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Evaluation of a calcium pre-molt and low-energy molt program: Effects on laying hen behavior, production, and physiology before, during, and after a fasting or non-fasting molt

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Molting has been traditionally induced in commercial laying hens by a period of feed withdrawal and light restriction. However, this practice has recently raised concern for the well-being of the laying hen and alternative non-fasting methods for inducing molt have been examined. Few studies have compared the behavior of the laying hen during a feed withdrawal molt to a non-feed withdrawal molt. Additionally, a pre-molt calcium treatment may result in a more efficient molt and has not previously been researched. Therefore, an experiment was designed to test a pre-molt calcium treatment and low-energy molting diets for their effects on behavior, performance, and physiology of the laying hen before, during, and after a fasting or non-fasting molt.

Thesis Organization

This thesis consists of four chapters: a general introduction with a literature review, two manuscripts of journal articles, and a general conclusion. The journal article manuscripts address the effects of the pre-molt calcium treatments and molting diets during and after an induced molt on the behavior and on the performance and physiology of the laying hen, respectively. Both manuscripts are formatted in the style required for submission to *Poultry Science*.

CHAPTER 2. LITERATURE REVIEW

In commercial laying hens, molting is induced to cause cessation of egg production and to extend the productive life of the hen. During this time feathers, are replaced, the reproductive tract regresses, and egg production ceases in order to provide the hen with a second laying cycle following molt. Molting is a natural process that is also called pause, rest, forced rest, and recycling (Berry, 2003). In commercial laying hens, it is most commonly referred to as an induced molt because the hens are brought out of egg production simultaneously. Traditionally, molt has been induced by feed withdrawal accompanied by light restriction throughout the molt period and sometimes water removal for up to 3 d (Berry, 2003). In molt programs, feed can be withdrawn for 4 to 14 d (Cunningham and Mauldin, 1996). However, this practice has raised societal concerns about its possible effects on physiological stress, immunology, social behavior, and overall well-being of laying hens.

The use of low-energy feeds as an alternative to feed withdrawal for inducing molt has been examined, as has the use of hormone analogs such as melengestrol acetate, progesterone, nicarbazin, and thyroxin (Biggs et al., 2003, 2004; Donalson et al., 2005; Landers et al. 2005). Industry groups have recommended that producers use only non-fasting molt programs after January 1, 2006 (United Egg Producers, 2008). The American Veterinary Medical Association (2005) policy on induced molting of laying hens states that “acceptable practices include reduction of photoperiod (day length) and dietary restrictions (including diets of low nutrient density) that result in cessation of egg production. Neither water nor food should be withdrawn." However, traditional feed-withdrawal molting programs have shown better molt and post-molt performance with increased egg production when compared to alternative low-energy diets (Biggs et al., 2003, 2004; Donalson et al., 2005). Additionally, the non-fasting molting programs have not proven to reduce stress and improve laying hen well-being compared to feed withdrawal (Biggs et al., 2004). Therefore, it is consumers' *perception* of hen welfare that is benefited and it seems to be at the cost of laying hen egg production.

Most wild bird species, including ducks, geese, and waterfowl, naturally undergo a period of molt. Voluntary anorexia occurs for many reasons including hibernation, egg incubation, territory or harem defense during breeding season, migration, molting, and any other time when eating interferes with an activity (Mrosovsky and Sherry, 1980). Specifically, fasting is important in king penguins who fast for 4 to 6 months (Stevens, 1996). Similar to molted laying hens, jungle fowl hens restrict feed intake during egg incubation and lose 10 to 20% of their body weight over a 20 d period (Mrosovsky and Sherry, 1980). At some point, laying hens would begin molting on their own, but not all hens would molt at the same time and this molt is incomplete which causes long periods of low egg production in the commercial flock and results in decreased profitability (Berry, 2003). For this reason, it is important for producers to induce a molt to allow all laying hens to go through this process simultaneously.

The practice of inducing molt in laying hens can increase profits from a flock by 1/3. (Holt, 2003). Molting allows producers to avoid decreased profits by molting their hens during times of lower egg prices and they can also avoid spent flocks by keeping their hens for a second egg laying cycle. A molted flock can be productive for more than one cycle with egg production and egg shell quality increasing rapidly post-molt resulting in increased profitability for producers. The molting period is defined as the time from the start of feed-withdrawal or low-energy diet until 50% egg production is reached post-molt and normally ranges from 5 to 9 wk (Swanson and Bell, 1974). A successful molt typically results in a 10 to 12% increase in post-molt egg production compared to pre-molt levels (Cunningham and Mauldin, 1996). A national survey conducted in 1999 by the USDA Animal and Plant Health Inspection Service (USDA, 2000) reported that 74% of the farm sites surveyed molted their last completed flocks, whereas 26% of the farm sites did not molt their last completed flock. Inducing molt provides many benefits to a producer and this survey showed that the majority of farms do choose to molt their laying hens.

Webster (2003) described three phases of the molting process. The first phase lasts only a few days and includes physiological and behavioral adjustments to reduce protein catabolism and energy expenditure. A temporary increase in plasma corticosterone may also be observed, as this increase stimulates gluconeogenesis to maintain plasma glucose levels. During the first 24 h of feed withdrawal, hens may also show an increase in aggression and during the first 48 h hens show an elevated level of activity and alertness. A rapid decrease in body mass will occur in phase one, as well as a decrease in peripheral body temperature. Phase two of a natural feed withdrawal molting is the longest, lasting up to several months in some species and more than 20 d in the chicken. In this phase, proteins are spared and lipids are broken down to provide energy. The hen will show an increase in resting behavior as they become less active. The third phase starts when the breakdown of protein accelerates. At this point the hen will eventually stop all physical activity. Webster (2003) explains that these three phases optimize the trade-off between the need to maintain plasma glucose levels to continue physical activity and the need to protect important body structures such as skeletal muscle and organs.

In addition to the changes described above, hens undergoing molt experience many physiological changes related to the cessation of egg production. One important change is the regression of the reproductive tract which results in loss of body weight. The ovary of the laying hen contains multiple small follicles plus about 9 preovulatory follicles arranged by size, with the largest follicle the next ovum (yolk) to be released for egg formation (Proudman, 2000). The growth of these follicles is due to estrogen and the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are produced by the ovary. Ovulation, or the release of this ovum to the oviduct, is the result of a surge of LH. This surge is restricted by a circadian rhythm and will occur only during an 8 h “open period” that falls during the dark cycle (Proudman, 2000). Wilson and Sharp (1973) reported that luteinizing hormone levels began to rise only after the lights went out or no more than 1 h after lights came back on. Therefore, with an egg laid once every 25 h, this surge in LH will occur later each night until there is no more dark period, which will result in no ovulation and no egg laid that particular day. Concentrations of LH peak 4 to 7 hours before ovulation occurs (Cunningham and Furr, 1972; Furr et al., 1973) and ovulation occurs about 30 min after the previous egg has been laid (Mountney and Parkhurst, 1995). Mountney and Parkhurt (1995) described the pathway of the ovum from the ovary to the vent. Once the ovum (yolk) is released from the ovary, it is captured by the infundibulum. This yolk consists of white yolk and yellow yolk and is made primarily of lipids and proteins. The white yolk is from the white follicle in the hen’s ovary, whereas the yellow yolk is deposited by lipoproteins from the bloodstream (Okubo et al., 1997). The yellow yolk is comprised of two parts: dark yellow that is formed during the day and light yellow that is formed at night when the plasma protein concentration is lower (Okubo et al., 1997). The lipids and proteins that make up the yolk are passed to the ovary from the liver via the bloodstream (Bellairs and Osmond, 2005). From here, the ovum moves to the magnum region of the oviduct and over a 3 h period the majority of the albumen, or egg white, is deposited from secreted proteins. Estrogen is involved with the control of albumen deposition (Scanes et al., 2004). Next, the egg passes into the isthmus for 1.25 h and the shell membranes are added. Finally, the egg spends about 21 h in the uterus where more albumen is deposited, as well as the egg shell. The shell is made mainly of calcium carbonate that is secreted in fluids by several glands onto the inner surface of the uterus and the salts in this fluid form the calcified shell (Okubo et al., 1997). This calcium comes from dietary sources or, if unavailable, from bone. If ovulation began at 7:00am, the egg would be in the uterus from about 11:30am to 8:30am the next morning. During this time, the egg shell is deposited; however, the majority of the shell is added during the dark period, which results in an increased demand for calcium during the night. The finished egg moves down the vagina of the hen to the cloaca and out the vent. During molt, a decreased supply of calcium in the diet will affect the formation of the egg shell and decreased levels of estrogen contribute to the cessation of egg production.

The gonadotrophic hormones FSH and LH are secreted in response to gonadotropin-releasing hormone from the anterior pituitary, which is regulated by a negative feedback loop by the steroids progesterone and estrogen. It is the disruption of this hypothalamic-hypophyseal gonadal axis that results in the interruption of egg production during molt (Koch et al., 2005a). Iwasawa et al. (2002) reported that sex steroid levels decreased quickly on the first day of a feed withdrawal molt and remained low throughout the molt period. In addition, these authors found that plasma levels of luteinizing hormone and progesterone were lower during the molt period compared to non-molted control hens. These changes affect egg production and result in the cessation of egg lay during molt. When comparing traditional feed-withdrawal to alternative non-fasting diets, the level of regression in ovary weight is comparable (Soe et al., 2007).

Bone density and mineral content can also be altered during molt. Mazzuco and Hester (2005a) measured bone density and mineral content of hens on a feed withdrawal molt and hens on a low-energy wheat middlings diet. Compared to pre-molt levels, the hens experienced a decrease of 35% and 18% in their bone mineral density, respectively. Their bone mineral content decreased by 39% and 27%, respectively. The non-molted control hens in this experiment maintained values similar to those of pre-molt levels. By d 126 post-molt, the bone mineral density and content levels were back to pre-molt values. Mazzuco and Hester (2005b) reported another study regarding skeletal integrity of hens on feed withdrawal for 10 d. The bone mineral density and content were measured in left-side tibia and humerus bones. Mazzuco and Hester (2005b) concluded the effect of molt was detrimental because the bone mineral density of the humerus never fully recovered after molt and the tibial bone mineral density recovered late in the second cycle. Mazzuco and Hester (2005b) also noted an increase in bone breakage following molt.

Egg production ceases during molt and can increase dramatically following molt. Biggs et al. (2003) reported results with 4 different molting diets: 4 and 10 d feed-withdrawal, a diet consisting of 98% wheat middlings and a diet consisting of 98% corn grain. Both lengths of feed-withdrawal resulted in cessation of egg production by d 5, wheat middlings by d 8, and corn reached a low of 1.2% by d 27 of molt. Post-molt, the 4-d-feed-withdrawal hens returned to production by d 12, wheat middlings hens by d 15, and 10-d-feed-withdrawal hens by d 23. The post-molt egg production was significantly higher for 10 d feed withdrawal and wheat middlings diets compared to the 4 d feed withdrawal and corn diets. Biggs et al. (2003) suggested the difference in production may be from effects of novelty or palatability causing the hens to consume less, which lowered egg production. The results show that the traditional feed withdrawal method is more effective at inducing molt than the low-energy wheat middlings diet and the corn diets.

Biggs et al. (2003) reported the effects of molt on egg weight. The hens on a 10 d feed withdrawal had significantly lighter eggs post-molt compared to the hens treated with a 4 d feed withdrawal or low-energy corn diet. The egg mass was significantly lower for the hens fed the corn diet and the 4 d feed withdrawal compared to both the 10 d feed withdrawal and the hens fed the low energy wheat middlings diet, due to lower egg production levels.

Egg quality is also affected by molt with post-molt egg quality improving compared to pre-molt levels. Specific gravity is a measure of egg shell quality and it is typically done by a floatation method with NaCl solutions varying in specific gravity (Damron, 1998). Biggs et al. (2003) found that hens molted on a corn diet had a lower specific gravity, and therefore a lower egg shell quality, in 6–8 wk and 13 wk post-molt, but there were no differences when measured in 41–44 wk post-molt. Biggs et al. (2003) determined that long-term post-molt specific gravity was not affected by the length of feed withdrawal (4 or 10 d) or by low-energy treatments (95% corn or 95% wheat middlings). The secretion of albumin, a protein found in egg whites, improves, resulting in a thicker egg white, and calcium secretion is more effective, resulting in a thicker and smoother egg shell (Bell, 2000).

Some farm managers molt their hens until they reach a desired body weight instead of using a set number of days and the issue of body weight loss is a key reason for public concern of hen welfare. Soe et al. (2007) reported a 32% body weight loss in fasted hens and a 21% body weight loss in non-fasted hens. In this study, feed intake of non-fasted hens fed low-energy diets consisting of corn, wheat bran, or corn gluten was still 40% less than control hens. Bar et al. (2003) reported a body weight loss of 22–25% in fasted hens. Biggs et al. (2003) reported a body weight loss of 11% for hens on a 4 d fast, 20% for hens on a 10 d fast, 15% for hens fed a corn diet, and 8% for hens fed a wheat middlings diet. Molt can result in extreme body weight loss determined by diet or length of feed withdrawal. However, at least 25% of this loss is from regression of the reproductive tract (Brake and Thaxton, 1979). This body weight loss is necessary for the “rest and rejuvenation” of body tissues and should not be viewed as a negative welfare issue (Ruszler, 1998).

Stress in molted laying hens is another reason for the public’s concern about the hens’ well-being. An increase in the ratio of heterophils to lymphocytes in blood samples can be a sign of a stressed hen (Soe et al., 2007). During stress, circulating heterophils increase and therefore result in a higher heterophil to lymphocyte ratio (Gross and Siegel, 1983; McFarlane and Curtis, 1989). Davis et al. (2000) showed that this ratio was significantly higher during a forced molt than during other times of the year. In addition, Soe et al. (2007), found that the heterophil to lymphocyte ratio was higher in a fasted group of hens compared to a non-fasted group. When comparing non-feed withdrawal molt treatments, Biggs et al. (2004) found no differences in the heterophil to lymphocyte ratio with a corn, wheat middlings, or distiller’s dried grain with solubles diet. The lack of difference in the ratios suggests that there may be little to no effect of treatments on the stress of a molted laying hen.

Molting may predispose the laying hen to multiple illnesses or harmful effects. These include kidney and adrenal damage, osteoporosis, paralysis, intestinal damage leading to *Salmonella*, and mortality. These issues can also affect wild birds that molt naturally. Osteoporosis has been reported for 16 species of wild birds and the effects have been observed in captive birds from the Paride family (Meister, 1951). It was reported that these birds did not perch at night, as usual, but sat on the cage floor due to their weakened legs. Whitehead (2004) explains that during molt there is a progressive decline in the amount of mineralized structural bone which leads to skeletal fragility and the possibility of fracture. The cause of osteoporosis is the need for calcium from bone. Bone is a storage place for calcium and egg shells have about 2 g of calcium in them, which is 10% of total body calcium (Loveridge, 1992). When the molted hens are not receiving calcium from their diets, they try to mobilize it from storage in the bone. This can also lead to paralysis in the laying hen. Weak bones, caused by the depletion of calcium, may break and cause the hen to become paralyzed.

Intestinal damage resulting in *Salmonella* is a major concern for commercial farms. Fasted hens have an increased susceptibility to colonization by *Salmonella* and this causes a concern for food safety (Berry, 2003). A decrease in the volatile fatty acids of the intestines of fasted laying hens is directly related to their increased *Salmonella* susceptibility, whereas non-fasted hens do not have a reduction in volatile fatty acids and reduced pH and therefore do not experience increased susceptibility to *Salmonella* (Cardona, 2001). Holt (1993) found that 50% of the fasted hens were shedding *Salmonella* 7 d after an oral dose of 3 to 10 *Salmonella* organisms, but the non-molted group of hens needed a much larger oral dose of 2×10^4 *Salmonella* organisms to have the same effect. This result suggests that hens molted by fasting are much more susceptible to intestinal damage that can result in *Salmonella* than non-molted hens. *Salmonella* can also be spread through the air, which is another concern for commercial farms. If *Salmonella* is present during molt in a small number of hens, it can be easily spread to nearby hens. Fasted hens were infected by airborne *Salmonella* from infected hens that were only 1 m away (Holt et al., 1998).

Hen mortality may be a concern for producers during an induced molt. Molting can weaken the immune system of the hen and may cause already weak hens to die. Biggs et al. (2003) found no difference in mortality across treatments of low-energy corn, low-energy wheat middlings, a 4 d fast, and a 10 d fast with mortality rates of 4.8, 1.2, 2.4, and 2.4%, respectively. Post-molt, the corn, wheat middlings, and 4-d-fast groups had a mortality rate of 4.8% and the 10 d fast group had a mortality rate of 3.6%. Keshavarz and Quimby (2002) reported low mortality (0 to 3%) during molt for feed withdrawal and low-energy diets, with the exception for high mortality (8%) in hens on a 1 d fast followed by a corn diet. There were no post-molt mortality differences. These studies suggest that mortality rates do not differ between a fasting molt and a non-fasting molt.

The need for an alternative to feed-withdrawal molt is due to consumer concern about the well-being and behavior of the molted laying hens. The question that needs to be addressed is whether or not the well-being of laying hens is actually negatively impacted by a molt program. Gakel calling, a specific vocalization in laying hens resulting from frustration, has been used to assess measures of hen welfare (Zimmerman and Koene, 1998). This gakel call has been found to be higher during molt in fasted hens compared to non-fasted hens (McCowan et al., 2006). This result suggests that a low-energy diet used to induce a molt may be more beneficial to the hen in terms of welfare. Regarding hen well-being, the following are criteria from Bell (2000) for a flock-friendly molting: no removal of feed, no “extreme” loss in body weight, no increased mortality, no injections or toxic substances, post-molt performance results comparable to pre-molt, and a method that is cost effective. There are a number of factors to consider when determining hen well-being, including aggression and hunger.

Studies that have examined the influence of molting on aggression in laying hens disagree on their results. Some studies show that increased aggression does not seem to last as hens quickly adapt to their environment. Webster (2003) found that aggressive behavior increased briefly during the first day of feed withdrawal only. Anderson (2004) found that feather pecking increased during a 2 wk feed withdrawal, but the frequency of aggression and submissive acts were significantly lower during the 2 wk molt. On the other hand, McCowan et al. (2006) reported that cage pecking increased in fasted hens and aggression increased in both fasted and non-fasted hens during molt. There needs to be more research to answer the questions that a molt causes increased aggression among hens and that there is a difference in aggression levels between fasted and non-fasted hens.

Hunger, or motivation to eat, has been measured as a way to evaluate the well-being of laying hens during molt. Hens subjected to feed withdrawal for 43 h were found to be faster than controls in reaching a preset force threshold needed to push open a door to access feed (Petherick and Rutter, 1990). Petherick et al. (1992) also found that trained hens on feed withdrawal will run an alleyway faster than control hens to get a feed reward. A study with broilers showed similar results when feed restriction caused them to work harder for feed and to eat faster compared to control hens (Savory et al., 1993).

There are multiple ways to induce a molt and, traditionally, molting has been induced by withdrawing feed and altering the photoperiod. Soe et al. (2007) used a 2-wk feed withdrawal molt followed by 1 wk of a commercial laying hen diet on alternating days and then allowed for ad libitum consumption. The feed withdrawal treatment caused a cessation of egg production within 8 d, whereas the non-feed withdrawal treatment decreased to 3.8% egg production by d 10. Bar et al. (2003) suggested 6 d of feed withdrawal may be enough to induce molt. Removal of feed also appeared to be a more efficient method compared to others (Bell, 2000). Removal of water can cause a more complete loss of feathers (Bell et al., 1979), but the added strain of water removal may be harmful to the hens. A 10 d feed withdrawal treatment with 2 d of water removal increased the number of soft-shelled eggs from 4% to 9% compared to treatments without water removal (Bell et al., 1976). Adjusting the photoperiod can also influence the efficiency of molt. A pre-molt lighting program can be used to replace the limited water intake for inducing molt (Ruszler, 1998). Ruszler (1996) used a short 4 d feed withdrawal in combination with light reduction to cease egg production by d 5 of molt. Brake and Carey (1983) recommend using a 24 h light program for 1 wk prior to a feed withdrawal molt. However, when this method was used with a diet supplemented with excess methionine there were no differences in egg production compared to a 17 h photoperiod (Ahmad et al., 1997).

Another traditional, but less common, method is altering the mineral or amino acid content of the diet to induce a molt. This alteration can include diets with low sodium (Na), low calcium (Ca), high zinc (Zn), high iodine (I), or the removal of the amino acid methionine. Berry (2003) stated that these alternative methods (with the exception of a high Zn diet) have not been consistent in the cessation of egg production. Altering minerals to induce a molt can also be costly, resulting in another problem with this method (Biggs et al., 2003). One option is lowering the amount of Na in the diet. A low-Na diet caused egg production to cease within 3 wk (Nesbeth et al., 1976). While cessation of egg production did occur, 3 wk is longer than other methods such as feed withdrawal, but not longer than low-energy diets. Nesbeth et al. (1976) also reported that a low-Na diet fed for 42 d caused an increase in egg weight and egg specific gravity, but had a 59% decrease in feed intake and a 19% loss in body weight during molt. A diet with 0% added Na did not affect feed intake compared to feed withdrawal, but cessation of egg production and body weight loss were not as complete as the 8 or 10 d feed withdrawal (Scheideler et al., 2003). Additionally, Bell et al. (1976) found that hens fed a low-Na diet during molt did not drop below 27% egg production. Results show that a low-Na diet used by itself to induce molt may not be the most efficient or cost-effective method to use.

Another way to induce molt is to lower the Ca levels in the diet. Calcium is used for egg shell formation in the laying hen, but it is also important for the release of gonadotrophic hormones which results in ovulation (Luck and Scanes, 1980). However, Douglas et al. (1972) reported that while this method appeared to induce a molt, it was not effective in improving specific gravity, caused some paralysis, and increased mortality up to 20% during molt. Berry and Brake (1987) also reported that a low-Ca diet did not result in differences in egg production or feed consumption compared to FW, high Zn, and control diets.

Increasing the amount of Zn in a diet can induce laying hens to molt by resulting in a decrease in feed intake which causes follicular atresia and a cessation of egg production (Shippee et al., 1979). A diet with 7.5 g/kg Zn (150 times the 1944 NRC recommended requirement) caused cessation of egg production within 1 wk and decreased feed intake by 50% (Shippee et al., 1979). This high-Zn diet did not result in any improvements in specific gravity, internal egg quality, or egg production compared to a feed withdrawal treatment. These results suggest that while the high-Zn diet may be effective at inducing molt, it is not any more effective than a feed withdrawal diet. Bar et al. (2003) found similar results when using a high Zn diet (20 g/kg Zn) that resulted in post-molt performance similar to that of a feed withdrawal treatment.

Adding high amounts of I to a diet has also been tested to induce a molt. Excess I in the laying hen can cause an enlarged thyroid, know as a goiter, and results in decreased egg production (Shemilt, 1982). However, it results in a second cycle that has lower egg production compared to non-molted controls (Wilson et al., 1967). When fed in the form of desiccated thyroid or sodium iodide, feed intake and egg production decreased (Asmundson et al., 1936). Asmundson et al. (1936) also tested diets high in I when fed as oyster shells, potassium iodide, iodo-salicyclic acid, di-iodotyrosine, or iodized olive oil and found they were not effective at decreasing egg production.

Finally, the removal of methionine to a laying hen diet has been used to induce molt. This method has been examined because of the relationship between protein (methionine) and feather growth and it may be possible that by altering the methionine level during molt, feather replacement is affected (Ahmad et al., 1997). According to the 2007–2008 Hy-Line W-36 Commercial Management Guide, the recommended level of methionine is around 0.4% of the diet for laying hens. An experiment that used only 0.05% added methionine to a laying hen diet had significantly smaller eggs than diets with 0.185% and 0.212% added methionine (Ahmad et al., 1997). This study reported that 0.05% added methionine to a laying hen diet was not efficient at inducing molt. Ahmad et al. (1997) also looked at third and fourth egg laying cycles following a molt and found that added methionine in the molting diet caused an increase in egg weight for the third cycle, but it resulted in little to no economic value (Ahmad et al., 1997), meaning this method for inducing molt did not result in long-term benefits.

A more recent method for inducing molt is the use of low-energy or low-nutrient feeds. These can include corn, wheat middlings, soybean hulls, cottonseed, corn distiller’s dried grains with solubles (DDGS), or alfalfa meal. Bell (2000) explained that in countries with regulations against feed withdrawal, low nutrient or low mineral diets are the only ones available. A diet consisting of 95% corn (3,172 kcal/kg ME) reached a low of 1.2% egg production by d 27 and a diet consisting of 95% wheat middlings (1,900 kcal/kg ME) ceased egg production by d 8 after molt (Biggs et al., 2003). Koelkebeck et al. (2006) found similar results when using a diet with 95% wheat middlings (1,900 kcal/kg ME) that caused cessation of egg production by d 8 and the laying hens stayed out of production until d 15. Additionally, laying hens fed this wheat middlings diet reached 50% egg production the soonest post-molt compared to a corn diet (3,172 kcal/kg ME) and the wheat middlings molt diet had the most similar results to the 10 d feed withdrawal treatment with no differences in egg production or post-molt mortality. Koelkebeck et al. (2006) concludes that molt diets with either wheat middlings or soybean hulls could be acceptable alternatives to a feed withdrawal molt. A diet consisting of 50% cottonseed meal (3,070 kcal/kg ME) caused a decrease in feed intake and a cessation of egg production after 5 d (Davis et al., 2002). When using corn distillers dried grains with solubles (2,343 kcal/kg ME), egg production never went below 18% (Biggs et al., 2004).

Another recent method for inducing molt is the use of hormone analogs and neuropeptides. These include melengestrol acetate, progesterone, and nicarbazin. Melengestrol acetate is an orally active progestin that down-regulates the hypothalamic–pituitary–ovarian axis, which leads to a reversible regression of the oviduct and the large yellow follicles, causing a temporary decrease in egg production (Koch et al., 2005a). At 4 and 8 mg/hen added to a diet, melengestrol acetate caused a reduction in egg production, but not at 0, 0.1, or 1 mg/hen (Koch et al., 2005a). The 4 and 8 mg/hen of melengestrol acetate also resulted in reduced reproductive tract weights, improved egg shell breaking strength, and increased shell thickness (Koch et al., 2005ab). Melengestrol acetate resulted in the greatest internal egg quality (measured as Haugh units) when 8 mg/hen were fed for 2, 4, or 6 wk compared to the 4 mg/hen post-molt (Koch et al., 2005b). When using hormone analogs, there is public concern about residues being left in eggs for human consumption. However, the melengestrol acetate found in the egg yolk from hens fed 4 or 8 mg/hen of melengestrol acetate was well below (3 orders of magnitude) the FDA's tolerance level of 25 ppb (Koch et al., 2005b). Melengestrol acetate is not currently approved by the FDA for use in laying hens to induce molt.

Progesterone is a hormone that is injected into laying hens to induce molt. Cessation of egg production occurred within 2 d with an injection of 40 mg/bird of progesterone (Adams, 1955). A 20 mg/bird injection caused cessation of egg production within 14 d (Shaffner, 1954). Injections of 0.5 or 1 mg/bird every day for 7 d also eliminated egg production (Gabuten and Shaffner, 1954). This method would be difficult to use on large commercial farms due to the amount of time it would take and the number of employees needed to inject every individual hen. Additionally, injecting hens with hormones may cause consumer concern.

When added to the diet, nicarbazin can induce molt. This hormone analog has been found to prevent follicle maturation, cease egg production, and cause partial regression of the reproductive tract (Weiss et al., 1960; Bar et al., 1978). Bar et al. (2003) reported that nicarbazin fed at 0.06% of the diet resulted in a moderate decrease in feed intake, minimal body weight loss, partial regression of the reproductive tract, and reduced mortality. However, these authors reported that egg production and egg shell quality were lower than alternative treatments and Bar et al. (2003) concluded that the use of nicarbazin is unlikely to be an adopted practice for inducing molt because it does not appear to be as effective as other methods.

Molting is an important process for commercial egg production and societal concerns for laying hen well-being has resulted in the need for an alternative to feed withdrawal to induce a molt. While there appear to be many alternatives available, one is needed that results in an effective and efficient molt without adversely affecting laying hen behavior. A recent method involves the use of low-energy feeds and these diets appear to result in a molt closest to that of the traditional feed withdrawal molt.

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CHAPTER 3. EFFECTS OF A PRE-MOLT CALCIUM AND LOW-ENERGY MOLT PROGRAM ON LAYING HEN BEHAVIOR BEFORE, DURING, AND POST-MOLT[1]

A paper to be submitted to *Poultry Science*

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Abstract

The objectives of this study were to compare the behaviors, postures, and heterophil to lymphocyte ratios (**H:L**) for laying hens when offered a Ca pre-molt treatment and low energy molt diets versus feed-withdrawal (**FW**) during and post-molt. A total of 144 Hy-Line W-36 hens (85 wk of age), housed 3/cage, (413 cm^2/hen) were used. Six treatments were compared in a 2 (fine versus coarse Ca pre-molt treatment) by 3 (FW, soybean hulls [**SH**], or wheat middlings [**WM**] molt

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diets) factorial design. 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 baseline. The Ca pre-molt treatment had no carryover effects during or post-molt for behaviors or postures. During molt, FW hens were more active and ate 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. 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 laying hen behavior compared to FW and did not increase H:L and could therefore be useful alternatives for inducing molt.

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

Introduction

In the egg laying industry, hens are exposed to an induced molt to extend the productive life of the hen 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 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). Industry groups have recommended that producers implement only non-fasting molt programs after January 1, 2006, 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) will no longer purchase eggs that have come from a commercial feed withdrawal program (Anonymous, 2000).

A number of studies have compared the effectiveness of feeding low-energy feeds, such as wheat middlings or soybean hulls, as an alternative to feed withdrawal for inducing molt (Biggs et al., 2003, 2004; Koelkebeck et al., 2006). Although feed withdrawal resulted in a more complete molt and better post-molt performance, Biggs et al. (2003, 2004) and Koelkebeck et al. (2006) concluded that the low-energy feeds were acceptable alternatives for inducing a molt with regard to laying hen performance. However, the non-fasting molt programs have not conclusively shown what effects they may have on laying hen behavior and have not concluded that stress is reduced for the laying hen. One study conducted by Biggs et al. (2004) found no differences in social behaviors between hens subjected to a 10 d fast and hens that were fed a wheat middlings molt diet. Alternatively, McCowan et al. (2006) reported increased aggression in both fasted and non-fasted hens during molt. An additional consideration is that feeding a calcium-deficient diet can inhibit ovulation and induce molt (Douglas et al., 1972; Hurwitz et al., 1975). It may be possible that a calcium pre-molt treatment will not allow the hen to mobilize sufficient calcium 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), but what effect this calcium pre-molt supplement may have on laying behavior and stress is unknown. Therefore, the objectives of this study were to compare the behavior and heterophil to lymphocyte (**H:L**) ratios of the laying hen kept in a cage system when offered (a) a calcium pre-molt treatment and (b) low-energy diets versus a traditional feed-withdrawal 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 144 Hy-Line W-36 laying hens (85 wk of age), weighing 1.7 ± 0.2 kg, were used. Laying hens were beak trimmed at a few days of age according to recommendations by the Hy-Line W-36 commercial management guide. Hens were housed 3 per cage (30.5 cm wide × 40.6 cm deep × 44.5 cm high), providing

413 cm^2/hen. Hens were able to see neighboring feed troughs, but were unable to reach them due to vertical plastic barriers between troughs. Hens were obtained from a single source and were considered to have a high health status. 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. All cages were located in 2 identical, light-controlled fan-ventilated rooms.

Treatments and Experimental Design

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 (fine versus coarse calcium treatment) by 3 (feed withdrawal [**FW**], soybean hulls [**SH**], or wheat middlings [**WM**] molt diets) factorial design.

Baseline Period. Hens were 85 to 87 wk of age during the 2-wk baseline period which was defined as the period before any experimental diets were applied and during which the hens had a 16-h photoperiod. A laying hen diet formulated to meet or exceed the recommendations for the Hy-Line W-36 commercial management guide (Table 1) and water was provided *ad libitum*.

Calcium Pre-Molt Treatment. Following the baseline period, the hens (at 87 to 88 wk of age) received either a combination of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or an all-fine $CaCO_3$ mixed into a commercial diet for 1 wk (Table 1). Both diets contained 4.61% Ca, so only the particle size of the Ca supplement differed between the 2 treatments. Water was provided, *ad libitum*, and the hens had a 24-h photoperiod (Carey and Brake, 1983).

During Molt. The 3 molt diets (FW, SH, or WM) were applied for a total of 28 d (hens at 88 to 92 wk of age). The hens fed the FW molt diet were deprived of feed for 7 d with free access to water followed by 21 d of skip-a-day feeding restricted to 60 g of feed (Table 1) per feeding day per hen. This diet allowed these hens to engage in feeding related behaviors during molt. The hens fed the WM and SH molt diets were provided feed and water for *ad libitum* consumption during the entire 28 d molt period with low-energy diets consisting of WM or SH (Table 1). Vitamins and minerals were added to the WM and SH molt diets to make acceptable diets for non-producing hens according to recommendations from the Hy-Line W-36 commercial management guide with the exception of energy content, and corn grain was added to the diets to improve flowability (Koelkebeck et al., 2006). Lighting was reduced to 8 h for the first 3 wk and was then increased to 12 h at the start of the last week of molt.

Post-Molt. Following the 4 wk of molt diets, the hens were given a commercial-type laying hen diet for egg-producing hens for 22 wk (hens at 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 (Table 1) from the Hy-Line W-36 commercial management guide. Hens were given free access to water and the lighting was gradually increased by 1 h each week until reaching a 16 h photoperiod.

Behavioral Equipment and Acquisition

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 before, during, and post-molt onto a DVR (RECO-204, Darim Vision®, Pleasanton, CA) at a rate of 30 frames/s. Behaviors and postures were recorded on d 7 (baseline), on d 23 and 49 (during molt), and on d 51 and 66 (post-molt) for 2 hours after lights came on in the morning and for 2 hours before lights went out at night (dependent on the photoperiod; Savory, 1980). This resulted in a total of 960 hours of behavioral recordings. The acquisition of laying hen behaviors and postures were collected by 2 experienced observers who viewed the DVD using a 24 h mode (1 frame/s) and recorded observational data using the Observer software (The Observer, Ver. 5.0.25 Noldus® Information Technology, Wageningen, The Netherlands) using a 1 min scan sampling technique (Biggs et al., 2004). Observers were trained by watching a practice video of the laying hens to familiarize themselves with the selected behaviors and postures. Their scoring was compared to each other to ensure similar results.

Behaviors and Postures

Two postures (sitting and active) and 5 behaviors (feeding, drinking, non-nutritive pecking, preening, and aggression), adapted from Webster (2000), were collected. Sitting was defined as a crouched posture with shanks or breast in contact with the cage floor. Active behaviors 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 ingestion of 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.

Physiological Stress

Blood was collected from a total of 189 laying hens at the end of the baseline period (9 hens; 86 wk of age), at the end of the Ca pre-molt treatment (9 hens from each of the 2 treatments; 87 wk of age), during the middle and end of the molt period (9 hens from each of the 6 treatments; 89 and 91 wk of age), and at the end of the post-molt period (9 hens from each of the 6 treatments; 113 wk of age). Blood was collected from the brachial vein into heparinized 15-mL centrifuge tubes. The tubes were stored on ice until analysis. Blood smears were made on a glass slide and were stained with a staining kit (Camco Quik Stain II, Cambridge Diagnostics

Products, Inc., Fort Lauderdale, FL). A total of 50 heterophils and lymphocytes were counted by a trained student and the number of heterophils were divided by the number of lymphocytes for the H:L ratio.Statistical Analysis

The experimental design was a randomized complete block design with treatments in a 2 × 3 factorial arrangement with 2 calcium pre-molt treatments and 3 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 ratios 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 and were subjected to a square root arcsine transformation process to achieve a normal distribution. Data were analyzed using the PROC MIXED procedure of SAS (SAS® Inst. Inc., Cary, NC) software for parametric data on a cage basis. The H:L ratios were analyzed with ANOVA using JMP with means of the molt diets assessed by Fisher's least significant difference. Experimental values of the H:L ratios were compared to baseline values using Dunnett's t-test. A P-value < 0.05 was considered significant in all comparisons.

Baseline Period. Location of cage within a room (2 rooms) was included in the initial model, but was not significant and was removed before final analysis. The statistical model included treatment and room, and the subplot included all 2-way interactions.

Calcium Pre-Molt Treatments, During Molt and Post-Molt Periods. Calcium treatment and the Ca treatment by molt treatment interaction were included in the initial model, but these parameters were not significant and were removed before analysis. The statistical model included molt treatment, period (during or post-molt), room (1 or 2), and all 2- and 3-way interactions, but no 3-way interactions were significant and were subsequently removed before final analysis. Cage was included as a random effect.

Results

Baseline Period

The percentage of time laying hens spent sitting, active, feeding, drinking, non-nutritive pecking, preening, and engaging in aggressive interactions were not different among treatments during the baseline period (Table 2). The mean H:L ratio during the baseline period is shown in Table 3.

Treatments During vs. Post-molt

The Ca pre-molt treatment had no carryover effect on the behaviors or postures of the laying hens during or post-molt. Drinking and aggression during and post-molt were not different among hens. Post-molt, laying hens engaged in more ($P < 0.001$) sitting and feeding related behaviors than during the molt period. Conversely, hens post-molt spent less time ($P < 0.05$) engaged in active postures, non-nutritive pecking, and preening behaviors (Table 4). During molt, hens assigned to the FW molt diet were more active ($P < 0.001$; Figure 1), but fed and drank less often ($P < 0.05$; Figures 2 and 3) than hens fed the SH and WM molt diets. Post-molt, the activity, feeding, and drinking levels of the hens were not different among molt diets. During molt, hens fed the SH molt diet preened less ($P < 0.003$) than hens fed the FW and WM molt diets. Post-molt, hens fed the WM molt diet preened more ($P < 0.003$) than hens fed the FW and SH molt diets (Figure 4). There were no differences among the 3 molt diets for H:L ratios during or post-molt and the values did not differ from baseline values, respectively. The Ca pre-molt treatments had no effect on H:L ratios during molt and they did not differ from baseline values. However, the fine-Ca pre-molt treatment resulted in lower H:L ratios compared to the coarse-Ca pre-molt treatment during the post-molt period, but neither treatment differed from baseline values (Table 3).

Discussion

Baseline Period

During the baseline period, hens assigned to the different Ca pre-molt treatments and molt diets did not show any differences in their time budgets. The time engaged by these hens in each respective posture and behavior is in agreement with previous research conducted on the laying hen (Anderson et al., 2004; Webster, 2000). Therefore, any differences reported in the subsequent molt and post-molt periods could be attributed to the molt diets. The mean H:L ratio during the baseline period was also in agreement with previously published literature for laying hens at this stage of their reproductive life (Biggs et al., 2004).

Calcium Pre-Molt Treatments

The Ca pre-molt treatment has not been previously researched for its possible effects on laying hen behavior or stress. The fine-$CaCO_3$ may seem deficient in Ca because of its smaller particle size which could result in less Ca availability to the individual hen at night when it is required for egg shell formation (Whitehead, 2004). However, the Ca treatment did not have a carryover effect on the laying hens behaviors and postures during or post-molt. However, this treatment may be beneficial when considering egg production in the next laying cycle and overall laying hen performance (Chapter 4).

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 on laying hen behavior during a traditional FW molt is conflicting. Webster (2000) stated that aggressive pecking within a cage was reported in 14% of all hens that were observed 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 molt. Anderson et al. (2004) reported that feather pecking increased during a 2 wk FW, but the frequency of aggression and submissive acts were lower during the same time period. Webster (2000) and Biggs et al. (2004) 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 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), the 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 days of observations during molt were averaged after being analyzed separately and producing the same results, which means the hens also did not show increased aggression on the first day of molt. Low levels of aggression can be genetically selected for by breeding a group of hens from the same cage with high productivity. This selection results in social hens that will be less aggressive and still highly productive (Muir, 1995; Weary and Fraser, 2004). Therefore, the differences seen in aggressive behaviors among studies may be due to the type of bird. 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, as in the present study.

Laying hens in non-cage systems spend 5 to 25% of their time engaged in foraging behaviors (Appleby et al., 1989), therefore caged hens that are unable to forage spend a great deal of 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 were performed less frequently in FW hens during molt compared to the SH and WM hens. Thiele and Pottguter (2008) 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. However, time spent engaged in feeding and drinking behaviors post-molt were not different among the treatments. The hens assigned to the FW molt diet spent more time preening during molt compared to hens fed the SH molt diet and compared to post-molt, whereas hens fed the other 2 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 themselves 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 feed deprived 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) attributed an increase in locomotion during a FW molt to hunger and the search for food. Furthermore, the hens returned to normal activity levels post-molt, once they were being fed a diet that met or exceeded their nutrient requirements.

The H:L ratios were not affected by the molt diets during or post-molt and they did not differ from baseline values which suggests the hens were not under additional stress and the molt diets were comparable in their effects. During the post-molt period, the fine-Ca pre-molt treatment resulted in lower H:L ratios compared to the coarse-Ca pre-molt treatment. This result would suggest the hens fed the fine-Ca pre-molt treatment were under less stress than the 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 an effect. Additionally, the values did not differ from baseline values.

During vs. Post-molt

Water is the most essential nutrient for life and an inadequate supply can result in devastating consequences such as overheating, dehydration, and, in the extreme case, death (Appleby et al., 2004; Thacker, 2000; Thiele et al., 2008). As expected, there were no differences during and post-molt for time engaged in drinking in the present study. Furthermore, hens during and post-molt did not engage in increased aggression. Although aggression can increase due to a disturbance such as withdrawing or changing 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, time spent engaged in aggressive behaviors may change.

Preening, activity, and non-nutritive pecking increased during the molt compared to post-molt. The increased preening may be due to sensitivity from the loss of feathers (Webster, 2000) or displacement behavior 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 with 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).

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. The hens engaged in more activity, non-nutritive pecking, and preening during molt, however, time spent in these behaviors decreased during the post-molt period suggesting temporary effects. Comparing the FW treatment to the low-energy diets, hens fed the WM or SH molt diets were able to be less active during molt. Additionally, the treatments had no effect on the H:L ratios of the laying hens suggesting minimal effects on stress. Therefore, with regard to laying hen behavior and 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

	Measure				
Period	ME_n, kcal/kg	CP, %	Ca, %	Non-phytate P, %	Na, %
Baseline	2,776	16.07	4.61	0.40	0.18
Calcium pre-molt[2]					
Fine Ca	2,776	16.07	4.61	0.40	0.18
Coarse Ca	2,776	16.07	4.61	0.40	0.18
During molt[3]					
FW	2,817	15.27	2.00	0.25	0.11
WM	2,198	13.09	2.00	0.25	0.14
SH	2,216	8.88	2.00	0.25	0.10
Post-molt					
First 2 wk	2,910	16.50	3.85	0.50	0.17
Last 20 wk	2,880	16.05	4.10	0.44	0.19

[1]Calculated values. Diets contained corn, soybean meal, vitamins, traceminerals, dicalcium phosphate, and calcium carbonate.
[2]Fine Ca was a 100% fine Ca supplement and coarse Ca was a 50% fine: 50% coarse Ca supplement added to the diet for a 1 wk pre-molt treatment.
[3]Three molting treatments were compared: 7 d feed withdrawal (FW), wheat middlings (WM) and soybean hulls (SH).

Table 2. Postures and behaviors of the laying hen during the baseline period (hens at 85 to 87 wk of age)[1]

	Treatment						
	Fine Calcium			Coarse Calcium			
Measure	**FW**[2]	**WM**	**SH**	**FW**	**WM**	**SH**	***P*-value**[3]
Postures, %							
Sitting	4.5 ± 0.02	4.1 ± 0.02	7.7 ± 0.02	5.7 ± 0.02	4.8 ± 0.02	5.8 ± 0.02	0.97
Active[4]	57.2 ± 0.02	55.2 ± 0.02	55.1 ± 0.02	59.2 ± 0.02	57.1 ± 0.02	59.6 ± 0.02	0.34
Behaviors, %							
Feeding[5]	23.0 ± 0.02	24.4 ± 0.02	22.8 ± 0.02	19.8 ± 0.02	22.8 ± 0.02	19.8 ± 0.02	0.21
Drinking	3.5 ± 0.01	3.1 ± 0.01	4.2 ± 0.01	3.6 ± 0.01	4.3 ± 0.01	3.8 ± 0.01	0.36
Non-nutritive pecking	0.3 ± 0.01	0.4 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.79
Preening	11.3 ± 0.01	12.9 ± 0.01	9.9 ± 0.01	11.3 ± 0.01	10.6 ± 0.01	10.5 ± 0.01	0.25
Aggression	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.01	0.71

[1]Values are least squares means ± SEM of 8 observations per treatment. All hens were observed using a 1-min scan sample.

[2]Three molting treatments were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).

[3]$P < 0.05$ was considered significant.

[4]Active postures included standing, standing on a cage mate, and comfort movements.

[5]Feeding behaviors included feeding from own feed trough and attempting to feed from a neighboring feed trough.

Table 3. Heterophil to lymphocyte ratios for laying hens during each period of experiment[1]

		Treatments							
		Ca pre-molt[2]		Molt[3]				*P*-values[4]	
Periods	**Baseline**	**Coarse**	**Fine**	**FW**	**SH**	**WM**	**SEM**	**Ca**	**Molt**
Baseline, %	40	–	–	–	–	–	0.04	–	–
Calcium pre-molt, %	–	45	41	–	–	–	0.09	0.59	–
During molt, %	–	42	46	42	44	46	0.04	0.36	0.59
Post-molt, %	–	47	40	46	43	41	0.04	0.01	0.42

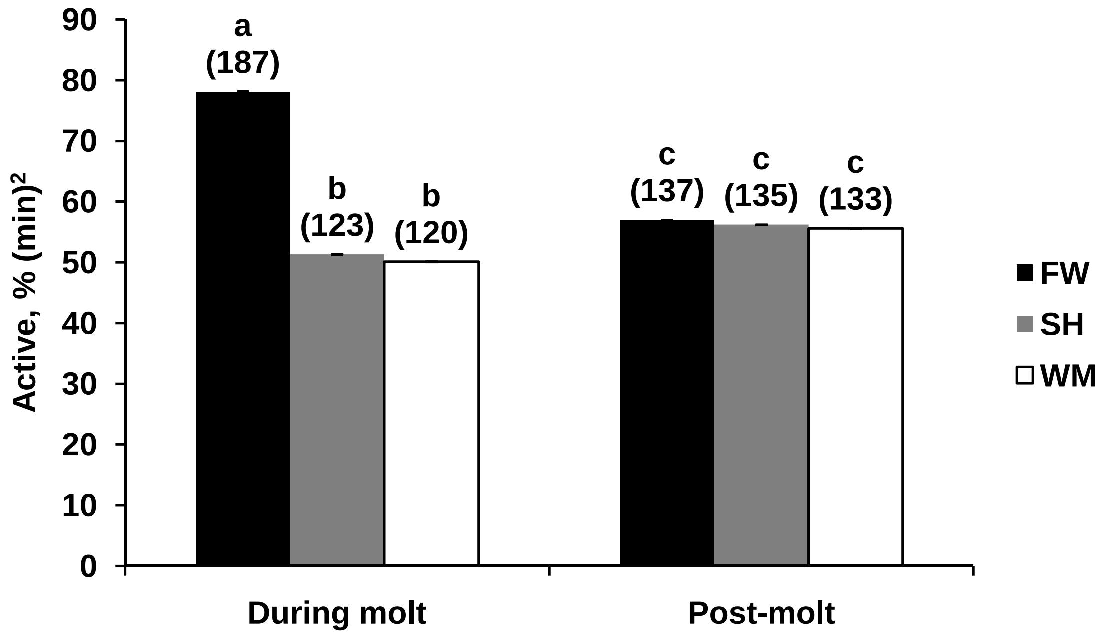
[1]Values are least squares means ± SEM of 9 observations per treatment.
[2]Calcium was supplied as either a combination of fine and coarse $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molting treatments were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]*P* < 0.05 is significant.

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

Measures, %	Period		*P*-value[2]
	During molt	Post-molt	
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

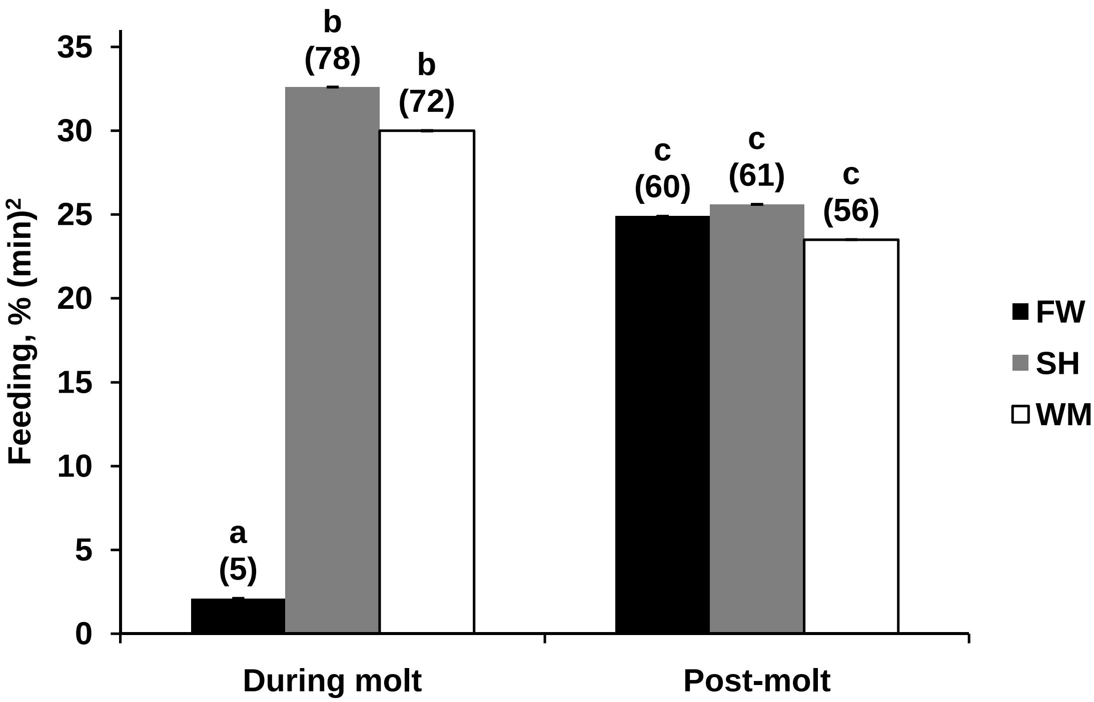
[1]Values are least squares means ± SEM of 8 observations per treatment. All hens were observed using a 1-min scan sample.
[2]$P < 0.05$ was considered significant.
[3]Active postures included standing, standing on a cage mate, and comfort movements.
[4]Feeding behaviors included feeding from own feed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

Figure 1. Active posture of the laying hen[1]



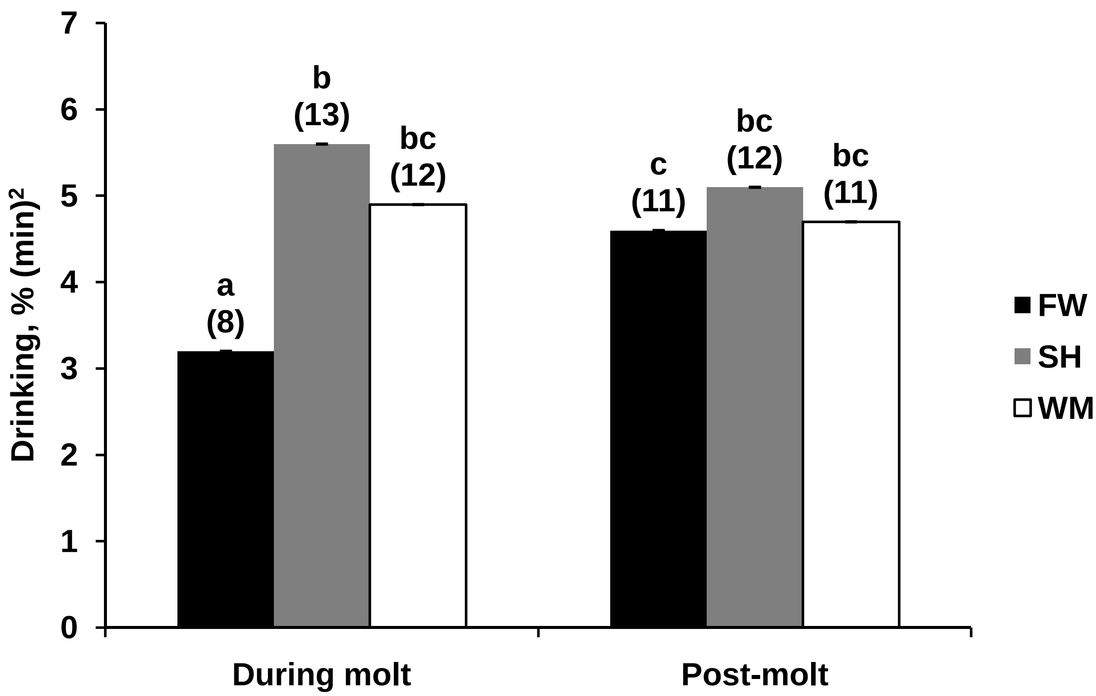
[1]Values are least squares means ± SEM with 8 observations per treatment. FW = feed withdrawal, SH = soybean hulls, WM =wheat middlings.
[2]Minutes are based on percentage of time over 240 minutes of observations.
[a-c]Superscripts above columns differ at $P < 0.0001$.

Figure 2. Feeding behavior of the laying hen[1]



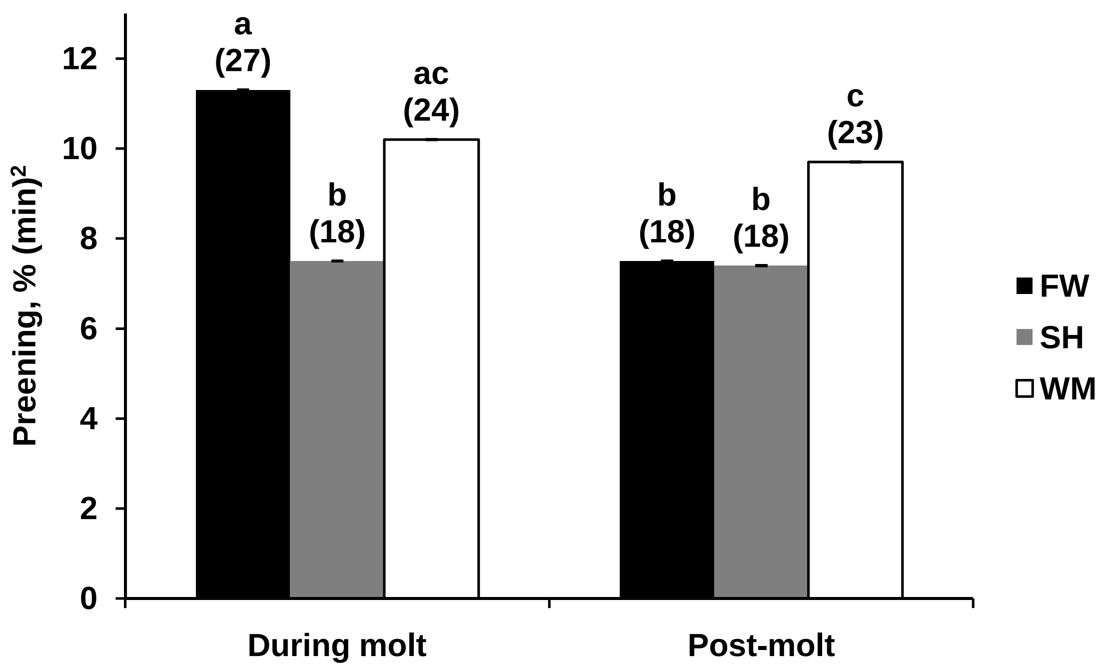
[1]Values are least squares means ± SEM with 8 observations per treatment. FW = feed withdrawal, SH = soybean hulls, WM =wheat middlings.
[2]Minutes are based on percentage of time over 240 minutes of observations.
[a-c]Superscripts above columns differ at *P* < 0.0001.

Figure 3. Drinking behavior of the laying hen[1]



[1]Values are least squares means ± SEM with 8 observations per treatment. FW = feed withdrawal, SH = soybean hulls, WM =wheat middlings.
[2]Minutes are based on percentage of time over 240 minutes of observations.
[a-c]Superscripts above columns differ at *P* = 0.004.

Figure 4. Preening behavior of the laying hen[1]



[1]Values are least squares means ± SEM with 8 observations per treatment. FW = feed withdrawal, SH = soybean hulls, WM =wheat middlings.
[2]Minutes are based on percentage of time over 240 minutes of observations.
[a-c]Superscripts above columns differ at $P = 0.0003$.

CHAPTER 4. EFFECTS OF A PRE-MOLT CALCIUM AND LOW-ENERGY MOLT PROGRAM ON LAYING HENS DURING AND AFTER A FASTING OR NON-FASTING MOLT[1]

A paper to be submitted to *Poultry Science*

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Abstract

The objectives of this study were to evaluate a Ca pre-molt treatment followed by a low-energy molt diet or a feed-withdrawal (**FW**) molt. A total of 981 Hy-Line W-36 laying hens (85 wk of age), housed 3 per cage, were used. Six treatments were compared in a 2 × 3 factorial design with 2 Ca pre-molt treatments (fine and coarse) and 3 molt diets: FW, soybean hulls (**SH**), and wheat middlings (**WM**). The coarse-Ca pre-molt treatment was a 50:50 mix of fine (0.14 mm mean diameter) and coarse

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(2.27 mm mean diameter) $CaCO_3$, whereas the fine-Ca was an all-fine $CaCO_3$. Both diets were formulated to contain 4.6% Ca, such that only the particle size of the $CaCO_3$ differed. Data were analyzed by ANOVA ($P < 0.05$ was significant). None of the treatments affected internal egg quality, however, quality improved post-molt compared to pre-molt values for all treatments. The fine-Ca pre-molt treatment was more effective than the coarse-Ca pre-molt treatment at decreasing egg production during molt and increasing it post-molt, regardless of the molt diet that was fed. The FW treatment resulted in the greatest decrease in production, whereas the SH diet resulted in lower egg production and ovary and oviduct weights during molt compared to the WM molt diet. During molt, differences seen among treatments in bone quality returned to baseline values post-molt, suggesting effects of molt are temporary. Therefore, a fine-Ca pre-molt treatment and a low-energy molt diet, particularly SH, can be useful alternatives to a FW molt.

Key Words: Calcium carbonate particle size, Laying hens, Molt, Egg production

Introduction

In commercial laying hens, molt is induced to cause regression of the reproductive tract and cessation of egg production which allows for a second laying cycle and extends the productive life of the hen (Webster, 2003). Additionally, egg production increases and egg shell quality improves post-molt compared to pre-molt values (Ruszler, 1998). In the US, molt is typically induced when hens are 65 wk of age with an egg production rate of 75%. Traditionally, molt has been induced by feed withdrawal (**FW**) ranging from 4 to 14 d, accompanied by a reduction in photoperiod (Cunningham and Mauldin, 1996; Berry, 2003). Molting using the FW method has raised societal concerns about possible effects on individual bird stress, alterations in their immune system, mineralization of structural bone, and their overall well-being (Holt, 1992; Webster, 2003; Whitehead, 2004; McCowan et al., 2006). The American Veterinary Medical Association (2005) and United Egg Producers (2008) have recommended that after January 1, 2006, producers implement only non-fasting molt programs, and some fast-food restaurants will no longer purchase eggs that have come from farms that use a FW molt program (Anonymous, 2000). Therefore, an alternative method to FW is needed.

A number of studies have compared the effectiveness of feeding low-energy feeds such as wheat middlings (**WM**) or soybean hulls (**SH**) as alternatives to FW for inducing molt (Biggs et al., 2003, 2004; Koelkebeck et al., 2006). These authors concluded that although the traditional FW resulted in a better post-molt performance, the low-energy feeds were nevertheless effective methods to induce molt.

A diet severely deficient in Ca (approximately 0.3%) has also been used to induce molt by inhibiting ovulation (Douglas et al., 1972; Hurwitz et al., 1975). The form or size of Ca provided is also important. Coarse Ca is solubilized more slowly from the digestive tract compared to powdered or fine Ca due to a larger particle size. This coarse Ca may provide the hen with more Ca for use in egg production and shell formation (Scott et al., 1971; Zhang and Coon, 1997). Therefore, it may be possible that a fine-Ca pre-molt treatment will not allow the hen to mobilize sufficient Ca from bone to meet the needs for eggshell formation and the production of the luteinizing-hormone surge that is needed for ovulation (Luck and Scanes, 1979, 1980; Johnson, 2000). Our hypothesis was that this fine-Ca pre-molt treatment would result in a more efficient molt by causing a more complete cessation of egg production. Therefore, the objectives of this study were to induce a molt with low-energy feeds or FW, to improve this molt with a Ca pre-molt treatment, and to examine effects on production, regression of the reproductive tract, and bone metabolism.

Materials and Methods

Experiments 1 and 2 were conducted simultaneously to evaluate effects of a Ca pre-molt treatment followed by a low-energy molt diet or FW. In Exp. 1, egg production and economics were examined. In Exp. 2, ovary and oviduct weight, bone-ash percentage, plasma Ca and inorganic P concentrations, and alkaline phosphatase (**ALP**) activity were examined before, during, and post-molt. The experimental design for both experiments was a randomized complete block design with treatments in a 2 × 3 factorial arrangement with 2 Ca pre-molt treatments (fine and coarse) and 3 molt diets (FW, SH, and WM). The periods included a 2-wk baseline period (hens from 85 to 87 wk of age), followed by a 1-wk Ca pre-molt treatment (hens from 87 to 88 wk of age), a 4-wk molt period (hens from 88 to 92 wk of age), and a 22-wk post-molt period (hens from 92 to 114 wk of age). All live-animal related procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals and Housing

Laying hens were housed 3 per cage (30.5 cm wide × 40.6 cm deep × 44.5 cm high), providing 413 cm^2 per 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. All cages were located in 2 identical light-controlled, fan-ventilated rooms.

Experimental Diets

All diets were formulated to meet or exceed the recommendations from the 2007 to 2008 W-36 Commercial Management Guide (Hy-Line International, Dallas Center, IA) using corn and soybean meal. During the 2-wk baseline period, all hens were given free access to water and a laying hen diet and had a 16 h photoperiod.

During the 1-wk Ca pre-molt treatment, Ca was supplied as either a 50:50 mix of fine (0.14 mm mean diameter; Unical S, ILC Resources, Des Moines, IA) and coarse (2.27 mm mean diameter; Unical S, ILC Resources, Des Moines, IA) $CaCO_3$ or as an all-fine $CaCO_3$ mixed into a laying hen diet (Table 1). Both diets were formulated to contain 4.6% Ca, such that only the particle size of the $CaCO_3$ differed between the 2 treatments. During this Ca pre-molt treatment, hens were given free access to feed and water and had a 24-h photoperiod.

During the molt period, the 3 molt treatments (FW, SH, or WM) were given for 28 d. The hens assigned to the FW treatment were deprived of feed for 7 consecutive days, followed by 21 d of skip-a-day feeding restricted to 60 g of feed (Table 1) per feeding day per hen. The hens fed either the WM or SH molt diet had free access to feed during the entire 28 d molt period (Table 1). All hens had free access to water at all times. No salt was added to either molt diet, however, vitamins, traceminerals, dicalcium phosphate, and calcium carbonate were added to the WM and SH molt diets and corn grain was added to improve flowability according to recommendations by Koelkebeck et al. (2006). During molt, lighting was reduced to 8 h at the start of the first week and was increased to 12 h on the first day of the last week of molt. The photoperiod during the 22-wk long post-molt period was gradually increased by 1 h each week until reaching a 16 h (Table 1).

Data Collection

Experiment 1. A total of 792 hens were used in Exp. 1. Egg production was recorded daily and eggs were inspected for damage (cracked, broken, thin-shelled, soft-shelled, or small eggs weighing less than 30 g). Eggs collected over a 24-h period each week throughout the experiment were saved for weight determination. Egg size distributions, based on egg weights, were determined by standards for jumbo, extra large, large, and medium sized eggs from the 2007 to 2008 Hy-Line W-36 commercial management guide. Egg mass was calculated as egg production × egg weight. During the 4-wk-long molt period, egg weight was determined during the first week only due to low egg production in the ensuing 3 wk. Feed consumption and body weight were recorded weekly until 4 wk post molt (hens were 95 wk of age) and were then recorded once every 3 wk. During molt, feed consumption and body weight of hens assigned to the FW treatment were recorded every other day during the skip-a-day feeding. Feed utilization was calculated as grams of egg mass divided by grams of feed consumed. During the molt period, daily ME intake was calculated as feed consumption × dietary ME content (kcal/kg) and damaged eggs (cracked, broken, thin-shelled, soft-shelled, and small) were recorded daily. Egg specific gravity was determined using eggs collected over a 24-h period twice before molt (hens were 86 and 87 wk of age, respectively) and 8 times post-molt (hens were 96, 97, 98, 99, 102, 105, 108, and 111 wk of age, respectively) by the floatation method described by Bregendahl et al. (2008). Eggs collected from a 24-h period once before molt (hens were 86 wk of age) and once post-molt (hens were 104 wk of age) were used for determination of Haugh units by the method described by Bregendahl et al. (2008). Egg components (yolk, albumen, and shell) of eggs collected over a 24-h period twice before molt (hens were 86 and 87 wk of age, respectively) and 6 times post-molt (hens were 96, 97, 98, 99, 108, and 113 wk of age, respectively) were determined according to Roberts et al. (2007). Mortality was recorded daily throughout the experiment.

Economics were evaluated as the return over feed cost, calculated from the cost of feeding the hens and the price obtained from the eggs. The feed cost was determined from the feed composition, feed consumption, and cost of feed ingredients obtained from the October 13, 2008 edition of Feedstuffs magazine (Chicago market). Because the mean egg weight of all eggs from the 6 treatments corresponded to large eggs (56 to 63 g), the egg value was calculated by multiplying the total egg count by the value of large eggs listed by the October 13, 2008 edition of Feedstuffs magazine (Chicago market).

Experiment 2. Blood was collected from a total of 189 laying hens at the end of the baseline period (9 hens; 86 wk of age), at the end of the Ca pre-molt treatment (9 hens from each of the 2 treatments; 87 wk of age), during the middle and end of the molt period (9 hens from each of the 6 treatments; 89 and 91 wk of age, respectively), and at the end of the post-molt period (9 hens from each of the 6 treatments; 113 wk of age). Blood was collected from the brachial vein into heparinized 15-mL centrifuge tubes. The tubes were kept on ice during transport to the laboratory where the blood was centrifuged (2,000 × *g* for 20 min at 4℃) and the plasma was stored at –80℃ until analysis. The plasma Ca concentrations were determined in duplicate using a digital flame analyzer at the Iowa State University College of Veterinary Medicine Pathology Laboratory. The inorganic P concentrations were determined in triplicate by the method of Gomori (1942) modified by Alexander et al. (2008) for use with a microplate spectrophotometer. The ALP activity in the plasma was assayed in duplicate according to the manufacturer's instructions using a QuantiChrom kit (BioAssay Systems, Hayward, CA) with a microplate spectrophotometer.

After blood was collected, hens were euthanized by CO_2 asphyxiation. Eggs in the reproductive tract, if any, were removed before recording fresh weights of ovaries and oviducts. The left-side humerus and femur bones were used to determine bone-ash percentages and were stored at –80° until analysis. The bones were boiled at 100℃ for 30 min before being manually cleaned of all soft tissue. Cleaned bones were dried in an oven (100℃) for 24 h, weighed, and dry-ashed in a muffle furnace at 700℃ for 24 h. Ash content was expressed as a percentage of the dry bone weight.

Statistical Analysis

The experimental design was a randomized complete block design with treatments in a 2 (Ca pre-molt treatments) × 3 (FW, SH, and WM) factorial arrangement. Cage location within the barn and initial body weight were used as the blocking criteria. Experiment 1 had 44 blocks and the cage containing 3 hens was the experimental unit, whereas experiment 2 had 11 blocks and the individual hen was the experimental unit. Data for both experiments were analyzed by ANOVA using JMP (version 6.0.3, SAS Institute, Inc., Cary, NC). For both experiments, Ca pre-molt treatment and block were the dependent variables for data collected during the Ca pre-molt treatment. During the molt and post-molt periods, Ca pre-molt treatment, molt treatment, the 2-way interaction of Ca treatment by molt treatment, and block were the dependent variables in the model. Values from the baseline period were used as covariates for analysis of egg weight, egg solids, and feed utilization. Egg size distributions (jumbo, extra large, large, and medium) were converted to numeric values (4, 3, 2, and 1, respectively) for analysis. The effects of the Ca pre-molt treatments were assessed using the main effect of the Ca treatment from the ANOVA table, whereas the effects of the molt diets were assessed by Fisher's least significant difference. Data from each period were compared to their respective baseline values using Dunnett's t-test. P-values ≤ 0.05 were considered significant and $P \leq 0.10$ was considered a trend in all comparisons.

Results

Experiment 1

During the 2-wk baseline period there were no differences in feed consumption, body weight, egg production, egg size distribution, egg mass, specific gravity, Haugh units, wet yolk, wet albumen, or wet shell percentages for hens assigned to the Ca pre-molt treatments or the molt diets. However, hens to be assigned to the SH molt diet had higher feed utilization compared to hens to be assigned to the FW molt diet, and higher egg weights compared to hens assigned to the WM molt diet. The hens to be assigned to the coarse-Ca pre-molt treatment had a higher percentage of egg solids compared to hens to be assigned to the fine-Ca pre-molt treatment during the baseline period (Table 2). As a result, baseline values were used as covariates in analysis of data from subsequent periods. During the Ca pre-molt treatment, there were no differences between hens allocated to the coarse or fine treatments for feed consumption, feed utilization, body weight, egg production, egg weight, egg size distribution, egg mass, specific gravity, wet yolk, wet albumen, wet shell, or egg solid percentages. However, hens from both Ca pre-molt treatments had lower feed utilization, egg production, and wet albumen percentages and higher egg size distributions, wet shell, and egg solid percentages compared to hens during the baseline period. There were no differences from the baseline period in feed consumption, body weight, egg weight, egg mass, specific gravity, or wet yolk percentages (Table 3).

During the molt period, the Ca pre-molt treatment did not affect feed consumption, ME intake, body weight, or egg weight. However, the fine-Ca pre-molt treatment resulted in lower egg production compared to the coarse-Ca pre-molt treatment during molt. Compared to baseline values, hens fed the fine-Ca pre-molt treatment had higher egg weights and both treatments resulted in lower feed consumption, body weights, and egg production. Hens assigned to the FW molt diet had the lowest feed consumption and egg production, whereas hens fed the WM molt diet had the highest feed consumption, ME intake, body weight, and egg production during molt. There was no difference among the molt treatments in egg weight during the first week of molt. Compared to baseline values, all 3 molt diets resulted in lower feed consumption, body weights, and egg production. Hens fed the SH molt diet had higher egg weights during molt compared to hens during the baseline period (Table 4).

The Ca pre-molt treatment had no effect on the number of days it took for hens to reach lowest egg production during molt. The hens fed the WM molt diet took the longest to reach lowest egg production during molt compared to the other 2 molt diets (Table 5). Hens fed the FW and SH molt diets reached 0% egg production (by d 8 and 21, respectively), whereas the hens fed the WM molt diet reached a low of 2.0% egg production by d 25 of molt. The counts for damaged eggs (cracked, broken, thin-shelled, soft-shelled, and small eggs weighing less than 30 g) during the molt period for each treatment are reported in Table 6.

During the first 2 wk post-molt, the Ca pre-molt treatments had no effect on feed consumption, body weight, egg production, or egg size distribution. The molt diets during this first 2 wk post-molt period had no effect on egg size distributions, but hens fed the WM molt diet had higher body weight and egg production compared to hens fed the other 2 molt diets and had a trend for lower feed consumption. Compared to baseline values, all treatments resulted in lower feed consumption, body weights, and egg production during the 2-wk post-molt period. During the next 20 wk post-molt, the fine-Ca pre-molt treatment resulted in higher egg production, egg size distribution, and egg mass compared to the coarse-Ca pre-molt treatment, and had a trend for higher feed consumption. There were no differences in feed utilization, body weight, egg weight, specific gravity, Haugh units, wet yolk, wet albumen, wet shell, or egg solid percentages. The hens fed the SH molt diet during the last 20 wk post-molt had the lowest feed consumption and body weight compared to hens assigned to the FW and WM molt diets, but there were no differences in feed utilization, egg production, egg weight, egg mass, specific gravity, Haugh units, wet yolk, wet albumen, wet shell, or egg solid percentages. Compared to baseline values, all treatments resulted in higher feed consumption, egg weights, egg size distributions, egg mass, specific gravity, and egg solid percentages during the last 20-wk post-molt. The fine-Ca pre-molt treatment and the FW molt treatment resulted in higher egg production compared to baseline values. All treatments except the WM molt diet resulted in higher feed utilization and all treatments except the SH molt diet resulted in higher body weights and wet shell percentages compared to values during the baseline period (Table 7). Post-molt, the Ca pre-molt treatment had no effect on how many days it took hens to reach 50% egg production. The hens fed the WM molt diet reached 50% egg production first compared to the other 2 molt diets (Table 5).

There were 3 mortalities during the baseline period (accounting for 0.38% of hens), 0 during the Ca pre-molt treatment, 4 during molt (accounting for 0.52% of remaining hens), and 27 post-molt (accounting for 4.15% of remaining hens). These hens are believed to have died for reasons unrelated to the treatments.

The Ca pre-molt treatments had no effect on overall (week 1 to 29 of experiment) egg income, feed costs, or profit per hen housed. The WM molt diet resulted in higher feed cost and had trends for higher egg income and profit per hen housed compared to the other 2 molt diets. During the post-molt period only (week 8 to 29 of experiment), the molt diets had no effect on profit per hen housed, whereas the fine-Ca pre-molt treatment resulted in higher profits compared to the coarse-Ca pre-molt treatment (Table 8).

Experiment 2

During the Ca pre-molt treatment, there were no differences between the fine- or coarse-Ca pre-molt treatment values for reproductive tract weights, bone ash percentages, plasma Ca, or inorganic P concentrations. There was a trend toward higher ALP activity in hens fed the fine-Ca pre-molt treatment. There were no differences between Ca pre-molt treatments and baseline values for femur-ash percentage, plasma Ca concentrations, or ALP activity. Hens that were fed the coarse-Ca pre-molt treatment had higher ovary and oviduct weights than during the baseline period. Hens fed the fine-Ca pre-molt treatment had lower humerus-ash percentage and higher plasma inorganic P concentrations compared to hens during the baseline period, but there were no differences from baseline values for birds fed the coarse-Ca pre-molt treatment (Tables 9 and 10).

During molt, there were no differences between the Ca pre-molt treatments for reproductive tract weights, bone-ash percentages, or blood parameters. There were no differences between the Ca pre-molt treatments during molt and baseline values for humerus-ash percentages or plasma inorganic P concentrations. Hens fed the coarse-Ca pre-molt treatment had lower ovary and oviduct weights, femur-ash percentage, and plasma Ca concentrations, and higher ALP activity compared to baseline values (Tables 9 and 10).

During molt, hens fed the WM molt diet had greater ovary weights compared to hens fed the SH molt diet, but ovary weights of hens fed the WM or SH molt diets were not different from hens assigned to the FW treatment. Hens fed the WM molt diet had greater oviduct weights and higher plasma Ca concentrations compared to hens fed the FW and SH molt diets. There were no differences in bone-ash percentages, plasma inorganic P concentrations, or ALP activity among the hens assigned to the 3 molt diets during molt (Tables 9 and 10).

When comparing values from the hens fed the molt diets during molt to baseline values, hens from all treatments had lower ovary and oviduct weights and plasma Ca concentrations, and higher ALP activity. Hens fed the FW and WM molt diets had lower femur-ash percentages during molt compared to hens during the baseline period. There were no differences in humerus-ash percentages or inorganic plasma P concentrations during molt for hens fed any of the molt diets and hens during the baseline period (Tables 9 and 10).

Post-molt, the hens fed the fine-Ca pre-molt treatment had heavier oviduct weights and there was a trend for heavier ovary weights compared to hens fed the coarse-Ca pre-molt treatment (Table 9). There were no differences post-molt in bone-ash percentages, plasma Ca and inorganic P concentrations, or ALP activity in hens fed the fine- or coarse-Ca pre-molt treatments (Table 10).

When comparing values for hens fed the Ca pre-molt treatment to baseline values post-molt, there were no differences in ovary weights, bone-ash percentages, plasma Ca concentrations, or inorganic plasma P concentrations. However, hens fed the fine-Ca pre-molt treatment had greater oviduct weights and higher ALP activity compared to hens during the baseline period, but there were no differences post-molt from hens during the baseline period when provided the coarse-Ca pre-molt treatment.

When comparing hens fed the 3 molt diets, there were no differences post-molt in ovary and oviduct weights, bone-ash percentages, or blood parameters. However, hens fed the WM molt diet had a trend toward lower plasma Ca concentrations compared to hens fed the other 2 molt diets. Hens fed the molt diets had no differences in ovary and oviduct weights or bone-ash percentages post-molt from hens during the baseline period. Hens fed the SH molt diet had higher ALP activity compared to hens during the baseline period, but none of the other blood parameters differed for hens fed the molt diets (Tables 9 and 10).

Discussion

Most wild bird species, including ducks, geese, and waterfowl, naturally undergo a period of molt. Jungle fowl hens restrict feed consumption during egg incubation and can lose between 10 to 20% of their body weight over a 20 d period (Mrosovsky and Sherry, 1980). At some point, laying hens would begin molting on their own, but not all hens would molt at the same time and the molt would be incomplete, resulting in longer periods of lower egg production for the producer and a subsequent decrease in overall profitability (Berry, 2003). For this reason, it is important for producers to induce a molt that allows all laying hens to go through this process simultaneously and effectively. A national survey conducted in 1999 by the USDA Animal and Plant Health Inspection Service (USDA, 2000) reported that 74% of the farm sites surveyed molted their last completed flocks, whereas only 26% of the farm sites did not molt their last completed flock. Molting a flock results in one-third of the profits from that flock (Holt, 2003) as molting allows producers to avoid decreased profits by molting their hens during times of lower egg prices and avoiding spent flocks by keeping their hens for a second laying cycle with a 10 to 12% increase in egg production and improved egg quality post-molt (Cunningham and Mauldin, 1996). Traditionally, molt is induced in commercial laying hens by a period of feed withdrawal, but this method is no longer recommended due to concern for laying hen well-being (United Egg Producers, 2008). However, the traditional FW molt results in a more complete molt and improved post-molt performance with an increase in egg production when compared to alternative low-energy diets (Biggs et al., 2003, 2004; Donalson et al., 2005). Therefore, non-FW alternatives need to be improved and, in the present study, our objectives were to evaluate a Ca pre-molt treatment followed by low-energy molting diets or a 7 d FW.

Calcium provided as coarse particles can improve egg shell quality and bone strength (Scott et al., 1982; Fleming et al., 1998; Scheideler, 1998; Whitehead and Fleming, 2000). The coarse-Ca particles are solubilized more slowly from the gizzard than fine-Ca particles which allows the hen to absorb dietary Ca at night during egg shell formation. This absorption can result in less Ca mobilized from bone stores (Scott et al., 1971; Zhang and Coon, 1997; Whitehead, 2004). Calcium is also necessary for the release of gonadotrophic hormones, including the surge in luteinizing hormone that results in ovulation necessary for egg production (Luck and Scanes, 1979, 1980; Johnson, 2000). In the present study, we tested if a fine-Ca pre-molt treatment would result in a more efficient molt by causing a faster decrease in egg production. Although the fine-Ca pre-molt treatment is not deficient in Ca, the hens' intestines will be devoid of Ca during the night when the hens do not eat. Therefore, the hens must rely on Ca mobilized from medullary bone for eggshell formation and this may result in a more complete cessation of egg production during the molt period. During molt, estrogen levels decrease, allowing osteoblasts to form structural bone, which improves skeletal integrity (Whitehead, 2004). Calcium is needed during bone formation and less intestinal absorption of fine Ca compared to coarse Ca may hinder the process of structural bone formation (Whitehead and Fleming, 2000). Therefore, the effects of the fine-Ca pre-molt treatment on physiological parameters such as bone-ash percentages and plasma mineral concentrations were evaluated in the present study.

During the Ca pre-molt treatment, there were no differences between hens fed the fine- or coarse-Ca pre-molt treatment for any production parameters, reproductive tract weights, bone-ash percentages, or blood measures. A possible rationale for seeing no differences is that the 1-wk period may have been too short and possible differences may not have occurred until the molt period. Hens fed the coarse-Ca pre-molt treatment had higher ovary and oviduct weights compared to hens during the baseline period. Plasma Ca concentrations are correlated with ovary and oviduct weights (Mirarchi, 1993) and the coarse-Ca pre-molt treatment may allow more Ca absorption in the hen, resulting in the higher ovary and oviduct weights.

Plasma Ca and inorganic P concentrations and ALP activity can be used to assess molt effects on bone metabolism (Hurwitz and Griminger, 1961; Reichmann and Connor, 1977). Plasma Ca and P ions from blood are deposited in bone tissue, with assistance from the enzyme ALP, for bone mineralization (Saladin, 2004). High plasma ALP activity and low Ca and P concentrations may indicate bone disease or fracture in laying hens. The hens fed the fine-Ca pre-molt treatment had a lower humerus-ash percentage and a higher inorganic plasma P concentration during the Ca pre-molt treatment compared to hens during the baseline period. These results may have been caused by the need for Ca stores in bone to mobilize for egg shell formation, because the fine Ca does not stay in the digestive tract as long as the coarse Ca, resulting in reduced bone mineral content (Guinotte and Nys, 1991; Whitehead, 2004). However, plasma Ca concentrations and ALP activity did not differ from baseline values during the Ca pre-molt treatment for either treatment. These results suggest that bone damage during the Ca pre-molt treatment was minimal for either treatment (Chute et al., 1961; Whitehead, 2004).

During molt, the hens assigned to the fine-Ca pre-molt treatment had lower feed utilization and egg production compared to hens fed the coarse-Ca pre-molt treatment. These results suggest the coarse-Ca pre-molt treatment did not cause a complete molt and the fine-Ca pre-molt treatment was more effective at reducing egg production and inducing molt.

The FW and SH molt diets were the most effective at quickly decreasing egg production during molt compared to the WM molt diet. Additionally, the hens fed the FW and SH molt diets reached 0% egg production, whereas the hens fed the WM molt diet did not, suggesting these hens did not go through a complete molt. Koelkebeck et al. (2006) and Biggs et al. (2004) also reported that hens fed diets with WM and corn did not reach 0% egg production, but these results disagree with Biggs et al. (2003) who reported that hens fed a WM diet resulted in 0% egg production by d 8 of molt. These differences in cessation of egg production may be due to energy differences in the diets because Biggs et al. (2003) used a 95% WM diet containing 1,900 kg/kcal ME, whereas the 75% WM diet in the present study contained 2,198 kg/kcal ME. The higher-energy diet used in the present study may be the cause for an incomplete cessation of egg production. Performance of the laying hen post-molt is related to the degree of regression of the reproductive tract during molt and an incomplete molt, such as the WM molt diet, can be a concern for producers if it results in lower egg production and egg quality post-molt (Ruszler, 1998).

The hens assigned to the FW molt diet had the lowest egg production during molt, followed by the hens fed the SH and then the hens fed the WM molt diet (which had the highest egg production). These findings agree with Biggs et al. (2003) and Koelkebeck et al. (2006) who both reported higher egg production during molt for hens fed a WM molt diet compared to hens assigned to a 10 d FW. The differences in egg production during molt may be due to the differences in feed consumption caused by novelty, palatability, or low energy contents of the diets (Biggs et al., 2003). The hens fed the WM molt diet had the lowest feed utilization and consumed the most feed during molt. These hens also consumed the most ME during the molt period compared to hens fed the other 2 molt diets. The hens assigned to the FW molt diet had the lowest feed consumption and egg mass compared to the other 2 molt diets. The lower feed consumption can be attributed to the limited feed provided during the skip-a-day feeding and the lower egg mass can be attributed to the low egg production of the hens assigned to the FW molt diet.

Body weight was highest during molt for hens fed the WM molt diet, which correlated with their higher feed consumption compared to hens fed the SH and FW molt diets. The hens fed the FW molt diet had higher body weights than the hens fed the SH molt diet and this increase may be due to the FW hens receiving a high-energy, high-protein diet every other day during the last 3 wk of molt which may have caused their higher body weight gain. These results agree with Biggs et al.

(2003) who reported body weight loss was lowest for hens assigned to a WM molt diet or a 10 d FW compared to a corn molt diet and a 4 d FW.

Hens assigned to all treatments had lower ovary and oviduct weights compared to hens during the baseline period which is expected due to the regression of the reproductive tract that occurs during molt (Berry, 2003). These results agree with Biggs et al. (2004) who reported ovary and oviduct weights decreased during molt for hens assigned to a 10 d FW, distiller's dried grains with solubles, corn grain, or WM molt diet. When comparing hens fed the molt diets in the present study, the hens fed the WM molt diet had the heaviest ovary weight compared to hens fed the SH molt diet and the heaviest oviduct weight compared to hens fed the FW or SH molt diet during the molt period. The higher weights for the hens fed the WM molt diet suggests their reproductive tract did not regress as fully as the hens fed the other molt diets, resulting in a less complete molt.

Whitehead and Fleming (2000) reported that molt resulted in a rapid decrease in medullary bone and resumption of structural bone formation. In the present study, the humerus-ash percentage was not different from baseline values for hens assigned to any of the treatments during molt. The humerus is a pneumatized bone rather than a medullary bone (Whitehead, 2004), so it may not be as affected when Ca stores are mobilized. However, the femur-ash percentage was lower than baseline values in hens fed the fine- or coarse-Ca pre-molt treatment with the FW or WM molt diets. Additionally, all treatments resulted in lower plasma Ca concentrations during molt and higher ALP activity compared to baseline. Calcium is mobilized from medullary bone during egg production for the formation of the egg shell and, during molt, the decrease in egg production results in lower plasma Ca concentrations (Whitehead, 2004). Therefore, this decrease in plasma Ca concentrations may be due to decreased egg production during molt rather than bone damage or disease. Also, the dietary Ca content of these diets was lower than the diet fed during the baseline period and may be the reason for lower plasma Ca concentrations.

The fine- and coarse-Ca pre-molt treatments had no effect on egg production, feed consumption, or body weight during the first 2 wk post-molt, which suggests there are no immediate effects when returning to production. However, the fine-Ca pre-molt treatment resulted in higher egg production and egg mass during the following 20 wk post-molt compared to the coarse-Ca pre-molt treatment which suggests it was more effective at increasing production long-term after the molt period.

Hens fed the WM molt diet had higher egg production and body weight compared to hens fed the other 2 molt diets during the first 2 wk post-molt and can be explained by the incomplete molt with higher egg production and body weights during the molt period. The effects of this incomplete molt can be seen during the following 20-wk post-molt when egg production, egg weight, and egg mass did not differ among the 3 molt treatments, meaning the hens fed the WM diet could not maintain their higher egg production. The treatments had no effect on Haugh units, specific gravity, wet yolk, wet albumen, wet shell, or egg solid percentages during the last 20 wk post-molt, which agrees with reports by Biggs et al. (2004), and suggests the treatments are comparable in their effects on interior egg quality post-molt.

Hens fed the WM molt diet reached 50% production the soonest post-molt (d 19) compared to hens fed the other 2 molt diets. These results are similar to those reported by Koelkebeck et al. (2006) with hens fed WM and 10 d FW molt diets reaching 50% production by d 15 and 19, respectively. It is speculated that the hens fed the WM molt diet reached 50% egg production before the hens fed the SH and FW molt diets because they did not decrease egg production as much during molt.

For the physiological parameters, none of the Ca pre-molt treatments or molt diets affected bone-ash percentages, plasma Ca and inorganic P concentrations, or ALP activity post-molt and they did not differ from baseline values. These results suggest that any changes during molt are temporary as the levels return to baseline values post-molt. However, oviduct weights were higher for hens fed the fine-Ca pre-molt treatment compared to hens fed the coarse-Ca pre-molt treatment and the oviduct weights also differed from the hens during the baseline period. There was a trend during molt for lower oviduct weights from hens fed the fine-Ca pre-molt treatment which suggests this higher weight post-molt is due to a more complete regression of the reproductive tract.

The fine-Ca pre-molt treatment resulted in higher profits per hen housed compared to the coarse-Ca pre-molt treatment when looking at the 22-wk post-molt period. However, none of the treatments differed in profits per hen housed during the entire 29-wk experiment. The WM molt diet did result in higher feed costs compared to the FW and SH molt diets, however, this higher cost was offset by higher levels of egg production during molt and the first 2 wk post-molt, resulting in similar profit levels for all 3 molt diets.

In conclusion, all-fine $CaCO_3$ added to a laying hen diet for 1 wk before molt was more efficient at inducing molt by causing a greater reduction in egg production during molt and higher egg production and oviduct weights post-molt compared to the coarse-Ca pre-molt treatment. Additionally, the fine-Ca pre-molt treatment did not appear to negatively affect bone-ash percentages or blood parameters and it resulted in a higher profit compared to the coarse-Ca pre-molt treatment during the post-molt period. The fine-Ca pre-molt treatment was successful regardless of what molt diet was used. In agreement with previous research, the FW molt diet resulted in the most complete molt with a greater drop in egg production and body weight. The SH molt diet was more effective at inducing molt compared to the WM molt diet by resulting in a greater reduction in egg production and ovary and oviduct weights during molt.

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Table 1. Chemical composition of the experimental diets[1]

Period and treatment	ME_n, kcal/kg	CP, %	Ca, %	Non-phytate P, %	Na, %	Lys, %	Met + Cys, %
Baseline (85 wk of age)	2,776	16.07	4.61	0.40	0.18	0.91	0.66
Calcium pre-molt (87 wk of age)[2]							
Fine	2,776	16.07	4.61	0.40	0.18	0.91	0.66
Coarse	2,776	16.07	4.61	0.40	0.18	0.91	0.66
Molt (88 wk of age)							
Feed withdrawal[3]	2,817	15.27	2.00	0.25	0.11	0.80	0.91
Wheat middlings	2,198	13.09	2.00	0.25	0.14	0.57	0.33
Soybean hulls	2,216	8.88	2.00	0.25	0.10	0.44	0.30
Post-molt							
First 2 wk *(92 wk of age)*	2,910	16.50	3.85	0.50	0.17	0.94	0.62
Ensuing 20 wk *(94 wk of age)*	2,880	16.05	4.10	0.44	0.19	0.91	0.62

[1]Calculated values. Diets contained corn, soybean meal, vitamins, traceminerals, dicalcium phosphate, and calcium carbonate.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]The 7 d feed withdrawal period was followed by restricted skip-a-day feeding for the ensuing 3 wk.

Table 2. Egg production and egg quality during the baseline period (hens at 85 to 87 wk of age) in Exp. 1[1]

Item	Treatments							
	Calcium pre-molt[2]		Molt[3]				*P*-values[5]	
	Coarse	Fine	FW	SH	WM	SEM[4]	Ca	Molt
Feed consumption, g/d	97.0	96.6	96.2	96.3	98.0	1.4	0.70	0.34
Feed utilization, g/g	0.460	0.457	0.445[a]	0.477[b]	0.454[ab]	0.012	0.76	0.02
Body weight, kg	1.68	1.68	1.68	1.67	1.69	0.01	0.55	0.18
Egg production, %	68.8	67.4	65.7	69.8	68.8	2.1	0.43	0.14
Egg weight, g	64.7	65.4	65.1[ab]	65.7[a]	64.5[b]	0.5	0.06	0.04
Egg size distribution[6]	2.5	2.6	2.5	2.6	2.4	0.1	0.40	0.17
Egg mass, g	44.7	44.3	43.0	45.9	44.6	1.3	0.71	0.10
Specific gravity	1.076	1.076	1.076	1.076	1.076	0.001	0.53	0.37
Haugh units	77	77	77	76	77	1	0.89	0.43
Wet yolk, %	28.8	28.5	28.5	28.6	28.9	0.4	0.30	0.62
Wet albumen, %	57.6	57.8	58.0	57.6	57.5	0.3	0.56	0.29
Wet shell, %	12.1	12.2	12.2	12.0	12.2	0.1	0.74	0.42
Egg solids, %	21.6	21.3	21.4	21.5	21.4	0.1	0.02	0.88

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]Pooled SEM.
[5]Overall *P*-value from the main effect of treatment.
[6]Egg size distributions based on egg weights. 4 = jumbo, 3 = extra large, 2 = large, 1 = medium.
[ab]Means within a row without a common superscript differ ($P < 0.05$).

Table 3. Egg production and egg quality during the Ca pre-molt treatment (hens at 87 to 88 wk of age) in Exp. 1[1]

Item	**Calcium pre-molt treatments**[2]			
	Coarse	**Fine**	**SEM**[3]	***P*-value**[4]
Feed consumption, g/d	98.7	97.1	1.3	0.13
Feed utilization, g/g	0.390*	0.412*	0.029	0.15
Body weight, kg	1.69	1.68	0.01	0.30
Egg production, %	63.9*	63.5*	2.2	0.81
Egg weight, g	65.5	65.5	0.5	0.98
Egg size distribution[5]	2.7*	2.8*	0.1	0.34
Egg mass, g	42.5	42.6	1.4	0.94
Specific gravity	1.076	1.076	0.001	0.69
Wet yolk, %	28.7	28.6	0.3	0.52
Wet albumen, %	57.1	57.2	0.3	0.87
Wet shell, %	12.4	12.5	0.1	0.85
Egg solids, %	22.4*	22.2*	0.2	0.24

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine Ca CO_3.
[3]Pooled SEM.
[4]Overall *P*-value from main effect of Ca pre-molt treatment; $P < 0.05$ was significant.
[5]Egg size distributions based on egg weights. 4 = jumbo, 3 = extra large, 2 = large, 1 = medium.
*Values differ from baseline ($P < 0.05$).

Table 4. Egg production and egg quality during the molt period (hens at 88 to 92 wk of age) in Exp. 1[1]

Item	**Treatments**								
	Calcium pre-molt[2]		**Molt**[3]				***P*- values**[4]		
	Coarse	**Fine**	**FW**	**SH**	**WM**	**SEM**[5]	**Ca**	**Molt**
Feed consumption, g/d	36.7*	36.0*	26.1[a],*	34.0[b],*	48.9[c],*	1.0	0.37	< 0.001
ME intake (kcal/d)	86.2	84.6	73.4[a]	75.4[a]	107.4[b]	2.1	0.37	< 0.001
Body weight, kg	1.39*	1.38*	1.37[a],*	1.35[b],*	1.45[c],*	0.01	0.19	< 0.001
Egg production, %	10.4*	8.4*	7.2[a],*	8.9[b],*	12.1[c],*	0.6	< 0.001	< 0.001
Egg weight, g[6]	65.8	66.1*	66.0	66.0*	65.5	0.4	0.66	0.85

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM). The 7 d FW was followed by restricted (60 g/hen) skip-a-day feeding.
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[6]Egg weight was only measured during the first week of molt due to low egg production.
[a-c]Means within a row lacking a common superscript differ ($P < 0.05$).
*Values differ from baseline ($P < 0.05$).

Table 5. Days to lowest egg production during molt and 50% egg production post-molt in Exp. 1[1]

Item	Treatments							
	Calcium pre-molt[2]		Molt[3]				*P*- values[4]	
	Coarse	Fine	FW	SH	WM	SEM[5]	Ca	Molt
Lowest egg production, d	9	8	6[a]	7[a]	13[b]	1	0.14	< 0.0001
50% egg production, d	23	23	24[a]	25[a]	19[b]	1	0.97	< 0.0001

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[ab]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 6. Counts of damaged eggs during the molt period in Exp. 1

Item	**Treatments**				
	Calcium pre-molt[1]		**Molt**[2]		
	Coarse	**Fine**	**FW**	**SH**	**WM**
Cracked eggs	46	40	26	32	28
Broken eggs	13	18	12	10	9
Thin shelled eggs	162	171	118	110	105
Soft shelled eggs	34	31	12	19	34
Small eggs[3]	7	2	0	2	7

[1]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[2]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[3]Small eggs weighed less than 30 g.

Table 7. Egg production and egg quality during the post-molt period (hens at 92 to114 wk of age) in Exp. 1[1]

Items	Treatments							
	Calcium pre-molt[2]		Molt[3]				*P*-values[4]	
	Coarse	Fine	FW	SH	WM	SEM[5]	Ca	Molt
First 2 wk post-molt (92 wk of age)								
Feed consumption, g/d	91.6*	92.0*	92.2*	93.4*	89.8*	1.5	0.70	0.06
Body weight, kg	1.44*	1.44*	$1.45^{a,*}$	$1.37^{b,*}$	$1.50^{c,*}$	0.01	0.84	< 0.001
Egg production, %	5.19*	4.82*	$0.89^{a,*}$	$0.93^{a,*}$	$13.2^{b,*}$	1.2	0.70	< 0.001
Egg size distribution[6]	2.7	2.7	2.7	3.0	2.5	0.1	0.95	0.77
Last 20 wk post-molt (94 wk of age)								
Feed consumption, g/d	103.2*	104.7*	$104.7^{a,*}$	$102.6^{b,*}$	$104.6^{a,*}$	0.9	0.06	0.03
Feed utilization, g/g	0.480*	0.491*	0.493*	0.483*	0.479	0.012	0.18	0.35
Body weight, kg	1.71*	1.72*	1.72^{a}*	1.69^{b}	$1.73^{a,*}$	0.01	0.23	0.003
Egg production, %	69.9	72.6*	71.6*	71.2	70.8	1.4	0.02	0.87
Egg weight, g	67.2*	67.5*	67.4*	67.2*	67.5*	0.3	0.31	0.53
Egg size distribution[6]	3.0*	3.1*	3.0*	3.0*	3.0*	0.1	0.04	0.95
Egg mass, g	48.8*	50.9*	50.3*	50.5*	48.7*	1.0	0.01	0.11
Specific gravity	1.081*	1.081*	1.081*	1.081*	1.080*	0.001	0.62	0.21
Haugh units	80*	80*	80*	80*	79	1	0.79	0.26
Wet yolk, %	28.2	28.4	28.4	28.2	28.3	0.3	0.48	0.81
Wet albumen %	57.4	57.0	57.0	57.0	57.3	0.4	0.28	0.60
Wet shell, %	12.4	12.5	12.5	12.3	12.5	0.1	0.91	0.09
Egg solids, %	21.9*	21.8*	21.9*	21.9*	21.8*	0.1	0.50	0.54

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[6]Egg size distributions based on egg weights. 4 = jumbo, 3 = extra large, 2 = large, 1 = medium.
[a-c]Means within a row without a common superscript differ ($P < 0.05$).
*Values differ from baseline ($P < 0.05$).

Table 8. Egg income minus feed costs during Exp. 1[1]

Items	Treatments						*P*- values[4]	
	Calcium pre-molt[2]		Molt[3]					
	Coarse	**Fine**	**FW**	**SH**	**WM**	**SEM[5]**	**Ca**	**Molt**
Egg income, \$/hen per 29 wk[6]	28.24	28.85	27.91	28.40	29.33	0.67	0.26	0.10
Feed cost, \$/hen per 29 wk[7]	0.30	0.30	0.30[a]	0.30[a]	0.31[b]	0.01	0.85	0.0001
Profit per hen-housed, \$/29 wk	9.38	9.58	9.27	9.43	9.74	0.22	0.26	0.10
Post-molt profit per hen-housed, \$/22 wk	7.85	8.14	7.90	7.94	8.14	0.18	0.05	0.38

[1]Values are least squares means of 44 observations per treatment.
[2]Calcium was supplied as either a combination of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]FW = feed withdrawal, SH = soybean hulls, WM = wheat middlings.
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[6]Egg income was based on 99.5 cents per dozen eggs produced obtained from Feedstuffs magazine (Chicago market, October 13, 2008).
[7]Feed cost was obtained from Feedstuffs magazine (Chicago market, October 13, 2008) based on feed consumption from week 1 to 29.

Table 9. Reproductive tract weights and bone ash-percentages in Exp. 2[1]

Items and periods		Treatments							
		Calcium pre-molt[2]		Molt[3]				*P*-values[4]	
	Baseline	Coarse	Fine	FW	SH	WM	SEM[5]	Ca	Molt
Ovary, g	41.3	–	–	–	–	–	2.0	–	–
Calcium pre-molt		50.9*	49.3	–	–	–	3.2	0.73	–
During molt		5.9*	5.6*	$5.3^{*,ab}$	$4.5^{*,a}$	$7.5^{*,b}$	1.7	0.74	0.03
Post-molt		42.3	48.3	45.8	42.9	47.3	3.6	0.06	0.49
Oviduct, g	52.1	–	–	–	–	–	5.2	–	–
Calcium pre-molt		66.4*	64.4	–	–	–	3.1	0.67	–
During molt		13.2*	12.2*	$10.9^{*,a}$	$11.0^{*,a}$	$16.2^{*,b}$	3.1	0.60	0.02
Post-molt		52.4	65.4*	61.2	55.1	60.3	4.1	0.001	0.31
Humerus bone-ash, %	64.6	–	–	–	–	–	0.7	–	–
Calcium pre-molt		62.0	61.0*	–	–	–	1.0	0.52	–
During molt		61.0	61.7	61.7	60.8	61.7	1.0	0.36	0.56
Post-molt		62.2	61.5	61.7	61.6	62.3	1.5	0.85	0.57
Femur bone-ash, %	55.2	–	–	–	–	–	1.4	–	–
Calcium pre-molt		55.1	54.6	–	–	–	1.0	0.75	–
During molt		51.5*	50.5*	50.9*	51.7	50.3*	1.1	0.22	0.39
Post-molt		52.9	53.3	53.2	52.5	53.6	1.6	0.72	0.79

[1]Values are least squares means with 9 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[ab]Means within a row lacking a common superscript differ ($P < 0.05$).
[*]Means within a row differ from baseline value ($P < 0.05$). *P*-value from Dunnett's t-test.

Table 10. Blood measures in Exp. 2[1]

Items and periods		Treatments							
		Calcium pre-molt[2]		Molt[3]				*P*-values[4]	
	Baseline	Coarse	Fine	FW	SH	WM	SEM[5]	Ca	Molt
Plasma Ca, mg/dL	29.6	–	–	–	–	–	2.3	–	–
Calcium pre-molt		33.1	35.0	–	–	–	4.3	0.58	–
During molt		13.7*	12.2*	11.4*,a	11.3*,a	16.2*,b	2.1	0.39	0.03
Post-molt		31.5	32.3	32.2	33.8	29.5	1.7	0.61	0.06
Plasma inorganic P, mg/dL	1.15	–	–	–	–	–	0.08	–	–
Calcium pre-molt		1.42	1.48*	–	–	–	0.08	0.58	–
During molt		1.06	1.01	0.96	1.07	1.09	0.05	0.36	0.16
Post-molt		1.32	1.36	1.33	1.41	1.23	0.10	0.42	0.64
Alkaline phosphatase (ALP), IU/L[6]	32.4	–	–	–	–	–	5.0	–	–
Calcium pre-molt		32.4	58.0	–	–	–	9.6	0.09	–
During molt		71.6*	65.5*	62.2*	66.0*	77.4*	8.8	0.40	0.21
Post-molt		41.4	48.5*	45.0	50.4*	39.3	5.5	0.12	0.14

[1]Values are least squares means with 9 observations per treatment.
[2]Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) $CaCO_3$ or as all-fine $CaCO_3$.
[3]Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
[4]*P*-values from main effect of Ca pre-molt treatment or molt diet.
[5]Pooled SEM.
[6]IU/L = international units per liter.
[ab]Means within a row lacking a common superscript differ ($P < 0.05$).
*Means within a row differ from baseline value ($P < 0.05$). *P*-value from Dunnett's t-test.

CHAPTER 5. GENERAL CONCLUSION

Conclusion

This study was conducted to evaluate the effects of a fine- or coarse-calcium pre-molt treatment with a 7 d feed withdrawal, wheat middlings, or soybean hulls molt diet on laying hen behavior, production, and physiology.

The molt diets did not adversely affect laying hen behaviors and postures during or post-molt. The hens fed the wheat middlings or soybean hulls were less active during molt compared to the feed withdrawal hens which may allow them to save energy for another laying cycle. Additionally, the molt diets did not result in an increased H:L ratio compared to baseline values suggesting minimal effects on stress. Therefore, the wheat middlings and soybean hulls molting diets can be useful alternatives to a feed withdrawal molt with regard to laying hen behavior and well-being.

The fine-calcium pre-molt treatment was more effective at inducing molt compared to the coarse-calcium pre-molt treatment. During molt, the fine calcium resulted in lower egg production and ovary and oviduct weights allowing for a more complete molt. Post-molt, the fine calcium resulted in higher egg production compared to the coarse-calcium pre-molt treatment. The fine-calcium pre-molt treatment was successful regardless of which molting diet the hen was fed. The hens assigned to the feed withdrawal treatment had the lowest egg production during molt. When comparing the low-energy molt diets, the hens fed the soybean hulls molt diet had a more complete drop in egg production and ovary and oviduct weights compared to hens fed the wheat middlings molt diet. Therefore, these low-energy diets, particularly soybean hulls, in combination with a fine-calcium pre-molt treatment may be useful alternatives to feed withdrawal for inducing molt in laying hens.

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