EFFECT OF DIFFERENT FEED PROGRAMS ON BROILER BREEDER BEHAVIOR AND REPRODUCTIVE PERFORMANCE

by

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(Under the Direction of Jeanna L. Wilson)

ABSTRACT

Selection for increased growth rate has led to an increase in body weight and appetite in broiler breeders. Feed restriction programs are essential to prevent excessive body weight, poor uniformity, and poor reproductive performance. The high degree of restriction is associated with behaviors indicative of feeding frustration, boredom, and hunger. Qualitative feed restriction is a method to alleviate these behaviors while limiting the growth rate of the birds. Soybean hulls were given as an addition to the feed on OFF feed day as an alternative feed program (ATD). The ATD feed program was compared to a standard skip-a-day program or a skip-a-day program with added fiber (soybean hulls) on the ON day. The results show an improvement in performance with: increase in body weight, improved uniformity, and increased egg production. Changes in the behaviors of the birds on the OFF day show the birds attained a level of satiety with a decrease in foraging and increase activity around the feeder and drinker. The results suggest the ATD feed program can potentially improve broiler breeder production parameters and improve the well being of the birds.

The level of feed restriction can also impact feathering of the birds. Changes in energy and protein in the diet can impact reproductive performance, feather cover, and mating behaviors. Changes in energy and protein of 100 kcal/g and 2% CP did not have an impact on the performance or feathering of the birds. The results suggest the changes in the diet balanced each other or were not different enough to have an effect on the birds.

INDEX WORDS: Broiler Breeders, Soybean hulls, Performance, Behavior, Well being, Feather

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DEDICATION

I dedicate this work to my beloved family. Thank you for your unconditional love and support in every step. To my mom, Rossemary Arnez, who has inspired me to be my very best. To my dad, Juan Carlos Aranibar, who has always been my role model of hard work and perseverance. To my brother, Juan Carlos Aranibar, who always finds a way to put a smile on my face. I love you all so much!

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CHAPTER 1

INTRODUCTION

Broiler breeder females today are the product of intensive genetic selection for fast growth rate, efficiency, and production traits. Selection for fast growth and meat yield in broilers has lead to a increase in voluntary feed intake on broiler breeders (Richards et al., 2010). Broiler breeders fed ad libitum consume above their energy requirement for growth, maintenance, and reproductive capacity leading to overweight birds, severe health and reproduction dysfunctions (lameness, premature death, poor laying performance and poor fertility) during the laying period are characteristic of overweight hens (De Jong and Guemene, 2011). Efficient reproduction, health, and livability of broiler breeders must be maintained while retaining the genetic potential for fast growth and high meat yield. However, reproductive competence and growth are negatively related production traits (Siegel and Dunnington, 1985). Therefore, limiting the growth rate of broiler breeders to allow for appropriate reproductive development becomes crucial for broiler breeder management.

Feed restriction is necessary to manage weight as to maintain health and reproductive competence in broiler parent stocks. Feed allocation is limited to attain a target growth curve that maximizes reproductive performance. The high potential for genetic growth leads to a high level of feed restriction. In commercially applied restriction programs food intake is restricted to about 25-55% of the intake of ad libitum fed birds of the same age during rearing (Savory et al., 1996) and is restricted to 50-90% of ad libitum intake of hens at the same age when they are in lay (Bruggeman et al., 1999). Despite the improvement in livability and reproductive dysfunctions, severe feed restriction leads to chronic hunger as well as other negative effects that impact broiler breeder welfare.

Numerous studies have shown that feed restricted broiler breeders show behaviors indicative of frustration, boredom, and hunger (De Jong and Jones, 2006). The prevalence of behaviors such as stereotypic object pecking, overdrinking and increased foraging are indicators of a lack of satiety in the birds (Hocking et al., 2001). The birds are considered to be actively seeking another food source. Increase in general activity and decreases in comfort behaviors are indicators of stress on the bird (De Jong et al., 2003). Changes in the stress level have been quantified by comparing corticosterone levels of the birds (Mormede et al., 2007), with feed restricted birds having higher corticosterone levels. These changes in the bird's behavior are a sign that the birds are experiencing an increase in levels of stress and hunger.

The breeder dilemma becomes: there are welfare issues of health if feed restriction is not imposed and there are welfare issues of hunger if it is (Decuypere et al., 2006). New strategies have to be implemented in management and selection to achieve the desired balance of productivity and welfare. One of the main strategies is to attempt to decrease the level of restriction by having a qualitative change in the diet. Qualitative restriction aims to increase feed volume by feeding a lower density diet. The increase in volume should lead to an increase in feeding time, increase gut fill, and thereby decrease feeding frustration. Previous studies have considered the use of higher fiber sources such as oat hulls, ground unmolassed sugar beet pulp, sunflower meal, and soybean hulls (Sandilands et al., 2014, Hocking et al., 2004, and De Jong et al., 2005). Most of the treatments increased the time spent eating and led to a lower number of birds performing non-feeding oral activities. However, the results were not consistent for all of the treatments and vary based on the level of inclusion in the diet and the fiber source used. In addition, there is also an impact on the birds' behavior and overall performance based on feeding frequency or feeding program (Moradi et al., 2013). The level of dilution, ingredient source, and feeding program that has the best effect on welfare is yet to be determined. Therefore, more research needs to be conducted evaluating more performance parameters and behaviors with inclusion of different ingredients at different levels.

The changes in feeding strategies and management practices due to the intensive level of genetic selection also have a direct impact on broiler breeder feathering. Feathering is greatly impacted by flock management, environmental conditions, bird density, feed management, flock health, body weight uniformity, and nutrition. Feathering plays a crucial role in insulation, feed intake and energy utilization (Emmans and Charles, 1977). In addition, feather loss can impact egg production in a commercial facility (Mills et al., 1988) and fertility due to its association to mating activity (Jones and Prescott, 2000). Therefore, understanding the impact on feather loss of changes in management and nutrition becomes extremely important for broiler breeder production.

The strategy to avoid excessive weight gain of broiler breeders, is to manage the energy and protein content of the diet. Changes in energy content in the diet have a direct effect on body weight, leads to changes in feathering production and have the potential to decrease fertility (Robinson et al., 1993 and de Beer and Coon, 2006). Changes in protein content impact body weight, breast yield, feathering, egg production and egg size (Harms and Ivey, 1992 and Lopez and Leeson, 1995). Therefore, it is important to evaluate the impact of the diets with different proportions of energy and protein levels in the diet have on the reproductive performance and feather cover of the birds.

In order to address some of the questions raised by the use of alternative approaches for managing broiler breeders a study was designed to explore possible feed regimes. The main objectives of this study were:

1. To evaluate the impact of an alternative feed program, in which soybean hulls are provided to the birds on a OFF feed day, compared to a standard skip-a-day feed program has on performance, behavior and plasma corticosterone levels.
2. To evaluate the impact of an alternative feed program, in which soybean hulls are provided to the birds on an OFF feed day, to a skip-a-day feed program with added soybean hulls on an ON feed day has on performance, behavior and water usage.
3. To evaluate the effect of an increase in energy and decrease of protein in a diet on reproductive performance, feather cover and its relationship to reproductive behaviors.

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CHAPTER 2

LITERATURE REVIEW

Genetic selection has played a key role in the advancement of poultry production. There has been tremendous genetic progress for growth and feed conversion through the efforts of primary breeding companies. Modern breeds have been heavily selected for high growth rate, breast-meat yield, and efficiency of feed conversion. This has left broilers vulnerable to welfare problems such as cardio-vascular diseases and lameness. The rapid growth rate and the change in feed intake have a negative impact on birds that are grown to adulthood to become broiler breeders. Due to genetic selection, broiler breeders rapidly become overweight leading to difficulty in mating, reduced levels of fertility, decreased egg output, and increased levels of aggression. In order to attenuate the fast growth and efficient gain, broiler breeders are feed restricted, and as genetic selection has increased these traits the levels of feed restriction have likewise increased leading to a new set of welfare problems. Broiler breeders are metabolically hungry since their needs are not met, changing their behavior and stress level. The breeder dilemma becomes: there is a welfare issue of health if feed restriction is not imposed and there is a welfare issue of hunger if it is (Decuypere et al., 2006).

EXCESS BODY WEIGHT MALES AND FEED RESTRICTION

Overweight roosters have reduced fertility and difficulty mating which has a negative impact on hen fertility. Male body weight influences the production of semen in males. Therefore, reproductive problems are associated with overweight and underweight males (McDaniel at al., 1981). Underweight males usually do not achieve target weight and are thereby less reproductively competent. In contrast, heavy males produce more semen and have higher spermatozoa levels at an early phase in production (Sexton et al., 1989). However, research shows that the consequence of overweight roosters is seen at a later stage during which a decline in fertility becomes more critical (Robinson and Wilson, 1996). A steeper decline in semen production and testes size was seen at 58 weeks in roosters that were heavier at the early phase of production (26-30 weeks) (Robinson and Wilson, 1996). Although semen production is benefited in the short term, over weight roosters become a concern for long-term production.

Body weight can also impact the behavior of broiler breeder males. Mating behavior is a key aspect of broiler breeder management since it can contribute or limit the level of fertility of the flock. In a series of four natural mating trials, body weight was negatively correlated to fertility (r=-0.39 to 0.09) (Wilson et al., 1979). Differences in fertility might be attributed to decreased sperm motility and lower mass of sperm cells (Morrison et al., 1997). The effect of body weight on mating behavior can also play a role on the decrease in fertility. Burke and Mauldin (1985) determined a negative relationship between body weight and mating activity. Overweight males have a decrease in the number of completed matings. The decrease in completed matings leads to a decline in fertility on the flocks.

Overweight roosters have difficulty mating due to physical constraints and courtship behavior changes. Excessive body weight has an impact on breast meat conformation and musculoskeletal problems on roosters. Breast yield in overweight males is increased compared to standard males (Fragoso et al., 2013). The change in the birds' conformation leads to a decline in complete copulations (Fragoso et al., 2013). Bilcik et al. (2005) showed that heavier males have a higher frequency of mating attempts without cloacal contact. Therefore, even though the birds are motivated to mate their conformation prevents them from successfully mating. Excessive weight on roosters leads to musculoskeletal problems such as destructive cartilage loss, dyschondroplasia, and ruptured tendons and ligaments (Hocking and Duff, 1989). These physical constraints make the roosters unwilling and unable to mate. Overweight roosters become more aggressive, changing their courtship behaviors leading to a decline in mating. Males adopt forced copulations as a mating strategy when females are not receptive (Jones et al., 2001). Heavier males have a higher frequency of forced matings (Bilcik and Estevez, 2005). The higher levels of forced mating can result in damage to the hens, decline in feathering, and an overall decrease in fertility.

The most common strategy to control male body weight and optimize reproductive performance is feed restriction. Research has shown that feed restriction of breeder males can be accomplished without negative effect on fertility and hatchability (Buckner et al., 1986). Broiler breeder males subject to feed restriction in the rearing phase are smaller than full fed males (Robinson and Wilson, 1996). The decrease in growth gives more time for males to reproductively mature prior to photo-stimulation. Scogin et al. (1980) found that the increase in feed in small allotments can significantly increase sperm cell numbers and semen volume. Therefore, the degree of restriction does not negatively affect the development of reproductive traits but can actually improve them.

Feed restricted males do not experience as big an increase in breast muscle and fat pad weight (McGovern et al., 2002). The conformation of feed restricted roosters becomes less of a concern in regards to mating activity and fertility. In addition, males with higher levels of feed restriction produce larger semen volume in the later phase of production (Brown and McCartney, 1986). The increase in semen volume becomes crucial in the later phase of production. As hens age the sperm storage ability of hens decreases (Pierson et al., 1988). Therefore, higher semen volume may lead to an increase in fertility in the later phase of production. Feed restriction is a viable method to control body weight of male broiler breeders and has the potential to positively impact fertility in the later phase of production.

EXCESSIVE BODY WEIGHT FEMALES AND FEED RESTRICTION

Overweight females have reduced levels of egg production, fertility, multiple ovulations, and decline in egg quality. Body weight has huge a impact on egg production. Robinson and Wilson (1996) showed that heavier females have lower levels of egg production. The researchers associate the decrease in egg production level to the lack of regulation of the ovarian hierarchy. The changes in regulation of ovarian hierarchy also lead to erratic laying problems, higher incidence of multiple-yolked eggs and shell quality problems (Robinson and Wilson, 1996).

Fertility is also impacted by body weight. Overweight broiler breeders have a reduced duration of fertility (Goerzen et al., 1996). Fertility is based on the hens' sperm storage capacity, sperm receptor, sperm transportation and oocyte health. The decline in duration of fertility can be related to an impact on the oocyte causing ovarian regression. The decline can also be associated to the effect body weight has on hormonal concentrations. Renema et al. (1999) found that the ovary morphology and hormone profiles vary based on the bird's body weight. The change in the hormone profile plays a huge role in the reproductive performance of the bird. Changes in hormone profile can cause birds to mature prior to photo-stimulation (Renema et al., 1999). This could lead to a difference in laying cycle diminishing the birds' reproductive capacity.

Egg quality and embryonic mortality is correlated to body weight. Embryonic mortality is high in the eggs of overweight hens (Robinson et al., 1993). Embryonic mortality is related to the nutritional status of the hen. A good nutritional status of the parent birds is crucial to the hen's transfer to the egg of an adequate, balanced supply of nutrients required for normal development of the embryo (Wilson, 1997). Therefore, nutritional excess seen in overweight hens could impact embryo mortality. Robinson et al. (1993) also reported egg calcification differences between overweight and standard hens. Overweight hens lay poorly calcified eggs leading to increased shell porosity, egg weight loss, and increased embryonic mortality.

Feed restriction is crucial in order to control weight gain of female broiler breeders to achieve desired levels of production. Restricting the quantity of feed reduces the weight of the ovary and the number of large follicles, the incidence of erratic ovipositions, defective eggs and multiple ovulations (Yu et al. 1992). The most critical period for limiting body weight increase is between 7-15 weeks (Bruggeman et al., 1999). This period requires the highest degree of feed restriction. Feed allocation is reduced to one-third of ad libitum-fed birds in rearing (de Beer and Coon, 2007) and up to 50- 90% in lay (Bruggeman et al. 1990). The high levels of feed restriction have an impact on behavior that will be discussed later in this review.

FEED RESTRICTION PROGRAMS

Feed restriction was successfully adopted by the poultry industry to control body weight of broiler breeders. However, the level of feed restriction and feed program differs among poultry companies. Reproductive performance and efficiency are different based on the different feed program. Most feed programs use similar weekly feed amounts as suggested by the breeding companies but differ in when they provide the feed. The four common programs are:

1. Every day (ED) – means birds fed every day.
2. 5/2 – means 5 feed days and 2 days with no feed.
3. 4/3 – means 4 days per week with feed and 3 days with no feed.
4. Skip-a-day (SAD)- means the birds are fed every other day.

Skip-a-day feeding is advantageous when feeding low amounts of a high-density feed. On feeding days the birds get a double ration of feed leading to a longer feeding period. This allows smaller birds to get access to feed, improving uniformity in the flock. The 5-2 and 4 –3 feed programs are a compromise between ED and SAD feed programs. These programs reduce the amount given on feed days compared to SAD. These programs are used during the later part of the growing period. The 5-2 and 4-3 feed programs decrease the prevalence of distended crops when too much feed is provided (Cobb Management Guide). No differences were found in regards to BW, frame size, uniformity, and egg production between SAD, 5-2, and 4-3 birds (de Beer and Coon, 2007). The 5-2 had significantly higher egg weights compared to the other birds under the other feeding programs. However, the differences did not have an impact on fertility.

Every day feeding programs are thought to be the more welfare friendly restriction program. However, research shows that ED under the same diet and restriction level might not be as beneficial compared to SAD. Birds on ED feed programs are still quantitatively restricted as SAD but are offered feed every day. Due to the levels of feed restriction the amount of feed offered is very small. Low amounts of feed lead to a short feeding time and body weight uniformity problems (Bartov et al., 1988). ED birds are consistently heavier than SAD (Leeson and Summers, 1985, Katanbaf et al. 1989a). The reduced level of feed efficiency in SAD birds is likely due to the nutrient turnover required for birds to store and mobilize nutrients on their off feed day (Richards et al., 2010). This can also relate to the difference in body composition with ED birds having less fat compared to SAD (de Beer and Coon, 2007).

The difference in body weight also impacts the age at sexual maturity. SAD birds are delayed in achieving sexual maturity compared to ED birds (Wilson et al., 1989). In regards to egg production, ED feeding can provide an advantage compared to SAD with higher levels of egg production and higher peaks in production (de Beer and Coon, 2007, Katanbaf et al., 1989b). Egg weights are also significantly different with SAD birds exceeding ED birds (de Beer and Coon, 2007). Both aspects may be related to the age at which the birds reached sexual maturity.

CONSEQUENCES OF FEED RESTRICTION

Behavior

Despite the benefits obtained through feed restriction such as increased livability and improved egg production, there is substantial evidence that feed restriction has negative effects on broiler breeder welfare. Numerous studies have shown that feed restricted broiler breeders show behaviors indicative of frustration, boredom, and hunger such as stereotypic object pecking, overdrinking, and pacing. (De Jong and Jones, 2006).

The level of general activity increases on quantitative restricted compared to ad libitum fed hens (de Jong et al., 2003, Hocking et al., 2001, Merlet et al., 2005). In order, to evaluate whether there is a negative impact on welfare it is important to evaluate individual behaviors. Non-feeding oral activities such as redirected pecking and stereotypy are considered negative behaviors. Pecking is often redirected to other birds leading to feather pecking problems. Feed restricted birds have higher levels of non-feeding oral activities then ad libitum birds (Hocking et al., 2004, Merlet et al., 2005, and Savory et al., 1992). The increase in pecking and foraging is also associated with feed seeking behavior. Van Emous et al. (2014) determined that higher foraging activity in birds is observed with birds that experience a certain degree of hunger. The increase of these behaviors is indicative of a lack of satiety in the birds.

The attention of the birds can also be redirected to other objects such as the drinker. Previous researchers found an increase in water usage on birds fed a restricted diet compared to ad libitum (Hocking et al., 2001, Savory et al., 1992, and Savory and Maros, 1993). The increase in water usage suggests that the birds might be experiencing boredom and are using the drinker as a distraction. However, other researchers suggest that the water to feed ratio is the main stimuli to water usage (Bennett and Lesson, 1989). They determined the amount of feed the birds consume plays a more significant role in water usage. However, water usage on birds might still provide an insight to the birds feed consumption and motivation.

Welfare aims to promote behaviors that are natural and that encourage a positive state in the animals. Comfort behaviors such as preening, dust bathing, and nesting are a good measurement of welfare for broiler breeders. Feed restricted birds exhibit less comfort behaviors (de Jong et al., 2003 and Putterflam et al., 2006). As the levels of feed restriction increases fewer amounts of comfort behaviors are exhibited suggesting a hunger related issue. There is also significant differences when comparing the number of birds exhibiting comfort behaviors on a ON and OFF feed day basis. There are more birds exhibiting comfort behaviors the day they are fed versus the day they do not receive any food (De Jong et al., 2005). The decrease in comfort behaviors shows the birds have a level of stress and hunger associated with the level of restriction.

Physiology

Feed restriction also has an impact on the physiology of the birds. Plasma corticosterone is released as a response to stress (Mason et al., 1968) and is commonly used to measure welfare. Research has conflicting results on the impact of feed restriction on plasma corticosterone. Some results showed higher levels (Hocking et al., 1988, Hocking et al., 2001, Kurbikova et al., 2001) and other showed no impact (Hocking et al., 2003, Savory et al., 1993). Corticosterone is not only released as a response to stress; it plays a role in various metabolic effects, resulting in an increase or maintenance in glucose. It also controls food intake with brain interactions and changes in response to exciting stimuli (D'earth et al., 2009). Therefore, the change in plasma corticosterone might not be directly related to the level of stress in the bird based on feed restriction level.

Heterophil: lymphocyte (H/L) ratio is another physiological measure to access stress. The level of dietary cortisone can induce lymphocytopenia and granulocytosis and affect the H:L ratio (Davison and Rowell, 1983). In the same manner as corticosterone measurement, research shows that the H/L ratio can either increase (Hocking et al., 2003, Savory et al., 1993) or maintain the same (Savory et al., 1996, Maxwell et al., 1992) in birds under feed restriction compared to ad libitum. Davis et al. (2008) indicates there is an inherent variation in white blood cell count among individuals limiting the utility of this method to measure stress. In addition, the lack of reference to evaluate H/L ratio makes it difficult to differentiate between acute and chronic stress levels. Therefore, there are inadequacies of the H/L method as a reliable stress indicator (Cotter, 2014).

Although there is conflicting data in regards to what is the best method to measure stress on a physiological level and whether we can define changes in welfare based on these measurements; there is a difference in the physiology of birds on quantitative levels of feed restriction. These differences combined with the changes in behavior are indicators that broiler breeders on restricted feed consumption exhibit negative signs of welfare.

There is no doubt that feed restriction has a positive effect on bird performance; however, it is important to acknowledge that there is an impact on the birds behaviors and the physiology of the birds. With genetics pushing for faster growth and efficiency in broiler breeders' progeny, the increasing levels of restriction have become a problem for the bird's welfare. In animal production worldwide, it is important that the welfare issues be addressed on every level of poultry production. New alternatives to the current feed restriction programs to diminish negative behaviors associated with stress and hunger need to be tested and changes implemented.

NEW STRATEGIES FOR BROILER BREEDER MANAGEMENT

The dilemma between production and welfare leads to the main question: Can the growth requirements of broiler breeder hens be aligned with good reproductive performances, health and welfare? New strategies in management and selection have to developed in order to achieve the desired balance. Some of the strategies currently discussed are the change in genetic selection by introducing dwarf lines and changing quantitative feed restriction to qualitative feed restriction by adding higher fiber or appetite suppressants.

Change in genetic selection

The use of slow growing bird lines is an alternative to decrease the negative aspects related to feed restriction. Some genetics companies are using a dwarf gene in an attempt to reduce feed restriction levels without impacting reproductive performance. Several genetic lines have been launched in Europe; these lines carry the sex-linked dwarfing gene in the females. The dwarf gene leads to lower levels of feed consumption (Merat, 1990). Therefore the birds can be grown on ad libitum feeding without becoming overweight and without decreasing performance parameters. Tona et al. (2004) compared two slow growing bird lines to the standard broiler breeder hen. They determined that slow growing birds could provide adequate egg production, however feed would still have to be restricted but in a less intensive manner. Welfare of the birds can be improved by replacing the standard food-restricted broiler breeders with food-restricted dwarf genotypes (Jones et. al., 2004). There is a decrease in the time spent on non-feeding oral activities and a decrease in H:L ratio in broiler breeders with dwarf genotypes compared to the standard broiler breeder line.

Although the same production parameters can be achieved there are several aspects that need to be taken into consideration with slow growing birds. The progeny from hens of dwarf lines (broilers) are not as heavy as the ones from the standard line. Therefore, it requires more resources for them to reach market weight and more time leading to lower number of broilers produced overall. In addition, economically it will be a lot more expensive to feed the hens. By reducing the levels of feed efficiency, the amount of feed required for hens to achieve desired body weights prior to photo stimulation will drastically increase. Changing the genetic lines of birds can reverse negative welfare aspects due to feed restriction; however, will require a change in management practices and impact economy of production leading to a more expensive meat product.

Qualitative Restriction

Another alternative to current feed restriction practices is to change from a quantitative to qualitative restriction. Several studies have evaluated the use of low-density diets (qualitative restriction) as a possible method to reduce stress and hunger.

The objective of these diets is to increase the feed intake time leading to a decrease in frustration. In addition, low-density diets may promote satiety through a more filled gastrointestinal tract, and thus feeling of hunger is reduced.

Sandilands et al. (2005) used supplements of calcium propionate (an appetite suppressant), oat hulls, or a combination to increase feeding time. They compared the birds on these diets to birds on a standard feed restriction diet. As expected they observed the time the birds spent eating increases for birds on these diets compared to the standard. The increase in time spent eating correlated to changes in behaviors of the birds. They observed a lower number of birds performing non-feeding oral activities. The same results were seen in different studies with researchers making a correlation between the increase in time spent eating to a decrease in pecking and stereotypy (de Jong et al., 2005 and Hocking et al., 2004). In fact, Hocking (2006) found an increase in number of birds exhibiting comfort behaviors on a high fiber diet versus the standard.

Alternate diets change the behavior of the bird and seem to go towards a more normal set of behaviors suggesting an improvement in welfare. Other researchers suggest the birds are not going towards natural behaviors but are only substituting the negative behaviors (Savory et al., 1996). The birds on alternate diets are redirecting their attention from pecking at each other to pecking at the feed (Mason et al., 2007). Therefore, by changing to alternate diets, we are not decreasing the feeding frustration we are just redirecting their attention to the feed. In order to prove there is a reduction in stereotypy rather than counting the number of birds performing the action a proportional decrease of the action needs to be evaluated (D'Eath et al., 2009).

In regards to physiology measurements, some researchers found low-density diets did not have an effect on plasma corticosterone concentration (de Jong et al., 2005 and Sandilands et al., 2006). Changes in H:L ratio were reported by Hocking et al. (2003). They showed that the H:L ratio can be decreased by feeding broiler breeders diets containing 50 g/kg of high fiber ingredients. However, the change in the H:L ratio is not consistent in all studies with some showing higher levels (de Jong et al., 2005, and Savory et al., 1996) or only having a difference at a specific week (Zuidhof et al., 1995). The differences in results show that other methods need to be used in order to evaluate stress levels on birds or the methods needs to be standardized.

In order to be able to accurately say whether or not these treatments improve welfare of broiler breeders more research needs to be conducted. We need to improve our understanding on how high fiber diets impact the bird and how it relates to the bird's behavior. In addition, the different ingredients and inclusion levels in the diets may impact the differences in results observed. Therefore, more research in these areas will allow us to get a better understanding on the impact of alternate diet and whether or not they improve welfare.

FEATHER LOSS AND PRODUCTION PARAMETERS

The changes in feeding strategies and management practices due to the intensive level of genetic selection also have a direct impact on the broiler breeder feathering. Feathering is greatly impacted by flock management, environmental conditions, bird's density, feed management, flock health, body weight uniformity, and nutrition. The changes in nutrition to manage birds' growth or improve well-being through dilution can have a negative effect on feathering. Katanbaf et al. (1989a) showed there is an increase in feather loss as levels of restriction increase. Therefore, it is important to take in consideration the effect of the diet on feathering and the impact it has on performance.

Body Weight and Feed Intake

Body weight is controlled in broiler breeders to optimize performance by achieving good peak production and consistency in lay. Body weight is also directly correlated to feather cover on laying birds; with heavier birds having better feather conditions than birds with lower weights (Renema et al., 2007). The lack of coverage contributes to body weight differences. By 40 weeks, feather loss deteriorates the weight of the hens (Damme and Pitchner, 1984). Feathers provide insulation for broiler breeders. There is a higher maintenance requirement for birds in a lower temperature (Neme et al., 2005). Birds with less feather coverage utilize more energy to regulate their temperature. Thereby hens with more feather loss use their energy for maintenance over growth and egg production making them smaller and can potentially decrease egg production compared to covered birds.

Feather cover can also impact feed consumption on laying hens. Feed consumption increases when feather cover decreases (Emmans and Charles, 1976). Tauson and Svennson (1980) determined that naked birds eat 41 g/bird more feed compared to fully feathered birds. During cold weather birds feed consumption can be up to 30% higher in hens with feather loss (Glatz, 2001). The increase in feed consumption might be attributed to energy maintenance for birds. The birds consume more feed in order to fulfill the increase in energy requirement on birds with feather loss. The increase in feed consumption for maintenance is substantial therefore it is important to monitor feather cover condition and determine causes of feather loss.

Egg Production, Mating Behaviors, and Fertility

Poor feathering has been associated with higher egg output. Mills et al. (1988) found a negative relationship between total egg production and overall feathering. The findings are consistent with previous research on layer hens (Hughes, 1980, and Tullett et al., 1980). The researchers link the level of egg production to the increase in feather loss to the abrasiveness caused by the cages or nests. The hens that have higher levels of production spend more time at the nests increasing feather loss. However, the difference in egg production might be attributed to changes in energy expenditure of the hens. The higher levels of egg production lead to difference in feed intake and higher energy requirements (Ivy and Gleaves, 1976). Therefore, if the amount of feed given to the bird does not increase based on their egg output, the bird will likely use some of the energy that could be used for feather maintenance on egg production. Therefore increase in energy expenditure on production might lead to a decrease in feather maintenance.

Feather loss has been directly related to mating frequency. The industry assumed that feather loss on the back of the hens meant they were more readily available to males (Jones and Prescott, 2000). Feather quality and the degree of feather coverage were theorized to be indicative of mating activity, because they result in feather damage and loss from the back of the hen. Females with feather loss were thought to be mated more frequently and have higher fertility levels. However, recent research suggests that feather loss causes a decline in mating attempts and completed copulations. In fact, Moyle et al. (2010) determined that hens with greater feather loss actually had fewer mounts by males. This relates to the hen's receptivity to be mated. Hen receptivity has a direct impact on mating behaviors (Casanovas and Wilson, 1998). Hens with high levels of feather loss are less receptive to be mated, actively avoid males leading to lower mating activity (Renema et al., 2007). The decrease in hen receptivity lead to increases in attempted and completed matings. Therefore, the decrease in feather cover leads to differences in hen receptivity and decline in mating activity.

There is a relationship between the degree of feather loss and fertility. There are no changes to the reproductive morphology of the birds when increased feather loss occurs (Renema et al. 2007). Therefore, changes in fertility due to feather loss are linked to the changes in mating activity. There is a direct impact of mating activity on fertility (Jones and Prescott, 2000). As mating activity declines so does the fertility level of the hens. The decline in mating activity attributed to a decrease in feather loss, leads to a decline in fertility.

CONCLUSION

Genetic selection for high growth and efficiency in broilers also impacts broiler breeders leading to overweight birds. Research shows that there has been an impact of genetic selection on feed regulation causing birds to have excessive levels of feed consumption. The high levels of feed consumption tied with the high efficiency leads to overweight hens and roosters that have a significant decline in production parameters and huge economic losses for the industry. Feed restriction prevents overweight broiler breeders. However, it has a negative impact on behaviors and physiological parameters. Although alternatives have been proposed to attenuate these effects, more research needs to be conducted in order to achieve an improvement in welfare. Feed restriction also plays a role in feathering. By changing the diets we are changing the birds nutrient expenditure. Thus, leading to feathering problems in the industry. Feather loss has an impact on body weight, feed intake, egg production, mating behaviors, and fertility. Therefore, maintaining adequate feather cover is crucial for broiler breeder management.

The answer to the broiler breeder dilemma still remains a mystery. However, the increase in research on feeding alternatives with focus on the behavior, productivity, and physiological changes will lead to a diet and feed program that limits the birds growth without having a significant negative impact on behavior or feather production.

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CHAPTER 3

IMPACT OF ALTERNATE FEEDING PROGRAM ON BROILER BREEDER PULLET BEHAVIOR, PERFORMANCE, AND PLASMA CORTICOSTERONE

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ABSTRACT

Broiler breeders are commonly feed restricted using some variation of skip-a-day (SAD) feeding to prevent excessive body weight gains and poor flock uniformity that results in low egg production and hatchability. While these feeding programs have improved flock performance in the past, the level of feed restriction has increased with genetic selection for feed efficiency. This project examined pullets that were offered a high fiber diet of soybean hulls (alternate day feeding; ATD) on the off day of a traditional SAD feeding program in comparison to the standard SAD program. The two dietary feeding methods each with 3 replicate pens of 210 pullets were tested. A sample of pullets was weighed weekly to adjust feed intake and maintain body weights at those suggested by the primary breeder. Feed allocations for the ATD pullets were reduced from 10 to 20 wks to compensate for an improvement in gain in these pullets. Body weights at 20 wks were not different with SAD averaging 1838 g vs 1852 g for ATD. Bird behavior was monitored via high definition video cameras photo period began and ended, respectively, each day (8 am to 4 pm). There were significant differences comparing the birds behavior on the ON and OFF feed day for each respective feeding treatment. At 22 wks of age, pullets were moved to lay cages and fed on a daily basis. Egg production through 40 wks was significantly improved in the hens fed under the ATD treatment (70.7%) when compared to the hens fed SAD (69.3%; P=0.03) as pullets. Overall, ATD females had greater weight gain on a similar feed allocation, and were more productive hens, which suggests improved bird wellbeing.

Key Words: Feeding Programs, Body Weight, Behavior, Corticosterone, Pullets

INTRODUCTION

Selection for increased growth rate has led to an increase in adult body weight and appetite for broiler and parent stocks (Zuidhof et al., 2014). Feed restriction is necessary to prevent excessive weight, maintain health and reproductive competence in broiler parent stocks. Broiler breeders fed ad libitum commonly have a decline in production and increase in mortality (Renema and Robinson, 2004). The still increasing growth potential of broilers, leads to a more severe feed restriction level for modern-day breeders. Feed intake is restricted to about 25-33% of the intake of ad libitum fed birds in rearing (De Jong et al., 2002).

There are productive and welfare implications of both ad libitum feeding and the current industry feed restriction program. The implication of overfeeding makes feed restriction programs the more welfare-friendly alternative to date. Despite the positive effect on overall hen health with feed restriction, previous research shows behaviors indicative of frustration, boredom, hunger, stereotypic object pecking and over drinking, and very high activity (De Jong et al., 2006). The level of feed restriction can also impact plasma corticosterone levels leading to elevated levels (Mormede et al. 2007). Increased corticosterone levels may be indicative of signs of stress or be related to the metabolic changes in the bird. In addition, the degree of fasting and stress may influence the immune and metabolic processes, based on gene expression of the birds (Sherlock et al., 2012).

Research has been conducted to decrease the prevalence of negative behaviors and decrease stress levels on feed restricted birds. Among some of the new management strategies are: environmental enrichment, scattering feed on the litter, and restricted every-day feeding. Those strategies aim to provide the birds with either object stimuli or provide feed every day to satisfy the bird's natural instinct to peck and forage (Leone and Estevez, 2008 and De Jong et al., 2005). Diluting the feed is another strategy that attempts to provide more feed with lower energy or crude protein levels (Zuidhof et al. 1995). The level of dilution and the ingredient that have a positive effect on welfare is yet to be determined. Ingredients with low energy and crude protein levels such as soy hulls, wheat midds, ground oats and pea hulls are commonly used as diluents.

The current study was conducted to investigate the effects of an alternative feed program to improve broiler breeder pullet welfare without negative influence in overall performance. Feeding soy hulls on an OFF feed day as an alternative feed (ATD) may have the potential to satisfy the need for the pullets to peck or consume something when rearing diet is provided. The theory was feeding soy hulls would decrease the prevalence of behaviors indicative of hunger or feeding frustration and have the potential to improve welfare of feed restriction programs.

MATERIAL AND METHODS

A total of 1260 one-day old (Cobb 700) pullets were raised in six floor pens in an environment-controlled poultry house. At 5 weeks of age, three replicate pens (7.3 x 4.6 m^2, 210 pullets) were allocated to each feeding treatment (630 birds per treatment). In rearing the pullets ate from a chain feeder and water was provided by a nipple drinker. All birds were banded at 8 weeks to track growth rate. The photoperiod to 22 weeks of age, was 23 hr of light: 1 hr of darkness (23L: 1D) for the first three days, followed by a 8L:16D pattern, until the birds were moved to lay cages at 22 weeks. The photoperiod increased to 14L:10D at 22 weeks of age and remained constant until the end of the study at 40 weeks of age. All birds were fed a common starter ration (1320 ME, 18% CP) for the first 3 weeks of age, followed by a grower diet (1280 ME, 15% CP) to 25 weeks of age. At 22 weeks of age hens were transferred into individual laying cages. The individual cages had a nipple drinker and a feeder pan. A standard breeder layer diet (1320 ME, 15.8% CP) was fed until hens reached 5% egg production at 25 wks. The amount of feed allocated was based on Cobb Breeder Management Guide Recommendations (Cobb,2014) and the body weights of the birds.

Experimental Design

During the first week of age, all birds were fed ad libitum. For the next 3 weeks all birds were fed limited amount daily to achieve primary target body weights. At 5 wk of age, birds were divided into two treatments: one treatment with birds fed under a standard skip-a-day (SAD) feed program while the remaining birds were fed on an alternate feed program (ATD). The SAD birds were fed a grower diet twice the daily feed amount every other day (ON day). The ATD birds were fed a grower diet twice the daily feed amount every other day (ON day) and soy hulls on the day they would otherwise not receive feed (OFF day). Four rooms were used to house the birds, with 1 or 2 pens per room. To avoid disruption to birds not being fed while others were, ATD and SAD birds were not housed in the same rooms. Two rooms housed the ATD (3 pens) birds and two rooms housed the SAD (3 pens) birds.

At 22 weeks of age all birds were weighed and 288 pullets (144 per treatment) were moved into individual cages in an environmentally controlled house. Treatments were randomly arranged throughout the house with 12 cages per group and 12 groups per treatment. The body weight and coefficient of variation (CV) of the birds selected was equivalent and reflected CV of each treatment at 20 weeks of age.

TMEn Determination

TMEn was determined according to the method of Sibbald (1976) as modified by Dale and Fuller (1984). Sixteen Single Comb White Leghorn roosters (60 wks of age) were fasted for 30 h to empty the digestive tract. Roosters were transferred to individual wire cages measuring 30.48 cm wide by 45.72 cm deep by 50.8 cm high. Each cage was equipped with a nipple drinker to provide free access to water and a stainless-steel excreta collection pan. Roosters were each precision-fed 30 g of soybean hulls. The roosters were subdivided into 2 replicate group of 8 birds each. Excreta were collected for 48 h post feeding. To estimate endogenous energy excretion 10 roosters remained unfed for 48 h collection period. All procedures were approved by the University of Georgia Animal Care and Use Committee.

Excreta were quantitatively collected from each individual pan, dried, and weighed. Crude protein and moisture of the feces and soybean hulls were determined (AOAC, 2006 and University of Georgia Agricultural and Environmental Laboratories), with gross energy of feed and feces determined with a bomb calorimeter (University of Georgia Agricultural and Environmental Laboratories). The gross energy of soybean hulls was obtained by averaging the values obtained from samples of the soybean hulls.

Growth and Productivity

A pullet sample weight was taken weekly (n=45) and all birds were individually weighed at 8, 12, 16, and 20 weeks of age during rearing and biweekly during lay. The CV for BW was calculated as a measure of flock uniformity (n=206). In the floor pens, during rearing, flock uniformity was calculated on per pen basis. The CV for the caged layers was calculated per group (n =12). Egg production in cages was monitored daily on a per hen basis (n=288) from 24 to 40 weeks. Egg production was calculated by taking the number of eggs laid per week as a percentage of hens housed per treatment.

Blood Sample Collection

At 8 weeks of age, 20 birds from each pen were randomly selected and marked by painting their backs as two groups (10 birds/group). The groups were divided based on sample time (24 h or 48 h after feeding). Blood samples were collected at 7:30 am before the light period started at 8:00 am at 8, 11, 16 and 20 weeks. Blood was collected from the brachial vein within one minute of physical contact with each hen. Blood samples were centrifuged at 1,000 x g at 4C for 30 minutes. Plasma was collected from each sample and frozen at -80C.

Plasma Corticosterone Determination

Plasma corticosterone concentration was determined using a corticosterone specific enzyme immunoassay (EIA) kit (Enzo Life Sciences, Plymouth Meeting, PA). An aliquot of plasma from each sample was mixed with steroid displacement reagent following the manufacturer’s protocol. To complete the displacement steroid extraction

25µL of sample was taken and mixed with 3 mL of anhydrous ethyl ether. The tube was allowed to incubate at room temperature for 30 min. The samples were placed in a -80C freezer for 5 minutes. The supernatant was then poured into another tube and allowed to dry overnight. The samples were suspended with AB15 provided in the corticosterone EIA kit. Corticosterone content in each sample was determined following the manufacturer's protocol. Duplicate corticosterone determination was made for each of the samples.

Behavioral Data

Video cameras (IR Network Camera, Hikvision Digital Technology) were mounted over each pen at 8 weeks of age. Videos were recorded onto a digital recording unit and transferred to external hard drives daily. Scan sampling was used to calculate the frequency of feeding (bird is feeder oriented), foraging, comfort behavior (dust bathing, preening, seating and wing flapping), walking, or drinking (Figure 1). Behaviors were observed during two consecutive days for each week at 8, 13, and 16 weeks of age. Each week was treated as the average of behaviors performed on the ON feed and OFF feed days. The nine days selected were uninterrupted days with no weighing, bleeding, pen maintenance or unplanned events. The behaviors were analyzed for the entire light period that day, with time 0 being time lights come on (8 am). A scan sampling was done every 10 minutes until the light were turned off (4 pm). The observation areas were as follows: around the feeder, the drinker and a demarked space in the open area (free space in the pen).

Body Composition by DEXA

Ten birds per treatment on the OFF feed day, before soybean hulls were provided, were randomly selected at 7,14, and 21 weeks for DEXA whole body composition and gene expression analysis that were measured by a dual-energy x-ray absorptiometry (DEXA, Prodigy, GE Healthcare, Chicago IL). Parameters for body composition were bone mineral density (BMD), bone mineral content (BMC), bone area, total tissue weight, fat weight, lean muscle weight, and fat%. A scan of the whole body was made and analyzed using small animal scan software (GE Healthcare, Little Chalfont, UK).

Gene expression using quantitative real-time PCR (qRT-PCR)

Gene expression was obtained to evaluate the impact of the alternate feeding program on the immune response and changes in metabolism of the birds. Total RNA was extracted from liver samples using TRIzol reagent (Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's instructions. The isolated total RNA was reverse transcribed using cDNA synthesis kit. Pairs of primers for each gene were designed and checked for target identity using the National Centre for Biotechnology Information (NCBI). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed in duplicate reactions including nuclease free water, the forward and reverse primers of each gene, cDNA and SYBR Green as a detector using CFX Connect ™ Real-Time PCR Detection System (Life Science Research, Bio-Rad, Hercules, CA). The data were generated using ΔΔCt method by normalizing the expression of the target gene to a housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the values were reported as fold changes of the expression of the target genes in the experimental groups compared with the negative control group. Target genes are IFN-gamma and IL-6 (Immune genes), Phophoenolpyruvate carboxykinase (PEPCK) and Glucose Transporter 2 (GLUT2) (glucose metabolism), and Acetyl-CoA acetyltransferase 2 (ACAT2) (lipid metabolism).

Statistical Analysis

Body weight, uniformity, egg production results were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Plasma corticosterone, behavior, DEXA, and gene expression results were compared using GLM (SAS, 2013, Cary, NC). Differences were deemed to be significant when the P-value was less than or equal to 0.05.

RESULTS

Body weight and Uniformity

BW was not significantly different at 5 wk when feeding treatment was imposed or at 20 wk age (Table 2). BW for 8, 12, and 16 wk was significantly different ($P<0.001$) between treatments. BW was not significantly different (Table 3) at each week during the laying period (26 to 42 wk).

The different feed programs (ATD vs. SAD) significantly affected uniformity at 8, 12, 16, and 20 weeks of age (Table 4). The ATD feed program significantly improved uniformity for each week ($p<0.05$) and the overall rearing period ($p<0.01$).

Feed intake and Digestibility

Feed intake was adjusted to meet recommended breeder target BW during rearing. Cumulative feed and cumulative CP intakes are shown in Table 5. Pullets under the ATD feed program consumed 3.2% less feed compared to the pullets under a SAD program. Crude protein intake was significantly different between the treatments; with pullets under the ATD feed program consuming more protein (coming from soyhulls on off feed day) and less energy (due to the reduced feed allowance) in an attempt to have similar BW (+77 g more crude protein and -42 kcal less energy). The calculated nutrient value that soy hulls provided the ATD pullet was approximately 7 kcal/kg. However, according to the results of our digestibility study the actual digestible nutrient intake was 3 kcal/kg. Feed intake during lay (25 – 40 wk) was the same with hens receiving the same feed.

Egg Production

Overall egg production was significantly different between treatments (P=0.039). Hens fed on a standard SAD program during rearing had lower mean egg production than hens fed on the ATD program (69.32 vs. 70.75) with most of the difference in egg production between 27-31 weeks of age (Figure 1).

Plasma Corticosterone

Plasma corticosterone levels are summarized in Figure 2. There was a significant difference between the treatments (SAD and ATD) for samples taken 48 h after feeding at 21 weeks, with the birds on ATD treatment having higher values than the SAD

($p<0.0214$). There were no significant difference between treatments for samples taken 24 h after feeding. There was also a significant differences between the sample day, with birds having significantly lower plasma corticosterone levels 24 h after feeding compared to 48 hours ($p<0.0001$).

Behavior Observations

Behavior traits by treatment and feed day during the rearing period are summarized in Figures 3 and 4. There were few significant differences between the treatments during the ON and OFF feed day at 8, 13 and 16 weeks of age (data not shown). However, there were significant differences comparing the behavior of the birds during the ON and OFF feed day within each treatment. The birds on the ATD feeding program did not have significant differences in percentage of birds at the feeders or exhibiting comfort behaviors. There was a significant difference in the percentage of birds foraging at 16 weeks with more birds foraging on the OFF feed day compared to the ON feed day on the ATD feed program. There were significant differences in drinking at 16 weeks as more birds were observed at the drinker line during the ON than the OFF feed day.

The birds on the SAD had significant differences between the percentage of birds at the feeder at 13 and 16 weeks. There was also a numerical difference at 8 weeks however there was no significant difference likely due to variation between samples. There was a higher percentage of birds at the feeder during the ON feed day than the OFF feed day for the SAD feed program. There were also significant differences in foraging behavior with more pullets foraging in the litter during the OFF than the ON feed day.

For the SAD feed program, the number of birds drinking was significantly higher at 8 weeks, with the ON feed day being higher than subsequent weeks. Comfort behavior was significantly different with higher levels during the OFF day at 13 and 16 weeks, but higher levels on the ON day at 8 weeks for the birds on the SAD feed program.

DEXA

At 7 wk of age, ATD showed significantly higher BMD, BMC, bone area, total tissue weight, and lean muscle weight compared to SAD (Table 6). At weeks 14 and 21 of age, there were no significant differences in body composition parameters between treatments.

Gene expression for immunity, glucose metabolism, and lipid metabolism

In order to determine the impact the feeding program had on the immune and metabolic (glucose and lipid) genes of the birds, a gene expression test was conducted. There were no significant differences in expression of key immune genes (IFN-gamma and IL-6) between birds fed on SAD or ATD treatments (data not shown). The ATD feeding program did not significantly increase PEPCK and Glut2 mRNA expression in the livers when compared to birds fed on SAD (data not shown). The ATD feed program significantly depressed ACAT2 mRNA expression compared to SAD feed program ($P<0.05$) (Figure 5).

DISCUSSION

Body Weight and Uniformity

In order to achieve target BW prior to lay the birds on the SAD treatment were fed more feed compared to the ATD birds. Mean body weights of birds fed on the ATD feed program did not differ significantly from pullets on SAD feed program at the end of the 20-wk rearing period. There were significant differences between treatments on previous weeks and overall rearing period ($p<0.001$). The difference between body weights in the rearing period might be attributed to increase in CP levels by the addition of soy hulls in the ATD program. The results of this study were in line with previous research that showed birds fed a lower amount of protein had higher feed intake to achieve target BW (Van Emous et al., 2015a, Hudson et al., 2001).

It is important to consider the level of total metabolizable energy and protein from soy hulls. There is very little information on the actual value of soybean hulls. The hulls are reported to contain about 10 – 12 % CP, 43% crude fiber and digestible energy content of 2070 kcal kg (Chee et al., 2005). However, the protein content on soybean hulls varies depending on processors, with research showing a range of 9.2-18% of CP (Cole et al., 1999). In addition, due to their high fiber content, soybean hulls are know to be poorly digestible by non-ruminant animals. Therefore, we conducted a TMEn analysis to determine the digestible protein and energy values for the soybean hulls used in the trial. We determined a 11.19% CP and TMEn of 658 Kcal /kg. Birds consuming soybean hulls did have significantly greater body weights causing a lower feed intake to achieve the desired body weight prior to photo-stimulation.

Uniformity provides an estimate of the variability in a flock and is a crucial measurement for broiler breeder management. Highly uniform flocks have better performance than more variable flocks, making it easier to meet the nutritional requirements of the uniform flock (Hudson et al., 2001). Feed restriction programs like SAD increases flock uniformity by increasing feeding time and decreasing feed competition (Bennett and Lesson, 1989) compared to birds on ED feeding program. The results in this study showed an improvement in uniformity for the birds on the ATD feed program compared to SAD feed program. The ATD program might have had a positive impact on uniformity due to the inclusion of soybean hulls on the OFF feed day. The soybean hulls increased the total crude protein level in the bird's diet. Previous researchers suggest that qualitative restriction improves the CV of body weight due to efficiency of nutrient utilization (Pinchasov et al. 1993). Birds on the ATD program are utilizing the extra nutrients for growth leading to higher body weights at rearing and improving uniformity. Morrisey et al. (2014) reported the same results with the greatest improvement in uniformity on the birds consuming a diet with soybean hulls used as filler.

Egg Production

There was a significant effect in overall egg production between the treatments (p=0.039). Since the CVs from rearing were maintained when the hens were transferred to cages, the differences in egg production might be attributed to the differences in uniformity. As noted in previous studies, better uniformity leads to higher egg production (Abbas et al., 2010). The difference in crude protein intake through rearing may have impacted egg production. Hocking et al. (2002) reported a decreased egg production when pullets were fed a low protein (10% CP) diet during rearing.

The increase in overall crude protein consumption by the ATD birds might have lead to the increase in egg production. Joseph et al. (2000) reported a decrease in early and late stage egg production when birds were reared on 14% CP diet compared to a 16% and 18% CP diet. However, both of our feed programs had crude protein levels above parent stock recommendations. Therefore differences in egg production might not only be attributed to changes in overall crude protein consumption.

Plasma Corticosterone

Plasma corticosterone levels play a key role in the stress response of the birds. Previous research shows that changes in plasma corticosterone are attributed to restriction in feed intake and are usually elevated during fasting (Mench 1991, Hocking et al., 1996, de Beet et al., 2008). De Beer et al. (2008) determined the differences in plasma corticosterone of birds on a every-day feed (ED) or skip-a-day feed (SAD) program during a 48 hour period. They determined peaks in corticosterone levels at 20 h and 48 h after feeding of the SAD birds. This contradicts our results, where corticosterone values are lower at 24 h compared to 48 h after feeding for both ATD and SAD feeding programs. The only difference between the studies was the feeding method; De Beer et al. (2008) fed the birds individually in cages while we fed the birds as a group with a chain feeder. Therefore competitive bird behavior could have an effect on the level of stress in the birds. Pullets that have to actively compete for feed may have a higher level of stress just prior to feeding (48 h after feeding) than birds individual fed.

We hypothesized a decrease in plasma corticosterone in the ATD fed birds. Birds on an ED feed program have lower levels of plasma corticosterone (Mench, 1991); therefore, we expected that giving the ATD bird soybean hulls on the OFF day might have a similar effect. The results were opposite with the ATD birds having higher levels of corticosterone compared to the SAD birds. This might suggest that the birds on the ATD feed program are more stressed then the SAD birds. However, differences in nutrient uptake have to be taken into account. The birds on the SAD feed program were given a greater volume of feed in order to reach adequate body weight prior photo stimulation (Table 5). Plasma corticosterone concentrations are a reflection of stress but also metabolic effect of feed restriction (De Jong et al., 2003). The birds on the ATD feed program have a lower level of total metabolizable energy, which could lead to a higher level of plasma corticosterone. Therefore, the higher level of plasma corticosterone might suggest that the birds were not necessarily more stressed on the ATD program compared to the SAD program but they were more feed deprived especially at the end of the rearing phase when the differences in corticosterone are significant (21 wk).

Behavior Observations

There were no significant differences in bird behaviors when comparing the different feeding treatments. When comparing the behavior within each treatment, we determined significant differences between the ON and OFF day behaviors. These differences for each treatment suggest that there was an impact of the feed program on the behavior of the birds.

The birds on the alternate diet had a similar percentage of birds around the feeder pecking at the feed on the ON and OFF feed day. The birds on the SAD had significantly different percentage of birds, with more birds on the ON than the OFF feed day. The additions of soybean hulls changed the birds' focus from other activities to the feeder on the ATD feed program. The difference in focus from other activities to the feeder has the potential to decrease stereotypic behaviors. Van Emous et al. (2015b) found that feeding birds a lower protein diet leads to an increase in feeding time and increase focus on the feeder. They found that the birds on the lower protein diet are a lot more tranquil and exhibit more comfort behaviors.

The percentage of birds foraging or pecking significantly changed when comparing the ON and OFF feed day of the birds. The birds on the SAD feed program have a significantly higher number of birds foraging on the OFF feed day compared to the ON feed day for all observation periods. Increased foraging represents a lack of satiety as it is characterized by food seeking activity (Dawkins, 1989). Therefore, the birds on a traditional SAD program may experience a degree of hunger when no feed is provided on the OFF day. In comparison, the birds on the ATD feed program did not have significant differences in foraging at 8 and 13 weeks of age. Soybean hulls may have increased the level of satiety and decreased feed-seeking activity. However, from the results of our study we could not determine whether we satisfied the birds' need to peck or nutritional needs. Previous research on diet dilution shows that by increasing feeding time you can change the pullets' behavior (Hocking et al., 2001, Van Emous et al., 2015b). The increase in feeding time leads to a decrease in foraging behaviors even though both groups are getting the same nutrient content. The birds on the ATD feed program had a significant increase in foraging by 16 weeks. We did not continue to increase the amount of soybean hulls, and perhaps by 16 weeks of age the amount of soybean hulls might not have been enough to satisfy birds foraging needs.

Previous studies found birds exhibit an increase in activity level as a result of feed restriction (Hocking et al., 1996). These studies suggest that the increase in hunger and feeding motivation leads to an increase in standing behavior and walking (de Jong et al., 2002). The percentage of birds walking increases leading to higher levels on the OFF feed day compared to the ON feed day. In addition, by feeding soybean hulls in the ATD feeding program should decrease feeding motivation leading to a lower activity level compared to the birds on the SAD feeding program. In our study, no significant differences were observed between the feed treatment and the feed day, which suggests that the ATD feeding treatment did not decrease feeding motivation.

The birds on the SAD feeding program exhibit more comfort behaviors on the OFF rather than the ON feed day from 13 and 16 weeks. The comfort behaviors are inverse at 8 weeks with a greater number of birds displaying comfort behaviors on the ON rather than the OFF feed day. The birds engage in comfort behaviors even though they do not receive any feed on the OFF feed day. These results suggest the birds get more comfortable or acclimated with the feeding program over time. This agrees with previous research that shows that birds on a SAD feeding program get used to the feed volume over time showing no differences in comfort behavior on the late phase of production (Morrisey et al., 2014). In contrast, the birds on the ATD feeding program do not have any difference in comfort behaviors between the ON and OFF feed day. The lack of difference suggests that the birds have similar behaviors on the ON and OFF feed day. Preening is also added to the number of comfort behaviors, however displacement preening might have been taking place. Displacement preening is considered a negative behavior and is different from normal preening (Duncan and Wood, 1972). Therefore, an over estimation of comfort behaviors might have been made since the distinction on the behaviors was not made during these observations.

DEXA and Gene Expression

The body composition of the pullets was only significantly different between the treatments at 7 weeks. There were no differences between the treatments at week 14 and 21. The lack of differences might be due to the fact we controlled feed intake based on the body weight gain of each group. The birds on the SAD and ATD feed program were fed different feed amounts in order to obtain similar body weight prior photo-stimulation (20 weeks). Sun and Coon (2005) showed that heavier birds have significant differences in body composition compared to light weight birds. Therefore, although there were significant differences in body weight at 14 weeks and for the overall period, the differences were not big enough to impact body composition of the pullets. In order to determine the impact of the feeding treatments on body composition both treatments would have to be fed the same amount despite differences that would have caused in body weight.

There was a significant difference in the gene expression of ACAT2 at 21 weeks with higher levels observed on the SAD feed compared to ATD feed. There were no significant differences at week 7 due to a large bird-to-bird variability (SEM=0.714). Therefore, increasing sample size would be recommended to compare gene expression measurements between treatments. The difference at 21 weeks suggests that the feeding treatment had an impact on lipid metabolism of the birds. Regassa et al. (2016) previously showed that the broilers exhibit lower levels of ACAT2 post feeding compared to birds on the fasted state. Our results contradict their findings, with birds fasted for a day (SAD) having higher levels then birds fed soybean hulls (ATD) on the OFF feed day. Fatty acid synthase catalyzes the synthesis of saturated free fatty acids from acetyl-COA (Lim et al., 2015). We did not measure expression of fatty acid, the difference in this enzyme might attribute to the differences in the ACAT2 gene in SAD and ATD treatments at 21 weeks. Therefore, additional research needs to be conducted in order to determine the cause for differences in gene expression.

From the results of the study, we can conclude that the addition of soy hulls on the off feed day of a standard skip-a-day program has an effect on overall performance and behavior. The alternate feed program improved productivity of pullets by: increasing body weight in rearing, improving uniformity and increasing overall egg production compared to the standard skip-a-day program.

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TABLE 3.1. Behaviors recorded of broiler breeder pullet[a].

Behavior	Description
Feeding	Pecking at the feeder
Drinking	Pecking at the nipple drinker
Foraging	Pecking and/or scratching the litter
Walking	Walking or running without performing other behaviors
Comfort	Preening, sitting, nibbling, stroking, dust bathing and wing flapping

[a] Behavior definitions modified from the ethogram of de Jong et al. (2005) .

TABLE 3.2. The mean body weight of birds (g) at 8, 12, 16, and 20 weeks of age and overall rearing period as affected by the skip-a-day and alternative feeding programs (SAD and ATD).

Weeks	SAD	ATD
8	793.4[b]	828.0[a]
12	1206.5[b]	1236.1[a]
16	1527.1[b]	1562.1[a]
20	1838.1[a]	1852.4[a]

[a-b] Treatments significantly different within period ($P<0.05$).

SAD= Skip-a-day feeding program; ATD= alternate feeding program (soy hulls).

TABLE 3.3. The mean body weight of birds (g) during lay at 26, 28, 30, 32, 34, 36, 38, 40 and 42 wk of age and overall laying period as affected by the different feeding programs (SAD and ATD).

Weeks	**SAD**	**ATD**
26	2490.3	2491.4
28	2933.9	2935.5
30	3196.1	3196.1
32	3453.2	3413.7
34	3622.6	3591.0
36	3758.1	3715.9
38	3830.4	3781.3
40	3852.2	3814.8
42	3768.0	3747.7

SAD= Skip-a-day feeding program; ATD= alternate feeding program (soyhulls).

TABLE 3.4. The coefficient of variation of body weight (%) at 8, 12, 16, 20 wk of age and overall rearing period as affected by the different feeding programs (SAD and ATD).

Week	**SAD**	**ATD**
8	14.00^{b}	12.74^{a}
12	14.54^{b}	13.25^{a}
16	14.52^{b}	13.23^{a}
20	14.85^{b}	13.31^{a}
Overall	14.48^{x}	13.13^{y}

$^{a\text{-}b}$ Treatments significantly differed within period (P<0.05).

$^{x\text{-}y}$ Treatments significantly differed within period (P<0.01).

SAD= Skip-a-day feeding program; ATD= alternate feeding program (soybean hulls).

TABLE 3.5. Cumulative feed, cumulative soy hull intake, crude protein (CP) intake and total metabolizable energy (TME) as affected by dietary crude protein level during rearing (2 to 22 wk of age).

Source	**SAD**	**ATD**
Feed (g/bd)	3753.9	3634.7
Soy hulls (g/bd)	---	983
CP intake (g/bd)	587.1	664.2
TME	10610.7	10568.3

SAD= Skip-a-day feeding program; ATD= alternate feeding program (soybean hulls).

TABLE 3.6. Body composition (BMD, BMC, bone area, fat%, total tissue weight, fat weight and lean muscle weight of SAD and ATD during rearing periods (7, 14, and 21 weeks)

Week 7

Treatment	BMD (g/cm^2)	BMC (g)	Area (cm^2)	Fat (%)	Tissue (lbs)	Fat (lbs)	Lean (lbs)
SAD	0.12^a	11.87^a	97.06^a	15.34	1.51^a	0.24	1.29^a
ATD	0.13^b	13.65^b	107.10^b	16.24	1.66^b	0.28	1.39^b
SEM	0.002	0.488	3.324	0.975	0.041	0.019	0.031
P value	0.015	0.015	0.042	0.517	0.013	0.148	0.031

Week 14

Treatment	BMD (g/cm^2)	BMC (g)	Area (cm^2)	Fat (%)	Tissue (lbs)	Fat (lbs)	Lean (lbs)
SAD	0.19	26.73	157.20	5.75	2.84	0.17	2.67
ATD	0.16	24.23	153.90	5.31	2.82	0.15	2.67
SEM	0.013	1.030	3.471	0.673	0.075	0.022	0.067
P value	0.288	0.097	0.513	0.647	0.836	0.574	0.956

Week 21

Treatment	BMD (g/cm^2)	BMC (g)	Area (cm^2)	Fat (%)	Tissue (lbs)	Fat (lbs)	Lean (lbs)
SAD	0.18	33.31	185.40	6.76	4.17	0.27	3.89
ATD	0.19	36.07	191.70	6.63	4.11	0.27	3.84
SEM	0.016	1.058	2.994	1.117	0.115	0.043	0.133
P value	0.108	0.073	0.152	0.933	0.688	0.964	0.783

SAD = skip a day. ATD = Alternative feed program (soy hulls).

FIGURE 3.1. Egg production curve as affected by the different feeding programs. Feeding programs are as follows: SAD= skip-a-day feeding program (—), ATD= alternate feeding program (soybean hulls) (- -). Each value represents the mean of the percentage of total eggs produced within each feeding program. The means for the overall period are 69.3 and 70.7 for the SAD and ATD feeding programs, respectively. There were significant differences (P=0.03) for the overall production between the feeding programs.

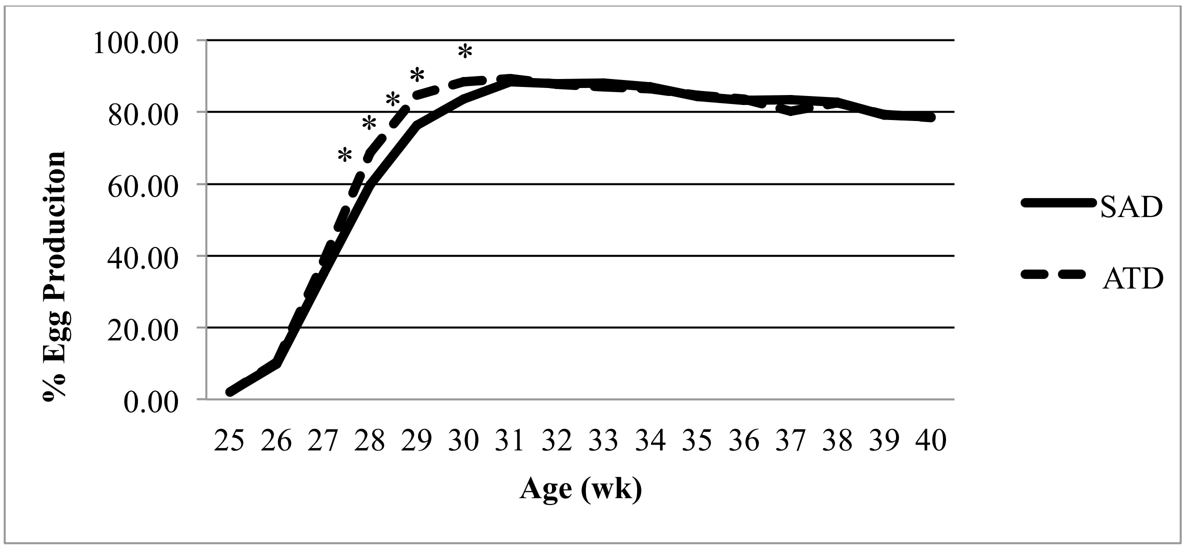

100.00
80.00
60.00
40.00
20.00
0.00
% Egg Produciton
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Age (wk)
SAD
ATD
*
*
*
*
*

FIGURE 3.2. Plasma corticosterone concentrations as affected by the feeding treatment (SAD or ATD). Feeding programs were as follow: SAD= skip-a-day feeding program, ATD= alternate feeding program (soyhulls). The samples were obtained 24 hours (OFF feed) or 48 hours after feeding (prior to feeding ON feed day). Each value represents the mean of 20 birds per treatment.

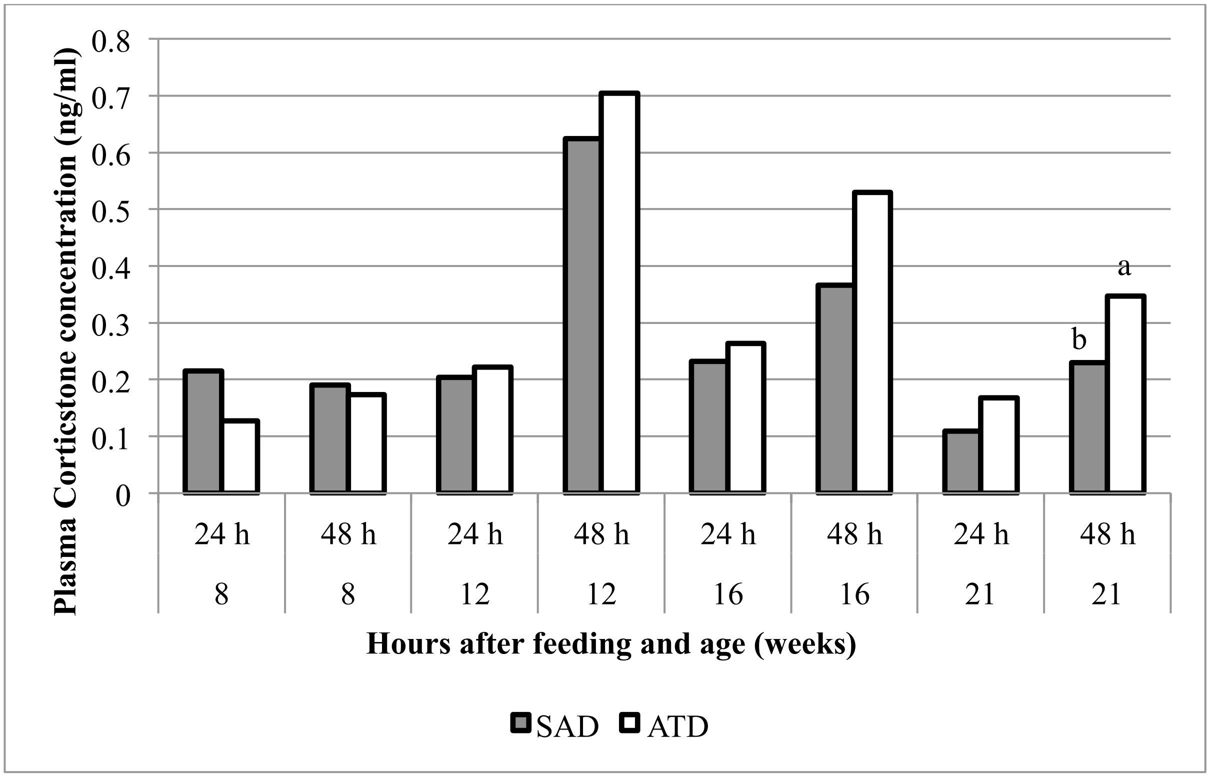

Plasma Corticstone concentration (ng/ml)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
24 h
48 h
24 h
48 h
24 h
48 h
24 h
48 h
8
8
12
12
16
16
21
21
Hours after feeding and age (weeks)
SAD
ATD

FIGURE 3.3. Effect of ATD feeding system (alternate program – soybean hulls) on the percentage of birds with observed behaviors over total number of birds in view (%). Data were collected from 3 observational areas (feeder, drinker and open area) in each of 2 ATD pens, every 10 min during the entire day (8:00 to 16:00). Observations occurred at 8, 13, and 16 wks for two consecutive days. ATD pullets were fed every day between 8:00 AM to 8:30 AM.

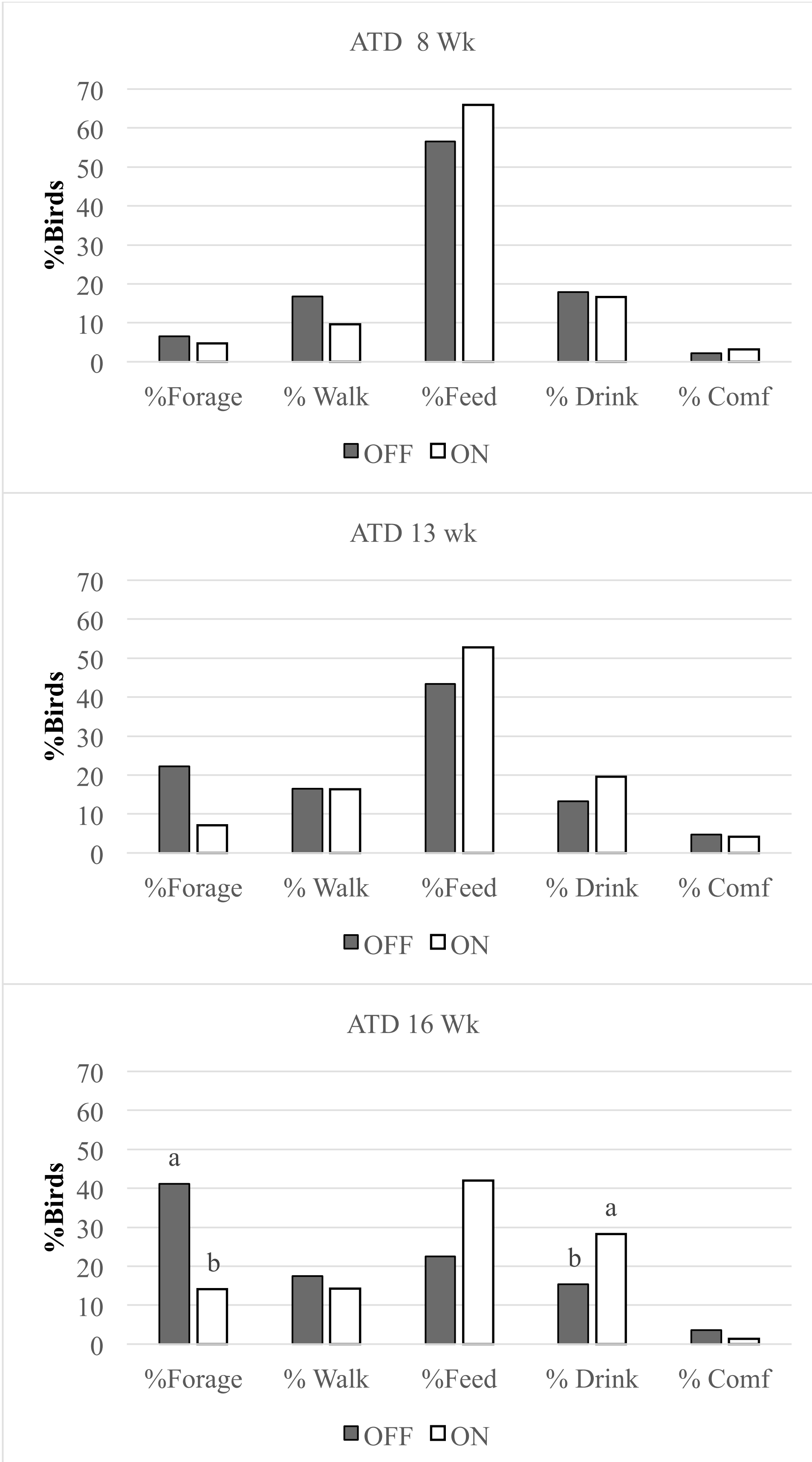

ATD 8 Wk
%Birds
70
60
50
40
30
20
10
0
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON
ATD 13 wk
%Birds
70
60
50
40
30
20
10
0
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON
ATD 16 Wk
%Birds
70
60
50
40
30
20
10
0
a
b
a
b
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON

FIGURE 3.4. Effect of SAD feeding system (skip-a-day program) on the percentage birds with observed behaviors over total number of birds in view (%). Data were collected from 3 observational areas (feeder, drinker and open area) in each of 2 SAD, every 10 min during the entire day (8:00 to 16:00). Observations occurred at 8, 13, and 16 wks for two consecutive days. SAD pullets were fed every other day (ON feed day) between 8:00 and 8:30 AM. Pullets on OFF feed day morning observations were done during feeding period.

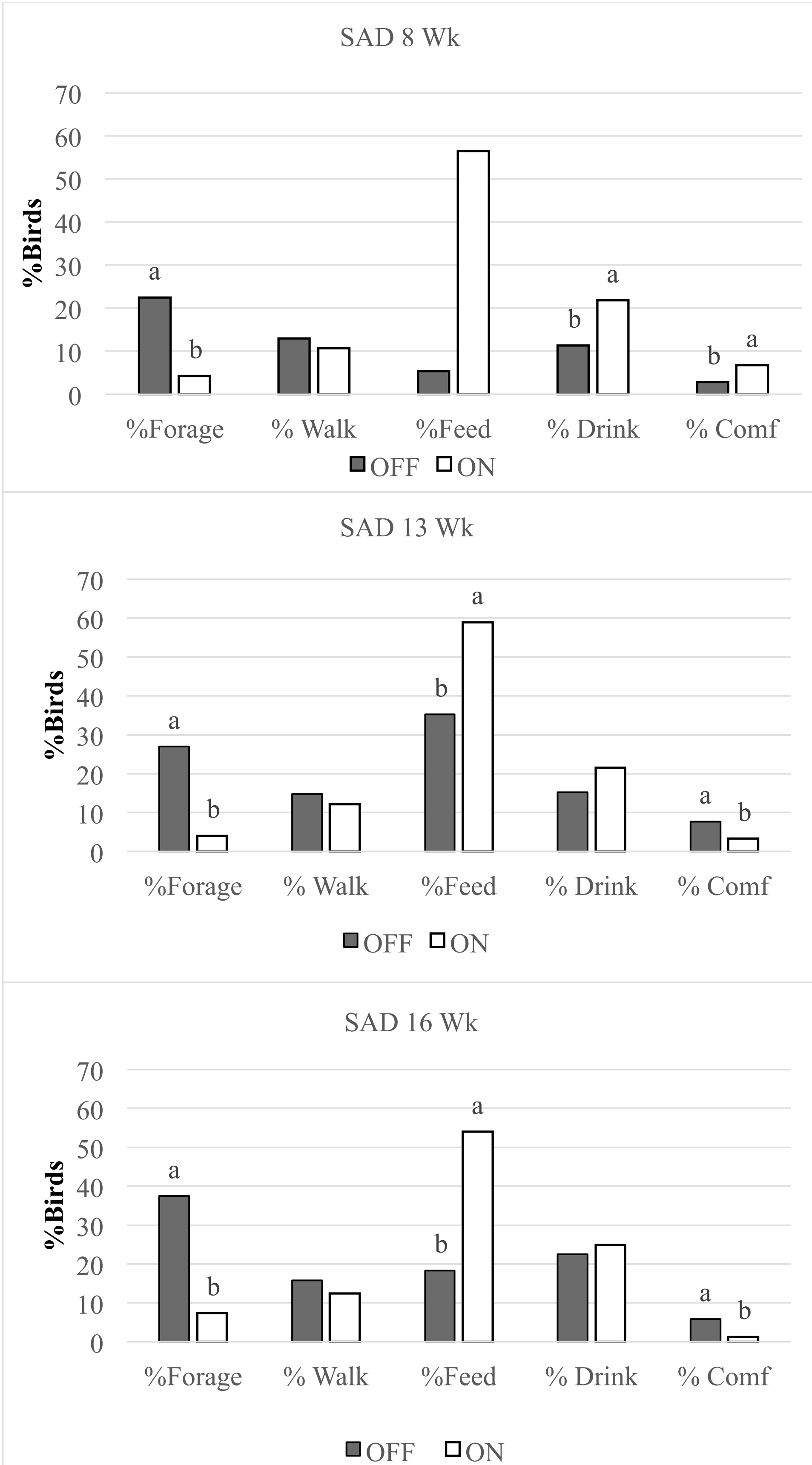
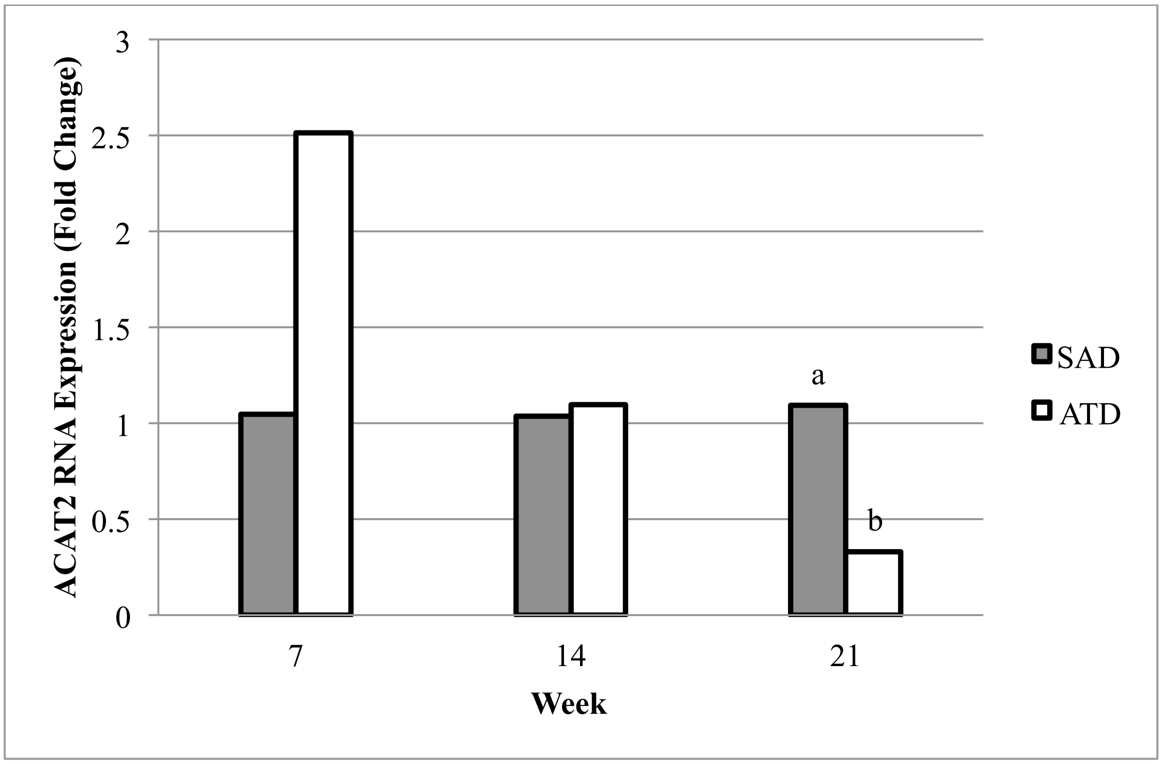

SAD 8 Wk
%Birds
70
60
50
40
30
20
10
0
a
b
b
a
b
a
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON
SAD 13 Wk
%Birds
70
60
50
40
30
20
10
0
a
b
a
b
a
b
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON
SAD 16 Wk
%Birds
70
60
50
40
30
20
10
0
a
b
a
b
a
b
%Forage
% Walk
%Feed
% Drink
% Comf
OFF
ON

FIGURE 3.5. Acetyl-CoA acetyltransferase 2 (ACAT2) gene as affected by the feeding treatment (SAD and ATD). Feeding programs are as follow: SAD= skip-a-day feeding program, ATD= alternative feeding program (soyhulls). The samples were on an OFF feed day prior to feeding. Each value represents the mean of 5 birds per treatment. There was a significant difference between the treatments at 21 weeks (P=0.0229).



ACAT2 RNA Expression (Fold Change)
3
2.5
2
1.5
1
0.5
0
7
14
21
Week
a
b
SAD
ATD

CHAPTER 4

IMPACT OF ALTERNATE FEEDING PROGRAM ON BROILER BREEDER PULLET BEHAVIOR, WATER INTAKE, AND PERFORMANCE

[1]Aranibar, C. D, M. Czarick, C. Usher, W. I. Daley, W. K. Kim, and J. L. Wilson. To be submitted to *Poultry Science*.

ABSTRACT

Broiler breeders are commonly feed restricted using some variation of skip-a-day (SAD) feeding to prevent excessive body weight gains and poor flock uniformity that results in low egg production and hatchability. While these feeding programs have improved flock performance in the past, the level of feed restriction has increased with genetic selection for feed efficiency. The objective of this project was to evaluate the effect of adding of soybean hulls to improve broiler breeder pullet welfare without a negative influence on overall performance. Soybean hulls were either added on top of the developer ration on the ON feed day (SAD) or the second feeding treatment received the same developer ration (same amount as SAD group) but were offered soybean hulls on the OFF feed day (alternative feeding, ATD). The nutrients given to the birds were the same when adding two days. Each feed treatment was replicated in 4 pens (n=215 pullets/pen). All pullets were weighed at 8, 12, 16 and 20 weeks of age and a sample of pullets (20% of the pullets) were weighed on the other weeks to adjust feed intake and maintain body weights to primary breeder targets. These data were analyzed by SAS SLICE using a significance level of $P \leq 0.05$. Body weight was significantly different at 12, 16 and 20 weeks of age with the weights at 20 weeks averaging 2135.5 and 2223.6 g for SAD and ATD birds, respectively ($P<0.0001$). Egg production was significantly increased ($P<0.001$) for birds on the ATD diet by 4%. Differences in behavior observations, feeding motivation and water usage was significantly different between the treatments. The birds on the ATD diet had significantly higher feed motivation and water usage on the ON feed day ($P < 0.001$). The birds on the ATD feeding program had significantly heavier liver and small intestine weights ($P<0.0001$). Differences in egg

production and body weight might be attributed to the differences in behavior and body morphology of the birds in each treatment. Overall, ATD birds had a greater weight gain on the same feed allocation, and hens from this pullet feeding treatment were more productive, which suggests improved bird wellbeing.

Key Words: Skip-a-day, alternate feeding, broiler breeders, soybean hulls, welfare, water usage

INTRODUCTION

Broiler breeder females today are the product of intensive genetic selection for rapid and efficient growth and a high rate of egg production (Renema and Robinson, 2004). Selection for fast growth and meat yield in broilers has lead to an increase in feed intake (Richards et al., 2010). Broiler breeders given unrestricted access to feed consume above their energy requirement for growth, maintenance, and reproduction. Broiler breeders fed ad libitum have the potential to become overweight, diminish their reproductive capacity, and develop musculoskeletal diseases that impair mating (McDaniel et al., 1981, Robinson and Wilson, 1996). Efficient reproduction, health, and livability of broiler breeders must be maintained while retaining the genetic potential for fast growth and high meat yield. However, reproductive competence and growth are negatively related production traits (Siegel and Dunnington, 1985). Body weight gain is limited by reducing feed intake through restriction. Feed intake is restricted to about 25-33% of the intake of ad libitum fed birds in rearing (De Jong et al., 2002). Feed allocation is limited to attain a target growth curve that maximizes reproductive performance.

The high level of feed restriction is associated with negative effects on bird welfare. Previous research has shown that feed restricted broiler breeders show behaviors indicative of frustration, boredom, and hunger (De Jong and Jones, 2006). These behaviors are evaluated by measuring the general activity level, non-feeding oral activities, and drinker use (Hocking et al., 2001, Merlet et al., 2005). In addition, feed restricted birds exhibit less comfort behaviors (De Jong et al., 2003). The changes in the bird's behavior are a sign that the birds are experiencing an increase in levels of stress and hunger.

Qualitative restriction is an alternative to current feed restriction programs. Qualitative restriction aims to increase feed volume by feeding a lower density diet. The increase in volume should lead to an increase in feeding time and decrease in feeding frustration. Sandilands et al. (2005) found an increase in feeding time and related it to changes in behaviors when birds were fed oat hulls. Previous research reported a decrease in stereotypic pecking and increases in comfort behaviors in birds on a high fiber diet (De Jong et al., 2005). The level of dilution, ingredient, and feeding method that has the best effect on welfare is yet to be determined. Data between researchers is conflicting; with some even suggesting that the inclusion of fiber just redirects the bird's attention and does not necessarily improve their wellbeing (Mason et al., 2007). Therefore, more research needs to be conducted evaluating more performance parameters with inclusion of different ingredients.

The current study was conducted to evaluate the effect the addition of soybean hulls to improve broiler breeder pullet welfare without negative influences in overall performance. The study evaluates only differences in the feeding program with no differences in the nutrient value, compared to the previous paper where differences between nutrient content and feed program were seen between the treatments (Chapter 3). Soybean hulls were either added on top of their feed ration for the ON feed day (SAD) or the soybean hulls were given as the OFF day feed for the alternative feed program (ATD). Soybean hulls have the potential to increase feeding time when given on the feed day or on the day they would not receive anything otherwise. Thus, decreasing the prevalence of foraging, pacing, on feather pecking behaviors that are indicative of hunger or feeding frustration and potentially improve bird welfare of feed restriction programs.

MATERIAL AND METHODS

A total of 1720 one-day old (Ross 308) pullets were raised in eight floor pens in an environment-controlled poultry house. At 5 weeks of age, four replicate pens (7.3 x 4.6 m^2, 210 pullets) were allocated into two groups based on feed treatments (860 birds per treatment). In rearing the pullets ate from a chain feeder and water was provided by a nipple drinker. The eight pens were divided among four rooms with 2 pens of the same feeding treatment per room. All birds were banded at 3 weeks to track growth rate. The photoperiod to 21 weeks of age, was 23 hr of light: 1 hr of darkness (23L: 1D) for the first three days, followed by a 8L:16D pattern, until the birds were moved to laying pens at 21 weeks. The photoperiod increased to 14L:10D at 21 weeks of age and remained constant until the end of the study at 40 weeks of age. All birds were fed a common starter ration (1320 ME, 18% CP) for the first 3 weeks of age, followed by phase feeding (explained later) the grower diet to 25 weeks of age. At 22 weeks of age hens were transferred into laying pens (2.4 x 3.6 m^2; 40 hens and 3 roosters per pen). Three roosters were added to each pen for fertility measurements. The laying pens had 2/3 of the floor space covered by raised slats and remaining 1/3 of the floor pen covered by pine shavings as litter. The hens were fed from ChoreTime breeder pans (4/pen) fitted with an exclusion grill to prevent the rooster from eating hen feed. The roosters were fed from a pan feeder suspended over the litter area. The pans were filled with a weighed amount of feed each evening during the last egg collection and raised to a height to prevent feeding.

The pans were hand lowered each morning at feed time (6:30 am). A standard breeder layer diet (1320 ME, 15.8% CP) was fed when hens reached 5% egg production at 25 wks and continued to the termination of the study at 40 wks. The amount of feed allocated was based on Ross Management Guide Recommendations (Ross, 2017) and the body weights of the birds.

Experimental Design

All birds were fed ad libitum during the 1st week of age. For the next 3 weeks all birds were fed a limited amount daily to achieve primary target body weights. The birds were switched at 3 weeks to a grower diet (2820 kcal/kg and 13% CP; grower 1, Table 1, Table 2, Figure 1). At 5 weeks of age, birds were divided into two treatment groups with half the birds fed on a standard skip-a-day-program (SAD) with the addition of soybean hulls, while the remaining birds were fed on an alternate feed program (ATD). The SAD birds were fed a grower diet at twice the daily feed amount suggested in breeder guide and soybean hulls (ranged from 10 g/bd to 15 g/bd) every other day (ON day). The ATD birds were fed the same grower diet and same amount of grower as fed to the SAD treatment (ON day) while the soybean hulls were fed on the OFF day (same amount fed to SAD, but on OFF day). At 5 wks the grower diet was adjusted to increase the volume of feed but still allow a reasonable growth rate (2620 kcal/kg, 13% CP; grower 2). At 18 wks the energy and the protein content was increased to achieve target body weights (2660 kcal/kg, 14% CP; grower 3).

At 22 weeks of age 480 birds (240 per treatment) were moved into laying pens (2.4 x 3.6m^2, 40 hens and 3 rooster per pen) in an environmentally controlled. The laying pen had 2/3 of the floor space covered by raised slats and remaing 1/3 of the floor pen covered by pine shavings as litter. The hens were fed from a ChoreTime breeder pans (4/pen) fitted with exclusion grill to prevent the rooster from eating hen feed. The roosters were fed from a pan feeder suspended over the litter area. Birds were no longer fed soybean hulls after being moved to the laying pens therefore feed formulation was adjusted at 22 weeks of age (2700 kcal/kg, 14.5% CP; layer) to allow high enough consumption to gain to target weight until birds were changed to a laying diet at 25 weeks. Birds were randomly assigned to one of 6 pens per treatment. The body weight and coefficient of variation (CV) was similar for both treatments.

Growth and Productivity

Pullet sample weights were taken weekly (n=40 per pen). All pullets were individually weighed at 4, 8, 12, 16, and 21 weeks of age during rearing. During lay, half of the birds per treatment were weighed weekly, and the following week the remaining three pens per treatment were weighed. The CV for BW was calculated during rearing as a measure of flock uniformity (n=215), and calculated on per pen basis.

Egg production was monitored daily on a per pen basis (n=6) from 23 to 40 weeks of age. Egg production was calculated by taking the number of eggs laid per week as a percentage of hens housed per pen.

Behavioral Data

Video cameras were mounted over each pen at 12 weeks of age. Videos were recorded on a digital recording unit and transferred to external hard drives daily.

Nine days (2 days/age) were observed through video recordings made on two consecutive days for each week at 16, 18, and 20 weeks of age. Video taken at 12 and 14 weeks was lost due to electronic storage issue. Scan sampling was used to calculate the number of birds feeding (bird is feeder oriented, pecking at the feeder), foraging (birds are pecking the floor), comfort behavior (dust bathing, preening, sitting and wing flapping), walking, and drinking (Table 3). Therefore, each week was treated as the average of behaviors performed on the ON feed and OFF feed days. The behaviors were analyzed for the entire light period each day, with time 0 being time lights came on (8 am). A scan sampling was done every 10 minutes until the end of the photoperiod (4 pm). The areas in which observations were made were: around the feeder, the drinker and open area (free space in the pen).

Feeding Motivation

The feeding motivation was determined by calculating the time it took for the pullets on the different feeding treatments to consume a determined amount of feed (same amount of feed for both treatments). The time feed was distributed was determined as time 0. The birds were given their daily ration and monitored to determine the time the feeder was empty. Feeding motivation was measured on every ON day from 17 to 21 weeks.

Water Data and Litter Moisture

Water usage was collected on a per pen basis from 6 to 20 weeks. Each drinker line was equipped with a low flow water meter (Omega FTB334D, OMEGA

Engineering, INC., Stanford, CT) at the incoming water source. The water meters were connected to Hobo U-30 (Onset U30-GSM, Onset Computer Corp., Bourne, MA) data logging unit set to record data each minute.

At 21 weeks two composite samples were taken from each pen for litter moisture analysis. The composite was taken from the litter under the center of the drinker line. The other composite was taken from 1.5 meters from the center of the drinker line in the middle of the pen. Two replicates were made from each composite. The samples were placed in pans and put in a drying oven at 75 C for 24 hours. Samples were weighed before and after drying (Shepherd, 2010). Percent litter moisture was calculated by dividing the difference of the before and after weight over the weight of the sample before the drying process.

Organ Weights and Reproductive Morphology

At 21 weeks of age, 5 birds per pen were randomly selected on the OFF feed day prior to providing soybean hulls. The birds were weighed and killed, and the proventriculus, gizzard, and liver were dissected and weighed. The intestines divided into duodenum, jejunum, and ileum and weighed. The organs weights were expressed as a percentage of body weight.

At 40 weeks of age, 10 birds per pen were randomly selected from each pen prior to feeding, and these birds were weighed, and killed. Ovary, oviduct, yellow follicles, breast muscle, and abdominal fat pad were dissected from the carcass and weighed. The reproductive organs and body parts weights were expressed as a percentage of body weight.

Feather Cover Data

Feather cover was evaluated for all the pens at 40 weeks of age. Birds were scored following a 5-point system similar to that used by Tauson et al. (2005). The scoring system focused on the condition of the back feathers as follows; Score 1: Bare back; Score 2: Bare back with feather cover tail area; Score 3: Obvious bare patches over mid back; Score 4: Small bare patch on back; Score 5: Complete feather cover with no bare patches (Figure 2).

Statistical Analysis

Body weight, egg production, uniformity, behavior, and water usage results were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Litter samples, feather cover scores, body and reproductive morphology, and feeding motivation results were compared using GLM (SAS, 2013, Cary, NC). Differences were deemed to be significant when the P-value was less than or equal to 0.05.

RESULTS

Body Weight and Uniformity

Body weight was significantly affected by the different feeding treatments (Table 4). Body weight was statistically different at 8, 12, 16, 20 weeks and the overall rearing period ($P<0.05$). There were no significant differences between the pens before the start of the feeding treatments (4 weeks). At 20 wks, pullets fed on ATD feeding program were 88 g heavier than the birds on the SAD feeding program. BW was not significantly different between treatments for the overall laying period (data not shown).

Uniformity was significantly affected by the feeding treatment at the highest level of feed restriction (12 weeks) (Figure 3). Uniformity was statistically different at 12 weeks, with a better uniformity for birds on the ATD feeding treatment (P=0.003). There were no significant differences at 4, 8, 16, or 20 weeks.

Egg Production and Fertility

Overall egg production was significantly different between treatments (P<0.01) (Figure 4). Hens fed on the ATD feeding program during rearing had a higher mean egg production (68.0% vs. 64.1%) than the hens on the SAD feeding programs with more significant differences between 25-28 weeks of age. There were no significant differences in fertility between the treatments on percent fertility (Figure 5).

Behavior Observations

Behavior traits by treatment and feed day at 16, 18, and 20 weeks are summarized in Figure 6. There were significant differences for both ATD and SAD feeding programs when comparing the bird behavior on the ON and OFF day. The percentage of birds at the feeder and drinker was significantly higher on the ON day versus the OFF day for both treatments. Pecking and comfort behaviors were higher on the OFF day than the ON feed day. There were significant differences between the feed programs when comparing them on a ON and OFF feed day basis. The birds on the SAD feed program spend more time at the feeder than the birds on the ATD feed program on an ON feed day. There were no significant differences on the OFF feed day. The birds on the ATD feed program spent more time at the drinker on the ON and OFF feed day than birds fed on a

SAD basis. The number of birds pecking the litter was significantly different on the OFF day with more SAD birds pecking the litter than the ATD fed birds. Walking and comfort behaviors were similar across feed programs and whether it was a ON and OFF feed day.

Water Data and Litter Moisture

Water usage for the feeding treatments from 6 to 20 weeks is summarized in Figure 7. There were significant differences in water usage between the ON and OFF feed day for both treatments (P<0.0001). The birds had higher water usage on the ON feed day than the OFF feed day. There were significant differences in water usage between the ATD and SAD feed programs during the ON feed day. The birds on the SAD feed program had higher water use than the birds on the ATD feed program at 7, 8, 12, 13, 14, 15, 17, 18, 19, and 20 weeks. Both feed programs had similar water usage on the OFF feed day. There were significant differences at 12 and 16 weeks, with the ATD program having higher water usage at 12 weeks and SAD program having higher water usage at 16 weeks. Water usage to feed intake ration was calculated daily (data not shown). There were no significant differences in the ratio between the treatments. The average ratio of water usage to feed intake was 2.25 and 2.10 for the ATD and SAD feed program on the ON feed day, respectively. There were no significant differences in the litter moisture between the treatments. The mean of the litter moisture level was of 29.2 and 27.3% for the ATD and SAD programs, respectively.

Feeding Motivation

The time the birds spent consuming the same amount of feed is summarized in Table 5. There was a significant difference between the SAD and ATD feed program at 17 and 18 weeks ($P<0.001$) with birds on the SAD feed program spending more time than the birds on the ATD feed program. There were no significant differences between treatments at weeks 19, 20, and 21. However, the birds overall mean for the SAD feed program were significantly different than those for birds on ATD feed program.

Body and Reproductive Morphology

The feeding treatments impacted the body morphology of the pullets but the differences did not continue through lay (Figure 8). There were significant differences between the liver, duodenum, jejunum, and ileum weight when expressed as a percentage of the body weight of the bird ($p<0.05$) at 20 weeks of age. The birds on the ATD feeding program had a smaller intestinal tract and liver compared to the birds on SAD feeding program. There were no significant differences on the proventriculus or gizzard weight between treatments. There were no significant differences in the reproductive morphology of the birds at 40 weeks. At 40 weeks, there were significant differences between the breast weights but not for the rest of the body morphology of the birds (data not shown). The birds on the SAD feeding program had greater breast weight than the birds on the ATD feeding program by 1.4%.

Feather Cover

No significant differences in feather cover between the treatments were observed. At 40 weeks of age all of the birds had a high cover score with the means being 4.34 and 4.17 for the SAD and ATD, respectively.

DISCUSSION

Body Weight and Uniformity

The birds on the ATD feeding program had a significant higher body weight than the birds on the SAD feeding program. Both feeding treatments were fed the same amount of feed and soybean hulls. Therefore, differences in body weight are only attributed to metabolic changes due to the feeding frequency and not to differences in nutrient intake (previous chapter). The results of this study agree with the results reported by Morrissey et al. (2014a) who determined that birds were heavier when feeding the feed ration everyday (ED) compared to skip-a-day (only feed ration). The difference in body weight might be attributed to the fact SAD are less efficient in the use of energy since nutrients have to be deposited and then remobilized for use on the off feed day (de Beer and Coon, 2007). Results from our study suggest that even by feeding a small amount of a low-nutrient ingredient (soybean hulls) on the off day, improved body weight gain over offering all the nutrients on the ON day in the SAD fed bird. Therefore, feeding frequency despite the diet or ingredient has a direct impact on body weight. The results of this study also agree with the results from the previous chapter (data not published) where we determined that the birds on the ATD feed program require less feed in order to achieve similar body weights.

The ATD feeding treatment did not negatively affected the uniformity of the flock, and was significantly improved at 12 weeks of age, which is the highest level of feed restriction and normally the age when the poorest CV's are observed. The difference between the groups decreased as the birds' age and the amount of feed increased (Figure 3). De Beer and Coon (2007) did not find significant differences in uniformity between the everyday (ED) and SAD birds. The difference in results might be due to the difference in the amount of feed the birds received. The birds on the ATD program received a larger volume of feed on the ON day, than the typical ED feed amount, potentially allowing for more birds to consume a more even amount of feed. Thereby, decreasing competition for feed and improving uniformity.

Egg Production

Egg production was significantly different between the ATD and SAD feeding treatments ($p<0.001$). Pullets transferred to the laying pens had similar mean body weight and CV (8.6 for the SAD and 9.0 for the ATD). The difference in egg production is only attributed to the feeding program in rearing. Previous research found that birds on the ED program have higher levels of egg production than the birds on the SAD program (de Beer and Coon, 2007 and Wilson et al., 1989). The results by the previous authors concur with our results that birds consuming feed daily (ED and ATD) produce more eggs. The differences in egg production might be attributed to changes in behavior and potential stress level. Higher levels of stress have been documented in broiler breeders under severe levels of feed restriction (Hocking et al., 1988 and Hocking et al. 2001). Shini et al. (2009) determined that elevated corticosterone levels significantly delay the onset of laying cycle and decrease egg production. The ATD program had the potential to change the birds' behavior and stress level and thereby improve egg production. However, the reproductive organs were not significantly different between feeding treatment when measured at the 40 wk, perhaps differences would have been observed if measured more frequently. The feeding treatment had a significant effect on egg production without changing the bird's long term reproductive morphology. Therefore, changes in egg production are not directly related to the changes in the birds' reproductive morphology.

Behavior Observations and Feeding Motivation

Behavior observations were significantly different, when comparing birds ON and OFF feed and when comparing the different feeding treatments. The birds spend more time around the feeder on the ON day than the OFF day in SAD and ATD fed birds. Both treatments got the highest volume of feed on the ON feed day; therefore more birds around the feeder was expected on the ON versus the OFF feed day. There was an increase in the percent of birds around the feeder in the SAD versus the ATD feed program on the ON feed day. The increase in the number of birds was likely reflective of the higher volume of feed provided the SAD birds (feed and soybean hulls – ON feed day).

The increase in the percentage of birds around the feeder also agrees with the feeding rate (time to consume daily feed amount) results. There was a significant difference in the time it takes the birds to consume the same amount of feed. The birds on the SAD feed program spend more time consuming the feed provided for the feeding motivation test. Since the birds on the SAD feed program spend more time consuming a set amount of feed, then it was expected that there would be a greater number of SAD fed pullets around the feeder on ON feed days. Feeding motivation data can be used as a measurement of the birds' interest to eat and varies based on feed program and the amount of feed given. Birds on high nutrient dense diet were highly motivated to eat and were considered chronically hungry (Savory et al., 1993). Sandilands et al. (2006) determined that birds on a high nutrient dense diet have a higher feeding motivation than birds fed a higher fiber diet. Their theory was that the birds on the high fiber diet are not as motivated to eat and consume less in the same amount of time when routinely offered a higher fiber diet because they have longer access to feed.

Previous results from our lab (unpublished data) show that the birds given soybean hulls on the OFF day spend a similar amount of time around the feeder on the ON and OFF day, while there were significantly greater number of birds at the feeder on ON versus OFF feed day when feed was offered only every other day (SAD). We suggested that the soybean hulls redirected the birds' attention to the feeder and might be a positive method of alleviating chronic hunger or provide a positive behavior outlet in broiler breeder pullets. Therefore, in this study we wanted to determine whether it was the increase in fiber or the feeding program that caused the difference in feeding behavior. We hypothesized that the birds on the ATD diet would not be as aggressive towards the feeder since they were given a stimulus (soybean hulls) the day before (OFF day) which could lead to a greater level of satiety or comfort. The feeding motivation test suggests that the birds on the ATD feed program are more aggressive towards the feeder, spending less time to consume the same amount of feed. Therefore, we might have fewer birds around the feeder for the ATD feed program than the SAD feed program on the ON feed day because they are eating their feed faster. These results show that the difference in behavior seen in the previous chapter (unpublished data) was more likely related to the increase in fiber than the feed program. In addition, in this study there were no differences in the number of birds around the feeder on the OFF feed day between the treatments. The lack of difference suggests that the birds are more satisfied from the previous day's feeding and are not as interested in feed even when offered a small stimulus (soybean hulls) to come to the feeder..

There were differences between the treatments in feeding motivation for weeks 17 and 18 but not for weeks 19 and 20. The differences between the weeks are likely due to the change in feed formulation after week 18. The birds in both treatments (SAD and ATD) were not achieving the desired body weight as suggested by Aviagen Management Guidelines. The diet was increased in energy and protein from 2620 kcal/kg and 13% CP to 2660 kcal/kg and 14% CP. The change in the diet might have lead to a certain degree of satisfaction leading to a decrease in feed motivation on the birds on the SAD feed program.

There are more birds around the drinker on the ON feed day compared to the OFF feed day for both feeding treatments. The increase in the percent of birds around the drinker on the ON versus the OFF feed day is likely due to the higher volume of feed. Water usage and therefore activity around the drinker area increases as the volume of feed increases (Hocking et al. 2001). The birds on the ATD feed program are given soybean hull on the OFF feed day. Soybean hulls might cause an increase in the birds motivation to drink causing the significant increase in the number of birds drinking on the

ATD program compared to the SAD feed program on the OFF day. The difference between the treatments on the ON feed day might be related to their feeding pattern. The birds on the SAD feed program spend more time around the feeder than the birds on the ATD feed program. Therefore, the number of birds at the drinker in the SAD pens will be decreased as they are spending most of their time in another activity (feeder). Although there are differences in percentage of birds around the drinker it might not directly represent water usage. Differences between water usage and percentage of birds might be different based on the intensity of drinking of the birds. In addition, the birds might not be drinking but are standing under the drinker and being counted as drinking.

The number of birds foraging is significantly different on the ON versus the OFF feed day for both the ATD and SAD feed program. The birds spend more time foraging on the OFF feed day than on the ON feed day. Foraging is characterized as food seeking activity caused by a lack of satiety in the birds (Dawkins, 1989). The difference in the number of birds foraging shows the birds are not as satisfied and possibly feed seeking on the OFF feed day when nothing or only soybean hulls are being offered. There is not a significant difference between the treatments on the ON feed day. Our results do not agree with previous research that showed higher foraging activity in birds with higher levels of feeding motivation (Van Emous et al., 2014). A higher number of birds foraging would be expected in the birds on the ATD feed program since they have a higher feed motivation. However, the lack of difference suggest even though the birds are more aggressive towards the feeder on the ON feed day they are somewhat satisfied with the amount of feed provided. In fact, the difference between the treatments on foraging activity on the OFF day suggests that the ATD feed program redirects the birds attention and might provide more satiety showing lower foraging numbers. The birds on the SAD feed program are foraging more and showing more feed seeking behaviors than the birds on the ATD feed program on the OFF day. As mentioned in previous paragraphs, the relationship between water consumption and feed intake on the OFF day might lead the birds to redirect their attention from feed seeking activities and a reduction in foraging.

Water Data

There was a significant difference in the water usage in the pullets on the ON feed day versus the OFF feed day. The birds consume more water on the ON feed day. The difference in water usage is related to the water: feed ratio. The pullets get all or most of their feed on the ON feed day therefore a higher amount of water use will occur on the same day. The water to feed ratio was 2.25 and 2.10 for the ATD and SAD feed program and were not significantly different between them. Bennett and Lesson (1989) reported similar water to feed ratios at 2.14 and 2.35 for birds on ED and SAD feed programs for broiler breeder pullets.

There were significant differences between the treatments on the ON feed day with the SAD birds using more water then the birds on the ATD feed program. Previous research has shown significant differences in water intake between birds fed different protein ratios, with the birds fed a higher protein ratio having higher water consumption (Hocking et al., 2001). They suggest the difference in water intake might be related to food intake. The birds on the lower protein diet consumed more feed than the birds on the higher protein (Hocking et al., 2001). Therefore, an increase in feed intake leads to an increase in water intake. The difference in feed intake might be the reason we found a significant increase in water usage on birds on the SAD feed program compared to the birds on the ATD feed program. The birds on the SAD feed program get their ration of soybean hulls on top of their feed allocation therefore have a higher water usage on the ON feed day than the birds on the ATD program. However there were no differences between the treatments on the OFF day. The quantity of soybean hulls provided to the ATD birds might have not been enough to significantly impact or increase water usage on the OFF feed day.

Hocking et al. (2001) also suggests that differences in water intake might be related to stress levels on the birds. Previous research shows that birds on a feed restricted diet spend less time resting and more time drinking (Hocking et al., 1996). We could attribute the difference in water usage on the ON feed day to a decreases in stress in the birds, however those differences are not seen on the OFF day where we expected to see a bigger difference between treatments. Therefore, boredom and hunger might not be the main stimuli to water usage patterns as suggested by Benson and Lesson (1989).

There is a difference between the water usage results and the percentage of birds around the drinker area. The behavior observations show higher number of birds around the drinker on the ON day compared to the OFF day for both feeding treatments, this agrees with the water usage data. However when looking at the behavior observations there were more birds around the drinker in the ATD than the SAD feed program, while we measured higher water usage on the SAD than the ATD on the ON feed day. Birds are counted as drinking when they are under the drinker; however, there is a chance that they are not drinking, causing a difference in water usage and behavior observation.

Another potential cause of difference is intensity of drinking by the birds. The birds on the SAD feed program might be triggering the drinker more times or faster causing a higher water usage but are only counted once in the behavioral data. To measure this type of potential differences, pens with fewer birds and camera's focused specifically on the drinker line would have to be utilized to determine the actual number of birds drinking and the number of times that they are triggering the nipple drinker.

Feather Cover

Feather cover is affected by several factors with one of them being feather pecking. This behavior is considered as a stereotypic behavior indicative of chronic hunger and frustrated feeding motivation (de Jong and Guemene, 2011). Morrissey et al. (2014b) determined that feeding the birds daily led to higher feather pecking than birds fed on a SAD program. We wanted to determine if the ATD feed program had an impact on feather pecking behavior and the birds' feather cover. We did not find significant differences in feather cover between the treatments. This may have been due to having both treatments consume high fiber levels (soybean hulls). Previous research has shown a decrease in the level of feather damage with increasing dietary dilution levels (Qaisrani et al. 2013). Differences in feather cover may have been observed if the birds were older; since feather condition worsens as birds age (Morrissey et al. 2014b).

Body Morphology

The relative weight of the liver and the small intestine of the birds were significant affected by the feeding program ($P<0.05$). The birds on the SAD diet had larger livers and small intestines than the birds on the ATD diet at 20 weeks. Differences between the treatments were observed even though the same nutrients were given to the birds over a two-day period. Therefore, changes in the organ morphology were directly attributed to the feeding frequency rather than the changes in the diet. De beer et al. (2007) found a relative increase in liver weight in pullets fed on a SAD compared to ED. Previous research found that consistency in nutrient supply has a major influence in liver size (Muiruri et al., 1975 and de Beer et al., 2007). By feeding the birds everyday there is more a fluctuation in liver size leading to a decrease in size. Differences in liver size in our research may be attributed to nutrient supply on the ATD fed pullets with the addition of soybean hulls leading to a fluctuation in the liver size.

The differences between the feeding programs were not carried on through the laying phase. The liver and small intestines were no longer different between the pullets on the SAD and ATD feeding at 40 weeks. Our results agree with previous research that found no significant differences in liver weights on hens fed on a SAD and ED feeding program (Ekmay et al., 2010). The authors suggest that the birds adapt to a new physiological state decreasing the levels of lipogenic characteristics (leading to a change in liver size) leading to lack of differences in liver size. Therefore, the results of our research suggest that the feeding program in rearing no longer influences the hens' liver size since they adapted to their new feeding regimen in lay.

From the results of study, we can conclude that the addition of soy hulls on the off feed day of a standard skip-a-day program has an effect on overall performance and behavior. The addition of the soybean hull on the OFF feed day improves productivity of pullets by: increasing body weight gain on the same feed allocation, improving uniformity, and increasing egg production. The alternate feed program changes the behavior of the pullets by decreasing foraging behaviors and water usage. The improvement in productivity and changes in behavior of the pullets suggests the addition of soybean hulls can lead to improve broiler breeder wellbeing.

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TABLE 4.1. Rearing and Laying Diets Ingredient Compositions

	Rearing 1	Rearing 2	Rearing 3	Rearing 4	Laying
Corn, Grain	69.19	59.72	59.98	60.00	53.04
Wheat Middlings	17.00	18.00	18.61	19.14	19.50
Soybean Meal -48%	4.50	5.50	9.50	10.02	14.44
Soybean Oil	0.00	0.00	0.00	0.00	3.41
L-Valine	0.00	0.08	0.08	0.00	0.00
Isoleucine	0.00	0.11	0.11	0.02	0.00
Limestone	0.59	0.93	0.90	0.92	6.68
Defluor. Phos.	1.96	1.51	1.53	1.49	1.21
Solka floc	3.00	2.07	2.00	0.00	0.00
Common Salt	0.20	0.25	0.25	0.24	0.24
Vitamin Premix	0.80	0.80	0.80	0.80	0.80
Mineral Premix	0.14	0.14	0.14	0.14	0.08
DL-Methionine	0.19	0.18	0.16	0.16	0.20
L-Lysine HCl	0.34	0.24	0.11	0.07	0.00
Tryptophan	0.00	0.00	0.00	0.00	0.00
Threonine	2.15	0.00	0.08	0.06	0.40
Wheat Bran	0.00	10.00	5.64	6.99	0.00
Argenine	0.00	0.12	0.12	0.00	0.00

TABLE 4.2. Rearing and Laying Diets Nutrient Composition

	Rearing 1	**Rearing 2**	**Rearing 3**	**Rearing 4**	**Laying**
Dry Matter	84.63	85.96	86.41	86.52	81.85
M.E.	2.82	2.62	2.66	2.70	2.80
Protein	13.00	13.00	14.00	14.50	15.20
E.E.	3.18	3.16	3.10	3.16	6.15
C18:2	1.86	1.84	1.80	1.84	3.33
C.F	2.97	3.98	3.71	3.92	3.19
Calcium	0.90	0.90	0.90	0.90	3.00
Total Phos.	0.73	0.75	0.73	0.75	0.63
Avail. Phos.	0.47	0.42	0.42	0.42	0.35
Ca:P=	0.02	-0.03	-0.03	-0.03	-1.15
K	0.47	0.59	0.63	0.66	0.65
Cl	0.22	0.23	0.21	0.20	0.18
Mn	149.38	160.91	248.47	160.78	154.29
Na	0.21	0.21	0.21	0.21	0.19
Zn	131.62	145.65	214.60	145.42	137.78
Choline	1.50	1.67	1.71	1.76	1.71
Folate	2.33	2.46	2.56	2.60	2.65
ARG	0.62	0.84	0.95	0.87	0.93
GLY	0.43	0.51	0.56	0.59	0.59
SER	0.50	0.58	0.65	0.68	0.70
GLY & SER	0.97	1.15	1.30	1.36	1.44
HIS	0.28	0.31	0.35	0.36	0.38
ILE	0.39	0.56	0.63	0.56	0.57
LEU	1.04	1.09	1.21	1.24	1.28
LYS	0.68	0.68	0.68	0.68	0.70
MET	0.38	0.38	0.38	0.38	0.43
CYS	0.21	0.23	0.25	0.26	0.26
TSAA	0.59	0.61	0.63	0.64	0.69
PHE	0.48	0.52	0.60	0.62	0.66
TYR	0.37	0.41	0.47	0.49	0.53
TAAA	0.85	0.93	1.07	1.11	1.19
THR	2.48	0.80	0.54	0.54	0.91
TRP	0.11	0.14	0.16	0.17	0.18
VAL	0.50	0.64	0.71	0.65	0.67

TABLE 4.3. Behavior observations on broiler breeder pullets[a].

Behavior	Description
Feeding	Pecking at the feeder
Drinking	Pecking at the nipple drinker
Foraging	Pecking and/or scratching the litter
Walking	Walking or running without performing other behaviors
Comfort	Preening, seating, nibbling, stroking, dust bathing and wing flapping

[a] Behavior definitions modified from the ethogram of de Jong et al. (2005).

TABLE 4.4. The mean body weight of birds (g) at 4, 8, 12, 16, and 20 weeks of age and overall rearing period as affected by the feeding program (SAD and ATD).

Weeks	**SAD**	**ATD**
4	453.90[a]	444.97[a]
8	765.00[b]	849.99[a]
12	1105.49[b]	1162.51[a]
16	1584.28[b]	1633.82[a]
20	2135.49[b]	2223.59[a]

[a-b] Treatments significantly different within period ($P<0.05$).

SAD= Skip-a-day feeding program; ATD= alternate feeding program.

TABLE 4.5. The mean time spent eating (min) at 17, 18, 19, 16, and 20 weeks of age and overall as affected by the skip-a day feeding program (SAD and ATD).

Week	SAD	ATD
17	58.6^{A}	43.4^{B}
18	63.3^{A}	45.5^{B}
19	43.1^{A}	42.3^{A}
20	46.0^{A}	41.3^{A}
21	42.3^{A}	44.9^{A}

SAD= Skip-a-day feeding program; ATD= alternate feeding program.

FIGURE 4.1. Nutrient relationship of phase feeding in rearing. Nutrients are as follows: M.E. =Metabolizable Energy, Protein= Crude Protein, and C. F.= Crude Fiber. Each value represents the nutrient amounts on the rearing diets 1 thru 4.

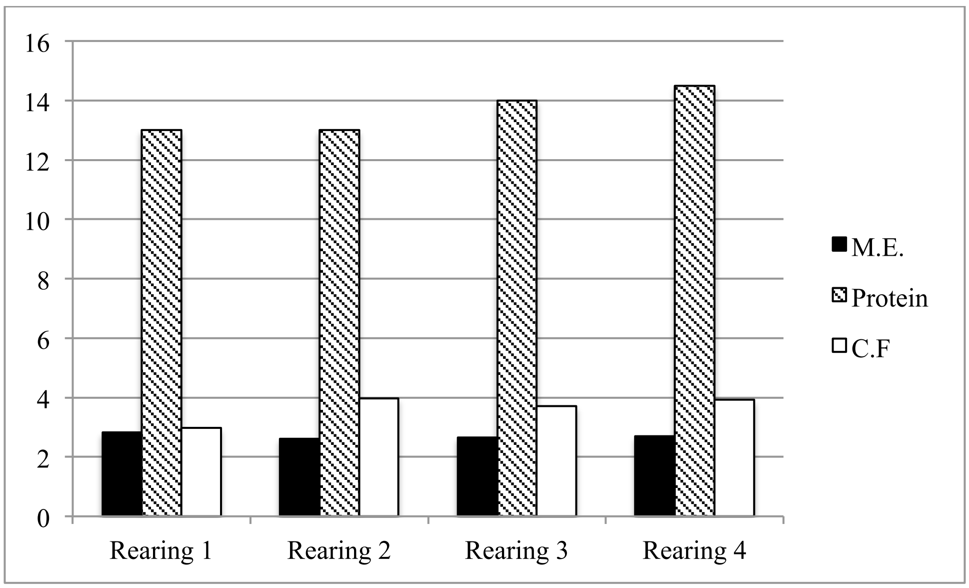

16
14
12
10
8
6
4
2
0
Rearing 1
Rearing 2
Rearing 3
Rearing 4
M.E.
Protein
C.F

FIGURE 4.2. Feather scoring scheme adapted from Tauson et al., 2005. The scoring system focused on the condition of the back feathers as follows; Score 1: Bare back; Score 2: Bare back with feather cover tail area; Score 3: Obvious bare patches over mid back; Score 4: Small bare patch on back; Score 5: Complete feather cover with no bare patches.

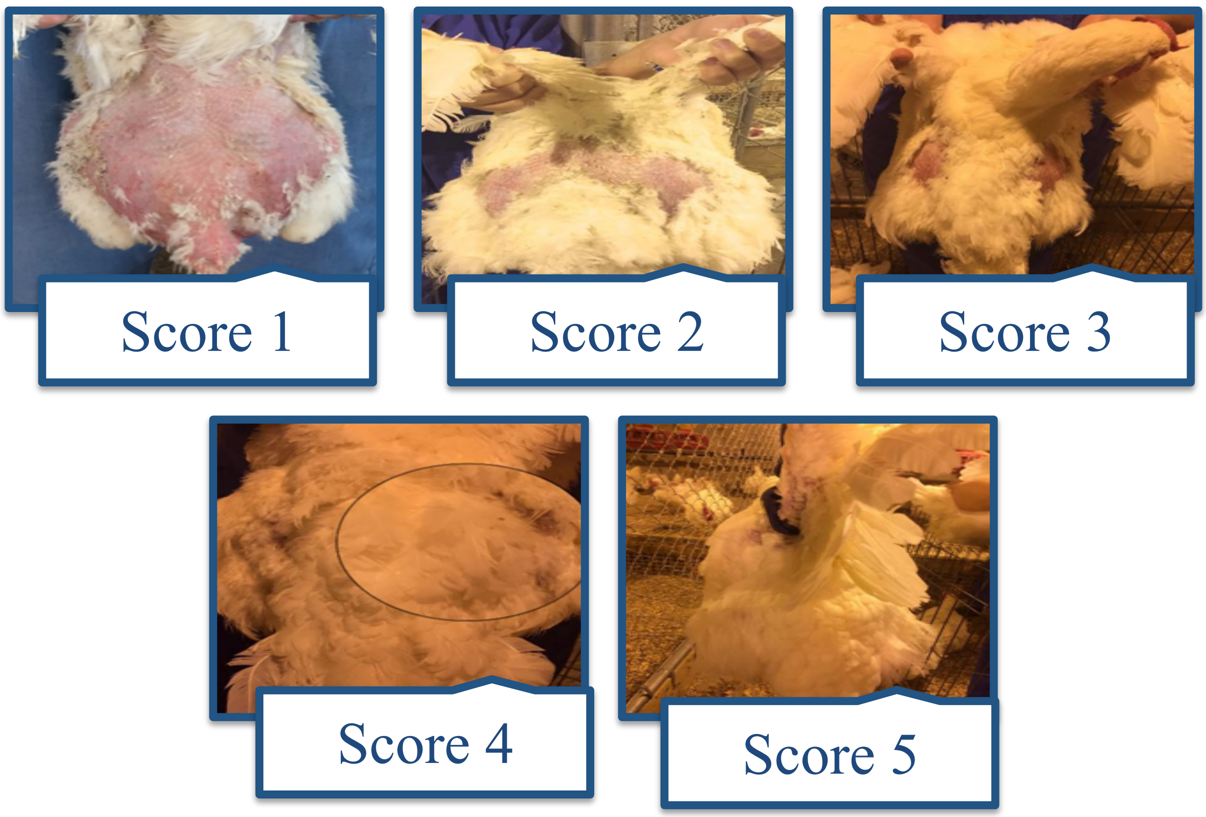
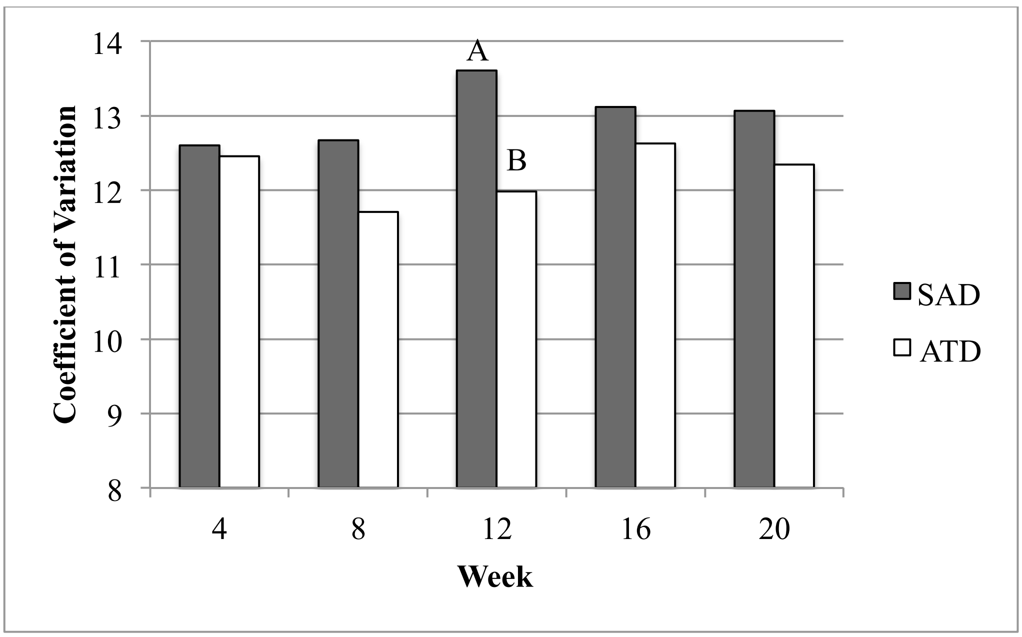

Score 1
Score 2
Score 3
Score 4
Score 5

FIGURE 4.3. Mean coefficient of variation in broiler breeder pullets at 4, 8, 12, 16, and 20 weeks as affected by the feeding program. Feeding programs were as follows: SAD= skip-a-day feeding program and ATD= alternate feeding program. Each value represents the mean of the coefficient of variation of the body weight in each feeding program. Means for different treatments with no common superscript (A-B) are significantly different ($P<0.05$). No superscript means no significant differences between treatments.



14
13
12
11
10
9
8
Coefficient of Variation
A
B
4
8
12
16
20
Week
SAD
ATD

FIGURE 4.4. Egg production was affected by the different feeding programs. Feeding programs are as follows: SAD= skip-a-day feeding program (—), ATD= alternate feeding program (- -). Each value represents the mean of the percentage of total egg produced within each feeding program. The means for the overall period ($p<0.001$) are 64.1% and 68.0% for the SAD and ATD feeding programs, respectively.


90
80
70
60
50
40
30
20
10
0
% Egg Production
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Age (wk)
SAD
ATD

FIGURE 4.5. Percent fertility at 30, 35, and 40 weeks as affected by the different feeding programs. Feeding programs are as follows: SAD= skip-a-day feeding program and ATD= alternate feeding program. Each value represents the mean of the percentage of fertile eggs on each feeding program. No superscript means no significant differences among treatments.

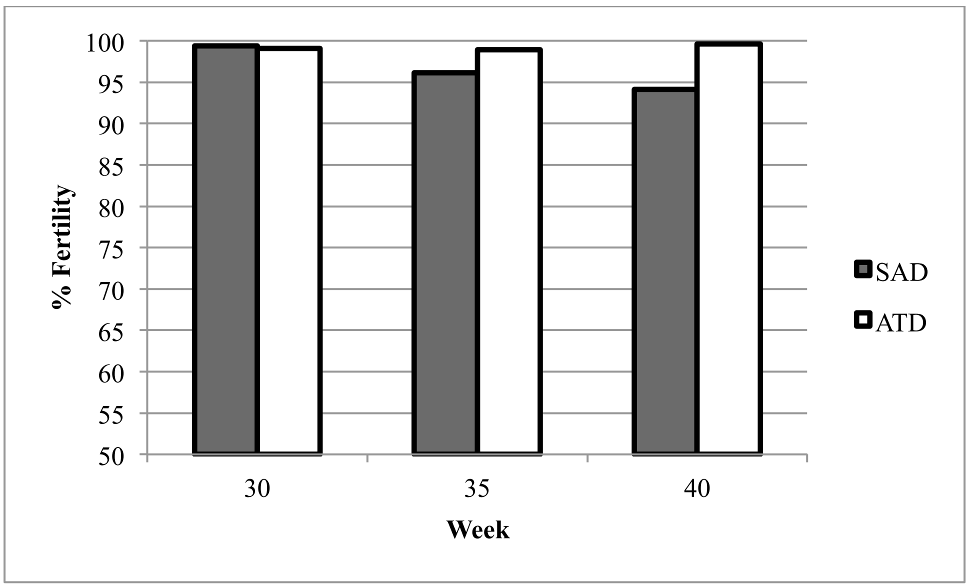
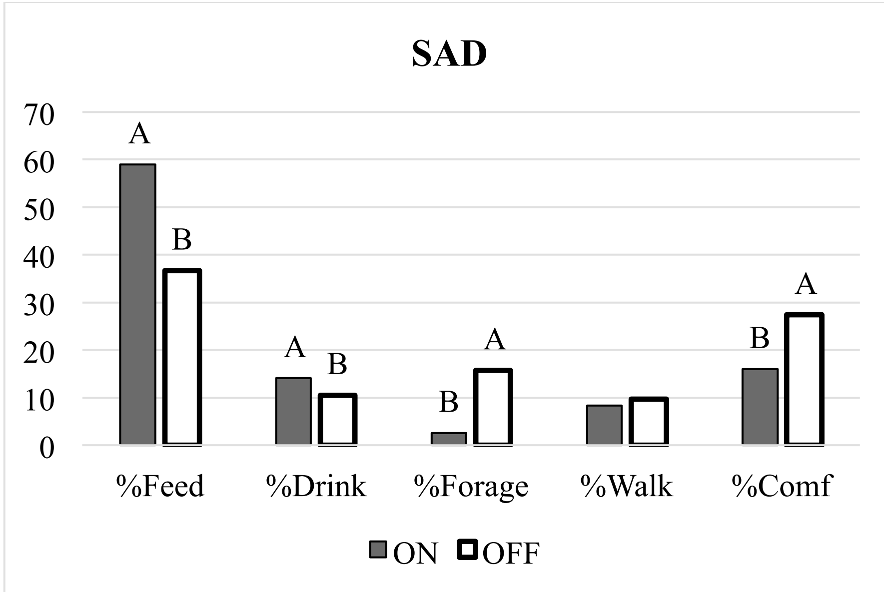
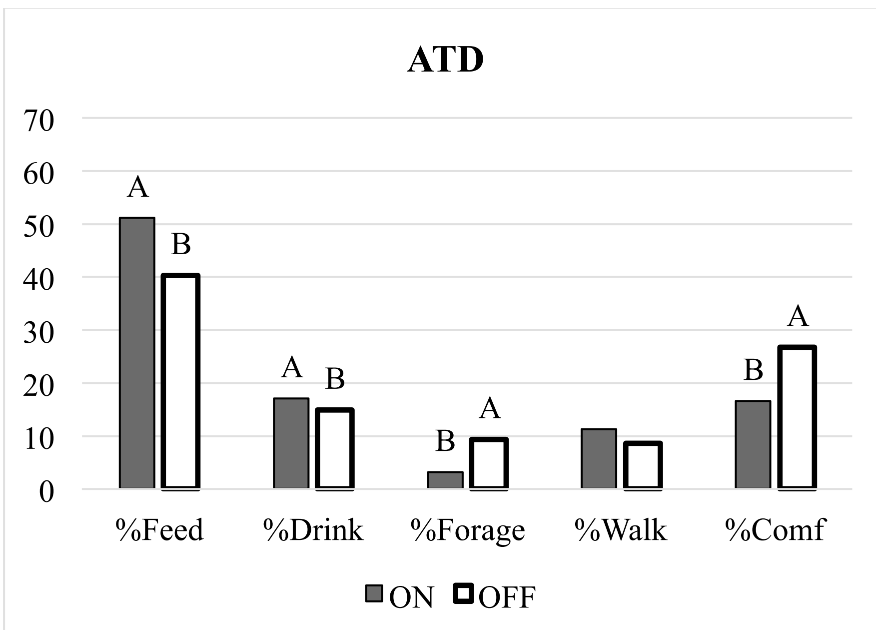
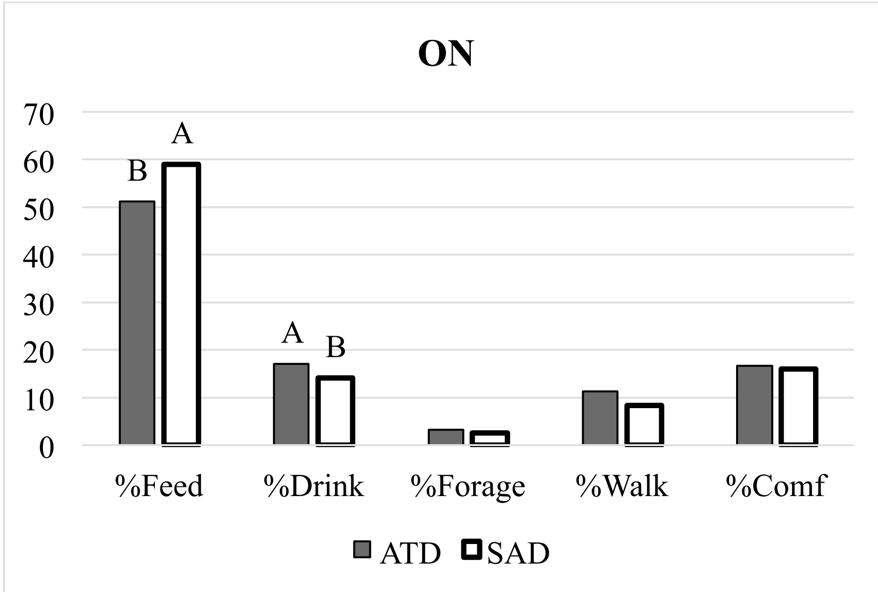

100
95
90
85
80
75
70
65
60
55
50
% Fertility
30
35
40
Week
SAD
ATD

FIGURE 4.6. Effect of feeding program ATD (alternate program – soybean hulls) and SAD (skip-a-day) on the percentage (%) of birds for observed behaviors. Data were collected in 3 observational areas (feeder, drinker and open area) in each of 2 ATD and SAD pens, every 10 min during the entire day (8:00 to 16:00). Observations occurred at 16, 18, and 20 wks for two consecutive days. ATD pullets were fed every day between 8:00 AM to 8:30 AM. SAD pullets were fed every other day (ON feed day) between 8:00 and 8:30 AM. Means with superscript (A-B) were significantly different ($P<0.05$). No superscript means no significant differences among treatments.


SAD
70
60
50
40
30
20
10
0
A
B
A
B
B
A
B
A
%Feed
%Drink
%Forage
%Walk
%Comf
ON
OFF


ATD
70
60
50
40
30
20
10
0
A
B
A
B
B
A
B
A
%Feed
%Drink
%Forage
%Walk
%Comf
ON
OFF


ON
70
60
50
40
30
20
10
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B
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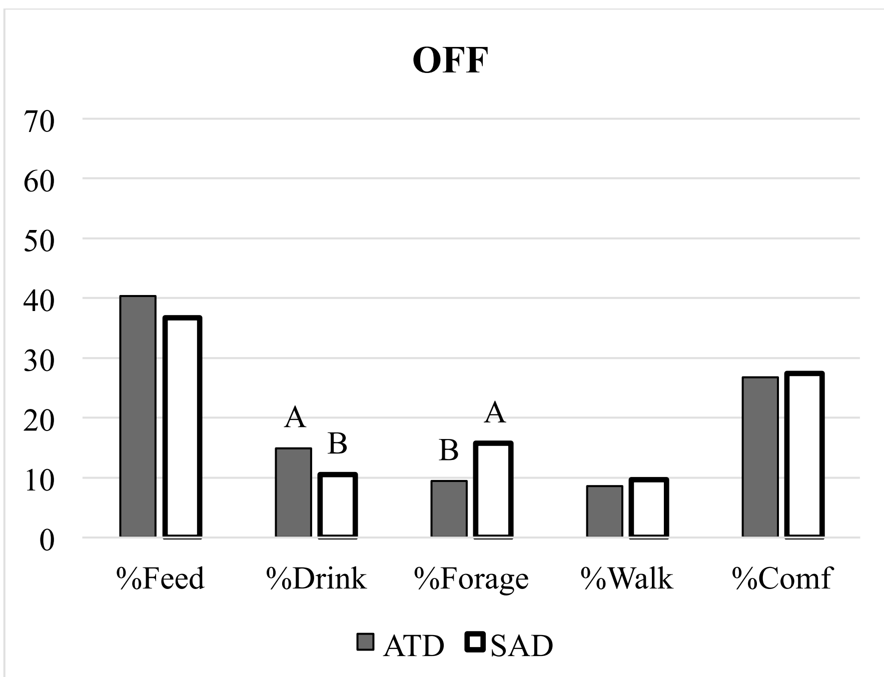
A
B
%Feed
%Drink
%Forage
%Walk
%Comf
ATD
SAD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A
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%Feed
%Drink
%Forage
%Walk
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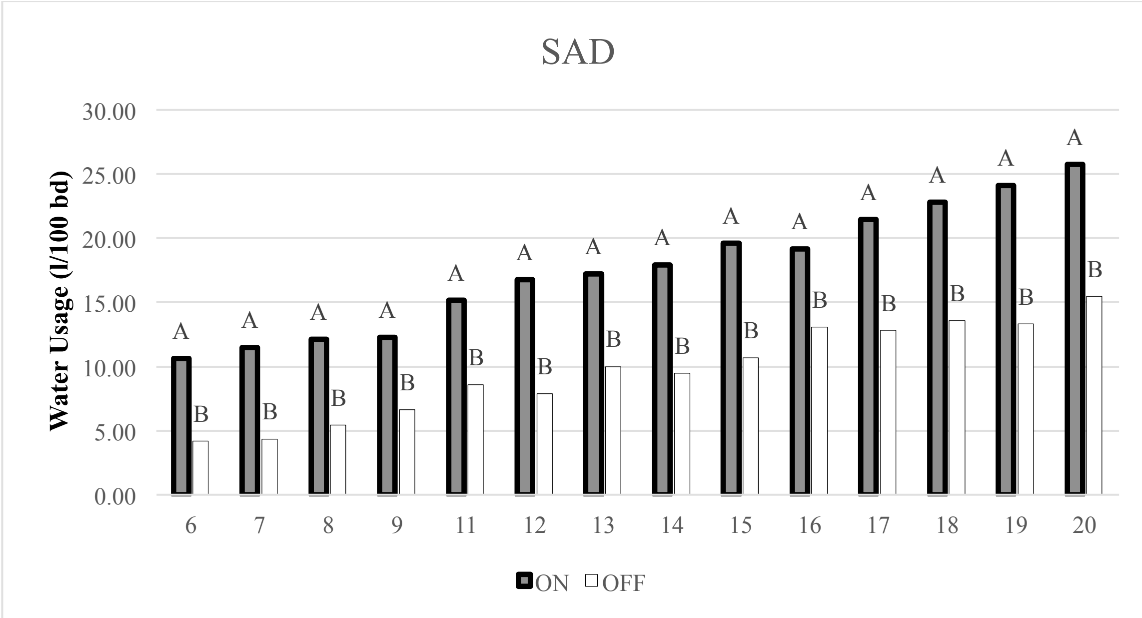
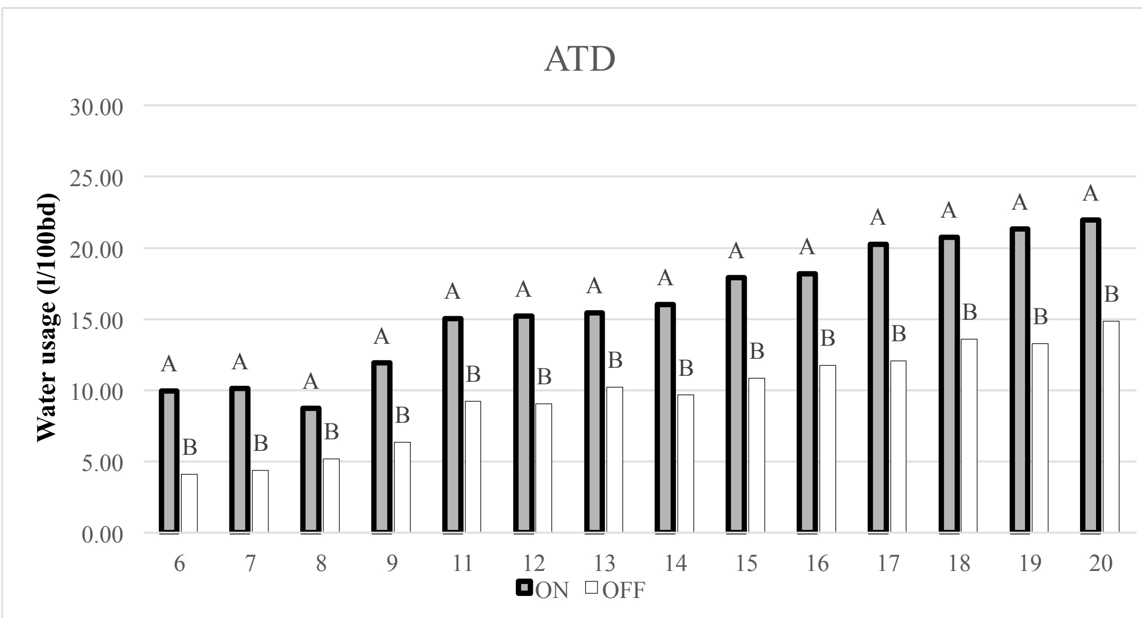
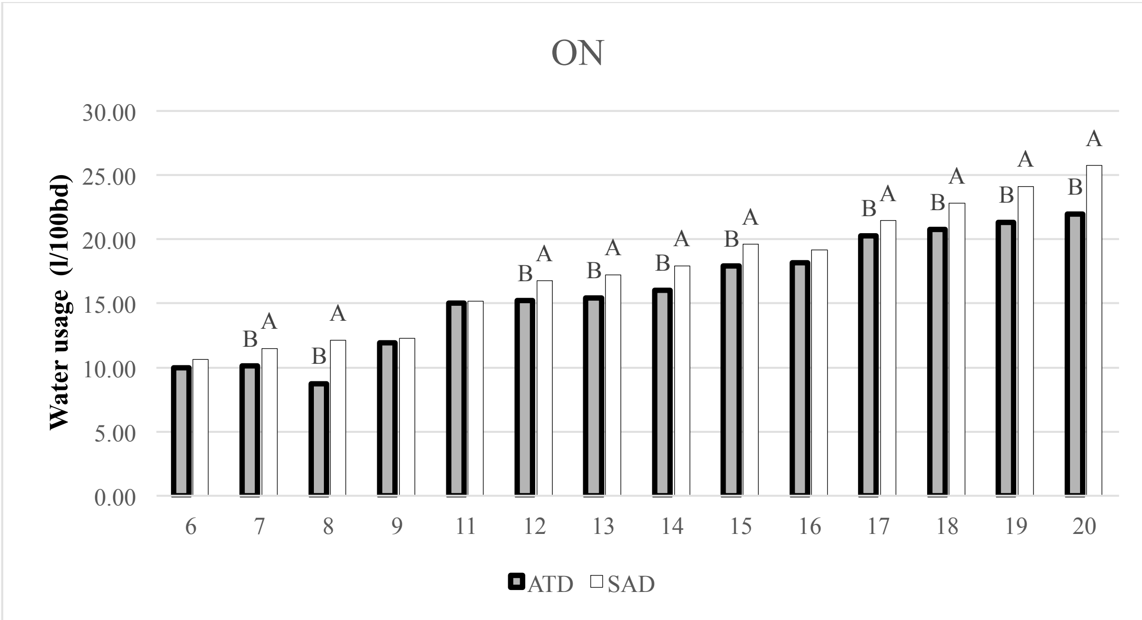
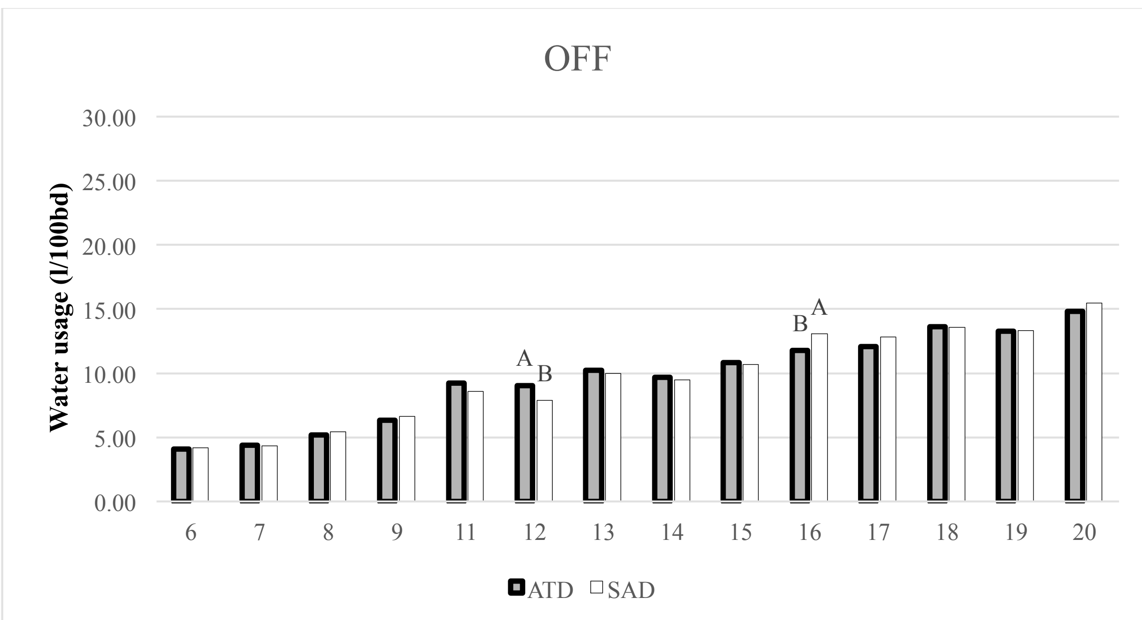
ATD
SAD

FIGURE 4.7. Effect of feeding programs ATD (alternate program – soybean hulls) and SAD (skip-a-day) on water intake of the pullets. Each value represents the mean of water intake in each feeding program. There was no data recorded for water usage on week 10 due to equipment issues. Means with superscript (A-B) were significantly different ($P<0.05$). No superscript means no significant differences among treatments.


SAD
Water Usage (l/100 bd)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
6
7
8
9
11
12
13
14
15
16
17
18
19
20
A
B
ON
OFF


ATD
Water usage (l/100bd)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
6
7
8
9
11
12
13
14
15
16
17
18
19
20
A
B
ON
OFF


ON
Water usage (l/100bd)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
6
7
8
9
11
12
13
14
15
16
17
18
19
20
A
B
ATD
SAD


OFF
Water usage (l/100bd)
30.00
25.00
20.00
15.00
10.00
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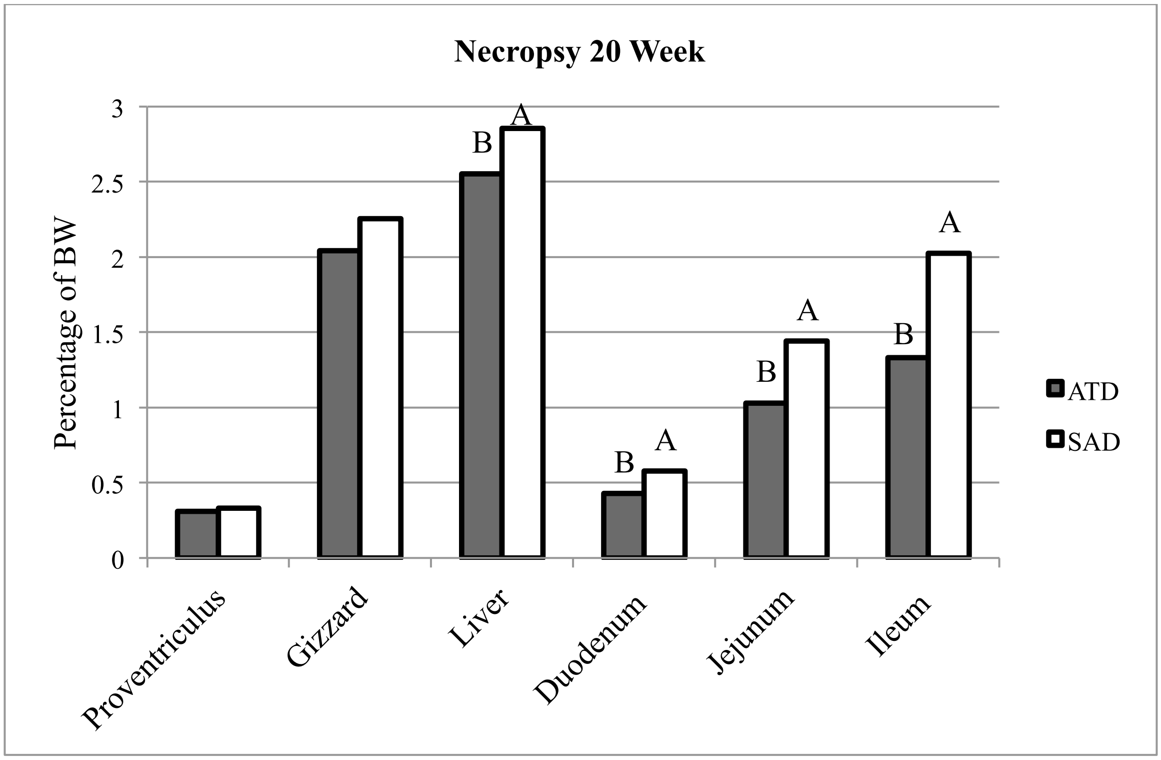
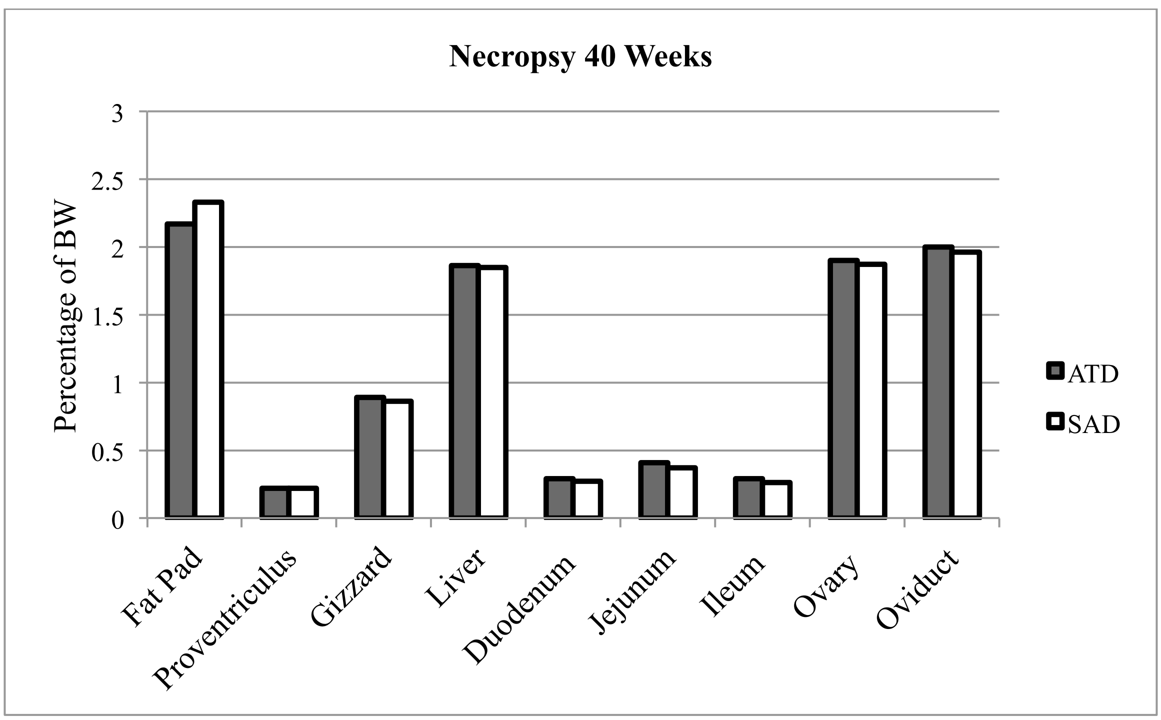
0.00
6
7
8
9
11
12
13
14
15
16
17
18
19
20
A
B
ATD
SAD

FIGURE 4.8. Overall necropsy data (proventriculus, gizzard, liver, duodenum, jejunum, ileum, and fat pad) as affected by the feeding programs (ATD and SAD) at 20 and 40 weeks. Each value represents the mean of the organ weight expressed as a percentage of the body weight of the bird. Means for different treatments with no common superscript (A-B) are significantly different ($P<0.05$). No superscript means no significant differences among treatments.


Necropsy 20 Week
Percentage of BW
3
2.5
2
1.5
1
0.5
0
A
B
A
B
A
B
A
B
A
B
Proventriculus
Gizzard
Liver
Duodenum
Jejunum
Ileum
ATD
SAD


Necropsy 40 Weeks
Percentage of BW
3
2.5
2
1.5
1
0.5
0
Fat Pad
Proventriculus
Gizzard
Liver
Duodenum
Jejunum
Ileum
Ovary
Oviduct
ATD
SAD

CHAPTER 5

EFEECT OF A HIGHER ENERGY AND LOWER PROTEIN DIET ON REPRODUCTIVE PERFORMANCE, REPRODUCTIVE BEHAVIOR, FERTILTY, AND FEATHER COVER OF BROILER BREEDERS[1]

[1]Aranibar, C. D., W. K. Kim. A. L. Owen, J. L. Wilson. To be submitted to *Poultry Science*.

ABSTRACT

Feathering is often a concern of broiler breeder managers with the assumption that feathering is directly correlated to mating behaviors and fertility of a flock. Nutrition plays a critical role in providing adequate energy and protein levels for egg production, feathering, activity and maintenance. Elevating energy levels has the potential to increase egg size and egg production but can potentially affect feather cover of hens. Based on industry observations, hens subject to elevated energy levels have increased feather loss. The objective of the study is to determine if an increase in energy and decrease in protein level would affect reproductive performance and feather condition among hens. A total of 420 Ross 708 broiler breeder hens were randomly assigned to 12 pens (n=35, 0.287 m^2/bird) with 6 of those pens allocated to one of two feeding treatments: high energy and low protein (**HL**, 3000 Kcal/kg, CP=13%) or low energy high protein (**LH**, 2900 Kcal/kg, CP=15%). Egg production, feather cover scores, mating behaviors, body weight and breast weight, abdominal fat pad weight and oviduct/ovary weights were measured during the laying period (21-62 weeks). Feather cover was visually evaluated and given a score of 1 to 5, with 1 being bare and 5 full feather cover. Data were analyzed using GLM (SLICE, SAS 9.4). Dietary treatments did not impact overall body weights or fertility from 23 to 62 weeks. The **HL** diet significantly increased egg production ($p<0.001$) compared to the **LH** diet, without having a significant impact on egg weights. There were no significant differences in ovary/oviduct weights, abdominal fat pad, or breast weight at 62 weeks. Feather cover scores were not significantly different between treatments ($p=0.09$). In this study, a slight increase in dietary energy increased egg production with no measured negative impact. The reduction of crude protein in the

laying diet appeared to have little impact on the hen’s performance or body measurements.

Key Words: broiler breeder, feathering, performance, behavior

INTRODUCTION

Genetic selection for faster growth and feed efficiency of broilers has led to changes in broiler breeder management. Broilers breeders are selected to produce fast-growing progeny; therefore have the potential to grow as quickly and efficient as their progeny. The high genetic growth potential leads to negative effects on reproductive performance and mortality when birds are fed ad libitum. Broiler breeders are feed restricted to ensure optimal health and maintain desired reproductive traits. Feed restriction can be quantitative or qualitative. Nutritionists and breeder managers have to adequately feed broiler breeders to achieve desired body weight and sustain egg production without reaching body weight that negatively impact reproductive traits. The main focus of feed formulation is energy and protein levels and reaching an adequate balance between them. Adequate developments of reproductive traits along with feathering play a crucial role in broiler breeder management. Feathering impacts energy utilization of broiler breeders and loss of feathering can negatively impact fertility.

Different Nutrient Levels on Broiler Breeder Performance

Many management aspects like feeding program, flock density, health of the flock, and photoperiod can impact flock performance. Energy is released during digestion and metabolism in the body. Energy is distributed in the body and used for growth, maintenance of tissues, immunological function, and egg production (Rabello et al. 2006). The nutritionist must establish the correct dietary energy level to allow the breeder manager to design a feeding program that fulfills the birds' requirements. Although specific daily energy recommendations are given by breeder management guidelines, actual values vary based on house temperature, phase of production, ambient environment, and feather condition.

To respond to changes in energy requirement through the laying phase, primary breeders suggest different feeding programs. However, the additional costs of storage in the mill and separate transportation and flow through the feed mill encourage the use of one layer diet. The problem becomes: how much energy is adequate for production. The high requirement of energy at peak egg production leads to diets with higher levels of energy than what is required by the hen. The high energy level results in overweight hens, impacts feathering production and have the potential to decrease fertility (Robinson et al., 1995).

Protein plays a critical role in body maintenance and egg production. Although changes in energy have a greater impact on body weight than protein (de Beer and Coon, 2006); elevated protein levels can increase body weight (Harms and Ivey, 1992) and impact body composition of the hen by increasing breast yield. Increasing protein levels up to 19%, increased egg production and egg size yielding more and bigger broilers (Lopez and Lesson, 1995). However, the increase in protein can lead to an overweight hen. Low protein can also have the negative impact on egg production and egg size. Protein levels below 12% in the diet have shown to decrease egg production and egg size (Lopez and Lesson, 1995).

Managing protein contents in breeder diets has become more important as levels of feed restriction have increased over time. By altering protein levels in the diet, breeder managers are able to increase the volume of feed without having a negative impact on performance (Van Emous et al., 2015). In addition, lowering the level of protein during lay can decrease breast fleshing in the late phase of production. Decreasing breast fleshing can improve fertility by altering body conformation and improve mating efficiency.

Broiler Breeder Feathering

Feather loss is a complex issue for the breeder industry. Feather production is greatly impacted by management of the birds in rearing. The quality and quantity of feathers produced in the rearing phase determines the amount of feather cover the birds have available during the lay phase. Therefore, the focus after rearing the birds is feather maintenance. Critical areas are: flock management, environmental conditions, bird density, feed management, flock health, nutrition and body weight uniformity. Feather cover plays a crucial role in insulation, feed intake and energy utilization (Emmans and Charles, 1977). In addition, drops in hatchability and fertility are thought to be associated with feather cover loss.

Feather loss can be impacted by egg production. Mills et al. (1988) determined a negative correlation between egg production and feathering. Increases in egg production decrease the amount of feather cover on birds. This key factor needs to be taken into consideration when comparing differences between flocks or treatments. While egg production is the single product of broiler breeder farms, the eggs have to be fertile to be of value to the industry. To assure optimum fertile eggs numbers, feather cover of the hens has to be a major concern in managing these flocks.

Another factor that plays a huge role in feathering is thought to be mating activity. It has been assumed that hens with more feather loss are mating more frequently and therefore have higher fertility levels than hens with lower feather loss (Jones and Prescott, 2000). However, recent industry data and studies suggest the contrary. Moyle et al. (2010) conducted a study where they compared pens with differences in mating frequency and hen back feathering. They determined that the hens with greater feather loss had actually fewer mounts by males (Moyle et al., 2010). The researchers did not determine a correlation factor between feather loss and fertility, however a difference in mating activity was present.

Altering the energy and protein levels have the potential to impact fat reserve, breast fleshing, egg production, reproductive organs, fertility and feather cover. An increase in energy levels can lead to greater body weight, increase in fat reserve and breast fleshing, and decline in feather cover. Decreasing protein can lead to a decline in egg production, decrease in fertility and decline in feather cover. Therefore, it is important to determine the impact nutritional deviations from broiler breeder guidelines have on overall bird performance. The current study was conducted to evaluate the effect of an increase in energy and decrease of protein in a diet on reproductive performance. In addition, effect of the higher energy and decrease protein diet on feather cover and its relationship to reproductive behaviors.

MATERIAL AND METHODS

At twenty weeks a total of 492 twenty-week-old Ross 708 female broiler-breeders were assigned to 14 pens (2.4 x 3.6m^2; 36 hens and 3 roosters per pen) to give similar body weight uniformity as the rearing pens. At twenty-five weeks, six replicate pens (n=36 hens/pen) were allotted per treatment (n=216). In addition, 1 pen of 30 birds per treatment was allocated to provide hens for morphology measurements (described later) thus reducing changes in bird density because of hen sampling. All birds were individually tagged and arranged to maintain the same uniformity across treatments. The pens were distributed across 2 rooms with 3 pens per treatment per room; the additional pen of each treatment was placed in a different room. The laying pens had 2/3 of the floor space covered by raised and remaining 1/3 of the floor pen covered by pine shavings as litter. The hens were fed from ChoreTime breeder pans (4/pen) fitted with exclusion grill to prevent the rooster from eating hen feed. The roosters were fed from a pan feeder suspended over the litter area. The pans were filled with a weighed amount of feed each evening during the last egg collection and raised to a height to prevent feeding. The pans were hand lowered each morning at feed time (6:30 am). The photoperiod at 22 weeks of age was increased to 14L:10D from the rearing photoperiod 8L:16D, and remained constant until the end of the trial at 62 weeks of age.

Experimental Design

Prior to starting the experiment diets, pens were standardized to an average weight as close to the overall mean (2093.1 g/bird) as possible. All birds were randomized into two treatments. The two experimental diets had different protein and energy content as follows: the HIGH diet (**HL**) had higher energy and lower protein values (3000 kcal/kg, CP=13 %, methionine 0.72%, and lysine 0.72%) compared to the LOW diet (**LH**), which contained lower energy and higher protein level (2900 kcal/kg, CP=15%, methionine 0.74%, and lysine 0.74%). Both treatments were fed the same amount of feed, determined based on Aviagen Broiler Breeder Guidelines (2014) and body weight of the birds.

Growth and Productivity

Three pens per treatment were weighed weekly. The following week the remaining three pens per treatment were weighed. Weekly average body weight per pen and treatment was calculated and used to determine feeding levels to sustain target weights.

Egg production was monitored daily on a per pen basis (n=12) from 23 to 62 weeks of age. Egg production was calculated by taking the number of eggs laid per week as a percentage of hens housed per pen. A sample of five eggs per pen was individually weighed weekly to monitor egg size based on treatment. Weekly average egg weight per treatment was calculated.

Body Morphology and Reproductive Morphology

At 22, 32, 42, and 52 weeks of age, 5 randomly selected hens per treatment were killed and weighed. At 62 weeks of age 60 hens per treatment were selected, killed and weighed. During each sample time, ovary, oviduct, yellow follicles, breast muscle, and abdominal fat pad were dissected from the carcass and weighed. The weights of the reproductive organs and body parts were expressed as a percentage of body weight.

Behavior Observations and Fertility Determination

Behavioral observations were made three times a week for two consecutive weeks every month. Each room (6 pens) was observed for 10 minutes after a five minute acclimation period. In the observational period, the number of attempted matings (**AT**) and complete matings (**CM**) were recorded per pen. Attempted mating in this case was defined as the rooster moves to the hen, making contact with her but does not make cloacal contact, so the mating was not successful. In CM the rooster and hen clearly have cloacal contact.

Eggs were collected for 4 days during the week that behavior observations and necropsy data were collected. The eggs were incubated by pen for fertility determination. Fertility was calculated by candling the eggs at 12 days of incubation. All clear and early dead eggs were removed, opened and classified as an infertile egg or an early dead embryo. These two indexes were similar to those used by Casanovas (1999).

Casanovas (1999) found a correlation (r=0.686) between fertility and the number of full copulation, for which he developed an index that would give more weight to full copulations (**CM**) over attempts (**AT**). The sexual activity index (**SAI**) estimates the interest of a male or amount of sexual activity (**SAI**= 0.5***AT** + 2***CM**).

Feather Cover Evaluation

Feather cover was evaluated in two-week intervals at 29, 30, 39, 40, 49, 50, 54, 55 weeks of age. Half of the birds (3 pens per treatment) were scored one week and the other half the following week. The scoring system was a 5-point system similar to that used by Tauson et al. (2005). The scoring system focused on the condition of the back feathers and was as follows; Score 1: Bare back; Score 2: Bare back with feather cover tail area; Score 3: Obvious bare patches over mid back; Score 4: Small bare patch on back; Score 5: Complete feather cover with no bare patches (Figure 1).

Statistical Analysis

Egg weight, body and reproductive morphology, and fertility results were compared using GLM (SAS, 2013, Cary, NC). Egg production, body weight, behavior, and feather cover results were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Differences were deemed to be significant when the P-value was less or equal to 0.05.

RESULTS

Growth and Productivity

Body weight was not significantly affected by the different dietary treatments (data not shown) with the mean of birds weight being 3695.4 and 3701.1 for the **HL** and **LH** diets, respectively. There were no significant differences in egg weight between the treatments (data not shown) with the mean of egg weights being 62.7 and 68.3 for the **HL** and **LH** diet respectively. Both treatments have similar trends for both measurements. The dietary treatment had a significant impact on egg production (P=0.015) (Figure 2). The **HL** diet had a higher level of egg production compared to the **LH** diet, with the hens on **HL** diet having more cumulative eggs.

Body Morphology and Reproductive Morphology

The treatments did not significantly impact the reproductive morphology of the hens (Table 1). There were no differences between the number of yellow follicles or ovary, oviduct, and breast percentage. However, there was a significant difference in fat pad percent between the treatments (P=0.023). The hens consuming the **HL** diet had a larger fat pad than the hens that consumed the **LH** diet.

Behavior Observations and Fertility

The means for attempted matings, completed copulations, and sexual activity per treatment and week are shown in Table 2. For the overall lay period, there were no significant differences in attempted matings, completed copulations, or the sexual activity index between the birds consuming the dietary treatments. In some of the weeks, there were significant differences between the treatments for **AT** and **CM;** however, there was no overall trend in the observations. When observations are summarized with the **SAI**, significant differences between sexual activity of the birds receiving the **HL** and **LH** diets are seen at the beginning of the observation period (32, 33, 39, and 42 weeks). The birds on the **HL** diet had a higher level of **SAI** than the birds on the **LH** for weeks 32, 33, and 39.

There were no significant differences on fertility. Both treatments had a high level of fertility with values over 95% through 51 weeks and then a sharp decline through 61 weeks. The levels of fertility decrease over time for both treatments with a significant decrease 51 weeks to 55 weeks (Figure 3).

Feather Cover

In Figure 4 and Table 3, the average feather cover score of hen's backs are presented. No significant differences in feather cover between the treatments were observed. The hens did lose feathers as expected with a decrease from an average of score of 5 at 29 weeks to an average of 2.5 at 62 weeks.

DISCUSSION

Growth and Productivity

Providing a higher level of energy and lower level of protein to the hens did not impact body weight for the period of 25 to 62 weeks of age. The results of this study agree with previous studies (Van Emous et al., 2015 and Sun and Coon, 2005) that did not find differences in body weight when feeding different levels of energy during the lay phase. Decreasing protein has the potential to decrease growth rate in chickens (Rezaei et al. 2004). However, the decrease from 15% in the **LH** diet to 13% in the **HL** diet fed during lay did not impact body weight in our study. The results coincide with those of Lesuisse et al (2017) who did not see an effect on body weight when feeding a lower level of protein in the lay phase. The lack of difference of body weight between the treatments could be attributed to differences in energy partition of each diet. The birds in the **HL** diet might have spent the extra energy in other aspects such as egg production rather than an increase in body weight.

Egg weight is influenced by many factors such as breed, age, and nutrition. Previous research suggests that increase of dietary energy and protein leads to the production of heavier eggs (Harms et al., 2000 and Joseph et al., 2000). Therefore it is important to determine the impact of a combination of a high energy and low protein diet has on egg weight. In this study, egg weight was not affected by the feeding treatments through the entire lay phase (data not shown). This finding does not agree with previous studies that reported an increase in egg weight as they increase energy during the late phase of production (Van Emous et al., 2015 and Sun and Coon, 2005). The difference in results might be attributed to the difference in protein content or perhaps the decrease in protein might have attenuated the effect of the higher energy level on egg weight.

There was a significant effect on overall egg production, with the hens on **HL** diet laying more eggs compared to the **LH** diet. Previous research did not report any differences in egg production by increasing energy or decreasing protein in the diet (Pishnamazi et al., 2011; Sun and Coon, 2005; and Joseph et al. 2000). The difference in results might be attributed to how the hens utilized the nutrients. Hens that produce heavier eggs tend to have lower levels of egg production. All the previous studies reported significant differences in egg weight but not in egg production. Therefore, the difference in this study could be due to the fact the hens used the increase in energy to produce more eggs instead an increase in egg or body weight.

Body Morphology and Reproduction Morphology

Body and reproductive morphology can be altered based on changes in a diet. The reproductive morphology (ovary, oviduct, and number of yellow follicles) were not affected by the treatments. There was a significant difference in egg production however the diet did not impact reproductive morphology. Changes in the layer diet can impact egg production but does not necessarily have a direct effect on the reproductive morphology. Joseph et al. (2002) determined differences in reproductive organ weights based on strain and photo stimulation but not crude protein. Changes in reproductive morphology are related to genetics, body weight at sexual maturity, age and age at photo stimulation (Reddish and Lilburn, 2004). Thus, reproductive morphology changes are more likely to be observed if differences in the diet start at early sexual development (Renema et al., 1999).

Breast weight was not significantly different between the treatments. The results agree with previous research by Joseph et al. (2002) who did not find significant differences in chest width, breast weight or pectoralis major or minor weights when feeding different protein levels. Decreasing protein content in the diet is a common strategy used to decrease or control breast fleshing of hens (Moran, 1979). Although crude protein values differed by 2% between diets the differences might have not been sufficient to impact breast weights. Aviagen parent stock nutrition specifications suggest a decrease of crude protein from 15% to 13% by 50 weeks to control breast deposition. Both of our diets are within the margin suggested by parent stock line. Therefore, to observe changes in breast weight or morphology differences protein content might need to be more drastic.

Abdominal fat pad weight was significantly different between the treatments with the **HL** diet being higher then the **LH** diet. The results of our treatment coincide with previous studies, where larger abdominal fat pad weights have been observed on higher energy and lower protein diets (Sun and Coon, 2005; and Lesuisse et al., 2017). Sun and Coon (2005) suggested differences in carcass composition might be associated with nutrient availability and utilization. Although there were no differences in body weight between treatments, there were differences in fat pad weights in this study, and perhaps the extra amount of energy was utilized to create a reservoir by the hens on the **HL** diet and accounts for their ability to lay more eggs. These results are similar to the improved egg production reported by Robbins (et al. 1988), who found that greater fat pad weights when consuming higher dietary energy were associated with significantly improved egg production. Since the hens are fed a higher level of energy, the hens on the **HL** diet can spend more energy and produce more eggs.

Behavior Observations and Fertility

Mating behaviors are influenced by the female and male status and have a direct correlation to fertility of the flock. There were no significant differences in overall trends between the treatments for attempted matings, completed matings, or the sexual activity index. There are several factors that contribute to the changes in the mating behaviors. Studies have shown that the body morphology (Kajer and Mench, 2003) can directly impact mating behavior. However, there were no differences in body morphology between the treatments in our study. Age also leads to a decline in mating behaviors, and this natural decline in sexual activity is seen in a study with a differences ranging from 5.3 to 2.6 and 4.0 to 1.5 from 32 to 56 weeks of age for the **LH** and **HL** diet, respectively. As males age they decrease in attempted and completed matings (Moyle et al., 2012). Hen receptivity also has a direct impact on mating behaviors (Casanovas and Wilson, 1998). Hen receptivity can be influenced by feather cover loss. Moyle et al. (2010) showed that hens with greater feather loss have fewer mounts by males. Feather cover was not significantly different between the treatments, therefore no differences in **AT**, **CP**, and **SAI** between treatments were expected.

Fertility of a flock is dependent on their reproductive status, interest in mating, and ability to mate. No significant differences in fertility were observed between the treatments for the overall period. This correlates to the lack of change in mating behaviors and reproductive morphology between the treatments.

Feather Cover

Disease, flock management, feather pecking and nutrition are related to feather loss. Energy and protein levels can impact feather growth and cover. There are no significant differences in feather cover scores between the **HL** and **LH** diets. Previous studies show that a decrease in protein content can lead to deterioration of feather condition (Li et al., 2017) leading to lower feather cover score in lay (Van Emous et al., 2015 b). In an earlier study, Van Emous et al. (2014) showed that increasing the energy content in the diet leads to poor feather cover. The lack of difference in this study compared to previous studies might be due to the magnitude of difference in energy. There was a 100 kcal difference in this study compared to as much as 400 kcal differences between the treatments in other studies. In addition, since feather cover is subjective as determined by visual observations and in this case the birds were scored over a long period of time, perhaps this subjective score was variable over time.

A higher energy and lower protein diet (**HL**) (3000 kcal/gr, 13% CP) compared to lower energy and higher protein diet (**LH**) (2900 kcal/gr, 15% CP) did not have a direct impact on the reproductive morphology, fertility, or mating behavior observations for the overall laying phase. Hens consuming the **HL** diet had greater egg production and abdominal fat pad deposition showing a positive impact of the dietary change without the negative impact of an increase in body weight.

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TABLE 5.1. Overall necropsy data (ovary, oviduct, breast, fat pad as a percentage of body weight and Number of Yellow Follicles) as affected by the feeding treatments (**HL** and **LH**).

	HL Diet [A]	**LH Diet**
Ovary (%) [B]	1.63	1.58
Oviduct (%)	1.50	1.51
Number Yellow Follicles	5.05	4.95
Breast (%)	22.14	20.45
Fat Pad (%)	2.39[a]	2.05[b]

[A] Abbreviation key: **HL** = 3000 kcal/kg, 13% CP, **LH**= 2900 kcal/kg, 15% CP.

[B] Each value within a treatment represents the mean of seventy birds.

[a-b] Means for different treatments with no common superscript are significantly different ($P<0.05$). No superscript means no significant differences among treatments.

TABLE 5.2. Influence of feeding treatment (**HL** and **LH**) on reproductive behavior..

WKS	TRT[A]	AT[C]	CP	SAI
32[B]	HL	1.83	1.56	4.03[b]
	LH	2.33	2.06	5.28[a]
33	HL	1.67[b]	1.22	3.28[b]
	LH	2.94[a]	1.72	4.92[a]
38	HL	1.89	1.28	3.52
	LH	2.43	0.87	2.94
39	HL	0.97	0.87	2.22[b]
	LH	1.68	1.45	3.74[a]
41	HL	1.72	1.33	3.53
	LH	2.22	1.39	3.89
42	HL	1.72	1.33[a]	3.53[a]
	LH	1.72	0.72[b]	2.31[b]
45	HL	2.39	0.94	3.08
	LH	2.06	0.78	2.58
46	HL	1.61	0.67	2.14
	LH	1.61	0.67	2.14
50	HL	2.78	0.89	3.17
	LH	2.94	0.78	3.03
51	HL	4.22[a]	0.78	3.67
	LH	2.94[b]	0.78	3.03
55	HL	2.11	0.39	1.83
	LH	2.06	0.56	2.14
56	HL	1.5[b]	0.39	1.53
	LH	2.72[a]	0.61	2.58

[A] Abbreviation key: **AT**= Attempts to mate, **CP**= Completed copulations, SAI= Sexual activity index (SAI=0.5*AT+2*CP), **HL** = 3000 kcal/kg, 13% CP, **LH**= 2900 kcal/kg, 15% CP.
[B] Each value within an age and treatment represents the mean of six pens.
[C] All behavioral measurements (AT and CP) based on 3 observational periods of 10 min in each pen. Observation times where from 1800 to 1930h.
[a-b] Means within an age and for different treatments with no common superscript are significantly different (P<.05). No superscript means or common superscripts means no significant differences among treatments.

TABLE 5.3. Feather cover scores thru lay period (29 to 62 wks.) as affected by the feeding treatment (**HL** and **LH**).

Weeks	HL Diet [A]	LH Diet
29	5	5
30	5	4.99
39	4.7	4.61
40	4.6	4.71
49	3.69	3.78
50	3.5	3.53
54	3.03	3.06
55	2.83	2.96
59	2.59	2.74
60	2.74	2.83
62	2.54	2.56

No significant differences between the diets (columns) were found. **HL** = 3000 kcal/kg, 13% CP, **LH**= 2900 kcal/kg, 15% CP.

[A] Each value within a treatment represents the mean of three pens.

FIGURE 5.1. Feather scoring scheme adapted from Tauson et al., 2005. The scoring system applied to the back feathers as follows; Score 1: Bare back; Score 2: Bare back with feather cover tail area; Score 3: Obvious bare patches over mid back; Score 4: Small bare patch on back; Score 5: Complete feather cover with no bare patches.

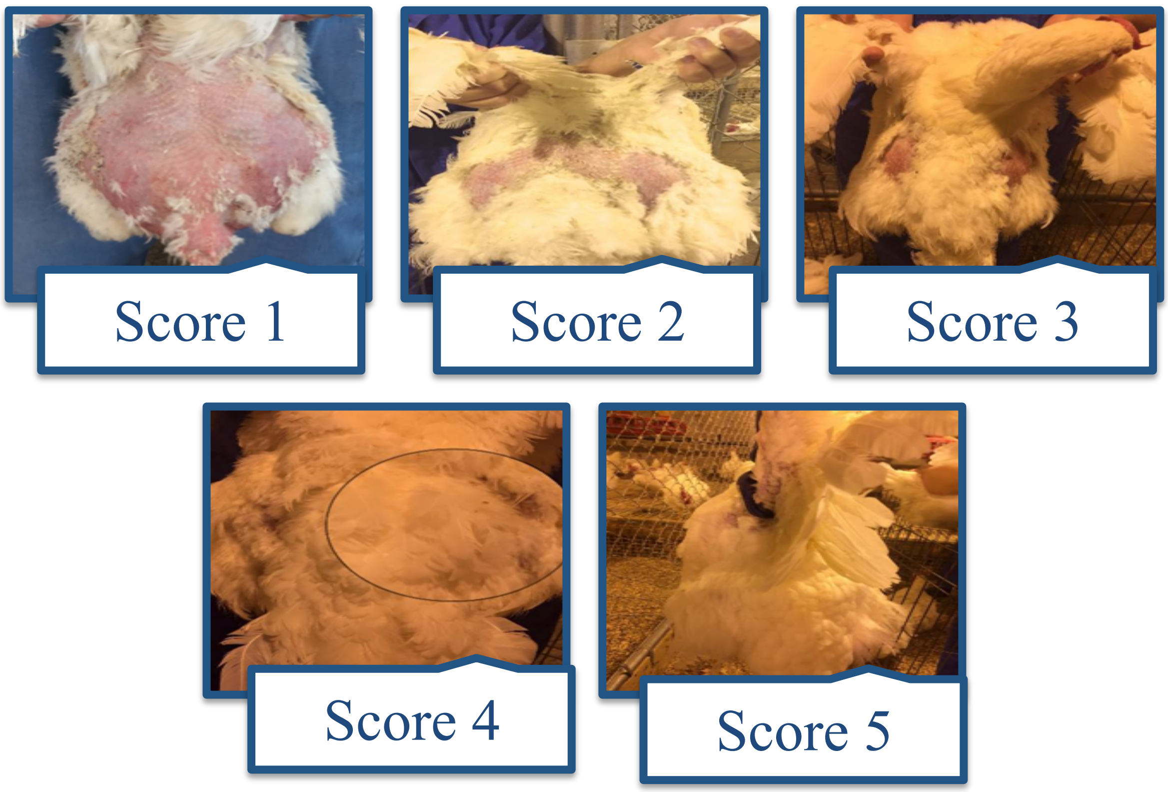
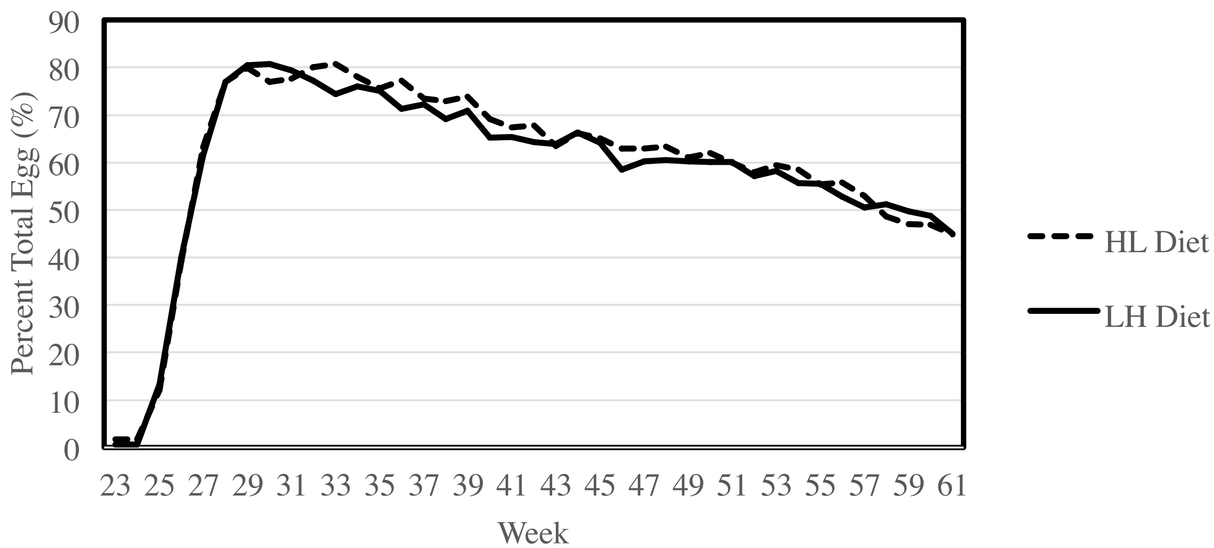

Score 1
Score 2
Score 3
Score 4
Score 5

FIGURE 5.2. Egg production curve as affected by the different feeding treatments. Feeding programs are as follows: = **HL** = 3000 kcal/kg, 13% CP (- -), **LH**= 2900 kcal/kg, 15% CP (---). Each value represents the mean of the percentage of total egg produced within each feeding treatment. There were significant differences (P=0.015) on the overall production between the feeding programs.



90
80
70
60
50
40
30
20
10
0
Percent Total Egg (%)
23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Week
HL Diet
LH Diet

FIGURE 5.3. Fertility changes from 32 to 61 week of age in natural mated broiler breeder hens with two different feeding treatments (**HL** and **LH** diets). Dietary treatments are as follow: **HL** = 3000 kcal/kg, 13% CP, **LH**= 2900 kcal/kg, 15% CP. Each value represents the mean of six pens. There were no significant differences in fertility due to the diet in this period (p=0.512).

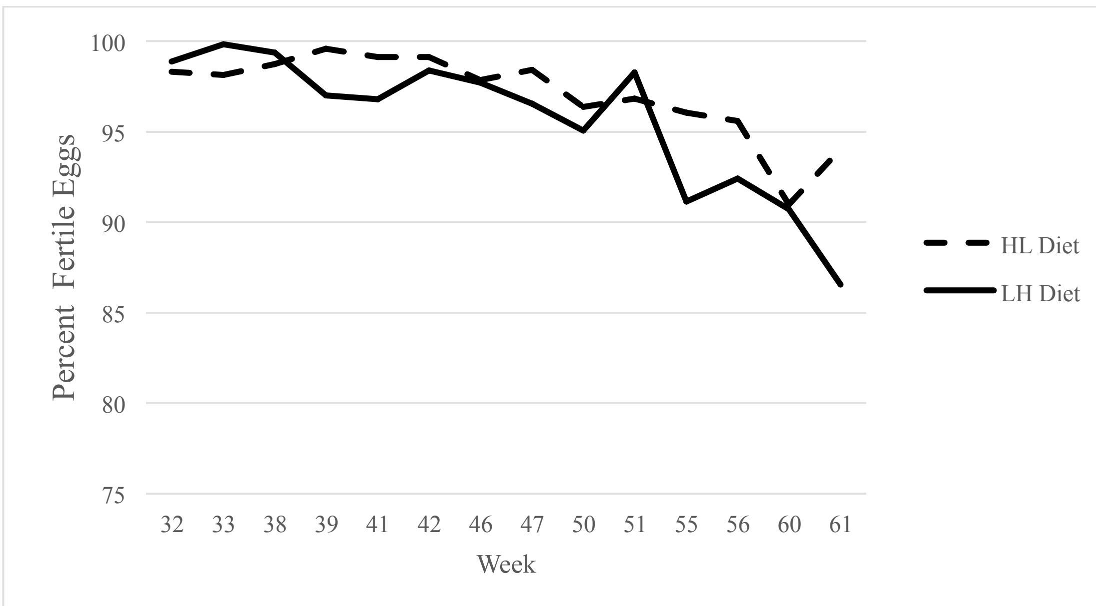
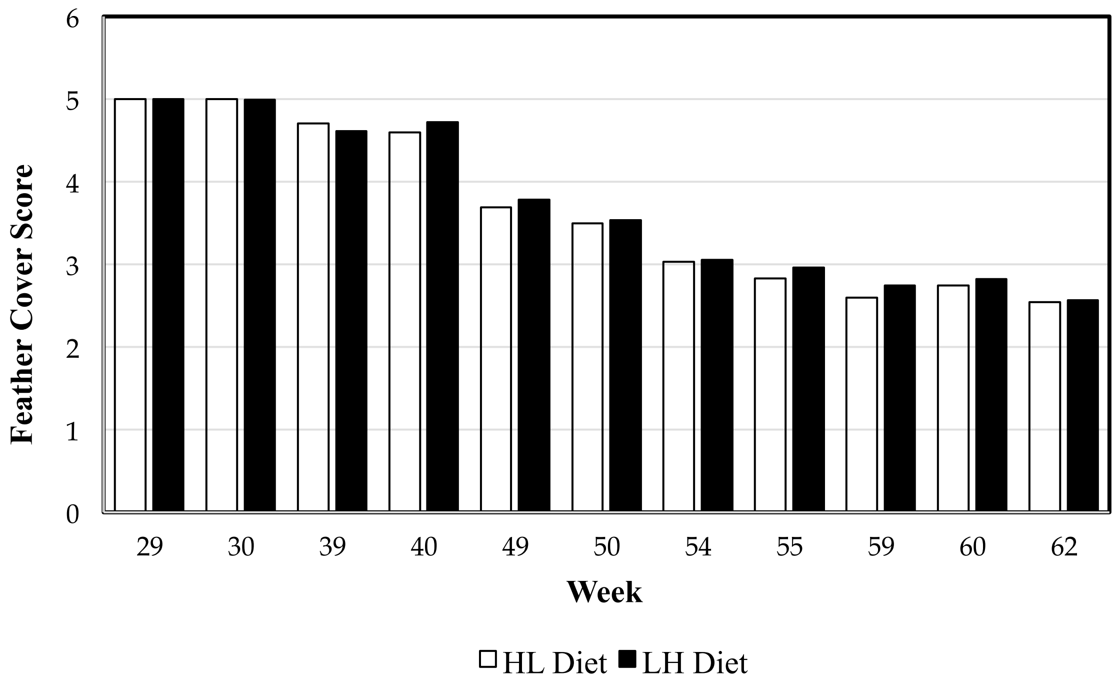

100
95
90
85
80
75
Percent Fertile Eggs
32
33
38
39
41
42
46
47
50
51
55
56
60
61
Week
HL Diet
LH Diet

FIGURE 5.4. Feather cover scores through the lay period (29 to 62 wks) as affected by the feeding treatment (**HL** and **LH**). Dietary treatments are as follows: **HL** = 3000 kcal/kg, 13% CP, **LH**= 2900 kcal/kg, 15% CP. Each value represents the mean of 3 pens. There were no significant differences between the treatments (p=0.108).



6
5
4
3
2
1
0
Feather Cover Score
29
30
39
40
49
50
54
55
59
60
62
Week
HL Diet
LH Diet

CHAPTER 6

CONCLUSION

Selection for increased growth rate has led to an increase in body weight and appetite in broiler breeders. Feed restriction programs have become essential to prevent excessive body weight, poor uniformity and poor reproductive performance. The high levels of feed restriction are associated with the prevalence of behaviors indicative of feeding frustration, boredom, and hunger. Qualitative restriction with high fiber diets has become an alternative to improve broiler breeder welfare while preventing excessive weight gain. In this study, we showed an improvement in production parameters and changes in bird behavior by adding soybean hulls on the OFF feed day. The alternate diet (ATD) program, which has the added soybean hulls on the OFF feed day, increases the body weight of the birds when compared to a standard skip-a-day (SAD) program. There is also an improvement in uniformity and increase in egg production for birds on the ATD program. We further studied the effect of the same ATD program but compared it to a SAD program that had the same amount of added soybean hulls on the ON day. The results were similar to those from the previous study on body weight, uniformity and egg production. Leading us to conclude that the differences in the production parameters are attributed to the feeding program rather than the differences in nutrients in the diets.

The potential cause for the differences in production between the diets might be attributed to changes in stress level and behavior of the birds. The ATD program changes the behavior of the birds on the OFF feed day with the ATD birds spending more time at the feeder and the drinker than the SAD fed birds. The increase in time spent feeding and drinking leads to birds spending less time exhibiting feed seeking behaviors (foraging). The changes in the time spent at the feeder and drinker shows a potential change in the birds focus. In addition, the decrease in foraging behavior might be indicative of a decrease in hunger levels or a sense of fullness in the birds. The changes in the behavior suggest an improvement in the birds' welfare when fed soybean hulls on the OFF feed day.

Feather cover is a concern for broiler breeder managers due to its relationship with reproductive performance. Changes in energy and protein can impact reproductive performance, feather cover, and mating behaviors. In this study we evaluated the effect of an increase in energy by 100 kcal/g and a 2% reduction in crude protein. There were no differences in reproductive morphology, fertility, feather cover, or mating behaviors between the treatments. Although previous research has shown that changes in energy and protein can impact reproductive performance and feathering; from the results of this study we concluded that the changes we made to the diets did not vary enough from the guidelines to have an effect. The increase in energy and the decrease in protein could also be balancing each other preventing significant differences to be observed. Further research concerning different levels and the interaction between energy and protein should be investigated.