EVALUATION OF THE USE OF ALFALFA DIETS AS AN ALTERNATIVE TO FEED DEPRIVATION FOR THE INDUCTION OF MOLT IN COMMERCIAL LAYING HENS

A Dissertation

by

KRISTIN L. LANDERS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2004

Major Subject: Food Science and Technology

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ABSTRACT

Evaluation of the Use of Alfalfa Diets as an Alternative to Feed Deprivation for the Induction of Molt in Commercial Laying Hens. (August 2004)

Kristin L. Landers, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Steven C. Ricke

Induced molting is process used by commercial producers to extend the reproductive life of a laying hen. Typically, producers deprive hens of feed for a period of 7 – 14 days while reducing the amount of light exposure to the hens. This allows for regression of the reproductive tract and for a second cycle of egg production to occur. However, induced molting by feed deprivation has been shown to increase the hen's risk of becoming infected with pathogenic bacteria, such as *Salmonella.* This increased risk could mean an increase in contaminated eggs or egg products, which causes concerns for public health. This combined with increasing pressure on egg producers from animal welfare organizations has prompted the investigation of diets that would provide available energy for the hens, while still inducing a molt that is economically advantageous to producers. Alfalfa, provided in meal or pelleted form, provides only ½ the metabolizable energy and ¼ of the calcium required of a laying hen that is reproductively active. Due to the decrease in nutrients, alfalfa was investigated as an alternative to feed deprivation. Studies were conducted to assess egg quality, egg production, consumer acceptance, and hen physiology.

DEDICATION

Diane Lynn Medvedev
June 18, 1946 – July 29, 1995

"I'll love you forever, I'll like you for always, As long as I'm living, My Mommy you'll be."
Love You Forever, by Robert Munsch

My mother cried whenever she read the book, Love You Forever, to me. As a 10 year old, I thought she was completely nuts. After all, who cries after reading a children's story? I now know that my mother cried because she knew what it was to give and to receive unconditional love, support, and kindness.

Mommy, thank you for teaching me what it means to truly love someone. Thank you for teaching me that empathy, compassion, and grace are things that are given freely to us from God to give to others. Thank you for sharing your life with me. You are never far from my heart.

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To my family: Agu and Peggy Medvedev and Eric and Kristina Medvedev. Thank you for all of your support over the past 8 years and for always giving me a place to come home.

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To Irene Zabala-Diaz. Thank you for commanding excellence out of me and for being my favorite friend. May you be playing meringue for your cells for many years to come.

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A special thanks to graduate students, past and present, who have made my life easier and the times much happier: Juliet Durant, Xin Li, Jeff Nutt, Cliff Froelich, Megan Kundinger, and Angela Kelley.

Lastly, thanks goes out to the love of my life, David Landers. Marrying you is the best decision I've ever made. Thank you for the laughter, the support, and all the fennema that goes along with it. I love you.

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CHAPTER I

INTRODUCTION

Molting is a process that occurs naturally in avian species either prior to or directly following the mating season (Mrorovsky and Sherry 1980). It is typically undergone to aid in replacement of feathers in wild birds, which aid in flight and can act as a secondary sexual characteristic. During a molting phase, birds will typically deprive themselves of feed for a period of several days until a significant loss of weight, as high as 35%, is achieved (Mrorovsky and Sherry 1980).

In the shell egg production industry, commercial laying hens can be induced into molt by manipulation of the hens' diets and lighting schedule. These manipulations often involve the removal of feed for a period of 7 – 14 days and changing the lighting schedule to one that allows less than 10 hours of light per day. This is often done at the end of a flock's first cycle of egg production, approximately 60 weeks of age. After the molt, hens will typically exhibit an increase in egg production and egg quality. This is advantageous, as producers can delay the need to replace flocks of older hens. The replacement of older flocks with younger pullets can be detrimental to the profitability of an operation since the facility would have to incur the price of rearing or purchasing more hens. Also, since younger hens produce more eggs, the law of supply and demand would dictate that the value of an egg would decrease. According to Bell (1996), an egg production increase of 1% would result in a 6% decrease in profits to egg producers.

This dissertation follows the format of Poultry Science.

Data compiled from 1994 to 1998 clearly showed that the trend that the price of eggs is a key determinant for whether or not flocks are molted, indicating that a shift in regional egg production indeed impacts the profitability of maintaining younger flocks (McDaniel and Aske, 2000). This trend highlights the importance of induced molting from an economic standpoint.

While induced molting has its definite egg production and business advantages, there may be some deleterious effects on hen health, which could ultimately increase the health risk to the consumers. According to work done by Holt (1992, 1994a,b) , laying hens molted by the use of a common feed deprivation methodology are more prone to infection with *Salmonella* Enteritidis, a human pathogen that has been widely implicated as a source of illness from the consumption of eggs. This research has centered around determining the specific risks associated with *Salmonella* Enteritidis and the induction of molt in commercial laying hens (Holt 1992, 1994a,b). If hens are challenged with *Salmonella* Enteritidis during a molt they have been shown to shed more of the organism in their fecal material, be an effective vehicle of infection to adjacent, non-challenged hens, and have decreased immunocompetence that would allow *Salmonella* to more readily invade and infect organs, including the ovary, more readily (Holt et al., 1992, 1994a,b). These risks, coupled with the increasing awareness of the practice of feed deprivation for the induction of molt by animal rights organizations, has prompted the investigation of diets that would effectively induce molt while providing the birds access to feed and possibly reduce the risk of *Salmonella* infection.

Alternative molting strategies have been in development for a number of years. In the early seventies, Creger used high levels of zinc, a known anorectic, to successfully induce molt in older laying hens. While this method was effective, the high levels of zinc created a toxicity in the hens, which resulted in an increase in mortality following the molt. This approach was modified by Breeding et al. (1992) by using two diets with moderately lower levels of zinc and calcium. This was done to decrease the toxic effects of zinc while still inducing a successful molt. Renewed interest in plant based diets with high fiber contents have been the most recent development in a dietary control strategy for the induction of molt. Diets composed of grape pomace (Keshavarz and Quimby, 2002), wheat middlings (Biggs et al., 2001; Seo and Holt, 2002), or alfalfa meal (Medvedev et al., 2001) have all been used as alternative molting diets. Both alfalfa meal (Kwon et al., 2001) and wheat middlings (Seo and Holt, 2002) have shown promise in reducing the rate of hens infected with *Salmonella* Enteritidis in both the gastrointestinal tracts and in organs such as the liver, spleen, and ovary. It is unknown whether this decrease in colonization is due to a healthier intestinal microflora, a low level of energy that is being used by the hen to maintain good immune function, or the constituents of the molting diets themselves. However, it is known that these strategies all bring about ovarian regression and some degree of weight loss, both key indicators for a successfully induced molt (Brake, 1992). The hormonal regulation of molting via both starvation and changes in the reproductive system are complex and not completely understood. Therefore, in order to truly comprehend the occurrences in the avian system that causes the phenomenon of molt, one must investigate the physiology of the

reproductive system , the intestine, and the blood of avian species both within and outside of the confines of a fasting state. Also, the reasons behind the existence of animal welfare movements should be discussed to further understand the treatment of laying hens from a layperson's perspective.

CHAPTER II

AVIAN REPRODUCTIVE AND GASTROINTESTINAL PHYSIOLOGY: A REVIEW OF LITERATURE AND ITS APPLICATION TOWARDS THE UNDERSTANDING OF INDUCED MOLT IN COMMERCIAL LAYING HENS

REPRODUCTIVE PHYSIOLOGY

Photoperiod and Egg Production

The reproductive system of a laying hen is regulated by a hen's exposure to light, a process known as photoresponsiveness. Exposure to light has stimulatory effects on production of Gonadotropin Releasing Hormone – I (GNRH-I), which controls the production of gonadotropins in the anterior pituitary (Sharp, 1993). There is a required amount of time that a bird must be exposed to light in order to initiate the hormonal responses necessary for ovulation. This is best described by the photoperiodic response curve. In laying hens, a minimum of 10 hours of light exposure is required for ovulation, with the maximum stimulation occurring after 12 hours of exposure (Sharp, 1984).

One of the major regulatory compounds associated with photoresponsiveness is melatonin. Melatonin is primarily released from the pituitary gland and eyes in Japanese quail (Underwood and Siopes, 1984). In the eye, ocular pacemakers, receptors for melatonin input, are connected to central oscillators in the hypothalamus which are regulated by melatonin output centers in the pituitary (Houdelier et al., 2002). High melatonin levels have been noted during periods of darkness and exposure to dim light in

pullets (Lewis et al., 2001), ducks (Zawilska et al., 2002), and quail (Houdelier et al., 2002). Higher levels of melatonin caused a suppression of leuteinizing hormone (LH) in castrated white leghorn roosters (Rozenboim et al., 2002). In these same roosters, it was noted that a constant administration of high levels of melatonin, 80mg/kg, caused a progressive inhibition of LH. The duration of this inhibition became longer as the time the birds were provided exogenous melatonin increased (Rozenboim et al., 2002).

General Avian Reproductive Anatomy and Follicular Development

Before a review of the hormonal regulation of avian reproduction, it is important to revisit the mechanisms surrounding follicular development and egg production. There are three stages of follicular development (Johnson et al., 2000) (Figure II-1). The first stage can take months to years to complete and involves an extremely slow rate of growth. Follicles ending the first stage of development are classified as being of 60-100 μm in diameter and are referred to as "small whites." The second stage of follicular development involves mobilization of yolk proteins such as vitellogenin and very low density lipoproteins from the liver into the developing follicle. A follicle will remain in the second stage for several months. Finally, a very rapid period of growth characterizes the third stage of follicular growth. Follicles will be in this stage for a period of 6 –11 days and will be the noticeably larger members of the follicular hierarchy. During this stage of development, yolk protein will be deposited into the follicle at a rate of 2g/day (Johnson, 2000).

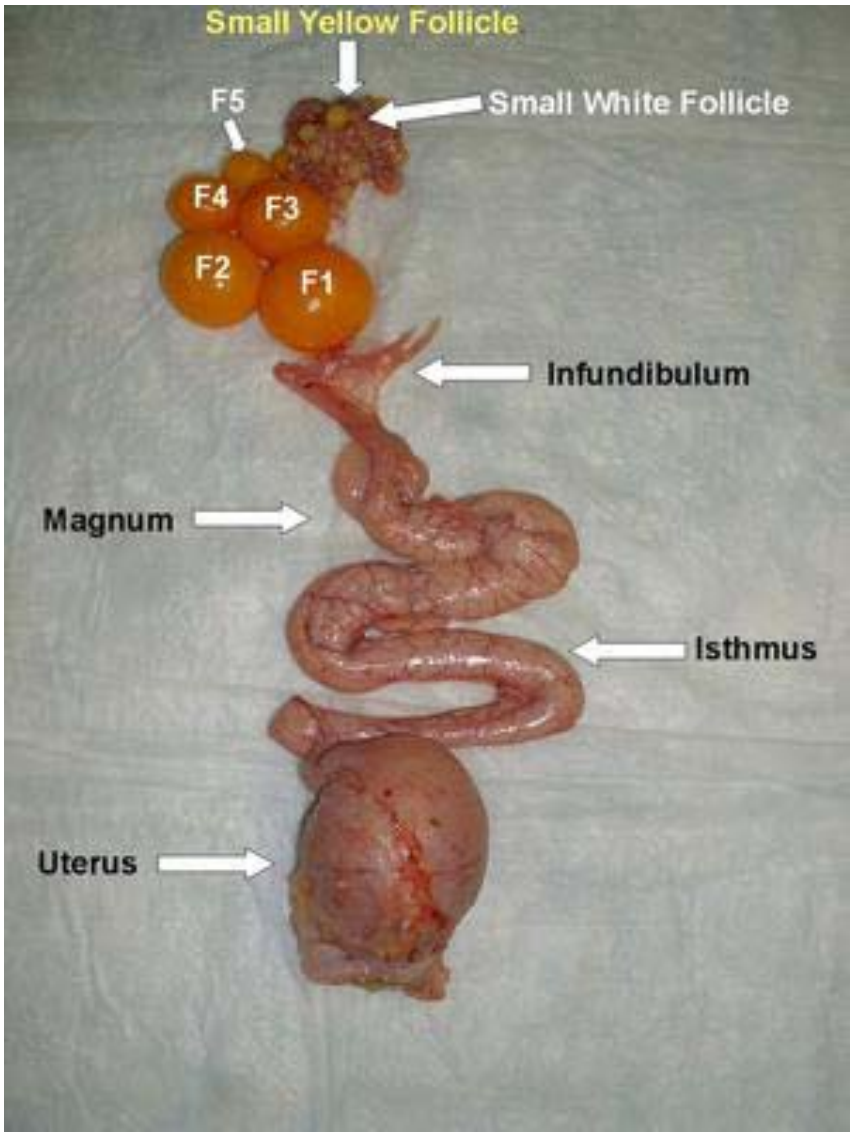

FIGURE II-1: The avian reproductive tract and follicular hierarchy. Original photograph by K.L. Landers and Z.R. Howard.

After a follicle has fully developed, there is a distinct timeline for the development of the egg and oviposition. Typically, ovulation of a mature follicle occurs every 24-26 hours in a bird that is in active egg production (Johnson, 2000). Just after ovulation, the follicle enters the infundibulum. While not directly attached to the ovary, the infundibulum's function is the uptake of the follicle for entrance into the reproductive tract. This step in egg development takes approximately 18 minutes (Johnson, 2000). Once in the oviduct, the developing egg spends 2-3 hours in the magnum. The magnum produces and secretes the albumen (Johnson, 2000). In the isthmus, inner and outer shell membranes are produced for a period of 1-2 hours (Johnson, 2000). Most of the time required for the development of the egg is needed for shell formation, which occurs in the uterus. The egg remains in the uterus for 18-26 hours where shell is laid at speeds up to 300 mg/hour (Johnson, 2000). Prior to shell formation, a fluid rich in ions and bicarbonate is pumped into the egg through the shell membranes in a process known as "plumping" (Salevsky and Leach, 1980). This created the "egg shape" and provides the platform for future shell deposition. After shell formation is complete in the uterus, the egg passes through the vagina where the waxy cuticle is laid on the exterior of the shell and is passed through the cloaca. Though the logistics of oviposition have been extensively observed and documented, there exists a complex relationship between follicular development, egg production, and steroidogenesis that should be more clearly investigated.

Gonadotropin Releasing Hormone (GNRH)

When discussing the function of GNRH, two hormones are of interest, GNRH-I and GNRH-II. These hormones are produced in the hypothalamus; receptors for these hormones have been located in the median eminence, a location where GNRH-I is secreted to mediate the production of gonadotropins such as Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and progesterone in the anterior pituitary (Sharp, 1993). GNRH-I regulates the production of these hormones via stimulation as photoresponsiveness. The increase in GNRH levels leads to preovulatory surge of (LH), the primary hormone responsible for the induction of ovulation of the F1 follicle. The levels of GNRH I or II in molted laying hens has not been documented. However, a photoperiodic responsiveness curve alluded to in the introduction does suggest why a lighting program is changed prior to the onset and during a molting phase. Since the minimum amount of light exposure is 10 hours, lowering the lighting program to below that level will aid in decreasing LH, which could lead to follicular atresia and a cessation of lay.

Short and long days are characterized by their stimulatory and inhibitory properties upon GNRH-I neurons in the hypothalamus (Sharp, 1993). Short days neither stimulate or inhibit GNRH-I production. Long days, however, are both stimulatory and inhibitory. The stimulatory effects on GNRH-I are immediate while the inhibitory effects can build over a period of several weeks. If the hens are exposed to the same lighting schedule for a long period of time, they can exhibit signs of photorefractoriness, which is typically noted by a decrease in egg production (Sharp, 1993). Since the

inhibitory effects of GNRH-I remain in the system for several days or weeks, changing the photoperiod during a molt can drastically improve the efficacy of the molt. A typical lighting programs for a molt would by 8h light:16h darkness, which is below the minimum amount of light required, according to the photoperiodic response curve (Sharp, 1984). Since there is no stimulatory effect on GNRH-I, all that remains is the inhibitory effects which would cause the bird to not ovulate (Sharp, 1993).

Luteinizing Hormone

LH is typically produced in the anterior pituitary. LH levels are responsive to changes in lighting schedule, as the change from a short day to a long day will increase LH levels (Wilson and Cunningham, 1981). A surge in LH also coincides with the development of secondary sexual characteristics such as reddening of the comb, the development of the ovary and reproductive tract, and medullary bone formation (Etches, 1990). Surges in LH generally occur 4-6 hours prior to ovulation with the lowest levels of LH occurring 11 hours prior to ovulation, which would normally be during darkness (Furr et al., 1973; Etches, 1990). LH also stimulates the production of other hormones important to follicular maturation. Incubation of small white, large white, and small yellow follicles with media that had been supplemented with LH caused a significant increase in androstenedione and estradiol production by the follicles (Robinson and Etches, 1986). The preovulatory LH surge has also been shown to a cause a large surge in progesterone production in the F1 and F2 follicles (Wells et al., 1985). Since the presence of LH is stimulatory to production of androgens and estradiol in smaller,

developing follicles, it can be assumed that receptors for LH can be found throughout the follicular hierarchy (Robinson and Etches, 1986). However, in a similar study using large yellow follicles, treatment with LH did not result in an increase in androgen production unless DHEA was added in the F1 follicle (Robinson and Etches, 1986). This could be indicative of a shutdown of the pathways responsible for the production of androgens in the higher order follicles (Robinson and Etches, 1986).

During a molt, LH drops to basal levels (0.4 ng/ml) in male emperor penguins (Groscolas et al., 1986). This was mirrored in male peking ducks (Jallageas et al., 1978) and willow ptarmigans (Stokkan and Sharp, 1980). This decrease in LH during molt was also seen in female white crowned sparrows and bar headed geese (Wingfield and Farner, 1979; Dittami and Hall, 1983).

Follicle Stimulating Hormone (FSH)

FSH is a gonadotropin produced in the anterior pituitary. FSH is more important to small, developing follicles than the larger follicles of the hierarchy (Etches and Cheng, 1981). This was confirmed by Wells and coworkers (1985). LH stimulation results in double the progesterone levels when compared to stimulation with FSH, signifying a decrease in importance to late stage developing follicle (Wells et al., 1985). However, it was also found that FSH stimulated estrogen secretion in the thecal cells of the F3 and F4 follicles (Onagbesan and Peddie, 1988).

FSH levels and molting is yet another area that is not well documented. One would expect levels of FSH to drop dramatically, in accordance to with the drop in LH,

especially since they are both regulated by GNRH. It has been shown that FSH requires exogenous calcium in order for it to be effective in stimulating estrogen production in the F3 and F4 thecal cells (Onagbesan and Peddie, 1989). This could lend credence to the use of low calcium molt diets, as a way to decrease FSH which would slow follicular development and ultimately result in a cessation of lay.

Progesterone

Progesterone is primarily produced in the granulosa of the large yellow follicles (Etches, 1990). Receptors for progesterone are present in the hypothalamus which allows for the positive/negative feedback relationship between progesterone and LH. This positive/negative feedback response can best be described by what must occur hormonally before a follicle can be ovulated. It has been shown that progesterone levels increase either prior to or in conjuction with the preovulatory LH surge (Furr et al., 1973; Peczely et al., 1980). This has been confirmed when quantifying hormone levels in the three largest preovulatory follicles, F1 – F3 (Shahabi et al., 1975). Progesterone levels peaked at 8 to 10 hours prior to the next scheduled ovulation, which is prior to when the preovulatory LH surge would occur (Shahabi et al., 1975). Progesterone also peaks synchronously with testosterone and estrogen in preovulatory follicles (Shabhabi et al., 1975) and with estrogen in the plasma of laying hens (Lague et al., 1975). Progesterone has also been implicated as being a hormone involved in the development of the reproductive tract of avian species. In Japanese quail, the first part of magnum growth is preceded by a decrease in plasma levels of progesterone (Pageaux et al., 1984). The

growth of the oviduct was rapid between 21 and 28 days of age. Prior to reaching 21 days of age, developing quail experienced a decrease in plasma progesterone, from 0.815 nmol/l to 0.502 nmol/l (Pageaux et al., 1984). Additionally, while the magnum is developing, progesterone receptors in the magnum increase substantially, from 5500 sites/cell at the beginning of development to 30,300 sites/cell at the onset of lay (Pageaux et al., 1986). This is logical as progesterone has been shown to stimulate production of egg white proteins (Oka and Schimke, 1969; Palmiter and Wrenn, 1971).

Progesterone is a hormone of importance when considering the conditions of an induced molt. Providing progesterone to birds either orally or through intramuscular injection can induce molt (Adams, 1955). Progesterone levels increased at the beginning of a feed deprivation induced molt (Szelenyi et al., 1983). These levels then decreased until day 35 of the molting procedure (Szelenyi et al., 1983). When the molt was hormonally induced with progesterone and T_3, progesterone levels were again high in the beginning of the molt, but decreased late in the procedure (Szelenyi et al., 1983). Progesterone levels in hens molted by feed and water deprivation were significantly higher than nonmolted hens by day 5 of the molting procedure (Pethes et al., 1981). Therefore, increased progesterone levels could be an initiator of molt in laying hens.

Androgens

Androgens are usually converted from progesterone in the thecal cells of large yellow follicles. Androstenedione is produced in significant amounts in the smaller follicles, although this is usually produced without the presence of progesterone

(Robinson and Etches, 1986; Culbert and Wells, 1986). The role of androgens in follicular development is certain since it is produced by small follicles, however, the specific role of androgens are not completely understood. In the larger yellow follicles, it may have a role as a being a conversion from progesterone. While progesterone was not detected in F2 and F3 follicles, higher levels of testosterone were seen (Shahabi et al., 1975). The high levels of progesterone are not converted to androgens in the F1 follicle, which could lead to the positive feedback response that would cause the surge in LH prior to ovulation (Robinson and Etches, 1986). Rises in testosterone levels in preovulatory follicles were seen at 4 and 8 hours after ovulation and peaked, in concert with LH, progesterone, and estrogen, at 4 to 6 hours prior to ovulation (Shahabi et al., 1975). In plasma, however, testosterone levels peak at 10 to 12 hours after ovulation had occurred (Shahabi et al., 1975).

Androstenedione levels have not been assessed in the molted laying hen. However, the production of testosterone, another androgen, was shown to decrease to basal levels in male emperor penguins during a molt (Groscolas et al., 1986). This contradicts, however, what occurs in the molted laying hen. Testosterone has been shown to increase late in the molting procedure, when molting was induced by either feed deprivation or hormonally with progesterone and T_3 (Szelenyi et al., 1983).

Prostaglandins

Prostaglandins are produced in the large yellow follicles and the post ovulatory follicle. Prostaglandin F2 α (PGF) and Prostaglandins E1 and E2 (PGE) have been

associated with the preovulatory surge of LH and have been implicated as a hormone involved in uterine contraction prior to oviposition. Turkeys injected with PGE had premature ovipostion within 13 minutes of the administration of PGE (Hammond et al., 1981). PGF and PGE have also been shown to increase the secretion of prolactin in immature males (Hall et al., 1984; 1985). Since prostaglandin plasma concentrations rise during oviposition, it is not surprising that they would be stimulatory to prolactin, the hormone responsible for broodiness in the domestic fowl (Hammond et al., 1981; Hall et al., 1985; Hall et al., 1984). PGF has also been shown to decrease the ovarian blood flow to the major preovulatory follicles, F1 – F5 (Scanes et al., 1982).

Triiodothyronine (T_3) and Thyroxine (T_4)

Although T_3 and T_4 are not gonadotropins, they do affect reproductive activity in the laying hen. Hens fed 59 mg/lb body weight of desiccated thyroid glands went through a natural molt more quickly than hens that were not fed thyroids (Cole and Hutt, 1929). T_3 levels were significantly lower in feed and water deprived hens when compared to fully fed controls (Brake et al., 1979; Pethes et al., 1981). However, T_4 levels in feed and water deprived hens were low at the beginning of the molt and increased to levels that were comparable to the fully fed control hens in the latter half of the molt (Brake et al., 1979). T_4 levels were also increased during the natural molt of female rooks (Peczely and Pethes, 1982). During the regrowth of feathers after two days of feed and water deprivation, increased levels of T_3 and T_4 were seen in both male and female Eurohyb chickens (Pethes et al., 1981). However, it has also been shown that the

release rate of thyroid hormones does not differ between molted and nonmolted hens (Tanabe et al., 1957).

THE INTESTINE

Since the intestine is the primary site of digestion and nutrient absorption, it can be assumed that starvation would alter the function of this organ. In order to discuss how starvation affects the intestine, a brief review of the structure of this organ as well as the cells that facilitate its function is warranted. There are four layers to the small intestine, the first of which consists of the squamous epithelium, peritoneum, and connective tissue with elastic fibers. Blood vessels and nervous tissue can be found where the mesenteries are formed (Hodges, 1974). The second layer is made of a thin muscular external layer followed by a much thicker circular layer of muscle. In between the layers of muscle, there is some elastic connective tissue and well developed nervous tissues (Hodges, 1974). In the third layer there is a very poorly developed layer of submuscosa, which is characterized by a thin layer of connective tissue and elastic fibers. There is also a nerve plexus in the submucosa which is akin to the Meissner's plexus in mammalian systems. The fourth layer consists of the intestinal mucosa. This is characterized by leaf-shaped villi that are in a zig zag pattern in the avian intestine. The crypts of Lieberkuhn are situated between the villi and take up most of the space between the villi and the third layer that has the muscularis mucosae. The remaining space is filled with lymph tissue, blood vessels, and connective tissues. This layer is the

innermost layer of the intestinal tract and is the location of production of cells that are important to digestion.

There are several types of cells that are of importance in the intestinal tract of animals. These cells originate in the crypts of Lieberkuhn where they are created via mitosis and differentiated (Toner, 1971). Mucous goblet cells can be found throughout the small intestine in avian species, while they are mainly located in the colon in mammals (Toner, 1971). Up to one half of the cell's area is taken up by mucous that is secreted by this cell. As the name implies, these cells are of goblet shaped and are found most often near the crypts, although they are also dispersed between chief cells and villi (Hodges, 1974). Their main function is to secrete a mucous containing both carbohydrates and proteins to aid in the lubrication of the digestive tract (Toner, 1971). The presence of paneth cells in avian species has been the subject of debate among researchers (Hodges, 1974). While they are not thought to occur in domestic fowl, they have been observed in other species of birds (Hodges, 1974). In mammals, the paneth cells contain lysozomes that may be of some antibacterial benefit to the small intestine (Toner, 1971). There are also thoughts that since the paneth cells are found in large numbers by the crypts that they may also have a function in cell production or differentiation (Toner, 1971). Enterochromaffin cells are basically endocrine cells in the digestive tract (Toner, 1971). They are located in the crypts and in small numbers on the villi (Toner, 1971; Hodges, 1974) and contain 5-hydroxytryptamine, or serotonin (Penttila, 1968). Serotonin was also found in increased amounts in the hypothalamus during feeding of chicks, suggesting that serotonin plays a role in the consumption of

food (Tachibana et al., 2001). This is substantiated by the fact that both male and female domestic fowl have serotonin concentrations in their small intestine (Gross, 1975). Several cells have also been found to be immunoreactive to serotonin in the avian small intestine (Yamanaka et al., 1989). In the rabbit, serotonin alters motility of the small intestine by intensifying the contractions of intestinal muscle (Salvador et al., 2000). Contraction frequency and intensity was also increased in equine small intestine samples (Weiss et al., 2002). When serotonin was provided intraluminally to rats, the transportation of fluids through the ileum and jejunum and absorption was decreased (McLean and Coupar, 1998).

The majority of carbohydrates, amino acids, fats, and electrolytes are absorbed in the small intestine (Denbow, 2000). Most of this absorption is facilitated by the chief cell, or primary absorbance cell. It is a long columnar cell that is the primary cell in the villus structure (Hodges, 1974; Toner, 1971). Along the membrane, microvilli are situated, forming a border around the cell. These microvilli are covered in small filaments that extend as far as 0.15 μm from the tip of the microvilli. The microvilli extend into the cell and are "rooted" in the terminal web. The terminal web is also the site within in the cell that provides junction points for communication between adjacent cells. (Hodges, 1974). The primary function of the 600-3500 microvilli on the cell membrane is to increase surface area for absorption of nutrients. According to Toner (1971), the exact figure of how much absorption is enhanced by the presence of the microvilli are unknown, although a good estimate would be 24 times that of what digestion would be without the presence of microvilli. The role of microvilli in the

efficiency of digestion would be more apparent in severely diseased individuals with a rapid cell turnover, as microvilli are more predominantly developed after a cell has matured (Toner, 1971).

The mode of digestion by the chief cell is not completely understood. Small vesicles in the membrane of the cell as well as the crypts could be used for the transport of nutrients, although this is not well documented (Toner, 1971). Disaccharidases and peptidases have been found along the brush border of microvilli. These enzymes are attributed to the "fuzzy coat" which surrounds the chief cell. This coat is primarily constructed of glycocalyx, which is resistant to proteolytic enzymes, an important feature of a cell that would function in the gastrointestinal tract (Ito, 1969). This coat tends to dissipate as the cell ages, suggesting that the layer is a protective agent to a functional digestive cell (Toner, 1971).

While the characteristic "zig zag" appearance to the avian intestinal villi is considered ideal, villi appearance may be altered due to age of the individual, site of the intestine studied, presence of any intestinal diseases, and diet (Toner, 1971). According to Toner (1971), in humans, villus structure differs between geographic regions. In Britain, a decreased amount of the finger shaped villi would indicate a sign of poor absorption and intestinal disease. However, a healthy individual in Africa can have several shapes to their villi, with infrequent amounts of the "normal" finger-like villi (Toner, 1971).

The main effect of starvation on the intestine concerns the structure of the villi and the covering of the intestinal tract with epithelial cells. In healthy individuals, crypts

would produce the cells needed for villi formation (Toner, 1971). During diseased states, the crypts can produce cells more quickly, to almost double the speed of when the individual is healthy. However, there will come a stage in the disease where the cells cannot be rejuvenated quickly enough to be replaced which would create holes in the epithelial lining. At this point, the structure of the villus could change to allow for complete coverage of the small intestine. While the finger like villus structure is the optimal arrangement of villi in the human intestine, this structure also has the highest cell requirement. Villi structured to be leaf shaped require only 25% of the cells normally needed for villi structuring. In advanced disease, where the villi appear to be flattened, only 3% of what is normally required is needed. This means that the crypts can produce cells quickly and still maintain coverage of the small intestine during disease states (Toner, 1971). There have been studies in birds that have demonstrated the same results: starvation results in a sloughing of epithelial cells at such a rapid rate that the crypts cannot produce cells quickly enough to replace them. This results in a conformational change in the structure of the intestinal muscosal layer. In white leghorn chicks, villus height, cell area, and the rate of cell mitosis by the crypt were significantly reduced when the chicks were deprived of feed for a period of 3 days (Shamoto and Yamauchi, 2000). Broilers that were subjected to feed deprivation for a period of 3, 5, or 7 days had similar reductions in villus height. After a 3 day fast, sloughing of intestinal epithelial cells was apparent. By day 7, the villi in the ileum appeared to be assuming a different, invaginated conformation and the total number of villi was lessened (Bayer et al., 1981). Bayer (1981) also observed morphological changes to chief cells in the duodenum after a

period of fasting (Bayer et al., 1981). This leads to the conclusion that a long term fasting state would be damaging to the intestine, thusly making the birds more susceptible to systemic infections with pathogens, such as *Salmonella*. This intestinal damage, therefore, may be one of the reasons that molted birds are more susceptible to *Salmonella* infection (Holt et al., 1992).

ANIMAL WELFARE

The most controversial aspect of induced molting by deprivation of feed involves the concept of animal welfare. Animal rights organizations have launched campaigns taking a stance against induced molting that have reached levels as high as the state legislatures in California and Illinois, where laws banning the use of induced molting in laying facilities have been introduced (California, 2000; Illinois, 2001). Animal rights groups have been quite outspoken about the commercial egg industry, and have used intense emotional language to cause consumers to empathize with the animals. For example, Ingrid Newkirk, the co-founder of People for the Ethical Treatment of Animals (PETA) described the life quality of a laying hen as this :

> The life of a 'layer' is even worse than that as a 'broiler' because it lasts longer and because layers' beaks are seared off with a hot wire. (…) This is done to prevent cannibalism that can occur in birds who are denied the room to establish a pecking order. The birds also experience chronic leg pain. They are kept in constant light to fool their bodies into churning out more eggs than is normal (a single egg on the breakfast plate is 22 hours of misery for the hen) and periodic 'false moltings' mean farmers withhold *all* food from birds for up to *fourteen days*. Their hunger must be indescribable as they drop not only their feathers, but up to 30 percent of their body weight (Newkirk, 1999).

Birds in nature deprive themselves of feed in order to induce molt (Mrorovsky and Sherry, 1980). In fact, studies by Mrorovsky and Sherry (1980) have demonstrated that birds will deprive themselves of feed during times of broodiness, even if feed is readily available. Therefore, if it is within a bird's physiological capability to undergo a molt, and thusly feed deprivation, then what is the ethical dilemma for producers to consider? Does physiology or the social conscience of our society drive the cause of animal welfare? To answer these questions, the theories of anthropomorphism and the physiological parameters scientists use to assess welfare in avian species will be reviewed.

Anthropomorphism occurs when human beings try to assign human behaviors or emotions to animals. This means of reasoning assumes that the animal would have the same response to a set of conditions as a human. For example, Newkirk (1999) offered several U.S. poultry industry leaders the opportunity to live in a caging system designed to mimic the conditions of a commercial laying facility for 24 hours. The thought was that if the people in authority could relate their feelings of being housed in a manner akin to that of a laying chicken, they would associate those feelings with the hens and change their ways of rearing their animals. While this example may seem to be a mere publicity stunt to raise awareness of PETA's stance on commercial laying facilities, the concept of using anthropomorphism to understand animal mental status is not entirely without merit. Caporael and Hayes (1997) wrote that the use of anthropomorphism in research could be a way of uniting the scientific community with animal rights activists. Since anthropomorphism "changes the way humans perceive animals, and limits and entrains

what actions are conceivable, undesirable, and essential," the authors feel that scientists allowing themselves to assign certain "human-like" behaviors to animals may help to bridge a gap between scientists, who are widely perceived by activists as being cold-hearted, and activists, who can be perceived as zealous (Caporael and Hayes, 1997).

Anthropomorphism is also a large part of a layperson's view on the way animals should be treated. College students at the University of Tennessee were given a questionnaire that asked for the respondent to give their opinions on 18 species of animals and their ability to experience mental states and sensations that would be consistent with a human-like experience (Herzog and Galvin, 1997). The researchers showed that on average, people were more likely to associate higher ordered mammals, such as dolphins and chimpanzees, and animals typically kept as pets, such as dogs and cats, with the ability to have at least a moderate level of consciousness. However, the results of the study concluded that people think that most animals, including invertebrates, had the potential to feel pain and suffering (Herzog and Galvin, 1997). Respondents noted on the questionnaire that any animal that could sense pain is deserving of moral consideration, or ethical treatment that would avoid any painful stimulus that was not necessary (Herzog and Galvin, 1997). This suggests that while it may be within the physiological capabilities of an animal to undergo certain stresses, consideration should be provided to them if these stresses could cause pain or suffering (Herzog and Galvin, 1997).

Researchers have devised ways to assess the mental state, or stress level of an animal. In hens, the most common way of quantifying stress is by the use of the

heterophil:lymphocyte ratio. An increase in heterophils can be indicative of a stress leukogram, which could predispose the bird to diseases (Gross and Siegel, 1983). There are also physical observations that could be conducted to ascertain stress level. Stressed birds are less likely to stand up quickly during tonic immobility studies (Jones and Faure, 1981). Birds undergoing stressful situations are also more likely to have ruffled feathers. While these methods are effective at determining stress levels of hens, they do not provide a complete picture of animal welfare. Therefore, there will continue to be speculation as to the mental status of a laying hen during a period of fasting. Since humans tend to think in an anthropomorphic manner, researchers must either search for alternatives to feed deprivation that satisfy the concerns of animal rights activists or they must devise ways to prove that since feed deprivation is physiologically viable in a hen, suffering of the animal is of minimal concern.

BLOOD

When considering the effects of starvation on an animal, researchers and clinicians are most likely to resort to analysis of blood chemistries and hematology to determine the animal's relative health. It is imperative to discuss what would be considered "normal" avian chemistry levels to the chemistry levels of a fasted bird to ascertain what the effects of starvation are on parameters that are measured through blood analysis. Typical analyses of avian blood would warrant investigation of the following parameters: uric acid, triglycerides, cholesterol, and glucose.

While normal values for galliformes are available, the ranges are often too wide to be useful due to the variation that exists based upon breed, age, and sex of the bird (Ritchie et al., 1994). Therefore, it would be considered more optimal to compare chemistry values from starved birds to the chemistry values of birds that were allowed access to relatively the same environmental conditions. When discussing relative literature to starvation and chemistry panels, comparison to normal values will be made within the controls of the experiments discussed.

Uric acid is a parameter that is used often when evaluating the health of a bird. It can be used to assess hydration as well as renal function (Phalen, 2000). An elevated level of uric acid could be a sign of proximal tubular damage or dehydration. However, the use of uric acid to quantify renal function is not always reliable since birds can have severe kidney dysfunction and still have normal uric acid levels (Phalen, 2000). Additionally, uric acid levels can be used to determine whether catabolism of amino acids is ongoing, when used concomitantly with plasma nitrogen levels (Simon, 1984). Uric acid levels also seem to be somewhat dependent on the length of feed deprivation. In chickens, feed deprivation for 48 hours did not affect uric acid levels significantly from fully fed birds (Radin et al., 1996). Additionally, higher uric acid levels were not noted in turkeys deprived of feed for 16 hours (Cason and Teeter, 1994). However, in leghorn hens that were fasted for 7 days, uric acid levels increased markedly by day 3 of feed deprivation during a molt (Buyse et al., 1995). Buyse stated that the changes in uric acid over the course of the molt signified changes in body conformation such as weight loss and the reduction in size of the abdominal fat pad (Buyse et al., 1995).

Triglycerides and cholesterol are of particular importance when discussing starvation in the laying hen. During a molt, triglyceride levels will decrease and cholesterol levels will increase when compared to a non-molted hen that is in production. This is because during reproductive activity, triglyceride levels remain high due to movement of lipids from the liver to the developing follicles (Tanaka et al., 1986). Since follicular development is halted during a period of fasting due to a marked decrease of circulating gonadotrophins, follicular atresia also occurs. This results in the absorbance of the lipoproteins in the follicles into the bloodstream, thusly increasing cholesterol levels (Barron et al., 1999). Another reason for the increase in cholesterol could be due to the formation of a new structure of HDL, known as HDL_R. HDL_R was first noticed in overfed chickens and was a sign of failure of yolk deposition (Walzem et al., 1994). This fraction has since been observed in Single Comb White Leghorns (SCWL) after 5 days of feed deprivation (Barron et al., 1999). Therefore, a concurrent decrease in triglycerides and increase in cholesterol could be interpreted as a sign of involution of the reproductive system and cessation of follicular development, and thusly, a successful molt. Research results seem to substantiate this thought. Tsaiya ducks that were in the first laying cycle, 10-14 weeks after onset of lay, or were young, growing birds, 8 – 12 weeks of age, were deprived of feed for 3 days. While both young and laying birds exhibited the characteristic decrease in triglycerides and increase in cholesterol, the laying birds displayed a marked decrease in triglycerides from 1456.43 mg/dl in fed birds to 247.03 mg/dl in fasted birds (Lien et al., 1999).

Levels of minerals such as magnesium, calcium, and phosphorus are also important when discussing egg laying birds. Calcium levels begin to increase in birds starting at hatch and will become static around sexual maturity (Combs et al., 1979). In a study by Parsons and Combs, the ionized calcium levels of both non-laying pullets and hens that were in production were assessed (1981). It was found that the ionized calcium levels of birds that were not in production were dependent on the time of day, with levels being the highest at 1.5 hours prior to the onset of light (Parsons and Combs, 1981). Birds that were in production had their highest levels of calcium production directly following ovipostion and their lowest levels were noted after a developing egg entered the uterus (Parsons and Combs, 1981). This is substantiated by Dacke and others' study of mature Japanese Quail (1973). Total plasma calcium levels of hens were lower at 9-14 hours post ovulation than at 0-7 hour post ovulation (Dacke et al, 1973). Also, hens genetically selected for a tendency for producing eggs with thick shells had a significantly higher level of total plasma calcium (Wideman and Buss. 1985). Additionally, hens intake more calcium on days when there is ovulation and oviposition (Hughes, 1972). While intake of food was highest early in the morning prior to oviposition, calcium intake was highest on the day of ovulation, with a peak intake as the developing egg entered the uterus (Hughes, 1972). Levels of phosphorus in chicken serum or plasma also seem to be dependent upon the reproductive cycle of the hen. (Miller et al., 1977;1978). Phosphorus levels were increased on the day of and following oviposition (Miller et al., 1978). Phosphorus levels peaked at around 4 hours prior to oviposition and were at their lowest at $\pm$ 2 hours of oviposition (Miller et al., 1977).

Magnesium levels in the hen seem to be positively correlated with calcium levels. Both calcium and magnesium were at their lowest levels when a developing egg was in the uterus of a leghorn hen (Hester et al., 1979; Kansal and Gangwar, 1982). Seasonally, magnesium and calcium levels were both at lower levels during the summer months due to decreased bone remineralization (Kansal and Gangwar, 1982).

Hematology is another method used to assess the health of a bird. One of the most common hematological assessments is the CBC, or complete blood count. This typically involves counting a set number of cells and calculating percentages of heterophils, monocytes, lymphocytes, basophils, and eosinophils. Another hematological test is the aforementioned heterophil:lymphocyte ratio, which is used to assess the stress level of the bird (Gross and Siegel, 1983). As with blood chemistries, published normal values for galliformes are available; however, the ranges are often too wide to be used diagnostically (Ritchie, 1994). For example, a normal value for percentage of lymphocytes can range from 45-75% (Ritchie, 1994; Shales and Shales,1983). Therefore, comparisons of values from hematological testing will be made with the controls.

There have been limited amounts of research conducted on the effects of feed deprivation on hematology. In young leghorns, birds that were fasted for two days had higher heterophil:lymphocyte ratios than birds that endured stresses such as handling and vaccination (Prabhakaran et al., 1997). Gross and Siegel found that an initial two day period of fasting resulted in an increased heterophil:lymphocyte ratio in laying hens (Gross and Siegel, 1986). However, subsequent periods of fasting using the same birds

caused no significant increase in the heterophil:lymphocyte ratio, which suggested that birds can adapt to periods of fasting (Gross and Siegel, 1986). This was further substantiated by Katanbaf and others who found that broiler breeders adapted to being fed according to an alternate day feeding regimen (Katanbaf et al., 1988; 1989). Heterophil:lymphocyte ratios increased after a 14 day induced molt in laying hens (Brake et al., 1982); however, long periods of fasting greater than 4 weeks did not cause a significant increase in heterophil:lymphocyte ratios in broilers (Maxwell et al., 1990). Longer periods of feed deprivation have been shown to increase the number of basophils, which suggests that while the heterophil:lymphocyte ratio can be used to assess mild or moderate stress levels, the presence of basophilia may be more indicative of severe stress levels in birds (Maxwell et al., 1992; Maxwell, 1993). In chicks fasted for 18 and 36 hours posthatch, heterophil:lymphocyte ratios were not significantly different from controls at 21 and 42 days following hatch, indicating that an increased ratio does not necessarily indicate a long term health risk (Gonzales et al., 2003). Additionally, birds with high heterophil:lymphocyte ratios in response to a stress are more likely to have progeny that exhibit the same tendency towards higher ratios (Al-Murrani et al., 1997).

RESEARCH OBJECTIVES

The factors of hen physiology, egg quality, and animal welfare discussed in the preceding pages details the need for an alternative to feed deprivation for the induction of molt. Alfalfa, provided in meal or pelleted form, provides only ½ the metabolizable

energy and ¼ of the calcium required of a laying hen that is reproductively active. Alfalfa has also been used as a feed additive in commercial poultry production as a source of xanthophyll pigments and as a protein concentrate. These factors are what allowed our lab to consider alfalfa as a possible alternative to feed deprivation for induced molting. Three experiments were conducted to assess the ability of alfalfa molting diets to induce molt. Firstly, hens were either provided *ad libitum* access to alfalfa or feed deprived for 9 days. Afterwards, ovarian weights were obtained from half the hens while the other half of the hens were allowed to enter into a second cycle of production. Egg production and egg quality were monitored for a period of time to assess how molting by alfalfa effects egg production and quality early in the second cycle. The second experiment involved sensory testing of eggs from a commercial flock that was molted using alfalfa. Eggs were received prior to the molt and 2 weeks into the second cycle of egg production from both feed deprived and alfalfa molted flocks. Mechanical evaluations such as shell strength, albumen height, yolk height, weight, and length were obtained. Additionally, two sensory panels were conducted using eggs from the flocks both before and after the molting procedure. Finally, studies were conducted to assess the physiological state of the hen during an induced molt using either an alfalfa diet or feed deprivation molting method. During the molting procedure, blood was drawn from birds via the jugular vein. Heterophil:lymphoctye ratios and chemistry parameters such as uric acid, glucose, total protein, ketone bodies, magnesium, phosphorus, calcium, triglycerides, and cholesterol were obtained. Additionally, after the molting procedure hens were sacrificed and organ weights were obtained for kidney,

heart, spleen, ovary, oviduct, intestine, pancreas, adrenal gland, and liver. These experiments have helped in the assessment of the use of alfalfa as an alternative to feed deprivation for the induction of molt.

CHAPTER III

EFFECT OF ALFALFA DIETS ON MOLT INDUCTION, POST-MOLT EGG PRODUCTION, AND EGG QUALITY IN COMMERCIAL LAYING HENS

SYNOPSIS

Molting is a process by which a hen's reproductive tract is rejuvenated prior to the beginning of a laying cycle. This process is often induced in commercial settings in order to extend the productive life of a flock of hens. The most common method for the induction of molt is feed deprivation for a period of several days. It has been noted that feed deprivation, while effective in inducing molt and allowing an adequate reproductive rest period for the hen, may cause deleterious effects on the animal. This has prompted the investigation of alternatives to feed deprivation for the induction of molt in commercial laying hens. Alfalfa, a fibrous feed with low metabolizable energy, may be provided to hens on an *ad libitum* basis. This study involved feeding alfalfa to hens to assess its ability to induce molt as well as to monitor post molt egg production and quality.

INTRODUCTION

In nature, birds undergo a process called molting once a year, where they lose up to 40% of their body mass and their reproductive systems regress (Mrosovsky and Sherry 1980). Upon completion of the molting process, the reproductive system is rejuvenated, allowing the bird to enter into an egg production cycle. In commercial systems, this

process is often induced in older table egg laying hens to increase egg production. In fact, according to the National Animal Health Monitoring System (NAHMS) Layers '99 report, 94.9% of commercial laying facilities in the western United States, which includes Texas, use induced molting as a means to increase flock productivity. The most widely used method of inducing molt is by the withdrawal of feed for 7 –14 days. This is an extremely efficient way to induce molt since it is easy to organize and allows the producer to avoid feed costs during the molting period (Brake 1992). However, it is becoming more apparent that a need for an alternative to this approach is needed. First, public awareness of molt induction by feed deprivation has increased over the years. More importantly, researchers suggest that molting by feed deprivation leads to greater colonization of the laying hen by *Salmonella* and may also increase the expression of virulence genes within the *Salmonella* chromosome (Durant et al 1999; Holt et al., 1992; 1994a,b).

According to North and Bell (1990), the most effective alternatives to feed deprivation for the induction of molt must be able to consistently molt the flock quickly, be cost effective, and not cause deleterious effects to egg production and quality during the second laying cycle. Researchers have attempted to find alternatives to feed deprivation that would fulfill these requests. Research has been previously directed towards formulating low energy basal diets that would induce molt when fed on a restricted basis (Brake et al 1979, Swanson and Bell 1974, Koelkebeck et al 1993, Rolon et al 1993). Recently, the use of insoluble plant fibers and byproducts have been investigated as well. Alternative diets have been developed from grape pomace

(Keshavarz and Quimby 2002) and wheat middlings (Biggs et al 2001, Seo and Holt 2001). The method used in this study involves feeding birds a single, insoluble fiber source, alfalfa. In the past, alfalfa has been investigated for use in commercial laying facilities as a means of pigmenting egg yolk (Guenthner et al., 1973). Alfalfa has also been investigated as a concentrated protein source for poultry (Tsigbe et al., 1987; Dale et al., 1984). It has been previously shown that alfalfa has one of the slowest passage rates in the avian gastrointestinal tract (Sibbald et al 1979 a, b; 1980). This is extremely advantageous, since alfalfa is readily fermented by indigenous microorganisms in the avian gastrointestinal tract (Ricke et al., 1982; van der Aar et al., 1983) and a slow passage rate would allow for a greater break down of feed and more extensive microbial fermentation. Due to the high protein content, alfalfa may promote a feeling of satiety in birds, which may reduce some of the stresses involved with feed deprivation.

The objectives of this study were to investigate as to whether alfalfa in pelleted or meal form would induce molt and to monitor egg production and quality as the hens entered the second cycle of production.

MATERIALS AND METHODS

150 SCWL hens aged 70-80 weeks were obtained from a commercial laying facility. Birds were placed 1 bird/cage at the Texas A&M University (TAMU) Poultry Science Research Center. The hens were provided *ad libitum* access to a complete layer ration and water for a period of 8 weeks. During this time, egg production was

monitored to insure that all hens were healthy and in active production. After the acclimation was complete, 116 hens were moved to different cages (placed 2/cage) for the molting procedure. Hens were divided into one of four treatment groups: Alfalfa meal, Alfalfa pellet, TAMU layer ration (nonmolted control) and feed withdrawal (negative control) (Table III-1). All treatments were allowed *ad libitum* access to water and their respective diets. Birds were placed on a lighting program of 8h light: 16h dark for one week prior to the beginning of the molt (Holt 1994). Treatments were placed throughout the house to ensure there was no variability in egg production or reproductive tract regression due to light stimulation.

Premolt egg quality data was also collected during the acclimation period. Exterior egg quality was graded according to defined USDA standards (USDA, 2000). Eggs were candled to determine thickness of the albumen and size of the air cell. Egg weight (g), circumference (cm), and length (cm) were determined. Interior quality including albumen height (mm) and yolk height (mm) were also measured.

During the molt, bird weights were monitored at day 1, 3, 5, 7, and 9. In accordance with Texas A&M University Lab Animal Care Committee (ULACC) animal use protocols, any hens reaching 25 % weight loss prior to the end of the trial (day 9) were removed from their respective diet. At the end of the molt, 58 birds were sacrificed and their reproductive tracts weighed. The remaining 57 birds were provided TAMU layer ration on an *ad libitum* basis. The light program was changed to 16h light: 8h dark to stimulate egg production. All egg parameters were monitored for 4 weeks after the

Table III-1. Treatments provided to birds in molting study

1	Feed Deprivation
2	*Ad Libitum* access to Alfalfa Meal
3	*Ad Libitum* access to Alfalfa Pellets
4	*Ad Libitum* access to a complete layer ration

end of the molting period. Egg production was monitored for 12 weeks after the end of the molting period.

Initially, n=32 for feed deprivation and n=28 for alfalfa meal, alfalfa pellet, and layer ration. Due to mortalities and the aforementioned sacrifice for ovarian measurements, only 57 birds were left for postproduction assessment with n = 16 for feed deprivation, n = 14 for both alfalfa meal and alfalfa pellet, and n = 13 for layer ration. Due to further losses, the last 8 weeks of egg production had n = 14 for feed deprivation, n = 13 for alfalfa meal, n = 14 for alfalfa pellet, and n = 12 for layer ration.

Data was analyzed using SAS v. 8.0 for windows (SAS, 2000). Analysis of variance was performed using the general linear model due to the differences in sample sizes. Means were separated by the Duncan procedure with significance denoted at $p < 0.05$.

RESULTS AND DISCUSSION

Body Mass

All treatments exhibited significant differences ($p < 0.05$) in percent body mass lost in this study. Feed deprived hens lost the most mass (23.3%), while alfalfa meal and pellet hens lost 18.87 and 15.2%, respectively (Figure III-I). Unmolted hens fed layer ration gained 2.31%. Hens that were feed deprived had similar weight losses to hens in previous studies that were molted by feed deprivation (Buhr, 1994). Collection and

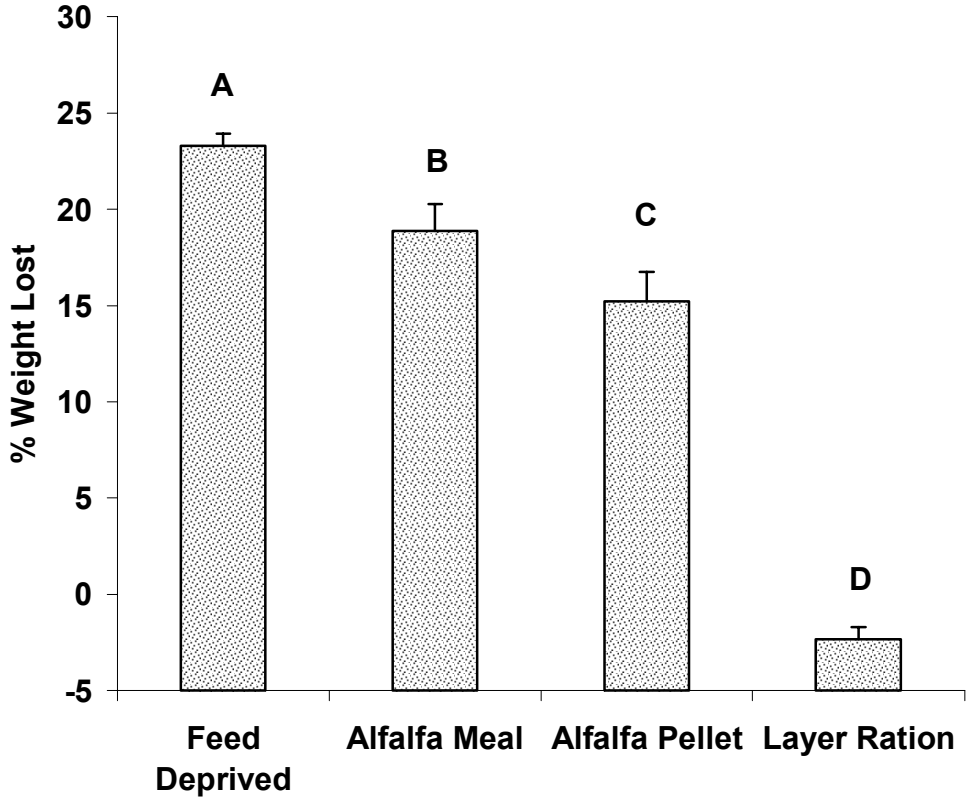

FIGURE III-1: Effect of molting treatments on percentage of body weight losses after 9 days. Bars represent average and standard error values for percent weight loss at the end of the molting period per methods ($p < 0.05$). * indicated that birds molted by feed deprivation lost significantly more weight than birds molted by alfalfa.

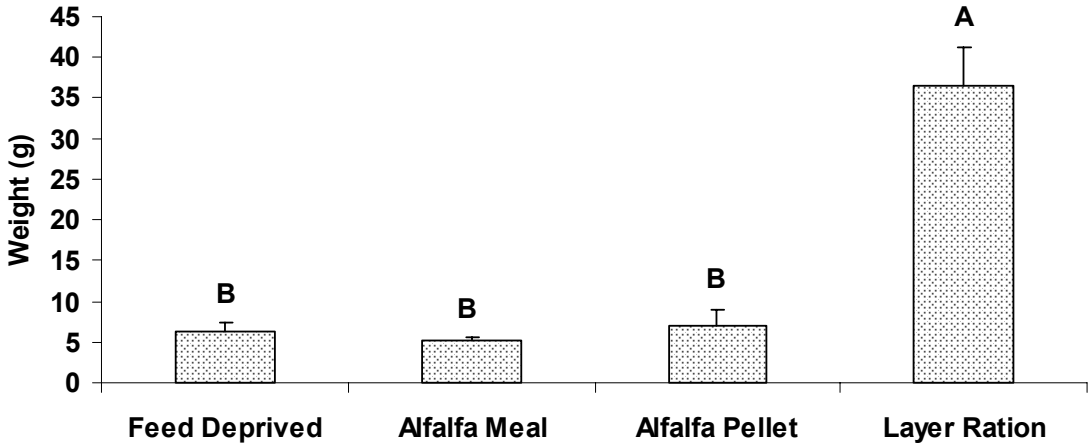

FIGURE III-2: Effect of molting diets on ovarian weight. Bars represent average and standard error values for ovarian weights measured at the end of the 9 day molt. Ovaries were resected and weighed in g according to methods ($p < 0.05$). * Suggests that birds molted by either alfalfa or feed deprivation had similar ovarian weights.

Table III-2. Egg quality response to molting treatments and the nonmolted control[1]

Diet	Length (cm)	Circumference (cm)	Yolk Height (mm)
Feed Deprivation	6.31 $\pm$ 0.10	14.33 $\pm$ 0.09	17.49 $\pm$ 0.50
Alfalfa Meal	6.49 $\pm$ 0.08	14.28 $\pm$ 0.10	17.61 $\pm$ 0.65
Alfalfa Pellet	6.49 $\pm$ 0.07	14.52 $\pm$ 0.10	17.65 $\pm$ 0.65
Control	6.43 $\pm$ 0.06	14.53 $\pm$ 0.13	17.23 $\pm$ 0.32

[1] – Means within a row with no common superscript differ significantly ($p<0.05$)

weight of intestinal tracts is warranted in future studies to see if the decrease in weight loss in alfalfa molted hens is due in part to presence of feed in the intestinal tract.

Ovarian Weight

Hens fed alfalfa in both pellet (7.03g) and meal (5.08g) form did not have significantly different ovarian weights when compared to hens that were feed deprived (6.22g) ($p > 0.05$). Unmolted hens fed layer ration had a significantly higher ($p < 0.05$) ovarian weight than all of the molted hen treatments. The ovaries from unmolted hens had a mean weight of 36.47g (Figure III-2). These values are comparable to the ovarian weights of a study done by Kwon and coworkers (2001), where alfalfa fed hens had a mean ovarian weight of 4.8g. However, in future studies it would be more advantageous to use birds that are younger in age. In commercial facilities, the most common time period to molt a flock of hens is at approximately 60 weeks of age, which coincides with the end of the first cycle of egg production (Brake, 1992). Older hens are more prone to involution of the reproductive tract due to feed deprivation which may have inadvertently biased this study. A younger sample group would allow for a more controlled assessment of ovarian regression during an induced molt with alfalfa.

Egg Quality

Yolk height, egg length, and egg circumference values were not significantly different ($p > 0.05$) across all treatments (Table III-2). Air cell values were also not significantly different ($p > 0.05$). Egg length and circumference values remained

virtually unchanged when pre and post molt data were compared. In a study involving the use of grape pomace as an alternative to feed deprivation for the induction of molt, little change in egg quality during the second cycle of production was also noted (Keshavarz and Quimby, 2002). In future studies, egg quality measurements should be taken throughout the second egg laying cycle as egg quality has been shown to decrease in the latter part of an egg laying cycle following a molt.

Egg Weight

Eggs laid by hens molted with alfalfa pellets (70.13g) weighed significantly more than eggs laid by hens molted by feed deprivation (65.34g) or alfalfa meal (65.55g). Eggs laid by unmolted hens (68.26g) were not significantly different from any of the treatments (Figure III-3).

Albumen Height

Eggs laid by hens fed alfalfa meal had an albumen height (6.21mm) that was significantly higher than unmolted hens (5.28mm). Eggs laid by hens that were feed deprived (5.89mm) and molted using alfalfa pellets (6.07mm) did not have an albumen height that was significantly different from either feed deprived or unmolted hens (Figure III-4).

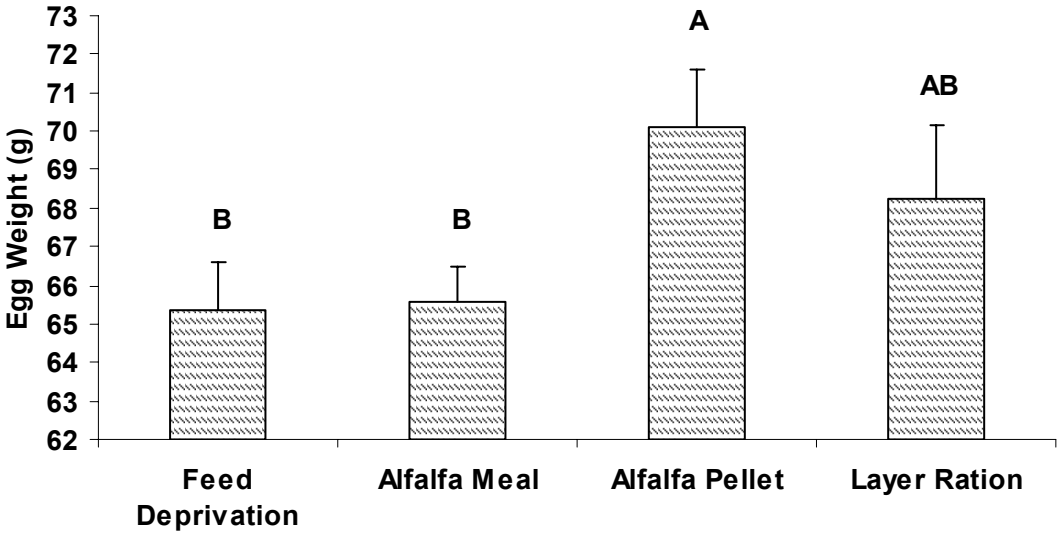

FIGURE III-3: Effect of molting treatments on egg weight during early second cycle production. Bars represent average and standard error values for the weight of eggs following the molt. Eggs were weighed and recorded in grams ($p < 0.05$).* Suggests that birds molted by alfalfa meal laid significantly larger eggs than hens that were molted by feed deprivation or alfalfa pellets.

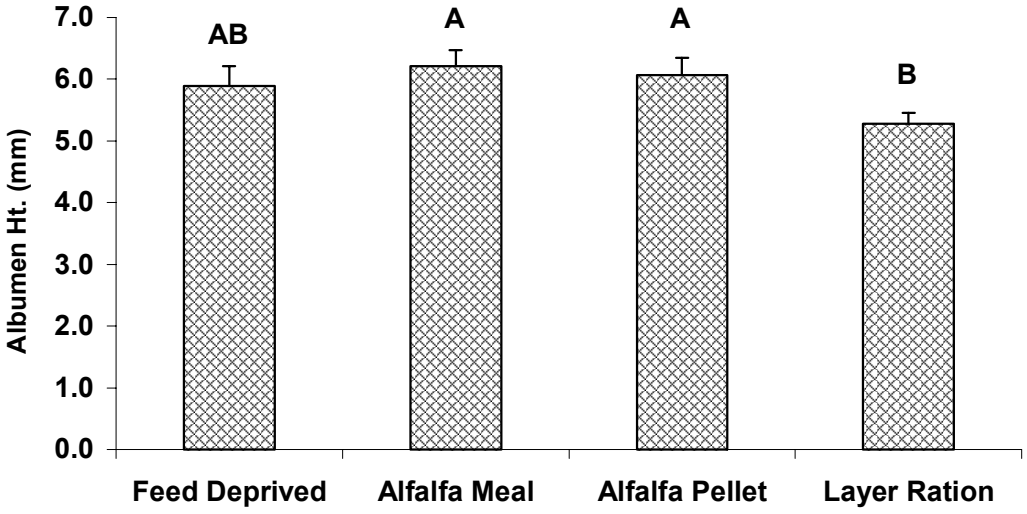

FIGURE III-4: Effect of molting treatments on albumen height in the early second cycle of production. Bars represent average and standard error values for albumen height taken from eggs laid up to two weeks after the molt. Albumen height was recorded in mm according to methods ($p < 0.05$). *Suggests that eggs laid by hens molted by alfalfa have comparable albumen heights to eggs from feed deprived hens.

Egg Production (0-7 weeks postmolt)

Unmolted hens fed layer ration had a significantly higher level of egg production (61.05%) than hens molted by feed deprivation (44.55%) 0 – 7 weeks after the molt. While hens molted by alfalfa pellet (50.46%) or alfalfa meal (49.09%) did not have significantly different levels of egg production from either feed deprived or unmolted hens, it should be noted that both of the treatments exhibited higher mean egg production than feed deprived hens. It is noticeable that these hens seemed to rebound more quickly than feed deprived hens. (Table III-3) (Figure III-5) As with egg quality, egg production should be measured for the entirety of the second egg laying cycle. Researchers have demonstrated that the latter part of an egg laying cycle is marked by a decrease in egg production (North and Bell, 1990).

Egg Production (8 – 12 weeks postmolt)

Hens fed alfalfa meal (83.38%) had a significantly higher egg production than hens molted by feed deprivation (70.57%). Unmolted hens (68.00%) and hens molted by alfalfa pellet (77.14%) showed no significant difference in egg production from 8 – 12 weeks after the molt. In the weekly analysis of production, it can be seen that hens fed alfalfa diets are continuing to increase egg production for up to 12 weeks post molt (Table III-3) (Figure III-6).

Table III-3. Effect of molting treatments on egg production in early in the second cycle and on date of reentry into egg production[1]

Diet	0-7 Week		8-12 Week		Date of Reentry
	(%)		(%)		(# of days)
Feed Deprivation	44.55 ± 4.96^{b}		70.57 ± 4.37^{b}		17.33 ± 1.63^{b}
Alfalfa Meal	49.09 ± 4.08^{ab}		83.38 ± 2.75^{a}		14.00 ± 0.89^{ab}
Alfalfa Pellet	50.46 ± 3.90^{ab}		77.14 ± 4.45^{ab}		11.62 ± 0.68^{a}
Nonmolted Control	61.05 ± 3.49^{a}		68.00 ± 4.59^{b}		N/A

[1] – Means within a row with no common superscript differ significantly ($p<0.05$)

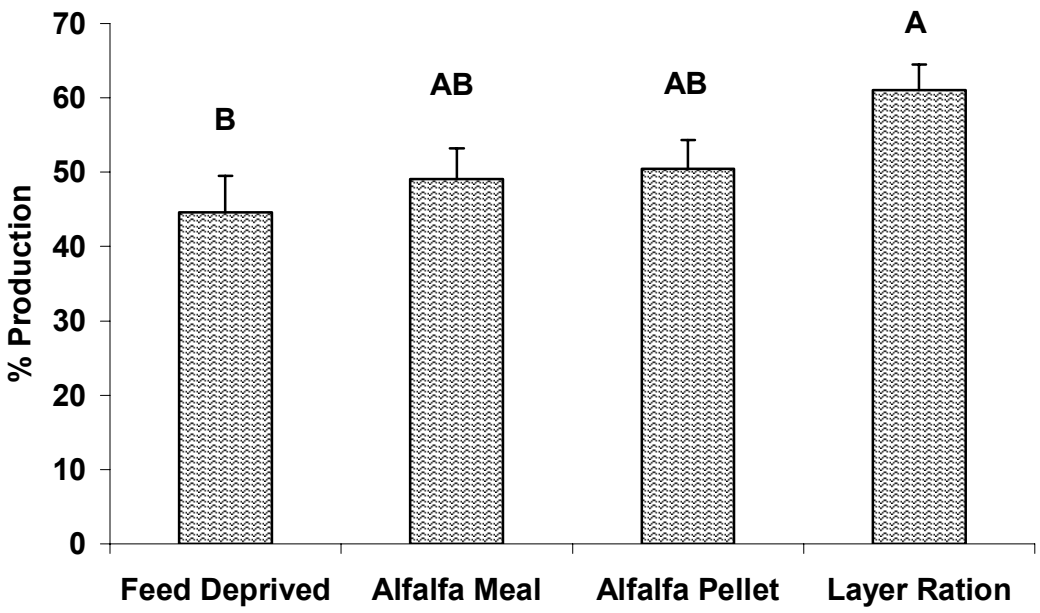

FIGURE III-5: Effect of molting treatments on early second cycle egg production, weeks 0 through 7. Bars represent average and standard error values for % egg production, which was calculated via the formula provided in the methods ($p < 0.05$). * Suggests that birds molted by feed deprivation or alfalfa had comparable egg production in the weeks following the molt.

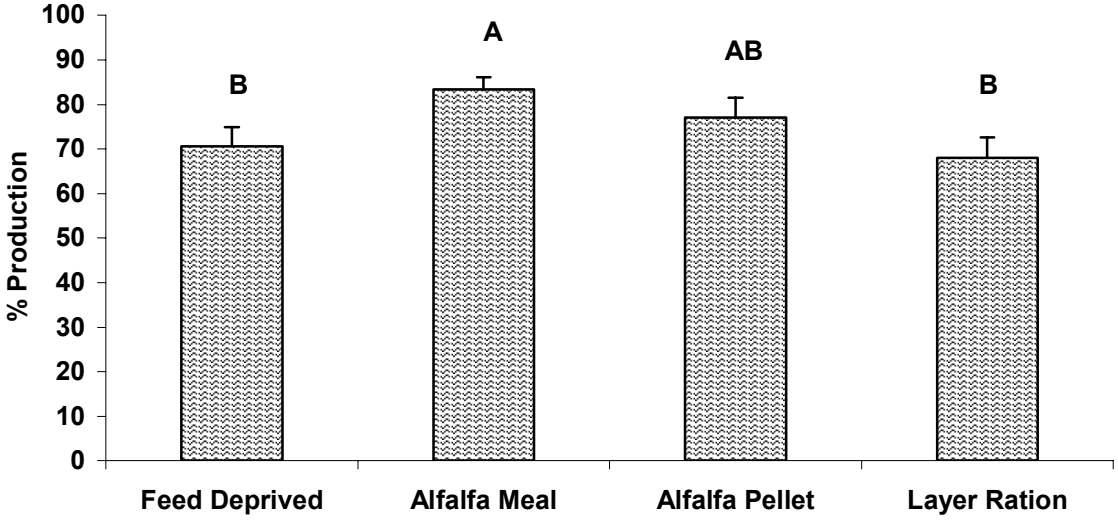

FIGURE III-6: Effect of molting treatments on early second cycle egg production, weeks 8 –12. Bars represent average and standard error values for egg production. Egg production was calculated with the formula provided in the methods ($p < 0.05$). * Suggests that hens molted with alfalfa meal had significantly higher egg production in weeks 8 – 12 of the second cycle.

Date of Reentry

Hens molted by alfalfa pellet (11.62 days) reentered production significantly faster than hens that were molted by feed deprivation (17.33 days). Hens molted by alfalfa meal did not exhibit a significant difference in the onset of egg production (14 days). This may be due to a theoretically higher amount of metabolizable energy in the pelleted diet due to the heat treatment involved in the preparation of the pelleted diet (Figure III-7).

CONCLUSIONS

Feeding alfalfa on an *ad libitum* basis for a period of nine days is effective at reducing ovarian size, allowing for a reproductive rest period for commercial laying hens. Hens fed alfalfa exhibited levels of egg production that were at least comparable to that of hens molted by feed deprivation and their eggs were of comparable quality when compared to eggs from hens molted by feed deprivation. Therefore, alfalfa may be useful to commercial laying facilities as an alternative to feed deprivation for the induction of molt.

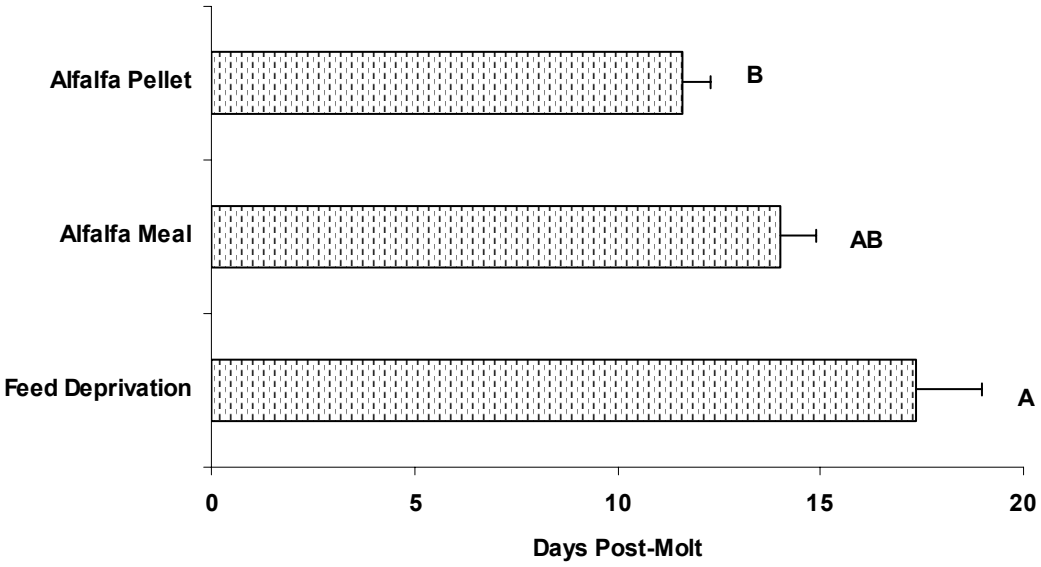

FIGURE III-7: Effect of molting treatments on date of reentry into egg production. Bars represent average and standard error values for reentry date, which was recorded that the first day an egg was laid following a molt ($p < 0.05$). * Suggests that birds molted by feed deprivation took significantly longer to reenter production than birds molted by alfalfa pellets.

CHAPTER IV

CONSUMER SENSORY AND MECHANICAL EVALUATIONS ON QUALITY ATTRIBUTES OF EGGS FROM COMMERCIAL LAYING HENS MOLTED BY ALFALFA

SYNOPSIS

Hens at a commercial laying facility were molted by both alfalfa and feed deprivation. After the hens had reentered production, eggs were collected and transported to Texas A&M University. Shell strength, albumen height, yolk height, weight, length, and yolk color were all tested using various mechanical techniques. The eggs were also sampled for testing by consumer sensory panels that assessed the desirability of the eggs' color and flavor/texture. Eggs laid by hens molted by alfalfa had a significantly higher ($p < 0.05$) level of green color (a*) as determined by Minolta colorimetry. Eggs laid by hens molted with alfalfa also exhibited significantly higher ($p < 0.05$) egg weights and length. In the consumer sensory test, there was no significant difference ($p > 0.05$) in color or flavor/texture scores in eggs from either feed deprived or alfalfa molted hens.

INTRODUCTION

Induced molting by feed deprivation is a practice that has been well researched. During the past several decades, commercial laying facilities have been using feed deprivation for a period of several days as a means of inducing molt to extend the productivity of a flock of laying hens (Brake, 1992). Consequently, the eggs laid by feed deprived molted hens have been used for human consumption for several years.

Due to increasing consumer pressures concerning the use of feed deprivation, alternatives for inducing a molt have been investigated. A single ingredient molting diet of alfalfa is such an alternative. Medvedev et al. (2001a,b) has previously demonstrated that birds in controlled experiments given ad libitum access to alfalfa have comparable ovarian regression and early second cycle egg production to birds that were molted by feed deprivation. Previous research on alfalfa has also been conducted to ascertain its merits as a feed supplement for the purpose of yolk coloration (Fletcher et al., 1985, Ganeshan and Kumar, 1989, Guenthner et al., 1973, Johns, 1986). This is an issue since a commercial producer would most likely prefer eggs of a uniform color. Additionally, different components of layer diets, such as menhaden oil, have been known to alter egg flavor (van Elswyk et al., 1992). In order to substantiate alfalfa as a viable alternative to feed deprivation for the induction of molt, it is important to verify that the diet does not impart any differences in aesthetics or organoleptic qualities. This requires measurement of interior and exterior egg quality in the early second cycle of production of both feed deprived and alfalfa molted hens. A previous study confirmed the effectiveness of

alfalfa molting, but measurements on egg parameters post molt were limited due to the small number of experimental hens (Medvedev, personal communication). The objective of this study was to determine if untrained consumers could detect a difference in taste/texture and yolk color of eggs laid by hens molted by alfalfa or feed deprivation.

MATERIALS AND METHODS

Molting Procedure and Egg Storage

Single comb white leghorn hens greater than 60 weeks of age were molted at a commercial laying facility by either feed deprivation or ad libitum access to alfalfa. The details of the molting procedure is proprietary information that was not released by the company. After the hens had reentered production, eggs were placed in a cooler at 4ºC , 97% relative humidity for a period of one week prior to the evaluations due to scheduling constraints for the consumer panels. Eggs were obtained prior to the molt, transported, and stored in the same way. One non-replicated trial was conducted before the molt and one non-replicated trial was conducted after the molt.

Egg Quality

Five parameters were used to assess exterior and interior quality of eggs, both before and after the molt. Yolk and albumen height were measured with a micrometer and expressed in mm. Yolk color was measured by Minolta Colorimetry. L* (lightness), a* (green to red), and b* (blue to yellow) values were recorded. Since the eggs were

stored, Haugh unit calculations were not performed due to an increase in variability attributed to the calculation (Silversides and Villeneuve, 1994). Albumen heights were used solely because of their high correlation to haugh unit measurements (Silversides et al., 1993). Length was measured using a caliper and was recorded in cm. Egg weight was measured using a balance and was recorded in grams to the nearest hundreth of a gram. Shell strength was assessed using an Instron machine and was recorded in kg force required to crack the shell surface. The egg was stood in its large end and placed on a level surface with a round indentation in the center measuring 1 cm at its narrowest point and 2.5 cm at its widest point. Force was applied using an anvil measuring 2.5 cm in width and 0.2 cm in thickness with a 50 kg load cell at a cross head speed of 50 mm/min to the narrow end of the egg (Hammerle, 1969).

Consumer Sensory Panels

Panels were held using untrained panelists for a 3 day period during the afternoon for both pre and post molt eggs. Scrambled eggs were used since no fat was added and flavor differences between scrambled and hard cooked would be minimal (van Elswyk et al., 1992). The panelists were subjected to a triangle test where they were randomly given 3 samples of scrambled egg (Roessler et al., 1948). The panelists rated each sample from 1 to 8, where each integer represented a degree of opinion ranging from extremely undesirable to extremely desirable, by marking an "X" on a 15 cm line (Figure IV-1). This line represented the evaluator's opinion of the egg sample based on

both flavor and texture. During these evaluations, the panelists were asked to select the two samples that were the most similar.

The panelists were asked to rate the color of 3 randomly selected raw yolk samples. The panelists were subjected to a triangle test where they were randomly given 3 samples of scrambled egg (Roessler et al., 1948). The panelists rated each sample from 1 to 8, where each integer represented a degree of opinion ranging from extremely undesirable to extremely desirable, by marking an "X" on a 15 cm line (Figure IV-1). During these evaluations, the panelists were asked to select the two samples that were the most similar. This was conducted to ascertain whether or not panelists could detect which samples were from birds molted by the same treatment.

Triangle test data was analyzed by assigning a numerical value to a panelist's response. If they correctly chose the sample that was not in the majority group, the value was 1. An incorrect response was scored with a 0 value.

Statistical Analysis

Statistical analysis was performed using SAS v. 8.0 (2000). Data were analyzed using molting treatment as the independent variable for each sensory and mechanical attribute tested. All sampling days were pooled due to a lack of significant difference between sampling days. For quality analysis, n = 30 for each parameter. For sensory panels, n = 66 (where n = 38 and 28, for alfalfa and feed deprived eggs as the majority sample, respectively) for flavor and n = 46 (where n = 25 and 21 for alfalfa and feed

Scrambled Egg Sensory Panel

I. Taste and Texture

Sample 1: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Sample 2: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Sample 3: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Which 2 samples are most similar in taste and texture? _____ and _____

II. Yolk Color

Sample 1: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Sample 2: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Sample 3: _____ (Write 3 digit number in blank)

1 2 3 4 5 6 7 8
(Extremely undesirable) (Extremely desirable)

Which 2 samples are most similar in yolk color? _____ and _____

FIGURE IV-1: Sensory panel data sheet.

deprived eggs as the majority sample, respectively) for color for the pre-molt trial. In the post-molt trial n = 49 (where n = 24 and 25 for alfalfa and feed deprived eggs as the majority sample, respectively) for flavor and n = 46 (where n = 27 and 26 for alfalfa and feed deprived eggs as the majority sample, respectively) for color. Analysis of variance using the general linear model was used to analyze all egg quality and sensory data. Mean separation was performed using the Tukey test. Significance was indicated by $p < 0.05$.

RESULTS AND DISCUSSION

Egg Quality

No significant differences were seen between flocks that were molted by alfalfa or by feed deprivation (Table IV-1). Albumen height, yolk height, shell strength, and Minolta b* (blue to yellow) and l* (lightness) values were not significantly different when alfalfa and feed deprived molting treatments were compared. Postmolt albumen height values were 5.99 mm for eggs laid by alfalfa molted hens and 5.94mm for hens molted by feed deprivation. Minolta values for alfalfa hens were a b* value of 45.07 and a l* value of 54.67 while feed deprived hens had a b* value of 43.54 and a l* value of 53.66. This suggests that egg yolks from alfalfa molted hens are comparable in appearance to yolks from feed deprived hens in terms of lightness and degree of yellow coloration (Table IV-2).

Eggs laid by alfalfa molted hens (64.08g) were significantly heavier ($p < 0.05$) than eggs laid by feed deprived hens (59.31g) (Table IV-1). However, earlier work by Medvedev et al. (2002) demonstrated that eggs laid by alfalfa meal molted hens were not

Table IV-1. Interior and exterior quality characteristics of pre-molt eggs[1,2]

Parameter	Pre-Molt Alfalfa	Pre-Molt Feed Deprivation [1]
Albumen Height (mm)	5.92 ± 0.21	5.87 ± 0.17
Yolk Height (mm)	17.85 ± 0.17	17.41 ± 0.16
Shell Strength (kg force)	2.70 ± 0.14	2.78 ± 0.11
Minolta b*	43.60 ± 0.45	43.39 ± 0.58
Minolta l*	54.86 ± 0.36	54.94 ± 0.33
Minolta a*	-3.62 ± 0.11	-3.38 ± 0.09
Egg length (cm)	5.72 ± 0.03	5.71 ± 0.14
Egg weight (g)	62.10 ± 0.83	61.19 ± 0.77

Means within a row with no common superscript differ significantly ($p<0.05$)

[2] – Values are a mean of n = 30

Table IV-2. Interior and exterior quality characteristics of post-molt eggs[1,2]

Parameter	Post-Molt Alfalfa	Post-Molt Feed Deprivation
Albumen Height (mm)	5.99 ± 0.27	5.94 ± 0.21
Yolk Height (mm)	19.60 ± 0.20	19.18 ± 0.17
Shell Strength (kg force)	3.36 ± 0.14	3.08 ± 0.17
Minolta b*	45.07 ± 0.55	43.54 ± 0.77
Minolta l*	54.67 ± 0.54	53.66 ± 0.38
Minolta a*	-2.18 ± 0.14[a]	-1.62 ± 0.11[b]
Egg length (cm)	5.83 ± 0.03[a]	5.66 ± 0.04[b]
Egg weight (g)	64.07 ± 0.62[a]	59.30 ± 0.70[b]

[1] – Means within a row with no common superscript differ significantly ($p<0.05$)
[2] – Values are a mean of n = 30

significantly heavier than feed deprived hens. Eggs laid by alfalfa molted hens (5.83cm) were also significantly longer ($p < 0.05$) than eggs laid by feed deprived hens (5.66 cm) (Table IV-2). Eggs from alfalfa molted hens are more likely to fall into the "extra large" (61.75 - 68.87) (53.33%) category, when using sizing guidelines described by the USDA (USDA, 1995). Eggs from hens molted by feed deprivation were most likely to have weights in the "large" range (54.63 - 61.74) (63.33%) (Table IV-2). Eggs from alfalfa molted hens also exhibited a significantly lower a* value (-2.18; $p < 0.05$) than eggs from feed deprived hens(-1.62) which is indicative of an increase in green color. While these values were significantly different, both values were numerically lower to that of the premolt data of both treatments.

Sensory Response

From both the premolt and postmolt consumer sensory panels, there were no significant differences found between molting treatment for taste/texture and color evaluations ($p > 0.05$) when using unseasoned scrambled eggs as the test sample for taste/texture evaluations and raw yolk for the color evaluation (Figure IV-2; Tables IV-3 and IV-4). During post-molt evaluation, eggs from alfalfa molted hens had a taste/texture value of 5.22 and a color evaluation of 5.45. This can be compared to eggs from feed deprived hens which were 4.91 for taste/texture and 5.20 for color. This indicates that the consumers displayed no preference towards eggs from either alfalfa or feed deprived molted hens.

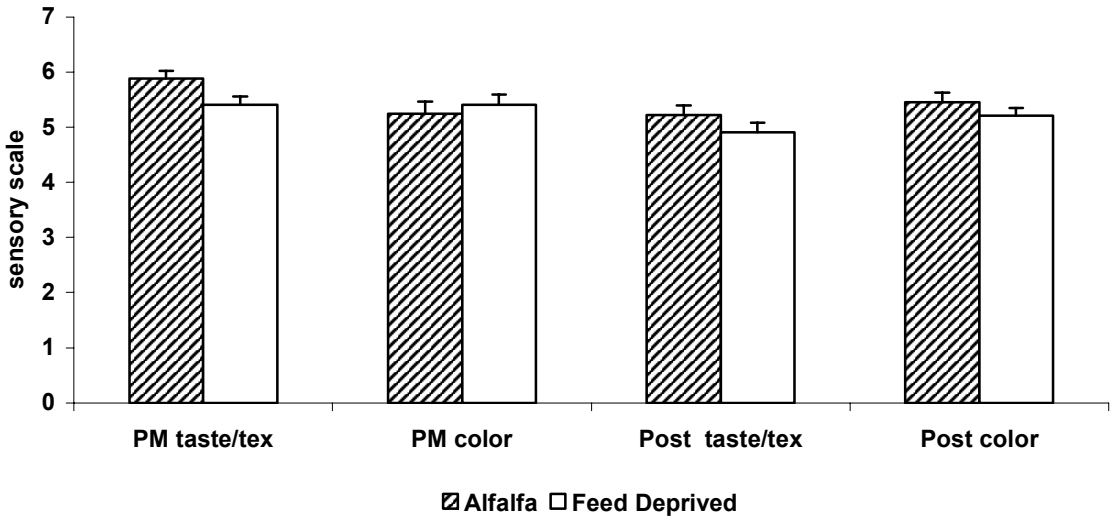

FIGURE IV-2: Effect of molting treatments on consumer sensory evaluation[1,2]. Bars represent average and standard error values of consumer acceptance. Respondents' sheets were scored in manner described in methods ($p < 0.05$). *Suggests that there are no significant differences in consumer preference for eggs from hens molted by alfalfa or feed deprivation.

[1] PM, Premolt
[2] Post, Postmolt

Table IV-3. Consumer sensory evaluations of eggs from hens molted by alfalfa and feed deprivation[1]

Parameter	Post-Molt Alfalfa	Post-Molt Feed Deprivation
Taste/Texture Rating (0 – 8)	5.22 ± 0.17	4.91 ± 0.17
Color Rating (0 – 8)	5.45 ± 0.18	5.20 ± 0.15
Taste/Texture Triangle Test Success Rate (%)	45.83 ± 10.39	32.00 ± 9.52
Color Triangle Test Success Rate (%)	40.74 ± 9.64	19.23 ± 7.88

[1] – Means within a row with no common superscript differ significantly ($p<0.05$)

Table IV-4. Consumer sensory evaluations of eggs from pre-molt hens

Parameter	**Pre-Molt Alfalfa**	**Pre-Molt Feed Deprivation**
Taste/Texture Rating (0 – 8)	5.89 ± 0.13	5.41 ± 0.16
Color Rating (0 – 8)	5.25 ± 0.22	5.40 ± 0.19
Taste/Texture Triangle Test Success Rate (%)	23.68 ± 6.99	42.86 ± 9.52
Color Triangle Test Success Rate (%)	48.00 ± 10.20	23.81 ± 9.52

[1] – Means within a row with no common superscript differ significantly ($p<0.05$)

Panelists could not discern which samples were different in both taste/texture and color evaluations, regardless of which treatment was the majority group. When two out of three samples were from alfalfa molted hens, the egg from feed deprived hens were correctly isolated 45.83% of the time for taste/texture and 40.74% of the time for color evaluations, compared to 23.68% ($p = 0.0711$) and 48.00% ($p = 0.6069$), respectively, for pre-molt evaluation. However, when two out of three samples were from feed deprived hens, alfalfa eggs were only isolated 32.00% of the time for taste/texture and 19.23% for color, compared to 42.86% ($p = 0.4254$) and 23.81% ($p = 0.7104$) in the pre-molt evaluation. This coupled with the lack of significant difference between consumer preferences for the eggs from the different molting treatments implies that consumer detection of differences between the two treatments is minor.

While few differences were noted in the results of this study, changes in the experimental design could have improved the ability of the sensory panel to detect differences in consumer acceptability. Taste and texture should have been evaluated on 2 separate lines, as they are separate attributes.

Consumer perception of eggs is an integral part of the decision making process that producers use when determining what dietary changes would be most advantageous to a commercial laying flock. In fact, the production and marketing sectors of the commercial egg industry are interrelated to ensure a product that meets both governmental regulations and economical expectations (Hunton, 1995).

Consumers desire eggs that exhibit high ratios of thick to thin albumen. Thick albumen also allows the yolk to appear more rounded, instead of the less desirable

"flattened" yolk (Hunton, 1995). Eggs from alfalfa molted hens appeared to have an albumen height that would be characteristic of a product that would be desirable to consumers. The consumer acceptability of the cooked product further supports that hens molted by this method do not lay eggs that would be deemed less acceptable by purchasers.

CONCLUSIONS

Due to increasing consumer pressure regarding animal issues, locating a viable, economically advantageous alternative to feed deprivation for the induction of molt is important to the commercial laying industry. This study on consumer sensory and mechanical quality attributes indicates that alfalfa shows promise as such an alternative.

CHAPTER V

THE EFFECT OF AN *AD LIBITUM* ALFALFA MOLTING DIET AND FEED DEPRIVATION ON HETEROPHIL:LYMPHOCYTE RATIOS AND SERUM CHEMISTRY PARAMETERS IN COMMERCIAL LAYING HENS

SYNOPSIS

SCWL > 60 weeks were molted using either *ad libitum* access to alfalfa or feed deprivation for a period of nine days. Blood samples were taken for the purpose of making blood smears for heterophil:lymphocyte ratio analysis and for various chemistry parameters. Differences between hens molted by alfalfa and feed deprivation are discussed below.

INTRODUCTION

Feed deprivation has been the preferred method for inducing molt in commercial laying facilities for several years. However, induced molting by feed deprivation has been shown to increase the bird's susceptibility to *Salmonella* infection (Holt, 1992). Molting by feed deprivation has also been shown to decrease immune function as decreased heterophil function and lower numbers of CD4+ T lymphocytes have been observed in feed deprived hens (Kogut et al., 1999; Holt, 1993). Feed deprivation has also been shown to cause an increased stress level in laying hens. Leghorn breeder hens had a 7.9% increase in corticosterone production by day 2 of a 10 day fast (Avrutina et al., 1974). Laying hens also exhibit behaviors indicative of frustration such as

displacement preening, gakel-calls, and pacing when deprived of feed (Zimmerman et al., 2000).

Due to the increased susceptibility to infections and the increase in stress associated with laying hens and induced molting by feed deprivation, alternatives to this practice are being investigated. The use of wheat middlings and alfalfa as alternative molting treatments has been documented to decrease the susceptibility of hens to *Salmonella* infections, when compared to feed deprivation (Seo and Holt, 2000; Kwon et al., 2001). Inducing molt with alfalfa meal has shown comparable results to feed deprivation in terms of egg production and egg quality (Medvedev et al., 2001, 2002). The objective of this study was to better determine the physiological status of laying hens molted by alfalfa. To accomplish the objective, various blood chemistry parameters and leukocyte percentages were conducted.

MATERIALS AND METHODS

Trial 1

SCWL hens approximately 80 weeks were obtained from a commercial laying facility. They were placed 2 birds/cage in wire layer cages, provided *ad libitum* access to layer ration and water, and acclimated for two weeks. Prior to the molting treatments, birds were subjected to an 8:16 lighting schedule. Birds were randomly assigned to one of three treatments: *ad libitum* access to layer ration (non-molted control), *ad libitum* access to alfalfa, and feed deprivation. All birds were allowed *ad libitum* access to

Table V-1. Treatments provided to birds in molting study

1	Feed Deprivation
2	*Ad Libitum* access to Alfalfa Meal
3	*Ad Libitum* access to a complete layer ration

water. All treatments were provided to birds for nine days (Table V-1). Blood samples were taken on day 0, before any treatments began and day 7, after the study began. Approximately 20mL of blood was taken from each hen through the jugular vein. Blood was collected in EDTA tubes for ketone body and blood smear preparation and in coagulation tubes for serum chemistry analysis. Serum chemistries analyzed included: glucose (minimum threshold = 20.0 mg/dl), calcium (1.0 mg/dl), magnesium (0.2mg/dl), phosphorus (0.5 mg/dl), uric acid (0.5 mg/dl), total protein (2.0 g/dl), cholesterol (50 mg/dl), and triglycerides (10 mg/dl). This analysis was performed at the Texas Veterinary Diagnostic Laboratory. Ketone body analysis was conducted using a kit provided by Sigma Laboratories. On day nine, 12 birds in each treatment were sacrificed by cervical dislocation. Organ weights were determined and expressed as a percentage of body weight on the following organs: liver, spleen, heart, intestine, ovary, oviduct, and kidney.

Trial 2

SCWL hens approximately 60 weeks of age were obtained from a commercial laying facility. They were placed 2/cage in wire layer cages, provided *ad libitum* access to layer ration and water, and acclimated for two weeks. Prior to the molting treatments, birds were subjected to an 8:16 lighting schedule. Birds were randomly assigned to one of three treatments: *ad libitum* access to layer ration (non-molted control), *ad libitum* access to alfalfa, and feed deprivation. All birds were allowed *ad libitum* access to water. All treatments were provided to birds for nine days (Table V-1). Blood samples

were taken on day 0 (before any treatments began) and day 8. Approximately 10mL of blood was collected from each hen through the jugular vein. Blood was collected in heparinized tubes for serum chemistries and blood smears for ease of sample preparation. Plasma chemistries analyzed included: glucose, calcium, magnesium, phosphorus, uric acid, total protein, cholesterol, and ketone bodies. Ketone body analysis was determined using a kit provided by Wako Diagnostics. On day nine, 12 birds in each treatment were sacrificed by cervical dislocation. Organ weights were determined and expressed as a percentage of body weight on the following organs: liver, spleen, heart, intestine, ovary, oviduct, kidney, and adrenals.

Statistical Analysis

Statistics were performed using SAS version 8.0 (SAS, 2000). The general linear model was used with Duncan's Multiple Range Test as the method for means separation. Significance was denoted at $p < 0.05$

RESULTS AND DISCUSSION

Serum and plasma chemistries from both trials signify that there are differences between hens molted by alfalfa and hens molted by feed deprivation. In alfalfa fed birds, calcium levels from pre-molted hens were not significantly different from molted hens in both trials, although the molted hens did have numerically lower calcium levels (Figures V-1 and V-2). However, in feed deprived hens, calcium levels in pre-molted

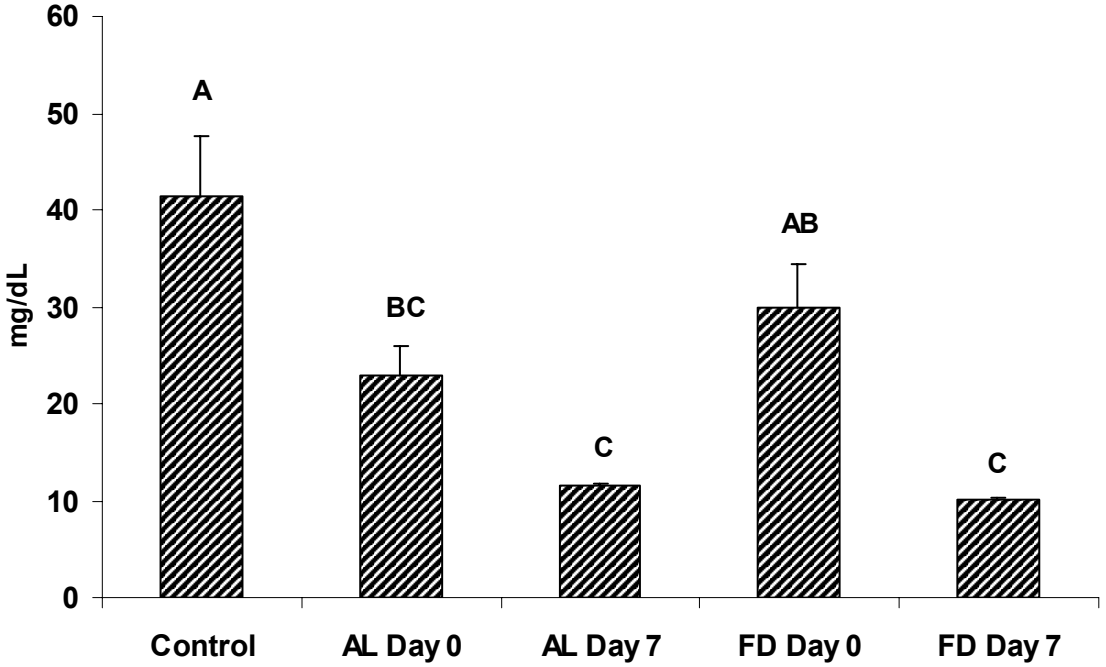

Figure V-1: Calcium levels of hens molted by alfalfa and feed deprivation (Trial 1). Bars represent average and standard error values for calcium levels (mg/dl) on the first and last day of the molt ($p < 0.05$). * Suggests that hens molted by either feed deprivation of alfalfa had significantly lower levels of calcium by day 7 of the molt.

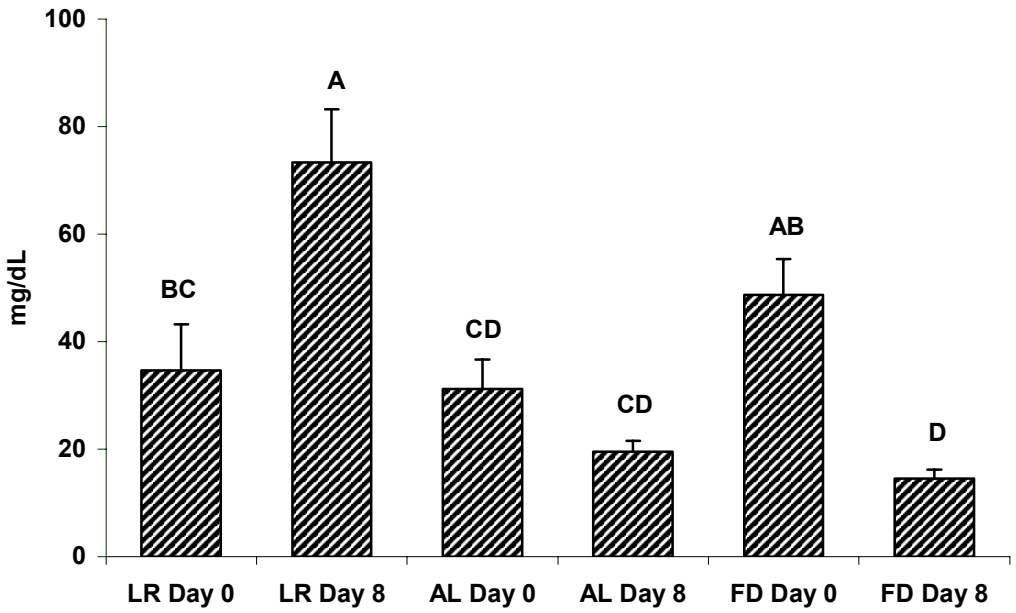

FIGURE V-2: Calcium levels of nonmolted hens (LR), hens molted by alfalfa (AL) and hens molted by feed deprivation (FD) (Trial 2). Bars represent average and standard values for calcium levels (mg/dl). Blood was taken on the first and last day and processed according to methods ($p < 0.05$). * Suggests that birds molted by feed deprivation had a significantly lower level of calcium on day 7 than the day 0 baseline.

hens were significantly lower than molted hens (Figures V-1 and V-2). Cholesterol levels of alfalfa fed hens were not significantly different in the first trial; however, in the second trial cholesterol levels were significantly higher in the molted hens (Figures 3 and 4). In feed deprived hens, cholesterol was significantly higher in molted hens than pre-molt hens in both trials (Figures V-3 and V-4). The latter trend is to be expected in molted hens, as the resorption of atretic follicles during the process of molt should increase the amount of circulating cholesterol in the blood. Additionally, failure to ovulate may also cause an insoluble fraction of cholesterol, known as HDL_R, which could also account for the increase in cholesterol (Walzem et al., 1994). Uric acid levels were not significantly different in either trial for alfalfa fed hens, while uric acid levels were significantly lower in feed deprived molted hens than either the pre-molt hens or the non molted control hens (Figures V-5 and V-6). Uric acid can be used to assess hydration or renal function, although birds with severe renal damage may not have decreased uric acid levels (Phalen, 2000). Thusly, the lower uric acid levels in feed deprived molted hens may be due to a decrease in water consumption during the molting process. Magnesium levels, which can also be used to assess kidney function, were significantly lower than the control or pre-molt values for both alfalfa and feed deprivation in Trial one; however, in Trial 2, the magnesium level of hens molted by alfalfa was only significantly different from the non molted control (Figures V-7 and V-8). Ketone bodies were significantly lower in alfalfa molted hens than feed deprived hens on day 7 of the molt in Trial one, which could mean that alfalfa fed hens are using some of the energy provided in the diet. However, in Trial 2, no significant differences

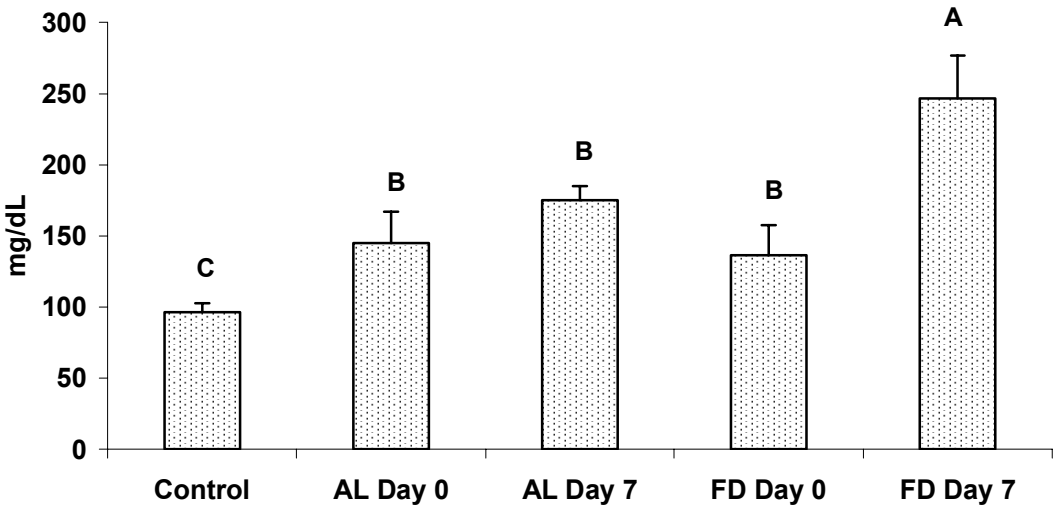

FIGURE V-3: Cholesterol levels of nonmolted hens (Control), hens molted by alfalfa (AL) and hens molted by feed deprivation (FD) (Trial 1). Bars represent average and standard values for cholesterol levels (mg/dl). Blood was drawn on the first and last day of the molt according to methods ($p < 0.05$). * Suggests that hens molted by feed deprivation had a significantly higher cholesterol level on day 7.

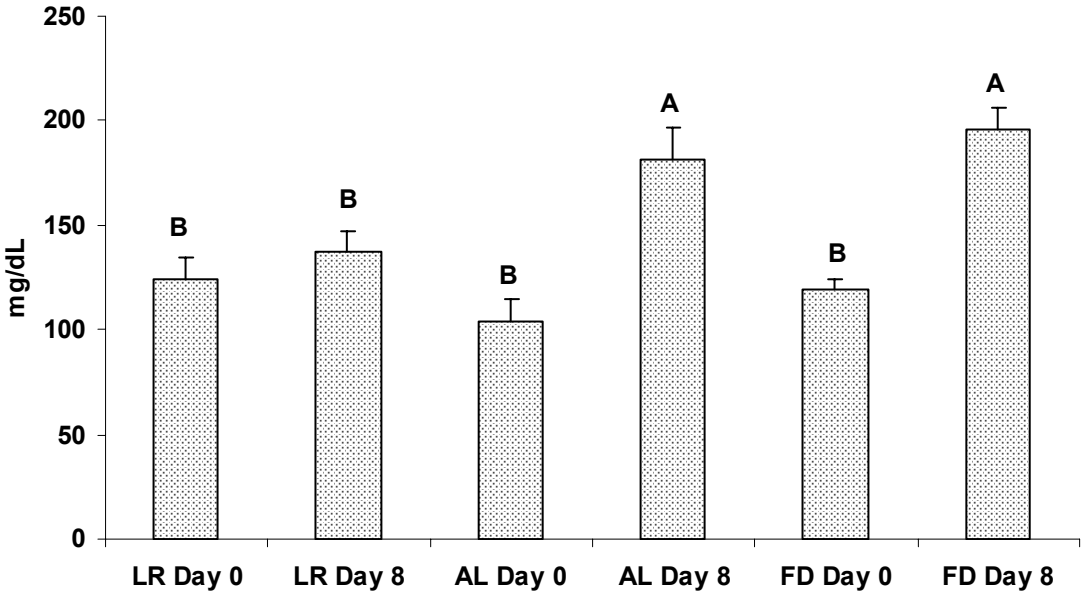

FIGURE V-4: Cholesterol levels of nonmolted hens (LR), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 2). Bars represent average and standard values for cholesterol levels (mg/dl). Blood was drawn on the first and last day of the molt according to methods ($p < 0.05$). * Suggests that hens molted by either alfalfa and feed deprivation had a significantly higher cholesterol level on day 8.

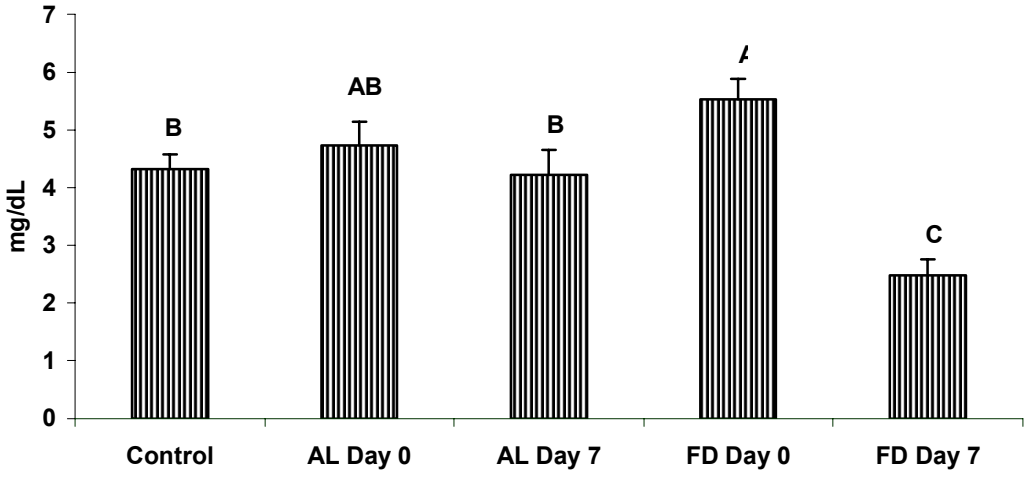

FIGURE V-5: Uric acid levels of nonmolted hens (Control), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 1). Bars represent average and standard error values for uric acid levels (mg/dl). Blood was drawn on the first and last day of the molt according to the methods ($p < 0.05$). * Suggests that birds molted by feed deprivation had a significantly lower level of uric acid on the last day of the molt.

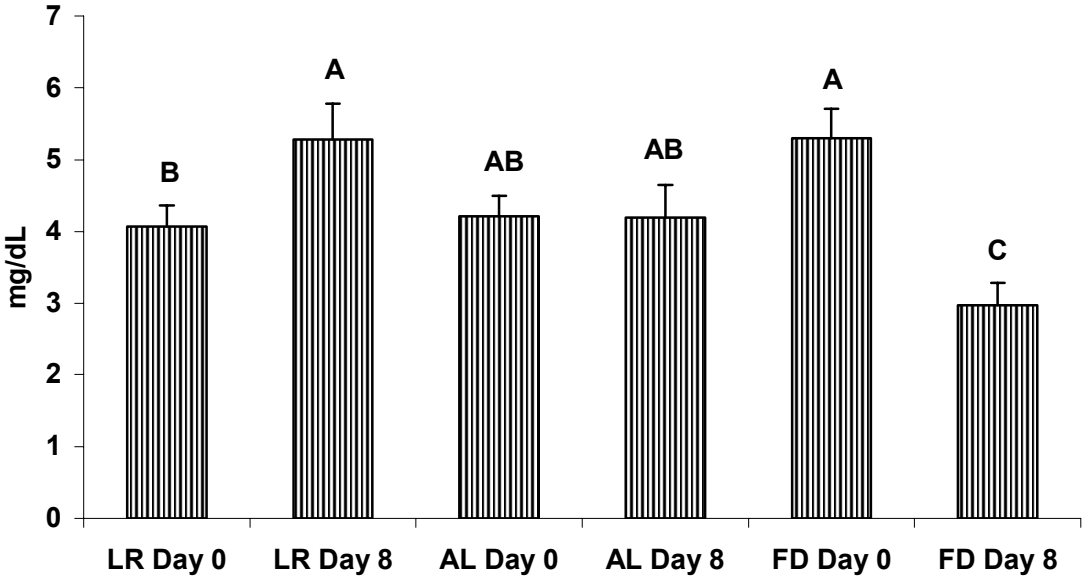

FIGURE V-6: Uric acid levels of nonmolted hens (LR), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 2). Bars represent average and standard error values for uric acid levels (mg/dl). Blood was drawn on the first and last day of the molt according to the methods ($p < 0.05$). * Suggests that birds molted by feed deprivation had a significantly lower level of uric acid on the last day of the molt.

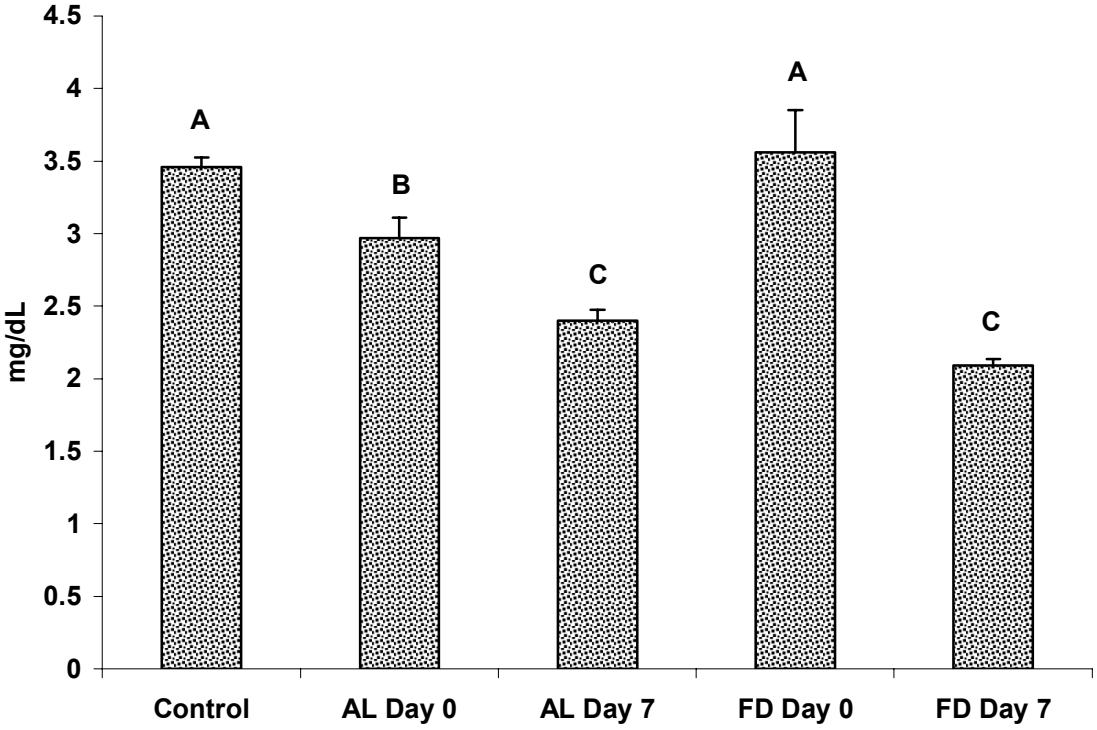

FIGURE V-7: Magnesium levels of nonmolted hens (Control), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 1). Bars represent average and standard error values for magnesium levels (mg/dl). Blood was drawn on days 0 and 7 according to the methods ($p < 0.05$). * Suggests that birds molted by either feed deprivation or alfalfa had a significant decrease in magnesium levels.

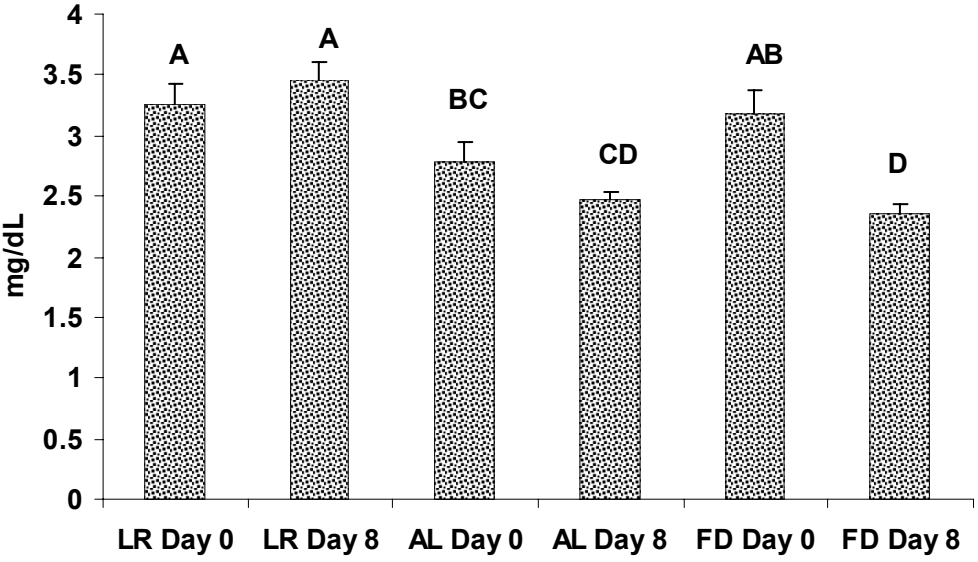

FIGURE V-8: Magnesium levels of nonmolted hens (LR), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 2). Bars represent average and standard error values for magnesium levels (mg/dl). Blood was drawn on days 0 and 8 according to the methods ($p < 0.05$). * Suggests that birds molted feed deprivation had a significant decrease in magnesium levels.

Table V-2: Serum chemistry parameters from hens molted by alfalfa or feed deprivation (Trial 1)[1]

	Control	Day 0 Alfalfa	Day 7 Alfalfa	Day 0 Feed Deprivation	Day 7 Feed Deprivation
Ketone Bodies	4.48±0.42^{C}	8.66±6.09^{C}	25.23±4.74^{B}	10.84±7.08^{BC}	46.89±5.20^{A}
Glucose	192.75±7.76^{B}	207.5±5.35^{B}	205.67±5.48^{B}	238.5±8.94^{A}	190.5±3.62^{B}
Total Protein	5.90±0.20^{B}	5.16±0.18^{A}	5.08±0.14^{A}	5.16±0.18^{A}	5.13±0.25^{A}

[1] – Means within a row with no common superscript differ significantly ($p < 0.05$)

Table V-3: Plasma chemistry parameters from hens molted by alfalfa or feed deprivation (Trial 2)[1]

	Day 0 Control	Day 8 Control	Day 0 Alfalfa	Day 8 Alfalfa	Day 0 Feed Deprivation	Day 8 Feed Deprivation
Ketone Bodies	414.06±52.61^{B}	474.48±56.10^{B}	349.28+36.07^{B}	1611.13+152.00^{A}	423.74+61.02^{B}	1841.36+220.35^{A}
Glucose	247.29+4.59	249.25+6.45	252.00+3.98	257.56+5.02	279.22+13.73	244.00+4.61
Total Protein	5.66+0.23	6.05+0.21	4.91+0.31	5.72+0.22	5.68+0.21	5.92+0.21

[1] – Means within a row with no common superscript differ significantly ($p < 0.05$)

triglyceride levels were only noted in trial. Birds molted by alfalfa had significantly lower triglyceride levels than that of the control, pre-molted hens, or hens molted by feed deprivation (Figure V-9). Triglyceride levels should decrease during the molt, as fatty acids are not mobilized from the liver for the purpose of follicular development. No significant differences were noted in any treatment for total protein or glucose (Tables V-2 and V-3).

The intestine was the only organ in which the weight from hens fed alfalfa differed significantly from hens molted by feed deprivation. Hens fed alfalfa had a significantly heavier intestine than that of hens molted by feed deprivation (Figure V-10). This could be due to the presence of feed in the gastrointestinal tract or the weight of the organ itself. Additionally, hens molted by either alfalfa or feed deprivation had significantly lower liver, ovary, oviduct, and pancreas weights as well as significantly higher spleen weights than non molted control hens (Figures V-11 to V-17). Birds kept under a normal lighting schedule had significantly higher heart weights than birds from either molting treatment in Trial 1; however, heart weights from molted hens did not differ significantly from the nonmolted control in trial 2 (Tables V-4 and V-5). In Trial 1, spleen weights did not differ significantly from the nonmolted control (Table V-4). In Trial 2, spleen weights from both molting treatments were significantly higher than the nonmolted control (Table V-5). There was no significant difference in adrenal weights, regardless of treatment (Table V-5).

Heterophil:lymphocyte ratios were significantly increased by the end of the molting treatments for hens that were feed deprived in both trials (Figures V-18 and V-

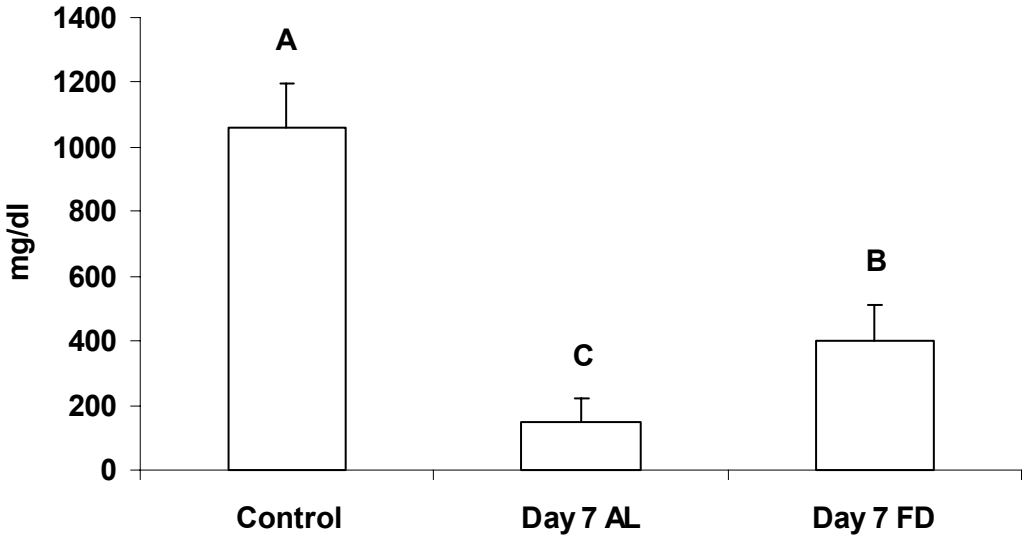

FIGURE V-9: Triglyceride levels of nonmolted hens (Control), hens molted by alfalfa (AL), and hens molted by feed deprivation (FD) (Trial 1). Bars represent average and standard error values for triglyceride levels (mg/dl). Blood was drawn on day 0 and day 9, according to the methods ($p < 0.05$). * Suggests that triglyceride levels are significantly decreased in birds molted by alfalfa and feed deprivation.

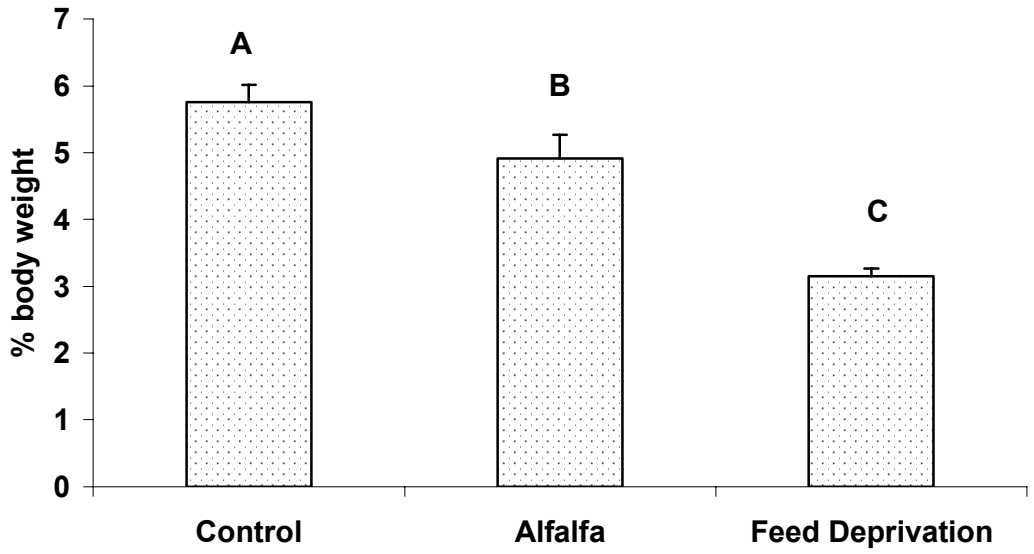

FIGURE V-10: Intestinal weights expressed as a percentage of body weight (Trial 2). Bars represent average and standard error values for intestinal weight, expressed as a percentage of body weight. Intestines were resected and weighed according to methods ($p < 0.05$). * Suggests that birds molted by either feed deprivation or alfalfa had a significantly lighter intestinal weight.

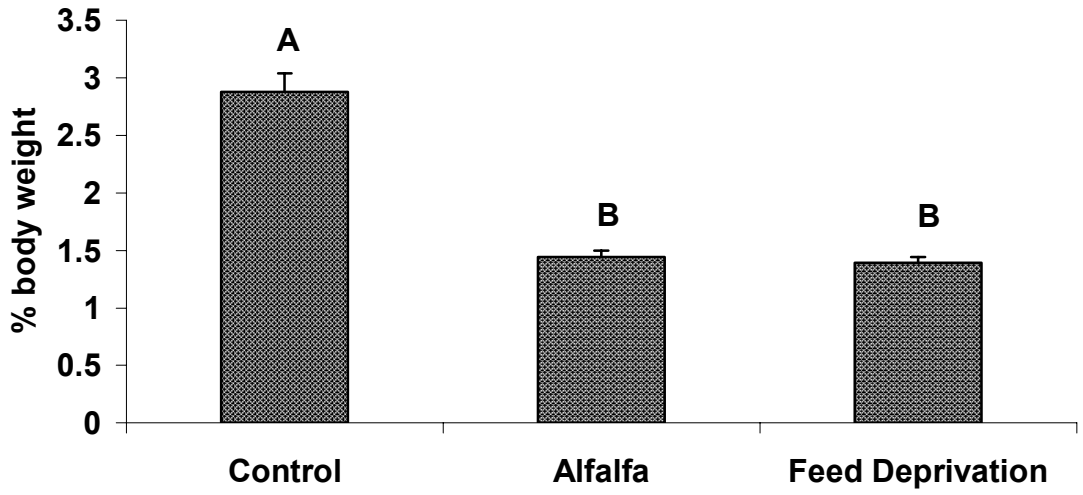

FIGURE V-11: Liver weights expressed as a percentage of body weight (Trial 1). Bars represent average and standard error values for liver weights, expressed as a percentage of body weight. Livers were resected and weighed in grams according to methods ($p < 0.05$). * Suggests that hens molted by feed deprivation have significantly lower amount of their body weight attributed to the weight of the liver.

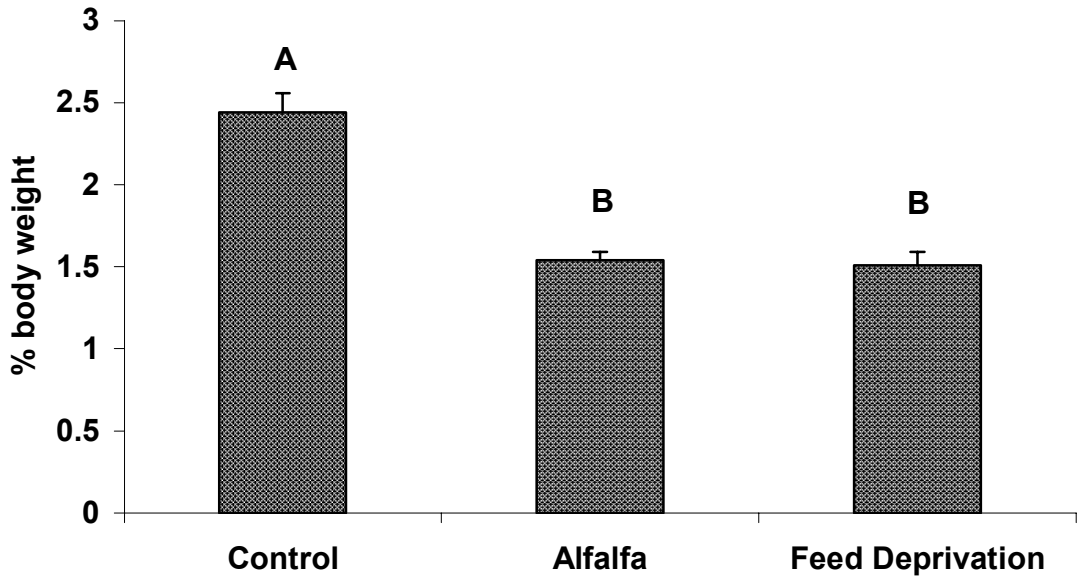

FIGURE V-12: Liver weights expressed as percentage of body weight (Trial 2). Bars represent average and standard error values for liver weights, expressed as a percentage of body weight. Livers were resected and weighed in grams according to methods ($p < 0.05$). * Suggests that hens molted by feed deprivation have significantly lower amount of their body weight attributed to the weight of the liver.

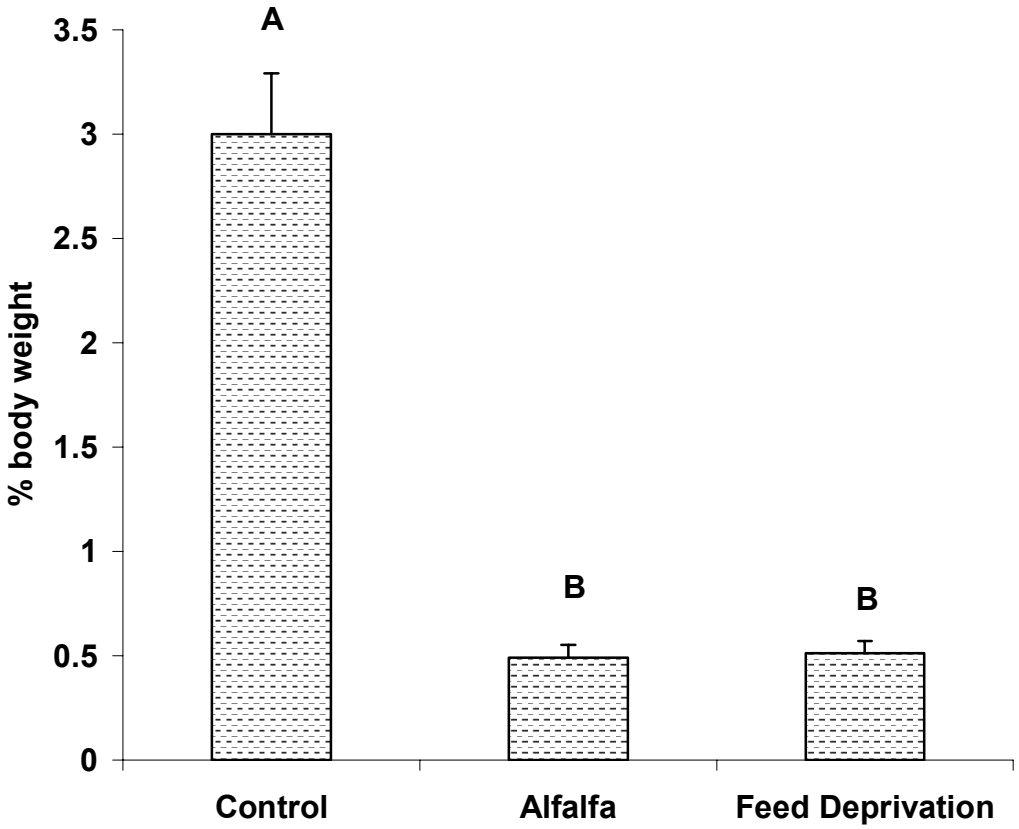

FIGURE V-13: Ovarian weights expressed as a percentage of body weight (Trial 1). Bars represent average and standard error values for ovarian weight expressed as a percentage of body weight. Ovaries were resected and weighed in grams according to methods ($p < 0.05$), * Suggests that birds molted by alfalfa and feed deprivation had significantly less of their body weight attributed to the weight of their ovaries.

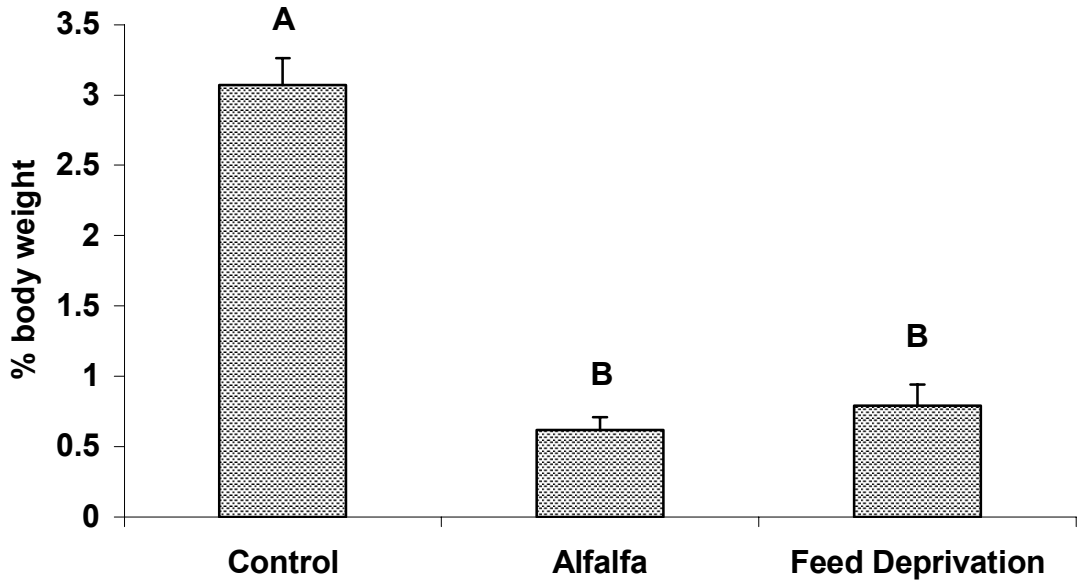

FIGURE V-14: Ovarian weights expressed as a percentage of body weight (Trial 2). Bars represent average and standard error values for ovarian weight expressed as a percentage of body weight. Ovaries were resected and weighed in grams according to methods ($p < 0.05$), * Suggests that birds molted by alfalfa and feed deprivation had significantly less of their body weight attributed to the weight of their ovaries.

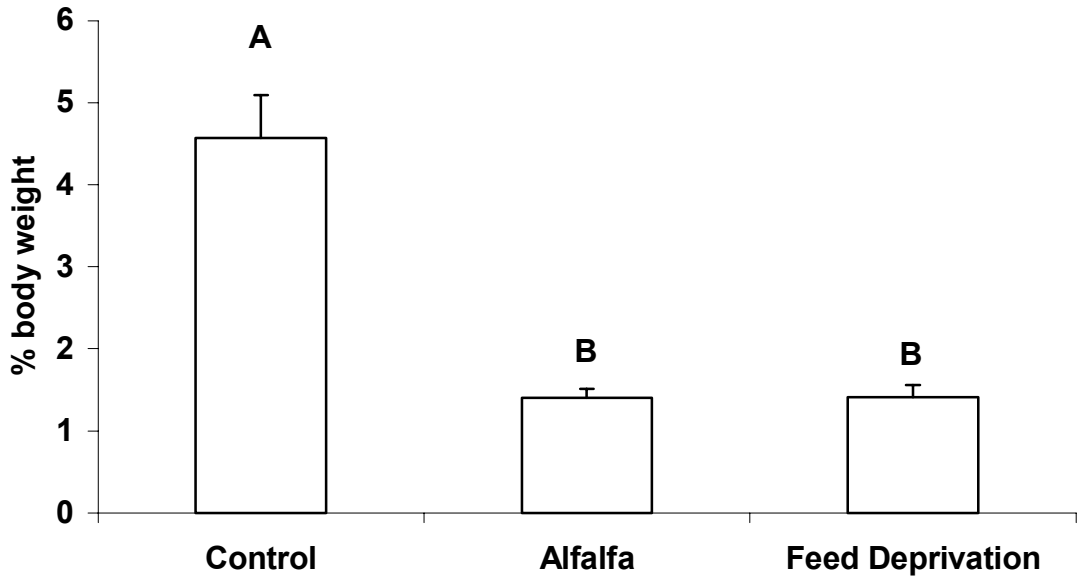

FIGURE V-15: Oviduct weights expressed as a percentage of body weight (Trial 1). Bars represent average and standard error values for oviduct weights expressed as a percentage of body weight. Oviducts were resected and weighed in grams according to methods ($p < 0.05$). * Suggests that birds molted by alfalfa and feed deprivation had significantly less of their body weight attributed to the weight of their oviducts.

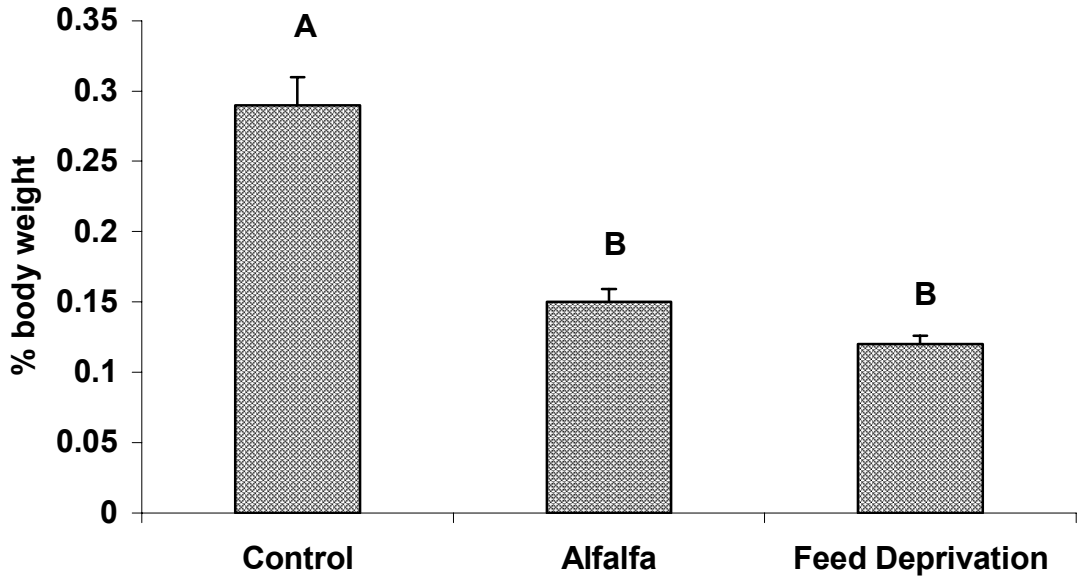

FIGURE V-16: Pancreas weights expressed as a percentage of body weight (Trial 1). Bars represent average and standard error values for pancreas weights expressed as a percentage of body weight. The pancreas was resected and weighed in grams according to methods ($p < 0.05$). * Suggests that birds molted by alfalfa and feed deprivation had significantly less of their body weight attributed to the weight of their pancreas.

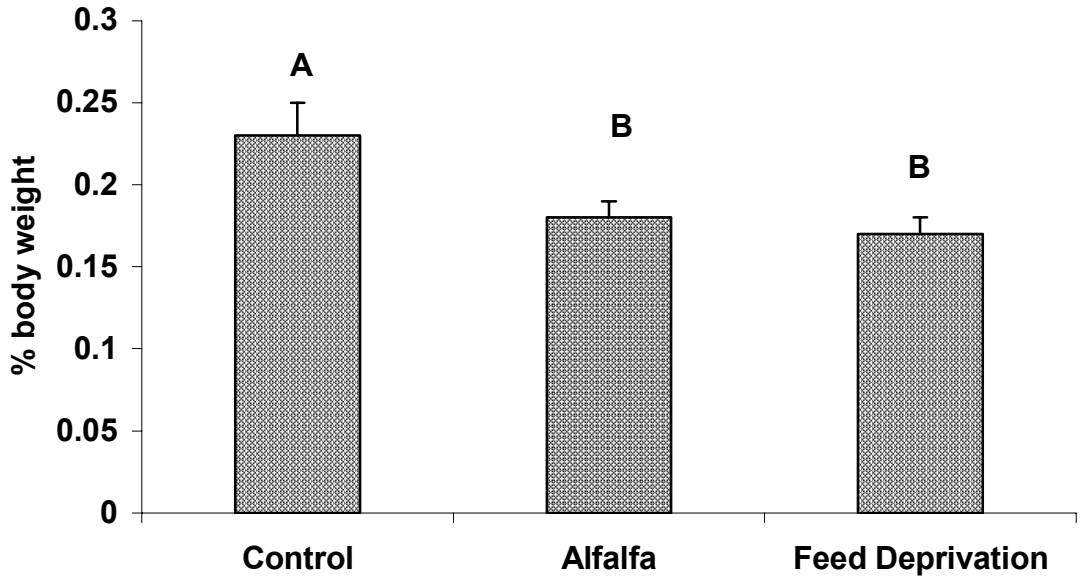

FIGURE V-17: Pancreas weight expressed as a percentage of body weight (Trial 2). Bars represent average and standard error values for pancreas weights expressed as a percentage of body weight. The pancreas was resected and weighed in grams according to methods ($p < 0.05$). * Suggests that birds molted by alfalfa and feed deprivation had significantly less of their body weight attributed to the weight of their pancreas.

Table V-4: Heart, spleen, and kidney weights expressed as a percentage of body weight (Trial 1)[1]

	Control	Alfalfa	Feed Deprivation
Heart	0.58 ± 0.03^{A}	0.43 ± 0.02^{B}	0.46 ± 0.01^{B}
Spleen	0.11 ± 0.006	0.11 ± 0.004	0.11 ± 0.006
Kidney	0.36 ± 0.01	0.28 ± 0.01	0.39 ± 0.07

[1] – Means within a row with no common superscript differ significantly ($p < 0.05$)

Table V-5: Organ weights expressed as a percentage of body weight (Trial 2)[1]

	Control	Alfalfa	Feed Deprivation
Heart	0.48 ± 0.01	0.48± 0.01	0.47± 0.02
Spleen	0.08 ± 0.004^{B}	0.10 ± 0.01^{A}	0.10 ± 0.004^{A}
Kidney	0.40 ± 0.01	0.39 ± 0.02	0.41 ± 0.03
Adrenal	0.007 ± 0.0006	0.008 ± 0.0006	0.007 ± 0.0008

[1] – Means within a row with no common superscript differ significantly ($p < 0.05$)

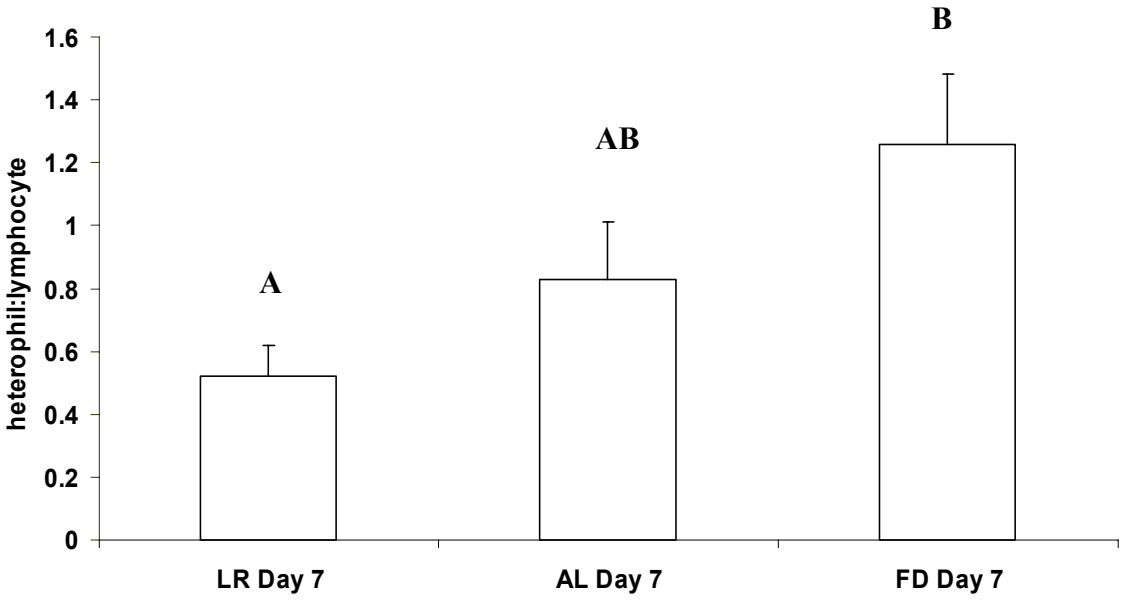

FIGURE V-18: Effect of diet on heterophil:lymphocyte ratio (Trial 1). Bars represent average and standard error values for heterophil:lymphocyte ratio. Blood smears were obtained on day 7 of the molt according to methods ($p < 0.05$). * Suggests that birds molted by feed deprivation have a significantly higher heterophiul:lymphocyte ratio when compared to birds that were not molted.

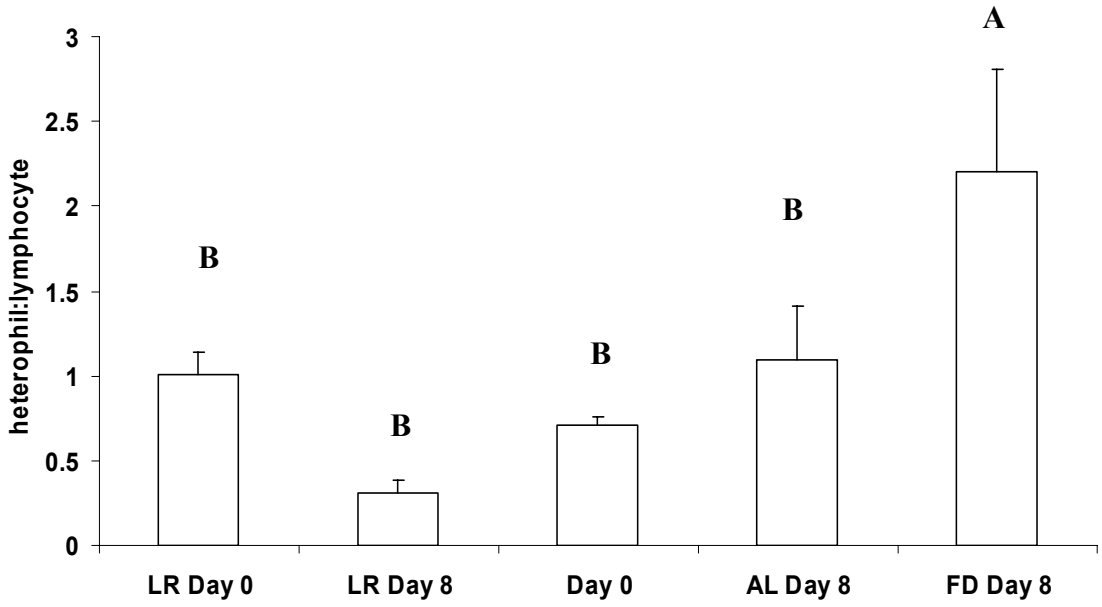

FIGURE V-19: Effect of diet on heterophil:lymphocyte ratio (Trial 2). Bars represent average and standard error values for heterophil:lymphocyte ratio. Blood smears were obtained on days 0 and 8 of the molt according to methods ($p < 0.05$). * Suggests that birds molted by feed deprivation have a significantly higher heterophiul:lymphocyte ratio when compared to birds that were not molted and birds molted by alfalfa.

19). Heterophil:lymphocyte ratios for hens molted with alfalfa were not significantly different from birds that were fed a complete layer ration in both trials (Figures V-18 and V19). In trial 1, hens molted by alfalfa had a heterophil:lymphocyte ratio of 083 $\pm$ 0.18 while hens molted by feed deprivation had a ratio of 1.26 $\pm$ 0.22. Birds molted by feed deprivation had a significantly higher ratio than that of the control (0.52 $\pm$ 0.10). In trial 2, results were similar. Hens molted by alfalfa had a lower heterophil:lymphocyte ratio (1.09 $\pm$ 0.32) than birds molted by feed deprivation (2.2 $\pm$ 0.61). Hens molted by feed deprivation had a significantly higher heterophil:lymphocyte ratio than both the non molted control (0.31 $\pm$ 0.01) and the day 0 baseline (0.71 $\pm$ 0.05).

CONCLUSIONS

Alfalfa effectively induced molt in both trials, as the ovarian weights were comparable to feed deprivation. However, differences in organ weights such as the intestine as well as differences in blood chemistry parameters for calcium, magnesium, uric acid, triglycerides, and cholesterol suggest that, physiologically speaking, hens molted by alfalfa differ from hens molted by feed deprivation. Increased levels of magnesium and calcium in hens molted by alfalfa may be due to the presence of these minerals in the diet or it could signify that the renal health of the birds may be better than that of birds that have been feed deprived. The decreased levels of uric acid in alfalfa molted hens could also signify greater renal health and hydration in alfalfa molted hens. Conversely, it could also mean that the birds have not adapted to the stressor of starvation. Levels of triglycerides and cholesterol in alfalfa molted hens seem to be in

accordance with what has been documented in other publications. Further research including corticosterone quantification, serotonin and melatonin levels, and avian behavior research during the molt will help to further elaborate on the physiological status of a hen molted with alfalfa.

CHAPTER VI

CONCLUSIONS

Molting by feed deprivation is a pressing issue in the commercial poultry industry due to increasing human health concern and the presence of animal activism. Finding an alternative to the use of feed deprivation for the induction of molt is of need in order to allow producers to continue providing eggs and egg products at competitive prices. An alternative such as alfalfa may be part of a solution to this problem.

In chapter III, it was shown that feeding older hens a diet of either alfalfa meal or alfalfa pellets was an effective way to induce molt, when ovarian weights were compared to hens molted by feed deprivation. These ovarian weights were consistent with what was seen in previous research using alfalfa as an alternative to feed deprivation for the induction on molt (Kwon, 2001). It was also shown that egg production and quality early in the second cycle of production from eggs laid by hens molted by alfalfa was comparable to the eggs from feed deprived hens. This is consistent with hens that were molted with destiller's dried grains (Biggs et al., 2002). While this is promising, using restricting sodium for the induction of molt also resulted in an increased level of egg production in the early part of the second cycle of egg production which tapered off in the later weeks of the second cycle (Scheideler et al., 2002). Further studies that look at production through the end of the second cycle are warranted to ensure that these production and quality levels remain comparable.

The information in chapter IV suggests that hens molted by alfalfa have eggs that are not only comparable in quality to eggs from feed deprived hens, but are also

indistinguishable when tasted by untrained consumers. Consumer perception of eggs is an integral part of the decision making process that producers use when determining what dietary changes would be most advantageous to a commercial laying flock. In fact, the production and marketing sectors of the commercial egg industry are interrelated to ensure a product that meets both governmental regulations and economical expectations (Hunton, 1995). The fact that perception did not seem to be altered was an important finding because it shows that using this molting technique in commercial facilities should not cause problems in consumer satisfaction with the organoleptic aspects of the product. Should additional sensory testing be warranted, comparions should be made independently to avoid confusion. Although, the successful triangle test should be sufficient.

Lastly, chapter V illustrates the physiological differences between hens molted by alfalfa and hens molted by feed deprivation. Our research has shown that differences in serum chemistry parameters may point to utilization of energy provided by the alfalfa or a resistance to adapt to starvation. However, the ketone body values for trial 2 suggest that the hens are physiologically enduring starvation when molted with alfalfa. Further research involving the physiological status of the hen is required to be able to fully answer this question. A particular area of interest will be stress levels of hens molted by alfalfa compared to hens molted by feed deprivation. The heterophil:lymphocyte data indicates that birds molted with alfalfa may have a stress level more consistent with that of a bird that is being fed a normal diet. This could be very important information, since producers would be able to use this as reasoning that molt induction is not as stressful as

animal welfare activists claim (Newkirk, 1999). Parameters such as vocalizations from hens and corticosterone levels will help in understanding the physiology of stress in the laying hen during an induced molt.

The issue of induced molting in laying hens is one of dichotomy. There is a need for producers to continue this practice in order to maintain normal operations. Conversely, this has become an intensely emotional issue for animal activists, and ultimately, the consumer. Researchers should continue to further study alternatives, such as alfalfa, in order to provide producers with a solution that is economically feasible and physiologically less stressful to the laying hen.

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Selected Abstracts:

Howard Z.R., K.L. Medvedev, R.W. Moore, S.G. Birkhold, and S.C. Ricke**.** Effects of storage time on growth of *Salmonella* Typhimurium in egg components. International Poultry Scientific Forum. Georgia World Congress Center, Atlanta, Georgia, January 20-21, 2003.

Medvedev K.L., R.W. Moore, C.L. Woodward, D.A. Landers, Z.R. Howard, J.A. Byrd, J. McReynolds, L.F. Kubena, D. Nisbet, and S.C. Ricke. Effect of alfalfa and feed deprivation molting techniques on various serum chemistry parameters in commercial laying hens. International Poultry Scientific Forum. Georgia World Congress Center, Atlanta, Georgia, January 20-21, 2003.

Li X., Z.R. Howard, I.D. Zabala, K.L. Medvedev, and S.C. Ricke. Development of research paper writing skills of poultry science undergraduate students taking food microbiology. Poultry Science Association, 91st Annual Meeting, August 11-14, 2002. Newark, Delaware.

Medvedev K.L., Z.R. Howard, S.G. Birkhold, and S.C. Ricke. Consumer sensory and mechanical evaluations of quality attributes of eggs from commercial laying hens Molted by alfalfa. Poultry Science Association, 91st Annual Meeting, August 11-14, 2002. Newark, Delaware.

Medvedev K.L., R.W. Moore, C.L. Woodward, D.A. Landers, Z.R. Howard, J.A. Byrd, L. Kubena , D. Nisbet, and S.C. Ricke. Effects of alfalfa and feed deprivation molting methods on leukocyte percentages in laying hens. Poultry Science Association, 91st Annual Meeting, August 11-14, 2002. Newark, Delaware.