FF) hens and the other treatments, because most of the control hens would have finished prelaying behavior and they would have already laid by 1200 h (Mills and Wood-Gush, 1985; Webster and Hurnik, 1990). Recordings were ended by 1400 h to avoid recording any increased feed intake by the control (FF) hens in anticipation of darkness (Savory, 1980).

The 24 hens were the subjects for the behavior analysis in each treatment. Seven different behaviors were adapted from Webster (2000) to assess the welfare of birds during the trial. Head movement was considered to be the rapid individual head movement of an alert bird. This is suggestive of visual surveillance by a bird of its environment. Feeder activity involved any pecking behavior that was directed toward the feeding trough while the focal bird was close enough to the trough to eat or anticipate of darkness (Savory, 1980).

Drinking was considered to be the apparent ingestion of water by pecking from the nipple waterers. Nonnutritive
pecking behavior was recorded when hens were pecking at anything other than the feed, which included pecking at the cage floor, sides, and their own feet. Walking was defined as the locomotion of the hen involving at least 1 step. Aggression was observed as any aggressive pecking behavior directed to another hen either in the same cage or in a neighboring cage. Aggressive behavior was reported as a one-zero sampling, in which the observer scores whether a behavior occurs (one) or not (zero) each interval the recordings were analyzed (Lehner, 1998). The data for the other 6 behaviors were recorded in the form of counts at 1-min intervals that were then summarized as the daily mean percentage of observations in which the subject was performing a particular behavior.

### Statistical Analysis

The average of each activity of the 3 hens in each cage per day was calculated, and the cage became the unit of observation (Webster, 2000). All data were subjected to arcsine square root transformation, after which all data were analyzed using a repeated measures design. PROC GLM (SAS version 8.3, SAS Institute Inc., Cary, NC) was used with treatment, time, treatment × time interaction, and chicken nested within treatments as factors. Chicken nested within treatment was the error term used to test for treatment effects. When significant \( P \leq 0.05 \), arcsine treatment × time interactions were found, least squares means were compared using least significant difference, and the untransformed percentage of means are displayed in the respective figures.

### RESULTS AND DISCUSSION

#### Egg Production

There were no mortalities in the any of the 3 treatments throughout the 9-d molt. The ALC hens stopped laying on d 6 of the trial the same time as the FW hens (Figure 1). As expected, the FF hens did not stop laying throughout the trial and produced significantly more \( P \leq 0.05 \) eggs than the FW and ALC hens. There were no significant differences \( P > 0.05 \) between the FW and ALC hens in the amount of eggs laid during the molt period. Biggs et al. (2004) observed that hens that molted on a wheat middlings diet stopped laying by d 6 of the trial, whereas Seo et al. (2001) reported that hens molted with wheat middlings stopped laying on d 7. When Keshavarz and Quimby (2002) induced a molt in laying hens using grape pomace, egg laying stopped by d 4 after the initiation of the molt.

#### Aggressive Behavior

Aggressive behavior was not observed in the FF or the ALC hens throughout the 9-d trial. One incidence of aggression between hens in separate cages was observed among the FW hens on d 8 (data not shown). Webster (2000) and Anderson et al. (2004) observed aggression in food-deprived hens, but the behavior declined by d 2. Aggressive behavior was observed by Duncan and Wood-Gush (1971) and Haskell et al. (2000) when they evaluated frustrated hens. McCowan et al. (2006) also found that hens molted by feed deprivation and hens molted while on a low-calorie diet displayed increased aggressiveness during the fast period.

#### Drinking Behavior and Head Movements

There were no treatment × day effects for drinking behavior \( P > 0.09 \), but the FF hens spent significantly more \( P \leq 0.05 \) time drinking than the other treatments (Table 1). The ALC and FW hens did not differ significantly in drinking behavior for the duration of the 9 d.
The reduced drinking behavior observed specifically in the ALC and FW hens could be due to decreased feed consumption. This is supported by Woodward et al. (2005), who observed that alfalfa-fed hens eat significantly less feed than full-fed hens. They also reported that full-fed hens drank more water than alfalfa-fed hens and feed-deprived hens. Webster (2000) examined the behavior of hens molted by feed deprivation and observed that the drinking behavior of these hens declined after the first few days of feed withdrawal.

No significant treatment × day effects in head movements \((P > 0.40)\) were observed among the 3 treatment groups throughout the 9-d trial. However, over the 9-d molt period, the FW hens exhibited more \((P \leq 0.05)\) head movements than the other treatments (Table 1). Webster (2000) observed increased levels of attentiveness among fasted hens, as indicated by increased head movement, an indication of their alertness during the molt. However, McCowan et al. (2006) reported no significant differences among treatments.

**Walking Activity**

There were significant treatment × day effects for walking \((P \leq 0.05)\). From d 1 through 7, FW hens walked less than ALC hens except on d 8 when FW hens walked more than ALC and FF hens (Figure 2). On d 8, FW hens walked more than ALC and FF hens. Reduced walking might be expected for FW hens, because these hens were not being fed and existed on energy reserves from the liver and adipose tissues (Cherel et al., 1988). During the molting period, most birds, whether in captivity or under natural conditions, decrease all locomotive activities (Murphy, 1996). Significant reductions in locomotor activity are common during the periodic molt of birds that results in energy savings that can almost compensate for energetic cost due to integumental loss and replacement during a natural molt in captive hens (Beckerton and Middleton,
The level of reduction observed in the FW did not occur in the ALC hens.

**Preening Activity**

The preening activities of the hens through the duration of the trial displayed significant treatment × day effects \((P \leq 0.05)\). On d 1, ALC hens preened more than the other treatment groups, but on d 4 and d 6, FW hens preened more than the FF hens (Figure 3). By d 7, both FW and ALC hens were preening more than FF hens, but all were the same on d 8 and 9. In general, it appeared that the FW and ALC hens spent an increased amount of time preening until the last few days of the molt period, whereas the FF hens did less. This may correspond to the timing of loss of feathers during the molt period. Shedding of the feathers was subjectively determined by the first signs of feathers under the cages on d 8 in the FW treatment and d 9 in the ALC treatment. Preening behavior can be stimulated by integumentary irritations, which can be an indication of feather push-out (Webster, 2000), as a displacement action in situations of conflict or frustration (Duncan and Wood-Gush, 1972) or as a comfort behavior (Nicol, 1989). This behavior is generally suppressed when hens are involved in other activities such as feeding (Budier, 1996). Webster (2000) observed the FW hens performed more preening behavior than the FF hens on d 8 to 10, but not at other times.

**Nonnutritive Pecking**

A significant day × treatment effect \((P \leq 0.05)\) was observed for nonnutritive pecking. On d 1, 8, and 9, FW hens did more nonnutritive pecking than the other groups, whereas on d 3, 5, and 6, FW and ALC hens exhibited higher levels of nonnutritive pecking (Figure 4). On d 2, 4, and 7, the FW hens displayed more nonnutritive pecking than the ALC hens, which in turn did more than the FF hens. The increased nonnutritive pecking observed in the ALC hens could have been because of the change of feed, because the behavior subsequently declined in these hens after the third day on the diet. The FW hens, however, continued increasing nonnutritive pecking behavior up to the end of the 9-d trial. When prevented from performing a specific activity such as eating, hens have a tendency to substitute or redirect one activity with another action (Cooper and Albentosa, 2003). Webster (1995) found an apparent arousal in behavior directed to foraging and feeding and believed that nonnutritive pecking was a typical response of chickens to feed deprivation. Savory and Fisher (1992) and Savory et al. (1992) also observed nonnutritive pecking behavior in egg-type pullets and growing broiler breeders when they were deprived of feed. Savory and Fisher (1992) concluded that nonnutritive pecking was a redirection of a consummatory response from a nutritive stimulus to a nonnutritive stimulus. Recently, McCowan et al. (2006) examined the behavior of molting hens on a low-caloric diet and observed that as the feed-deprived hens exhibited increased incidence of cage pecking, the behavior also increased in the nonfast-induced treatment from the rate that was observed premolt. Accordingly, the nonmolted hens did not display increased cage pecking in their study. Appleby and Hughes (1991) reported that even in the

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**Figure 3.** Percentage of observations of preening behavior of laying hens while on an alfalfa crumble diet. Results represent the daily mean percentage of observation spent in the activity from the 3 groups of hens in the experiment. ■ = hens fed an alfalfa crumble diet; ◆ = hens molted by feed withdrawal; ▲ = nonmolted control hens. \(n = 24\) hens per group. Treatments within the same day with different letters are significantly different at \(P \leq 0.05\).
presence of available food, birds spent most of their day foraging.

**Feeder Activity**

From this study, it was not possible to tell if the hens were actually eating the feed or merely pecking at the feeding trough. Because of this, a paradox arose in which the FW hens were also scored for the amount of time they spent feeding. We interpreted this score not as the amount of time they spent checking the feeding trough. A significant day × treatment effect ($P \leq 0.05$) was observed for feeder activity. On d 1, 3, and 9, ALC and FF hens spent more time at the feeder than the FW hens, whereas on d 5, FF hens spent more time than the other treatment groups (Figure 5). On d 2, 4, 6, 7, and 8, the FF hens spent more time at the feeder than the ALC hens, which in turn spent more there than the FW hens. It is apparent that a rapid decline in feeder activity occurs in hens experiencing complete removal of feed. Similar behavior responses were observed by Webster (1995, 2000), who reported that hens molted by feed withdrawal spent progressively less time visiting the feeder as the molt period progressed. It was also observed in this study that as hens spent less time at the feeder, they spent more time involved in nonnutritive pecking activities, which was consistent with reports by Webster (2000), who observed that the reduction in feeder-related activities by FW hens was accompanied by an increase in nonnutritive pecking. Duncan and Wood-Gush (1972) believed that hens needed less than an hour per day to eat sufficient food when a complete ration was provided.

It appeared that some adjustment to alfalfa as a feed source occurred as feeder activity initially declined then increased toward the end of the molt period. However, feeder activity from d 1 and onwards was always greater than FW, indicating that the birds were responding to the presence of feed. The reduced feeding behavior in ALC birds compared with FF birds could be the result of reduced intake due to unpalatability of saponins present in alfalfa (Matsushima, 1972; Sen et al., 1998), slow passage rates, or both. Sibbald (1979) reported that when compared with other feed ingredients, alfalfa exhibited the slowest passage rate in chickens, requiring more than 24 h to be cleared from the gastrointestinal tract of the chickens. He concluded that this gave the birds a feeling of satiety, causing them to reduce their intake. When Donalson et al. (2005) evaluated the utilization of different ratios of alfalfa and layer ration for molt induction, they observed that hens fed a diet containing 100% alfalfa meal ate less than 100 g/hen during a 9-d trial, versus twice as much for hens fed a diet containing 90% alfalfa plus 10% layer ration and 4-fold more for hens fed a diet containing 70% alfalfa plus 30% layer ration.

**Conclusions**

Concerns of reduced welfare have been an issue regarding induced molting by feed deprivation. Behavioral pri-
Figure 5. Percentage of observations of feeder behavior of laying hens while on an alfalfa crumble diet. Results represent the daily mean percentage of observation spent in the activity from the 3 groups of hens in the experiment. ■ = hens fed an alfalfa crumble diet; ◆ = hens molted by feed withdrawal; ▲ = nonmolted control hens. n = 24 hens per group. Treatments within the same day with different letters are significantly different at \( P \leq 0.05 \).

Priorities during an induced molt can be an effective way of assessing the welfare of hens while they are fasting (Webster, 2000). In this study, the behavior responses indicative of welfare were at least intermediate in the hens that were molted using alfalfa when compared with the FF hens and the FW hens. The hens from the commercial stock that were utilized in this study did not exhibit displacement behavior, stereotypy, or aggression that is indicative of frustration, and these hens were given the opportunity to eat even though initially they did not accept the diet. Based on nonnutritive pecking and feeder activity, it appeared that once hens adjusted to the alfalfa diet, their behavior approximated hens fed a nonmolt diet. However, in addition to behavior observations, metabolic and immune indicators need to be monitored to determine whether high-fiber diets can decrease physiological stress in hens while they are undergoing a molt and if long-term effects beyond the molt period occur.

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REFERENCES


