Reduction of Stress Through Early Photostimulation in Broiler Breeders and Effects of Dietary Essential Oils on Broiler Meat

by

RENATA HOSKOVA

(Under the Direction of Andrew Benson)

## ABSTRACT

As the human population grows, poultry production must improve to accommodate it and the increasing interest in animal welfare. A design of skip-a-day vs spin-feeding and 15wk vs 21wk photostimulation determined if early photostimulation or everyday spin feeding lowered hunger stress in broiler breeders (BB). SPIN21 had significantly ($P<0.05$) higher corticosterone than SAD15 during rearing. At 15wk, SAD21 had significantly ($P<0.05$) higher corticosterone than SAD15. These results indicate rearing BB on an advanced growth curve (early photostimulation) causes a reduction in stress. Additionally, the use of antibiotic growth promoters is slowing in broilers, creating a need for alternatives. Broilers were raised on four diets, three containing mixtures of essential oils. EO's did not significantly impact breast meat characteristics, but differences in live weight were seen between EO treatments. These differences were most likely seen due to immune-stimulating effects of BioStrong.

INDEX WORDS: Broiler breeder, Photostimulation, Broiler, Essential Oils

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# REDUCTION OF STRESS THROUGH EARLY PHOTOSTIMULATION IN BROILER BREEDERS

## Chapter 1

## Broiler Breeders

### *1.1 Industry Importance*

Broiler breeder hens must be efficient at two conflicting things: rapid growth and a high rate of egg production (Renema and Robinson, 2004). Because of decades of genetic selection for rapid growth rate and meat production, both male and female broiler breeders have experienced reproductive problems (Siegel and Dunnington, 1985). The adverse effects of rapid growth on reproductive fitness in the chicken has been known for over 50 years (Maloney et al., 1967; Jaap and Muir, 1968). Using feed restriction in broiler breeders decreases reproductive disorders and excessive body weight gain (Renema and Robinson, 2004). In the industry, it is considered essential for the well-being of the birds (Katanbaf et al., 1989a) and for the production of eggs and chicks (Katanbaf et al., 1989b). However, if a female broiler breeder is raised using the feed restriction necessary to ensure reproductive efficiency and good livability, other aspects of welfare may be affected (Mench, 1993; 2002). On the other hand, a full feeding environment leads to obese birds and a general decrease in reproductive fitness (Katanbaf et al., 1989a). When reduced livability and increased leg problems are combined with these other

problems, birds have a hard time reproducing themselves (Whitehead, 2000). In order to increase welfare, industry has felt pressure to eliminate or modify feed restriction; however, the degree to which feed restriction should be relaxed has not been agreed upon (Renema and Robinson, 2004).

*1.2 Rearing*

Broiler breeder rearing is very similar to broiler rearing. Parameters for both broiler and broiler breeder rearing focus on target body weights. Rearing of broiler breeder pullets, however, also has to take reproduction into account. Broiler breeder target body weights have not changed much over the past 40 years, even though broiler growth has increased dramatically due to genetic potential, because target body weights for broiler breeders are restricted to improve bird welfare and reproductive performance (Renema et al., 2007a). Various studies have been conducted to find what body weight or type of growth curve results in the best performance in the production house (Sun and Coon, 2005; Wilson et al, 1995; Robinson et al., 2005; Robinson et al., 2007; Renema et al., 2007b; Zuidhof et al., 2007; Harper, 2008; De Beer and Coon, 2009). Investigated growth curves were within 20-25% of primary breeder target weights at housing or the curves converged at conventional photostimulation of 21 weeks of age. These studies have found that pullet rearing body weight programs have influence on hen body weight, body conformation, age at first egg, settable eggs, and weight of eggs obtained during the production period (England et al., 2014).

## *1.3 Feed Restriction*

### *1.3.1 General Overview*

To achieve better bird health and reproductive fitness in commercial broiler breeders, skip-a-day, 5-2, or 4-3 feeding programs are used during rearing rather than providing a restricted amount of feed on an every-day basis (Cobb-Vantress, 2005). Skip-a-day diets produce significant differences from an every-day diet in endocrine and metabolite profiles even though the same amount of feed is consumed (Richards et al., 2010). Not feeding pullets for 24-hour periods, as is done in skip-a-day, 5-2, or 4-3 feeding programs, sets up a state of repeating fasting-refeeding cycles (de Beer and Coon, 2007). This is most likely what leads to the reduced growth efficiency seen in skip-a-day birds when compared to every-day fed birds. Feed restriction has been shown to reduce body weight, delay sexual maturity, improve ovarian function with fewer multiple ovulations, increase egg production, reduce the number of abnormal eggs, and decrease mortality during the laying period (Renema and Robinson, 2004). However, the positive effects of feed restriction on bird health and reproductive fitness come with a negative impact on bird welfare. Furthermore, consumers are increasing their push for better animal welfare, leaving the broiler breeder industry in need to find an alternative to these commonly used feed restriction methods.

### *1.3.2 Feed Intake and Body Weight*

*Ad libitum* broiler breeder pullets spend most of their time eating and resting, while feed restricted pullets spend most of their time scratching and pecking in the litter (Hocking et al., 1993). Numerous studies have shown that feed restricted broiler breeders display behaviors,

such as overdrinking, pacing, and stereotypic pecking or pecking without any known benefit, which are indicative of frustration, boredom, and hunger (Mench, 2002; Savory, 1989). Aggressive pecking or feather licking in feed restricted birds is also a problem in the industry (Aviagen, 2014). Broiler breeder eating behavior was found to be controlled more by satiety mechanisms rather than by hunger mechanisms, whereas laying hen eating behavior was found to be equally influenced by hunger and satiety mechanisms (Bokkers and Koene, 2003). Due to this, *ad libitum*-fed broiler breeders that were found to consume 22% of their total daily intake during the dark period indicate that they are in a continually hungry state (Hocking, 1993). Broiler breeder females fed *ad libitum* from hatch have been shown to have more than double the body weight of feed restricted females by photostimulation age (Katanbaf et al., 1989a; Yu et al., 1992a).

When *ad libitum* feed access was allowed during the 7 to 15-week period, much greater body weight gains were seen when compared to restricted birds than during the 16 week to first egg period (Bruggeman et al., 1999). When *ad libitum* feeding begins at photostimulation, hens weigh 26% (Renema et al., 1999a) to 33% (Yu et al., 1992a) more by sexual maturity than feed restricted hens. Hens that are changed to *ad libitum* feeding may initially eat more feed than birds that have long-term *ad libitum* access to feed (Hocking, 1996). However, feed intake does decrease to a consistent level within 4 days (Robinson et al., 1993b) and when the degree of feed restriction becomes more relaxed, feed intake relative to body weight is similar for both *ad libitum* and feed-restricted hens (Renema and Robinson, 2004). However, the number of chicks produced per unit of feed consumed has been shown to be four times higher in feed restricted hens compared to *ad libitum*-fed hens (Hocking et al., 2002a).

### *1.3.3 Sexual Maturity Age*

From the time of photostimulation, hens reach sexual maturity faster when they are fed *ad libitum* compared to restricted feeding (Pym and Dillon, 1974; Robbins et al., 1986; Ingram and Wilson, 1987; Katanbaf et al., 1989b; Yu et al., 1992b; Bruggeman et al., 1999; Renema et al., 1999a). The onset of sexual maturity has been shown to be advanced by 1.0 week (Ingram and Wilson, 1987) to 8.1 weeks (Katanbaf et al., 1989b). Such a wide range exists due to age at photostimulation, degree of feed restriction, or genetic factors because all of these factors may influence the ability of pullets to respond to photostimulation (Renema and Robinson, 2004). However, this accelerating effect can be reduced by photostimulation at later ages (Robinson et al., 1996). Additionally, heavier birds, which have a higher lipid content, usually come into production earlier (Summers and Leeson, 1983). Sexual maturity depends more on age (hypothalamic maturity) in *ad libitum* fed broiler breeders and on body weight and carcass traits in feed-restricted birds (Katanbaf et al., 1989b). Body weight and age are both indicators of the responsiveness of the reproductive hypothalamo-pituitary-gonadal axis (Renema and Robinson, 2004). Due to this, rapid onset of sexual maturity from photostimulation in pullets fed *ad libitum* rather than feed-restricted most likely means the internal signal for reproductive development is a metabolic one (Renema et al., 1999a).

### *1.3.4 Ovarian Morphology*

Overfeeding of hens selected for growth during sexual maturation will most likely result in excess production of LYF, which will arrange in multiple hierarchies (Hocking et al., 1987; 1989; Katanbaf et al., 1989b; Yu et al., 1992b; Renema et al., 1999b). This will then increase production of unsettable, or abnormal, eggs (Jaap and Muir, 1968). *Ad libitum* feeding started

early in the egg production period causes a significant increase in both ovarian yolk mass and carcass lipid content (Robinson et al., 1991a; Yu et al., 1992a, 1992b; Renema et al., 1999a, 1999b). However, the effects of overfeeding on the ovary are less severe with increasing age (McGovern et al., 1997). This may be because of decreased sensitivity of the ovary to extra feed availability (Hocking, 1996). Additionally, within birds of similar body weight, birds that consumed more feed had more LYF (Hocking, 1993). The faster onset of sexual maturation in *ad libitum* fed pullets may have adverse effects on the normal development of mechanisms that control ovarian LYF numbers (Renema et al., 1999b). In order to address this problem, reduction of nutrient intake to slow the rate of sexual development needs to take place.

### *1.3.5 Egg/Chick Production & Laying Patterns*

*Ad libitum* fed broiler breeders come into production earlier but do not lay as many eggs as feed-restricted hens (Robbins et al., 1986; 1988; Katanbaf et al., 1989b; Robinson et al., 1991a; O'Sullivan et al., 1991; Yu et al., 1992b). Higher weight birds produced more eggs than lower weight birds, however, higher weight birds also produced fewer settable eggs because of high levels of unsettable egg production (Udale et al., 1972). Unsettable eggs include membranous, thin-shelled, double-yolked, or abnormal-shelled eggs (Renema and Robinson, 2004). Feed restriction during the rearing phase, production phase, or both, caused significantly reduced erratic laying (oviposition outside the prime laying period) compared to birds fed *ad libitum* throughout both phases (Yu et al., 1992b). In *ad libitum* broiler breeder hens, defective egg production due to multiple ovulation mainly happens in the early weeks of production, disappearing by peak production (30 to 32 weeks of age). Broiler breeder hens have periods of consecutive oviposition days, which are interrupted by one or more pause days (Renema and

Robinson, 2004). *Ad libitum* fed birds were found to have a prime sequence length of 62% of the length for restricted birds (Robinson et al., 1991a). This reduced sequence length of *ad libitum* birds leads to a higher incidence of first of sequence eggs. First of sequence eggs have a higher likelihood to undergo embryonic death due to the stop in ovulation over the pause day(s) (Robinson et al., 1991b; Fasenko et al., 1992). This, in turn, decreases hatchability of eggs produced by the *ad libitum* fed birds.

*Ad libitum* fed hens have a reduced chick production due to poor egg production as well as reduced fertility, hatch of fertile eggs, and embryonic viability (Yu et al., 1992b). Fertility seems to be the same between both feed-restricted and *ad libitum* hens when mated to restricted males (Hocking et al., 2002a), however, hatchability is reduced in *ad libitum* fed hens because of late embryonic death. Decreased chick production may also be due to poor male fertility and from incomplete matings resulting from both male and female obesity and physical impairment (Renema and Robinson, 2004). When hens were fed *ad libitum* for 8 weeks, beginning at 52 weeks of age, a 21.2% reduction in duration of fertility was reported when compared to feed restricted birds (Goerzen et al., 1996). *Ad libitum* fed hens from photostimulation also experienced mean hatchability values of 60% to 82% for the production period of 24 to 49 weeks of age after artificial insemination (Renema et al., 1999c).

### *1.3.6 Bird Health and Stress*

Broiler breeders have low immune-responsiveness because of negative selection pressure from growth efficiency selection (Siegel et al., 1984). Various studies have found that *ad libitum* feeding of broiler breeders causes higher death rates due to bacterial infection (Katanbaf et al., 1989a; Pym and Dillon, 1974; Yu et al., 1992a; Hocking et al., 2002a). In addition to this, slat

and nest-box areas may be too high for obese birds, leading to an increased likelihood of floor eggs (Renema and Robinson, 2004). In feed restricted breeder pullets, plasma corticosterone concentrations were found to be elevated (Hocking et al., 1993). This high concentration was worse on non-feed days in a skip-a-day diet; so, moving to a daily feeding diet would most likely decrease the stress associated with fasting (Renema and Robinson, 2004). The plasma heterophyl:lymphocyte (H:L) ratio was also elevated at 8, 12, and 16 weeks of age in feed restricted birds, but these differences were gone by 24 weeks of age (Hocking et al., 2001). Another factor that may introduce stress to broiler breeder pullets is season. Spinu et al. (2003) found that low air temperature imposed a greater stress on the hens than bird density.

### *1.3.7 Bird Welfare*

Even though feed restriction has been shown to have positive effects on both health and reproductive capability, evidence suggests that, during rearing, feed restriction has negative effects on pullet welfare. In addition to higher stress, feed restricted broiler breeder hens consume their feed ration in a short amount of time, leading to an increase in aggressive behavior (De Jong et al., 2002). Thus, equal access to feed is not allowed by the more aggressive birds, leading to lowered flock uniformity, because the more aggressive birds get more of the available feed. The impact of feed restriction on bird welfare includes chronic hunger and displaying of abnormal behaviors, such as hyperactivity, abnormal pecking, pacing, over-drinking, and feather licking (Savory et al., 1993). These behaviors are characteristic of hunger and frustration of feeding motivation (Hocking et al., 1993; 1996; Savory and Maros, 1993; Savory and Kostal, 1996; Mason and Mendl, 1997; Savory and Mann, 1997; Mench and Falcone, 2000). It has been shown that birds fed on a skip-a-day diet exhibit more feather pecking during rearing (Morrissey

et al., 2014). The increase in feather licking and pecking, as well as maintenance of back feathers, associated with feed restriction are concerns in today's broiler breeder management (Huber-Eicher & Weschsler, 1997). Although a skip-a-day diet was intended to allow for more equal access to feed, this practice can create welfare issues for a substantial amount of the flock.

There has been an increase in consumer demand for animal welfare and more "natural" rearing conditions, so, spin feeding could be a way to please consumers because it allows birds to exhibit their natural foraging behavior. Spin feeding is a popular strategy in some areas of Europe and Canada (Van Middelkoop et al., 2000), but is not commonly used in the United States. Spin feeding stimulates foraging behavior and allows for slower feed intake (Hubbard, 2012; Montiel, 2016). Stereotypic pecking is also reduced, which shows that this strategy may reduce boredom and/or frustration (Lawrence and Terlouw, 1993). Due to an increased interest in animal welfare by millennial consumers (Vanhonacker and Veerbeke, 2009), spin feeding should be researched for its potential use in the broiler breeder industry in the United States. Evidence suggests that modern consumer beliefs align with their purchases. Market shares for selected products with humane labeling rose from 1 to 9 percent by the end of 2013 (WATTAgNet.com). Additionally, 70 percent of companies have published animal welfare policies on their websites, which is an increase of 24% from 2012. The purchase of welfare friendly animal products is predicted to highly impact the poultry industry (FAWC, 2006; Freedom Food, 2007).

### *1.4 Impact of Stress*

Stress has an important role in helping an animal to survive the acute challenge to homeostasis that is known as a typical stressor. However, an excess of stress can have

devastating effects on metabolism, vascular function, growth, tissue repair, immune defenses, and the health of some neurons. The most reported negative effects of prolonged stress are disruptions in reproduction and behavior (Wingfield and Sapolsky, 2003). However, the impact of stress on broiler breeders is difficult to report because adrenocortical sensitivity to stress may vary as a function of social status (Sapolsky, 1992; Schwabl, 1995; Schwabl et al., 1985; Creel, 2001), body condition (Wingfield, 1994), infection (Dunlap and Schall, 1995), and other factors (Dauphin-Villement and Xavier, 1987). Additionally, chronic stress can be assessed by baseline corticosterone concentrations and the heterophil:lymphocyte (H/L) ratio (Freeman et al., 1981; Maxwell et al., 1992; Savory et al., 1993, 1996; Hocking et al., 1996). At this time, conflicting studies exist. Some report a higher H/L ratio in restricted birds as compared to *ad libitum*-fed birds (Maxwell et al., 1992; Hocking et al., 1993, 1996; Savory et al., 1993), while others do not (van Niekerk et al., 1988; Katanbaf et al., 1989a; Maxwell et al., 1990; Savory et al., 1996; Hocking et al., 1999). In addition, feed restricted broiler breeders had higher baseline corticosterone concentrations in some studies (Hocking et al., 1993; Savory et al., 1996; Savory and Mann, 1997), whereas no differences were found between restricted and unrestricted birds in other studies (Savory et al., 1993). Stress was found to affect body temperature and heart rate, as well as the circadian rhythm (Tornatzky and Miczek, 1993; De Jong et al., 1999). The main role of corticosterone is to help regulate blood glucose levels (Mench and Falcone, 2000), and body temperature and heart rate might increase because of an increased metabolic rate (Rothwell and Stock, 1983). Therefore, plasma corticosterone concentrations may be affected by both metabolic and stress effects of feed restriction.

*1.5 Photostimulation*

Typical lighting programs for broiler breeders have an 8 to 10-hour photoperiod during the rearing stage. At about 21 weeks, a 12 to 14- hour photoperiod is implemented. From there, weekly increases are made to reach a maximum of 15 to 16-hours by 25 to 30 weeks. The broiler breeder's response to photostimulation depends on two factors: target body weight needed for sexual maturation and dissolution of juvenile photorefractoriness. Juvenile photorefractoriness is the inability to respond positively to a stimulatory photoperiod (Lewis et al. 2003). Sexual maturity is slowed in photorefractory birds, like broiler breeders, when they are not given short days during the rearing period, or when they are transferred to long days before the complete dissolution of juvenile photorefractoriness. The amount of time needed to dissipate juvenile photorefractoriness in broiler breeders depends on the degree to which growth is restricted. This means that more relaxed feed restriction allows birds to reach sexual maturity at a younger age (Lewis, 2007). Therefore, an advanced growth curve allows for earlier dissipation of juvenile photorefractoriness. Lewis at al. (2007) produced a model that indicated the lower limit for uniformly stimulating sexual development within a flock that has been allowed maximum or near maximum growth is around 10 weeks of age. This is in line with the 2 months needed to dissolve juvenile photorefractoriness in *ad libitum* fed seasonal birds (Follett, 1991).

Several studies report that broiler breeders can be successfully photostimulated for reproduction six to eight weeks prior to the more standard photostimulation of about 20 weeks of age (Proudfoot, et al. 1984; Dunn and Sharp, 1992; Ciacciariello and Gous, 2005; Lewis and Gous 2006). Furthermore, the onset of reproduction can be sped up in broiler breeders by allowing them to reach the threshold body weight for reproduction sooner. In a recent study, sexual maturity was advanced without a reduction in egg production in hens grown to threshold

body weight (2.1 kg) and photostimulated at 15 weeks of age versus hens photostimulated and reaching threshold body weight at 20 weeks of age (Ciacciariello and Gous, 2005). Although there was only one replicate room for the treatments in this research study, the results indicate potential economic increases because of the reduced time birds are maintained in rearing facilities and the expansion of the time hens are producing by 6 weeks.

### *1.6 Summary*

Broiler breeders have been bred for two purposes: reproduction and fast growth. In most cases, if a broiler breeder is fed to achieve fast growth, then its reproductive efficiency will be lowered. Because of this, feed restriction management techniques are used when raising broiler breeder pullets. Even though feed restriction has many positive effects on bird health and reproduction, bird welfare is severely decreased when these management techniques are employed. Consumers today buy meat that is raised "naturally," thus, spin feeding, which encourages the birds' natural foraging behavior may be a solution to the broiler breeder paradox. With increased stress due to chronic hunger and frustration in feed restricted broiler breeders, reproductive efficiency, most likely, is lowered. To increase broiler breeder egg and chick production, photostimulation earlier than 21 weeks of age may be employed. Because the modern consumer has already influenced change in the poultry industry through the emergence and growth of the cage free egg market, the broiler breeder industry will most likely not remain unaltered.

# Chapter 2

## Statement of Purpose

It is well established that stress has a negative impact on reproduction. Most commercial farms that raise pullets to mature broiler breeder hens use the skip-a-day feeding technique, which causes the birds stress due to chronic hunger and frustration. However, conflicting research exists in regards to plasma corticosterone concentrations between feed restricted and *ad libitum* fed pullets. Additionally, most commercial farms choose to photostimulate their broiler breeder hens at 21 weeks of age. If pullets were fed every day to obtain the ideal body weight for photostimulation (2.1 kg) earlier, at 15 weeks of age, then broiler breeder production would have the potential to increase due to a longer amount of time to lay eggs and a decrease in hunger-associated stress. This research investigates the impact of skip-a-day feeding and earlier photostimulation on stress within pullets raised to broiler breeder hens via the ELISA corticosterone assay using plasma.

# Chapter 3

## Materials & Methods

### *3.1 Animals*

A total of 2400 female one-day old Cobb 500 broiler breeder chicks were brought to the University of Georgia Poultry Research Center. Three replicate pens (7.32 X 4.6 $m_2$, 200 pullets) were allocated into one of four groups (600 pullets per treatment): every-day-spin (SPIN) with early photostimulation at 15 days (15-P), SPIN with standard photostimulation at 21 days (21-P), skip-a-day (SAD) with 15-P and SAD with 21-P. Rearing pens were separated into different rooms by treatment to reduce variation incurred through SAD birds either hearing or seeing the SPIN birds eat on the SAD off-feed day. All pullets were grown on pine shavings in rooms with heat and evaporative cooling. Birds were fed a standard corn/soybean meal starter mash diet *ad libitum* through 2 weeks of age, then they were switched to a pelleted breeder developer diet on either a skip-a-day (SAD) schedule using chain-feeders, or a SPIN schedule, using hand broadcast feeding for improved distribution. Pullets were fed the pelleted breeder developer diet until target body weight, approximately 2,100 g, was reached at either 15 (15-P) or 21 (21-P) weeks. Water was provided *ad libitum* from nipple drinkers. Day length decreased from 24L:0D to 8L:16D from 2-7 days of age, remaining at 8L:16D until photostimulation at 15 or 21 weeks. Photostimulation day length was 14L:10D. Pullets were wing-banded at 3 weeks of age, weighed every 3 weeks, and feed allotments were adjusted to maintain target growth curves. At either 14

or 20 weeks of age, depending on photostimulation age, birds were selected based on uniform weight distribution and rearing treatment (SPIN-15P; SPIN-21P; SAD-15P; SAD-21P), and placed into 36 total pens with 38 hens per pen. There were 9 replicates per treatment, with 342 hens per treatment for the laying period.

*3.2 Blood Extraction*

Blood extraction commenced when the pullets reached 10 weeks of age. At 10, 15, 21, 24, 26, and 30 weeks of age, 30 birds from each treatment were randomly selected for blood sample collection. Blood samples were collected at 7 h, 24 h and 48 h (SAD only) post-feeding. Approximately 3 mL of blood was collected from the brachial vein and immediately placed into individual glass vacutainers (Becton, Dickinson, and Co., Franklin Lakes, NJ) containing EDTA as an anticoagulant and stored on ice. Blood samples were collected within one minute of physical contact with each hen to avoid corticosterone levels being influenced by handling stress (Romero and Reed, 2005). Blood samples were centrifuged at 1,000 x g at 4º C for 10 min. Plasma was collected from each sample and frozen at -80º C.

*3.3 Corticosterone Ether Extraction*

Because plasma samples that were collected were less than or equal to 300 µl, two sets of 18 X 150 mm glass culture tubes were labeled for each sample. Plasma was pipetted into one of the appropriately-sized and labeled tubes and covered with foil. Samples were then heated in a 65ºC water bath for 1 hour to denature proteins that may have interfered with hormone extraction and were allowed to cool to room temperature. In the fume hood, 5X volume of diethyl ether was added to each sample (e.g. 1.5 mL diethyl ether to extract 300 µl plasma) and each sample was

vortexed for 30 seconds. Samples were covered with foil and allowed to stand for approximately 2 minutes until fractions separated. Following separation, the samples were incubated at -80$^{o}$C for approximately 5 minutes or until the lower aqueous phase was frozen. In the fume hood, the ether fraction was decanted into the second labeled tube for each sample. This was done quickly to ensure that the frozen phase did not thaw and decant with the ether fraction. Once the ether fraction was decanted, the extracted plasma was allowed to thaw.

The above process was conducted once more, for a total of two ether extractions of each plasma sample. Tubes that contained the ether fraction, and thus the steroids, were allowed to stand overnight in the fume hood to ensure ether was fully evaporated. As the ether was drying down, samples were periodically vortexed to rinse down any steroid that may have stuck to the side of the tube. During this time, 10 mL of 10X buffer concentrate (Cayman #400060) was reconstituted with 90 mL UltraPure water to make up 1X ELISA buffer, which was then stored at 4$^{o}$C. Each sample was reconstituted with 1X volume ELISA buffer to reduce the risk of undetectable levels of hormones in the extract. Samples were then vortexed for 30 seconds, covered with foil, and placed in a 65$^{o}$C water bath for 1 hour, vortexing for 15-20 seconds every 15 minutes to rinse down any steroids from the sides of the tube. Once vortexed, samples were allowed to stand for approximately 5 minutes to drain to the bottom of the tube and were transferred to a labeled 1.7-mL polypropylene microcentrifuge tube. Extracted plasma samples were stored at -80$^{o}$C.

*3.4 Corticosterone Enzyme-Linked Immunosorbent Assay (ELISA)*

Seven ELISA plates were set up and read. Each plate contained one total activity well (TA), two blank wells (Blank), two non-specific binding wells (NSB), three maximum binding

wells ($B_0$), and an eight-point standard curve run in duplicate (S1-S8). For each sample well, 50 µl of sample was added to produce the ELISA plates that can be seen in **Figures 1-3.** Corticosterone ELISAs were conducted based on Cayman Chemical (Ann Arbor, MI) instructions. Each plate was read at a wavelength of 405 nm in a SpectraMax ELISA plate reader. Plates were read when the absorbance of the blank subtracted $B_0$ wells were in the range of 0.3-1.5 A.U. Prior to graphical analysis, the data was transformed using the following equation: (Sample or standard bound – average NSB)/ (average $B_0$ – average NSB). To obtain $\%B/B_0$, these values were multiplied by 100. Inter- and intra-assay %CV can be seen to the right.

| Treatment | Inter-Assay %CV |
|---|---|
| SAD15 48 10 WK | 19.57 |
| SPIN15 24 10 WK | 28.70 |
| SAD15 7 10 WK | 36.05 |
| SPIN15 7 10WK | 10.48 |
| SAD15 48 15 WK | 38.07 |
| SPIN15 24 15 WK | 11.11 |
| SAD21 48 10 WK | 18.52 |
| SPIN21 24 10 WK | 37.11 |
| SAD21 7 10 WK | 6.92 |
| SPIN21 7 10 WK | 25.37 |
| SAD21 48 15 WK | 25.41 |
| SPIN15 24 21WK | 49.18 |
| SAD15 24 21WK | 25.77 |
| SPIN15 24 26WK | 26.09 |
| SAD15 24 26WK | 83.98 |
| SPIN21 24 24WK | 24.56 |
| SAD21 24 24WK | 36.33 |
| SPIN21 24 30WK | 17.88 |
| SAD21 24 30WK | 13.02 |
| SAD21 48 20WK | 10.11 |
| SPIN21 24 20WK | 35.95 |
| SPIN21 24 15WK | 12.79 |
| Pool High | 12.98 |
| Pool Low | 7.29 |

| Treatment | Intra-Assay %CV |
|---|---|
| SAD15 7 15WK | 15.81 |
| SPIN15 7 15WK | 22.24 |
| SAD21 7 15WK | 36.38 |
| SPIN21 7 15WK | 37.31 |

*3.5 Statistics*

ELISA corticosterone data was first tested for an interaction (SAD vs SPIN*15 vs 21) using effects tests in JMP. When no interaction between the two factors was found, the data were subjected to ANOVA and analyzed via Tukey-Kramer HSD means comparisons for all pairs using JMP. The corticosterone ELISA data were analyzed in groups based on hours post feeding (7, 24, or 48) as well as weeks of age (10, 15, 20, 24, 26, and 30). Differences were considered to be significant when $P<0.05$.

**Figure 1.** Set-up of ELISA plates 1-3.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TA | S1 | S1 | SAD15 48 10 WK | SAD15 48 10 WK | SAD15 48 10 WK | SAD15 48 10 WK | SAD15 48 10 WK | SAD15 48 10 WK | SAD21 7 10 WK | SAD21 7 10 WK | SAD21 7 10 WK |
| | T END | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| B | Blank | S2 | S2 | SPIN15 24 10 WK | SPIN15 24 10 WK | SPIN15 24 10 WK | SPIN15 24 10 WK | SPIN15 24 10 WK | SPIN15 24 10 WK | SAD21 7 10 WK | SAD21 7 10 WK | SAD21 7 10 WK |
| | nothing | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| C | Blank | S3 | S3 | SAD15 7 10 WK | SAD15 7 10 WK | SAD15 7 10 WK | SAD15 7 10 WK | SAD15 7 10 WK | SAD15 7 10 WK | SPIN21 7 10 WK | SPIN21 7 10 WK | SPIN21 7 10 WK |
| | nothing | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| D | NSB T | S4 | S4 | SPIN15 7 10 WK | SPIN15 7 10 WK | SPIN15 7 10 WK | SPIN15 7 10 WK | SPIN15 7 10 WK | SPIN15 7 10 WK | SPIN21 7 10 WK | SPIN21 7 10 WK | SPIN21 7 10 WK |
| | 100 ul EIA | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| E | NSB T | S5 | S5 | SAD15 48 15 WK | SAD15 48 15 WK | SAD15 48 15 WK | SAD15 48 15 WK | SAD15 48 15 WK | SAD15 48 15 WK | SAD21 48 15 WK | SAD21 48 15 WK | SAD21 48 15 WK |
| | 100 ul EIA | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| F | $B_0$ T + AS | S6 | S6 | SPIN15 24 15 WK | SPIN15 24 15 WK | SPIN15 24 15 WK | SPIN15 24 15 WK | SPIN15 24 15 WK | SPIN15 24 15 WK | SAD21 48 15 WK | SAD21 48 15 WK | SAD21 48 15 WK |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| G | $B_0$ T + AS | S7 | S7 | SAD21 48 10 WK | SAD21 48 10 WK | SAD21 48 10 WK | SAD21 48 10 WK | SAD21 48 10 WK | SAD21 48 10 WK | SPIN15 24 15 WK | Pool High | Pool High |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| H | $B_0$ T + AS | S8 | S8 | SPIN21 24 10 WK | SPIN21 24 10 WK | SPIN21 24 10 WK | SPIN21 24 10 WK | SPIN21 24 10 WK | SPIN21 24 10 WK | SAD15 48 15 WK | Pool Low | Pool Low |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |



**Figure 2.** Set-up of ELISA plates 4-6.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TA | S1 | S1 | SPIN15 24 21 WK | SPIN15 24 21 WK | SPIN15 24 21 WK | SPIN15 24 21 WK | SPIN15 24 21 WK | SPIN15 24 21 WK | SAD 21 48 20 WK | SAD 21 48 20 WK | SAD 21 48 20 WK |
| | T END | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| B | Blank | S2 | S2 | SAD15 24 21 WK | SAD15 24 21 WK | SAD15 24 21 WK | SAD15 24 21 WK | SAD15 24 21 WK | SAD15 24 21 WK | SAD 21 48 20 WK | SAD 21 48 20 WK | SAD 21 48 20 WK |
| | nothing | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| C | Blank | S3 | S3 | SPIN15 24 26 WK | SPIN15 24 26 WK | SPIN15 24 26 WK | SPIN15 24 26 WK | SPIN15 24 26 WK | SPIN15 24 26 WK | SPIN21 24 20 WK | SPIN21 24 20 WK | SPIN21 24 20 WK |
| | nothing | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| D | NSB T | S4 | S4 | SAD15 24 26 WK | SAD15 24 26 WK | SAD15 24 26 WK | SAD15 24 26 WK | SAD15 24 26 WK | SAD15 24 26 WK | SPIN21 24 20 WK | SPIN21 24 20 WK | SPIN21 24 20 WK |
| | 100 ul EIA | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| E | NSB T | S5 | S5 | SPIN21 24 24 WK | SPIN21 24 24 WK | SPIN21 24 24 WK | SPIN21 24 24 WK | SPIN21 24 24 WK | SPIN21 24 24 WK | SPIN21 24 15 WK | SPIN21 24 15 WK | SPIN21 24 15 WK |
| | 100 ul EIA | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| F | $B_0$ T + AS | S6 | S6 | SAD21 24 24 WK | SAD21 24 24 WK | SAD21 24 24 WK | SAD21 24 24 WK | SAD21 24 24 WK | SAD21 24 24 WK | SPIN21 24 15 WK | SPIN21 24 15 WK | SPIN21 24 15 WK |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| G | $B_0$ T + AS | S7 | S7 | SPIN21 24 30WK | SPIN21 24 30WK | SPIN21 24 30WK | SPIN21 24 30WK | SPIN21 24 30WK | SPIN21 24 30WK | SAD21 24 24 WK | Pool High | Pool High |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |
| H | $B_0$ T + AS | S8 | S8 | SAD21 24 30 WK | SAD21 24 30 WK | SAD21 24 30 WK | SAD21 24 30 WK | SAD21 24 30 WK | SAD21 24 30 WK | SPIN21 24 24 WK | Pool Low | Pool Low |
| | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul | 50 ul |

**Figure 3.** Set-up of ELISA plate 7.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TA | S1 | S1 | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK |
| | **T END** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| B | Blank | S2 | S2 | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SAD15 7 15WK | SPIN15 7 15WK |
| | **nothing** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| C | Blank | S3 | S3 | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK |
| | **nothing** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| D | NSB T | S4 | S4 | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SPIN15 7 15WK | SAD21 7 15WK | SAD21 7 15WK |
| | **100 ul EIA** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| E | NSB T | S5 | S5 | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK |
| | **100 ul EIA** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| F | $B_0$ T + AS | S6 | S6 | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SAD21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK |
| | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| G | $B_0$ T + AS | S7 | S7 | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | Pool High | Pool High |
| | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |
| H | $B_0$ T + AS | S8 | S8 | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | SPIN21 7 15WK | Pool Low | Pool Low |
| | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** | **50 ul** |

# Chapter 4

## Results

Within the 7 hours post feeding 10 weeks of age group, SPIN21 had significantly ($P<0.05$) higher corticosterone levels than SAD15, SPIN15, and SAD21. SAD15, SPIN15, and SAD21 did not have significantly different corticosterone levels from each other. Within the 24 (SPIN) and 48 (SAD) hours post feeding 10 weeks of age group, SPIN21 had significantly ($P<0.05$) higher corticosterone levels than SAD15. SPIN15 and SAD21 did not have significantly different corticosterone levels from any of the other treatment groups. Within the 7 hours post feeding 15 weeks of age group, SPIN21 again had significantly ($P<0.05$) higher corticosterone levels than SAD15 but did not have significantly different corticosterone levels from SPIN15 or SAD21. SAD15 also did not have significantly different corticosterone levels from SPIN15 or SAD21. Within the 24 (SPIN) and 48 (SAD) hours post feeding 15 weeks of age group, SAD15 had significantly ($P<0.05$) lower corticosterone levels than both SAD21 and SPIN21. SPIN15 did not have significantly different corticosterone levels than SAD15, SAD21, and SPIN21. Additionally, SAD21 and SPIN21 did not have significantly different corticosterone levels from each other.

An analysis was performed between SAD15 48HR 15WK, SPIN15 24HR 15WK, SAD21 48HR 20WK, and SPIN21 24HR 20WK to determine any difference in corticosterone levels at the time of photostimulation. Within this group, SAD15 and SAD21 had significantly

($P<0.05$) lower corticosterone levels than SPIN21. No significant difference in corticosterone levels was found between SAD15, SPIN15, and SAD21 as well as between SPIN15 and SPIN21. After photostimulation, there were two groups of corticosterone levels compared: the 21/24 weeks of age group and the 26/30 weeks of age group. The first age group corresponds to first lay of the 15 week stimulated group and first lay of the 21 week stimulated group, respectively. Within this 24 hours post feeding 21 and 24 weeks of age group, no treatment had significantly different corticosterone levels from the others. This pattern of no significantly different corticosterone levels continues to 4 weeks after lay, as can be seen in the 24 hours post feeding 26 and 30 week age group.

**Figure 4.** Plasma corticosterone concentrations of broiler breeder pullets 7h post-feeding at 10wk of age. Letters are used to represent significant (P<0.05) differences.

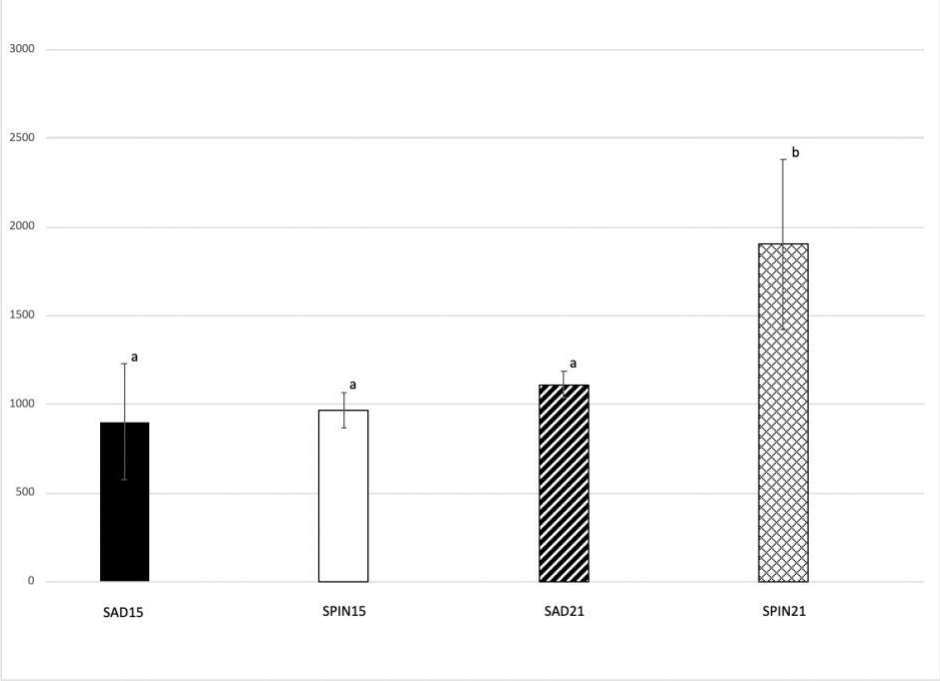



**Figure 5.** Plasma corticosterone concentrations of broiler breeder pullets 24/48h post-feeding at 10wk of age. Letters are used to represent significant (P<0.05) differences.

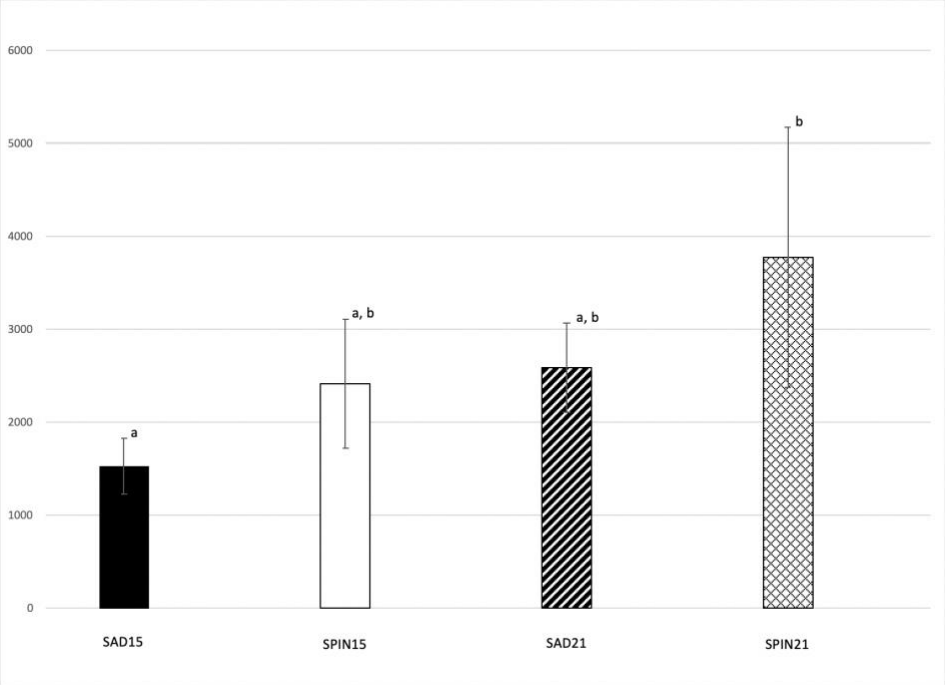


**Figure 6.** Plasma corticosterone concentrations of broiler breeder pullets 7h post-feeding at 15wk of age. Letters are used to represent significant (P<0.05) differences.

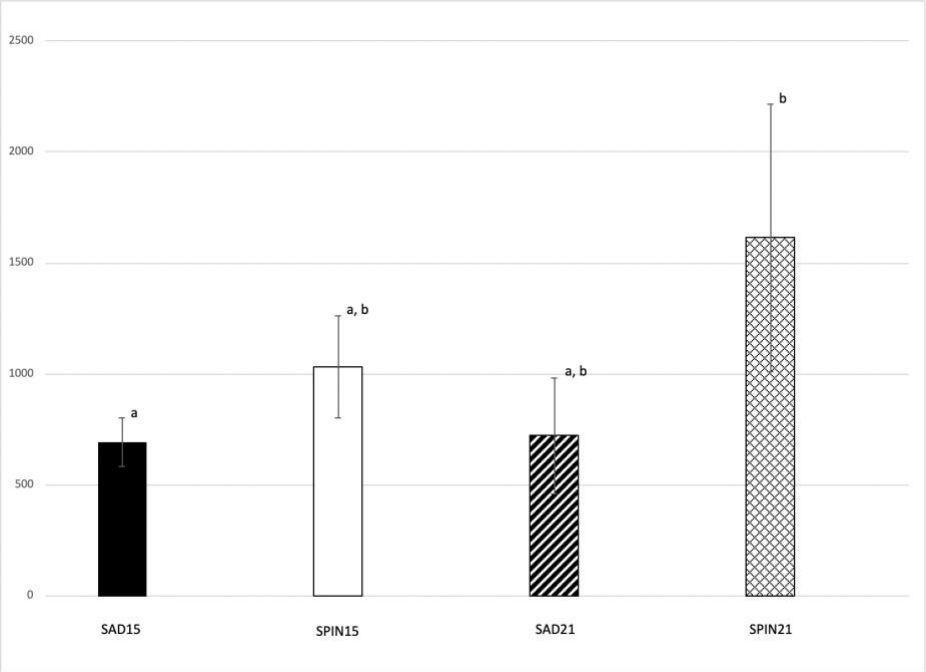



**Figure 7.** Plasma corticosterone concentrations of broiler breeder pullets 24/48h post-feeding at 15wk of age. Letters are used to represent significant (P<0.05) differences.

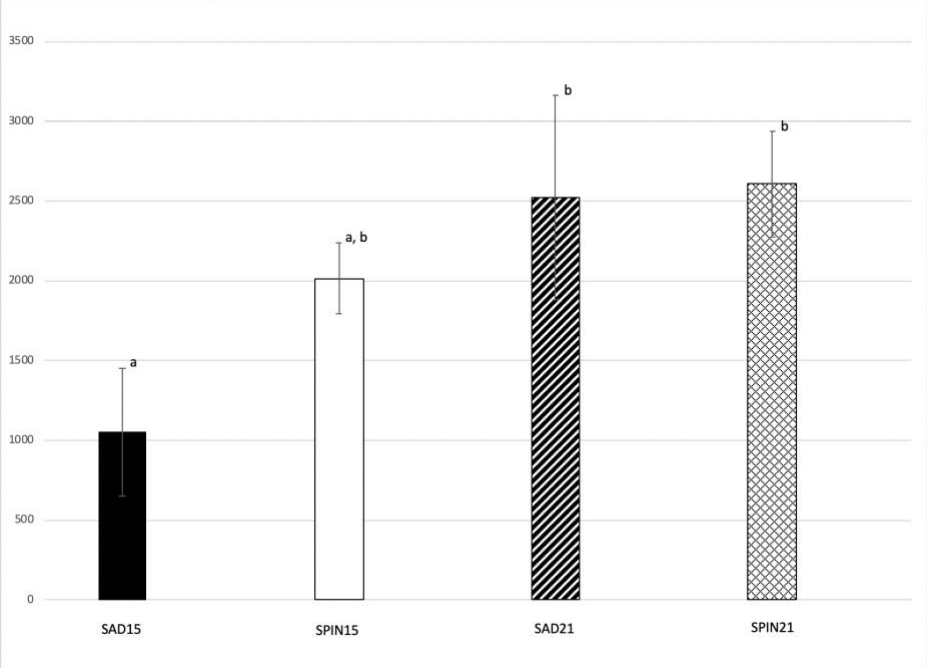


**Figure 8.** Plasma corticosterone concentrations of broiler breeder pullets one week before photostimulation. Letters are used to represent significant (P<0.05) differences.

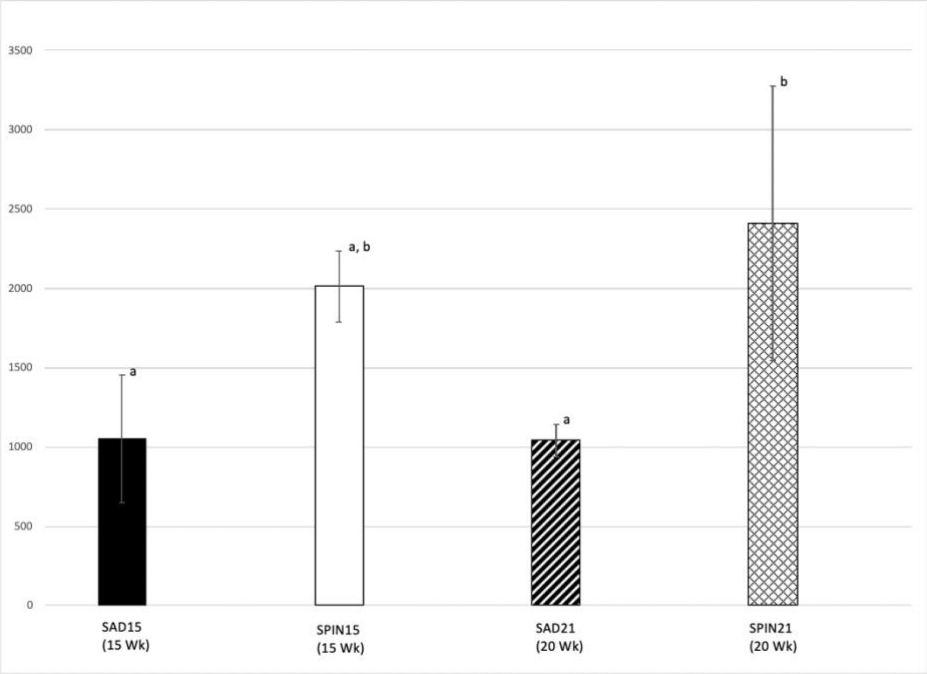



**Figure 9.** Plasma corticosterone concentrations of broiler breeder pullets at the time of first lay. Letters are used to represent significant (P<0.05) differences.

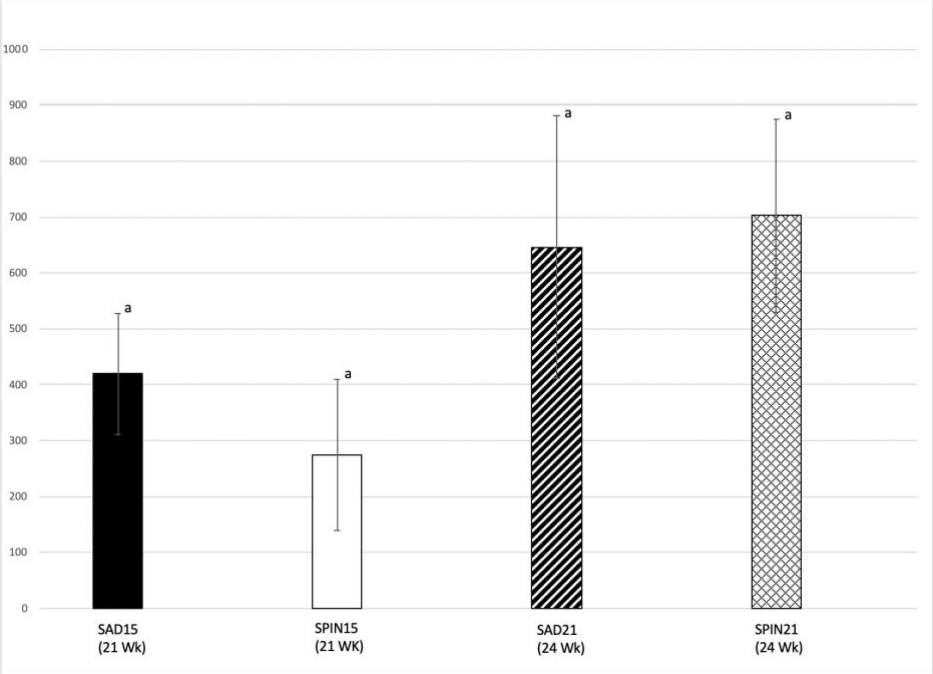
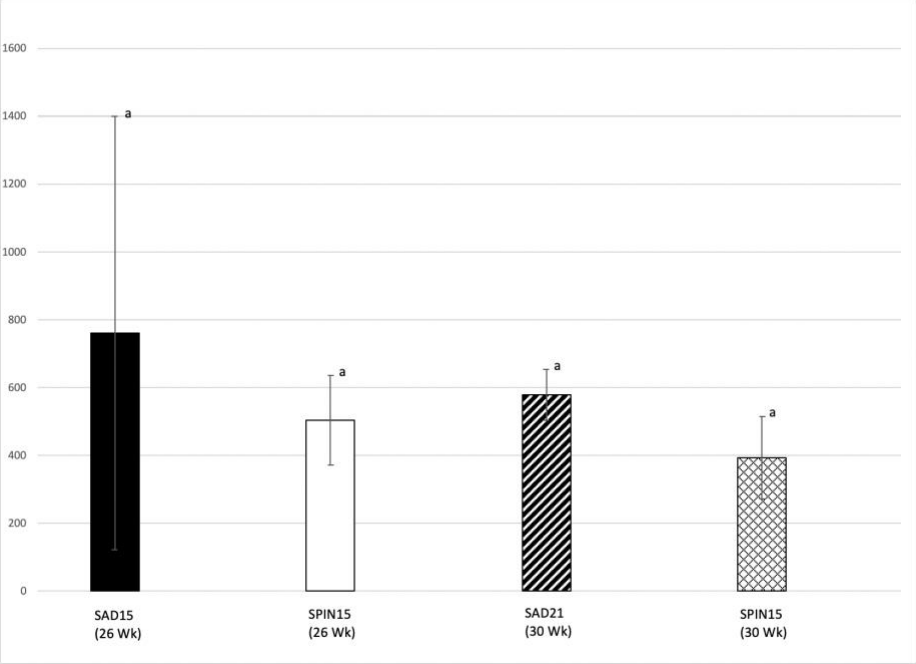


**Figure 10.** Plasma corticosterone concentrations of broiler breeder pullets 4Wk after first lay. Letters are used to represent significant ($P<0.05$) differences.



# Chapter 5

## Discussion

There have been conflicting studies regarding plasma corticosterone concentrations in ad libitum fed versus feed restricted broiler breeder pullets. In some studies, feed restricted broiler breeders had higher baseline corticosterone concentrations (Hocking et al., 1993; Savory et al., 1996; Savory and Mann, 1997), whereas no differences were found between restricted and ad libitum fed birds in other studies (Savory et al., 1993). However, the findings from this study do not support either of the evidence that exists in regards to plasma corticosterone concentrations in ad libitum fed versus feed restricted broiler breeder pullets. From the start of plasma collection to the age of photostimulation, SPIN21 had a significantly higher plasma corticosterone concentration than SAD15. This is speculated to be due to the association of human presence with feed in the SPIN21 pullets. This means that every time someone entered the room, the SPIN21 pullets were expecting to be fed, whereas the SAD15 pullets were used to only getting feed every other day. Thus, the SAD15 pullets most likely did not make the association that humans equal feed. Another reason that SPIN21 pullets had a higher plasma corticosterone concentration than SAD15 may be due to the fact that spin feeding requires the bird to use its natural behavior to forage for feed, and thus, the bird must look throughout all of the shavings to find feed, which may be a stressor.

The fact that SPIN21 plasma corticosterone concentration was higher than SAD15 throughout the whole rearing period shows that birds fed on a skip-a-day diet and an advanced body weight growth curve to achieve photostimulation at 15 weeks of age rather than the traditional 21 weeks of age experience less stress than birds fed on an everyday spin diet on a traditional body weight growth curve. Additionally, at 15 weeks of age, SAD21 had a significantly higher plasma corticosterone concentration than SAD15, most likely due to SAD15 pullets being able to achieve a fuller crop than the SAD21 pullets on days they were fed. SAD15 pullets may have achieved a fuller crop because they got fed more feed since they were on an advanced body weight growth curve. Feeding broiler breeder pullets on an advanced body weight growth curve can reduce stress associated with feed restriction. After photostimulation, at first lay and 4 weeks after first lay, each treatment group got switched to the same feeding regimen; thus, no significant differences in plasma corticosterone concentrations were found. Switching to an everyday feeding regimen at photostimulation for all treatment groups meant that, at first lay and even 4 weeks after first lay, stress levels were very similar in all treatment groups.

The main conclusion that can be made is that feeding broiler breeder pullets on an advanced body weight growth curve with SAD feeding to photostimulate at 15 weeks of age rather than a traditional body weight growth curve leads to reduced stress in the birds during rearing. Broiler meat that comes from these broiler breeders could be marked as "humanely raised" and sold for a higher price due to increased consumer concerns about agricultural animal welfare. Additionally, the data presented show that spin feeding pullets did not improve welfare, as assessed by corticosterone analysis. Thus, further research is needed to evaluate different

strategies of everyday feeding that can improve broiler welfare by reducing the stress associated with feed restriction in pullets.

## Chapter 6

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# EFFECTS OF DIETARY ESSENTIAL OILS ON BROILER MEAT

## Chapter 7

## Dietary Essential Oils

### *7.1 Industry Importance*

Within the animal production industry, antibiotics have been used for decades. Some antibiotics were used therapeutically to increase the health and well-being of animals, however, most antibiotics were being used for preventative purposes and to improve growth rate and feed conversion efficiency (Huyghebaert et al., 2011). Antibiotics that were used for the latter purpose are called antimicrobial growth performance promoters. Approximately up to 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Jang et al., 2007). With the increase of microbes that are resistant to antibiotics used to treat human and animal infections, the European Commission (EC) has banned the marketing and use of antibiotics as growth promoters in feed (Huyghebaert et al., 2011). Because of threats to both human and animal health, which arise from increased pathogenic resistance to antibiotics and the accumulation of antibiotic residues in animal products and the environment (Bampidis et al., 2005), antibiotic growth promoters are now being removed from animal diets. The removal of antibiotic growth promoters around the world has led to a number of animal performance problems, feed conversion increases, and a rise in the incidence of certain animal diseases, such

as necrotic enteritis (Wierup, 2001). Because of these emerging issues, the demand for alternatives to antibiotics that can be used as prophylactic and growth promoting agents has increased (Puvača et al., 2013). Performance and disease problems have been limited due to therapeutic antibiotic use and alternatives for antibiotic growth promoters are now being used as feed additives (Lee et al., 2004).

Alternatives to growth promoters that have the same beneficial effect as antibiotic growth promoters include exogenous enzymes, organic acids, probiotics, prebiotics, and herbs and essential oils (Stanaćev et al., 2008; 2011a). Dietary enzymes in poultry target non-starch polysaccharides (NSPs) because the viscous nature of NSPs causes anti-nutritive effects. These enzymes work by reducing the viscosity of the digesta in the small intestine, increasing the rate of digesta passage and nutrient digestion (Bedford and Classen, 1992). NSP degrading enzymes have also been shown to reduce the proliferation of pathogenic bacteria such as *Clostridium perfringens* (Jackson et al., 2003). Another alternative that decreases the incidence of subclinical necrotic enteritis is organic acids. In addition to their antimicrobial effects, organic acids also stimulate epithelial cells to proliferate and differentiate (Dalmasso et al., 2008). Probiotics in poultry have also been shown to increase the bird's resistance to enteropathogenic bacteria, such as *C. perfringens.* Probiotics contribute to immunological modifications in the gut and anatomical and physiological changes in the intestinal cell wall structure as well (Nurmi and Rantala, 1973; Hofacre et al., 1998). Prebiotics may have a similar mode of action as probiotics because they selectively stimulate the growth or metabolic activity of intestinal microbiota species, such as *Bifidobacteria* and *Lactobacillus spp* (Gibson and Roberfroid, 1995). Although most of the above alternatives' mode of action has been determined, the mode of action for herbs

and essential oils is difficult to elucidate because of a wide variation in composition due to biological factors, manufacturing, and storage conditions (Huyghebaert et al., 2011).

## *7.2 General Overview*

### *7.2.1 Introduction*

Essential oils are plant derived aromatic and volatile liquids. Essential oils have been used as perfumes and flavors for foods and beverages (Djilani and Dicko, 2012). They are biosynthesized in plant organs as secondary metabolites. Some examples of this include flowers (jasmine, rose, violet, and lavender), herbs, buds (clove), leaves (thyme, eucalyptus, salvia), fruits (anis, star anise), twigs, bark (cinnamon), and more. Most essential oils have a lower density than water and are hydrophobic, which allows them to be easily separated from the aqueous phase by decantation (Asbahani et al., 2015). Essential oil exceptions that do have a higher density than water include sassafras, vetiver, cinnamon, and clove. Generally, they are also lipophilic and soluble in alcohol, non or weakly polar solvents, waxes and oils. Most essential oils are colorless or pale yellow, with the exception of chamomile, which is blue (Djilani and Dicko, 2012). They have low extraction yields (about 1%) because many essential oil techniques include a heating step, which causes essential oil loss since they are volatile oils. Due to this and the fact that only 10% of plant species synthesize them, essential oils are a rare substance (Svoboda and Greenaway, 2003).

### *7.2.2 Essential Oil Classes*

Essential oils are usually mixtures of volatile compounds, whose chemical compositions and concentrations of individual compounds are variable (Lee et al., 2004). For example, the

concentrations of two predominant components of thyme essential oils, thymol and carvacrol, have been reported to range from as low as 3% to as high as 60% of total essential oils. A main cinnamon essential oil, cinnamaldehyde, makes up approximately 60 to 75% of the total oils. Essential oils represent less than 5% of plant dry matter and are made up of mainly hydrocarbon terpenes (isoprenes) and terpenoids. Due to the presence of olefinic double bonds and functional groups like hydroxyl, aldehyde, and ester, essential oils are readily oxidizable by light, heat, and air (Skold et al., 2006; 2008). Essential oils can be classified into two main categories: terpenoids and phenylpropanoids or hydrocarbonds and oxygenated compounds (Andrade et al., 2011; Akhila, 2006). Monoterpenes (10 carbon atoms) make up more than 80% of essential oil composition. Some terpenes have been shown to have beneficial effects against cancer, malaria, and heart disease, while others show insecticidal properties (Bakkali et al., 2008). Isoprenoids are oxygenated derivatives of hydrocarbon terpenes and include alcohols, aldehydes, ketones, acids, phenols, ethers, and esters. Thymol and carvacrol are classified as monoterpenoids or isoprenoids. Phenylpropanoids make up the least amount of essential oils, with approximately 50 phenylpropanoids being described to date (Lee et al., 2004). They are only found in special cases, such as eugenol, trans-cinnamaldehyde, piperine, capsaicin, and clove.

### *7.2.3 Genetic & Epigenetic Factors Affecting Chemical Composition*

Hydro-distillation, steam, and steam/water distillation are the most common methods of essential oil extraction. Novel methods of essential oil extraction include solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction, and phytonic process (Da Porto et al., 2009). Chemical compositions of extracted oils differ based on the extraction technique. Hydro-distillation and steam-distillation yield oils high in terpenes,

whereas the supercritical extracted oils yield oils higher in oxygenated compounds. Additionally, essential oil fragrance and chemical composition is impacted by geo-climatic location and growing conditions (soil type, climate, altitude, and amount of water available), season (before or after flowering), time of day at harvest, and genetics. This means that the same species of plant can produce similar essential oils with different chemical compositions, resulting in various therapeutic activities. Chemical composition variations led to chemotypes, which are distinct populations within the same species of plant that produce different chemical profiles for a specific class of secondary metabolites (Djilani and Dicko, 2012).

### *7.2.4 Essential Oil Applications*

Essential oils are readily applied in food products, drinks, perfumes, pharmaceuticals, and cosmetics (Anwar et al., 2009). Because essential oils have applications in so many industries, production and consumption are increasing very fast (Lawless, 1995). World production of essential oils varies from 40,000 to 60,000 tons yearly, making up a market of approximately 700 million USD (Verlet, 1994). Essential oils produced for industry use are mainly from orange, cornmint, eucalyptus, citronella, peppermint, and lemon (Hunter, 2009), whereas those produced for domestic use include lavender, chamomile, peppermint, tea tree oil, eucalyptus, geranium, jasmine, rose, lemon, orange, rosemary, frankincense, and sandalwood. Main worldwide producers of essential oils are Brazil, China, USA, Indonesia, India, and Mexico and major consumers are the USA, EU, and Japan (Djilani and Dicko, 2012).

### *7.2.5 Bioavailability of Essential Oils*

Bioavailability is the fraction of an administered dose of drug that reaches the systemic circulation and can be used for a specific function and/or stored. Intravenous bioavailability is 100%, with other routes of administration having decreased bioavailability. For example, oral administration has decreased bioavailability because of gastro-intestinal metabolism, incomplete absorption, or first-pass metabolism. High bioavailability is achieved if the compound administered has high absorption and low renal clearance. Additionally, it has been found that essential oils cross the blood-brain barrier and interact with receptors in the central nervous system, where they affect biological functions such as relaxation, sleep, digestion, etc. (Djilani and Dicko, 2012). Most essential oil components are metabolized and eliminated through either the kidneys (polar compounds) or the lungs ($CO_2$), For example, oral administration of (-)-menthol saw a 35% renal excretion as menthol glucuronide (Bronaugh et al., 1990). Similar findings have been reported with thymol, carvacrol, limonene, and eugenol (Buchbauer et al., 1993). Fast metabolism and short half-life of the active compounds of essential oils leads to a minimum risk of accumulation in body tissues (Kohlert et al., 2000; 2002).

### *7.2.6 Therapeutic Benefits of Essential Oils*

Essential oils can be supplied to the body via feeding of aromatic herbs, spices, and dietary supplements. Dietary essential oil sources include orange and citrus peel, caraway, dill, cherry, spearmint, black pepper, and lemongrass. Absorption through skin, inhalation, and ingestion are the three ways essential oils can enter the body (Djilani and Dicko, 2012). Lipid soluble essential oils pass through the skin membranes easily, allowing fast diffusion into the systemic circulation (Adorjan & Buchbauer, 2010). A faster way that essential oils can enter the

body is through inhalation. They are highly volatile oils; thus, they can be inhaled easily (Margaris et al., 1982). Due to potential toxicity of some essential oils, oral ingestion has not been studied extensively. Ingested essential oils are absorbed and then delivered to the rest of the body via blood circulation. Essential oils act through three modes of action: biochemical (pharmacological), physiological, and psychological (Djilani and Dicko, 2012). If acting through the biochemical pathway, essential oils chemically interact in the bloodstream with hormones and enzymes. Essential oils may also act on a specific physiological function. If inhaled, the olfactory region of the brain is triggered to send out chemical and neurotransmitter messengers, which in turn alter the mental and emotional behavior of the person (Buchbauer et al., 1993). Lavender and lemon EOs are known for their relaxant properties. (Djilani and Dicko, 2012).

### *7.3 Broiler Nutrition*

Broilers are usually fed on an *ad libitum* basis to allow for rapid development to market size. Although broilers are marketed at a wide range of ages and body weights, males can be grown to 2.8 to 3 kg. Because of this wide range, it is hard to establish a single set of requirements for all broiler production (NRC, 1994). However, the 1994 NRC does have recommended requirements for broilers. Dietary requirement for lysine to maximize yields of breast meat of broilers is greater than that needed to maximize weight gain (Acar et al., 1991). Many nutrients in broiler nutrition are interdependent, making it difficult to convey requirements for one without thinking of the other. Examples of this include lysine and arginine and calcium, phosphorus, and vitamin $D_3$ levels. Age and gender of broilers may also affect requirements. There is a slight difference in nutrient requirements between males and females when expressed

as a percentage of the diet. Additionally, as age increases, requirements for most nutrients decrease (NRC, 1994).

Amino acid requirements are important to monitor in order to support the rapid growth of broilers. The recommended total sulfur amino acids (methionine + cystine) requirements for 0 to 3 weeks of age is 0.87% of the diet (Nelson et al., 1960; Hewitt and Lewis, 1972; Boomgaardt and Baker, 1973b,c; Woodham and Deans, 1975; Attia and Latshaw, 1979; Robbins and Baker, 1980a,b; Wheeler and Latshaw, 1981; Baker et al., 1983; Mitchell and Robbins, 1983; Thomas et al., 1985). For 3 to 6 weeks of age, TSAA requirements were kept constant with previous recommendations (Graber et al., 1971; Holsheimer, 1981; Wheeler and Latshaw, 1981; Mitchell and Robbins, 1983). Recommended requirements for arginine are 1.25% and 1.1% for the 0 to 3 and 3 to 6 weeks of age periods, respectively (NRC, 1994). Lysine requirements for 0 to 3 weeks of age were reduced from 1.2% to 1.1% of the diet (Edwards et al., 1956; Boomgaardt and Baker, 1973a,b; Woodham and Deans, 1975; McNaughton et al., 1978; Burton and Waldroup, 1979). For 6 to 8 weeks of age, the lysine requirement is 0.85% (NRC, 1994). Tryptophan requirement for 0 to 3 weeks of age was reduced to 0.2% from 0.23% (Wilkening et al., 1947; Griminger et al., 1956; Klain et al., 1960; Boomgaardt and Baker, 1971; Hewitt and Lewis, 1972; Woodham and Deans, 1975; Steinhart and Kirchgessner, 1984; Smith and Waldroup, 1988a). The threonine dietary requirement is set at 0.8% for broilers at 0 to 3 weeks of age (Uzu, 1986; Robbins, 1987; Thomas et al., 1987; Bertram et al., 1988; Smith and Waldroup, 1988b; Austic and Rangel-Lugo, 1989). Leucine, isoleucine, and valine requirements for 0 to 3 weeks of age were established through various studies (Almquist, 1947; D'Mello, 1974; Woodham and Deans, 1975; Thomas et al., 1988). Additionally, phenylalanine, tyrosine, glycine, and serine requirements for weeks 0 to 3 were based on studies with purified diets (Fisher et al., 1957;

Klain et al., 1960; Dean and Scott, 1965; Sasse and Baker, 1972; Coon et al., 1974; Baker et al., 1979) as was the histidine requirement (Klain et al., 1960; Dean and Scott, 1965; Baker et al., 1979). A dietary need for proline was established by Greene et al., 1962 and Graber et al., 1970. Requirements for isoleucine, leucine, valine, phenylalanine, glycine, serine, histidine, and proline have been estimated for 3 to 6 and 6 to 8 weeks of age from the amino acid:lysine ratio in 0 to 3 weeks of age (NRC, 1994).

Research surrounding mineral and vitamin requirements focuses on either their economic value or the likelihood of encountering a dietary deficiency in practical diets. Calcium requirements were kept constant with previous requirements (NRC, 1994). Phosphorus requirements for 3 to 6 and 6 to 8 weeks of age were reduced (O'Rourke et al., 1952; Waldroup et al., 1963b, 1974a; Twining et al., 1965; Sauveur, 1978; Yoshida and Hoshii, 1982a; Tortuero and Diez Tardon, 1983). Requirements for potassium are between 0.25% and 0.30% (Leach et al., 1959). Sodium and chlorine requirements have increased for the 0 to 3 weeks of age period (NRC, 1994) and they decrease with increasing age (Edwards, 1984; Hurwitz et al., 1973). The requirement for iron is around 80 mg/kg (Davis et al., 1968; McNaughton and Day, 1979) and the requirement for copper is 8 mg/kg (McNaughton and Day, 1979). In a corn-soybean meal diet, requirements for zinc exceed 40 mg/kg (Edwards et al., 1959; Lease et al., 1960; Zeigler et al., 1961). Iodine requirements are based on research conducted by Creek et al., 1957. Selenium is recommended at a concentration of 0.15 mg/kg (Jensen et al., 1986).

Vitamin A requirements range from 900 to 2,200 IU/kg and Vitamin D requirements for maximum growth are consistent among studies (NRC, 1994). Vitamin E requirement for increased immune response are likely higher than the amount needed for growth (Tenerdy and

Nockels, 1973; Colnago et al., 1984). Vitamin K requirement is around 0.5 mg/kg (Nelson and Norris, 1960; 1961b). The riboflavin requirement at 0 to 3 and 3 to 6 weeks of age remains unchanged at 3.6 mg/kg. Pantothenic acid requirement for broilers is 5 mg/kg (NRC, 1994). Niacin requirement estimates range from 25 to 35 mg/kg (Ruiz et al., 1988a; 1990). The Vitamin B12 requirement is around 0.01 mg/kg (Looi and Renner, 1974; Rys and Koreleski, 1974). Biotin requirement estimates range from 0.15 to 0.20 mg/kg and folic acid requirement estimates range from 0.35 to 0.50 mg/kg (NRC, 1994). Pyridoxine is required in the broiler diet at 2.3 to 3.5 mg/kg and must be increased with an increase in dietary protein (Gries and Scott, 1972a; Daghir and Shah, 1973). Linoleic acid is required at 1% of the broiler diet (Balnave, 1970).

Water is one of the essential nutrients of broilers. Environmental temperature, relative humidity, salt and protein levels of the diet, growth rate, and the individual bird's ability to resorb water in the kidney all influence water intake (Medway and Kare, 1959). Because of this, water requirements for broilers are highly variable. Water deprivation for 12 hours or more slows growth of young poultry and water deprivation of greater than 36 hours results in an increase in mortality of both young and mature broilers (Bierer et al., 1965a,b; Haller and Sunde, 1966; Adams, 1973). Additionally, water restoration after long periods of water deprivation may cause "water intoxication," leading to death (Marsden et al., 1965). Antibiotics (5-25 mg/kg) and copper (150 ppm) are sometimes included in a broiler diet to improve growth rate, feed efficiency, and livability (Stokstad et al., 1949; Coates et al., 1951; Libby and Schaible, 1955; Milligan et al., 1955; Bird, 1968; Begin, 1971; Morrison et al., 1974).

## *7.4 Biological Effects of Essential Oils in Feed*

### *7.4.1 Proposed Mechanisms of Action*

Essential oils may exhibit a range of potentially beneficial properties including antimicrobial, antioxidant, antiviral, antiparasitic, and a digestive stimulant (Khattak et al., 2014). Anethole is known for promoting appetite or aiding in digestion (Muchtaridi et al., 2010). Another stomachic active compound is eugenol, which is also antiviral, antimicrobial, and antifungal (de Paoli et al., 2007; Koba et al., 2011). Additionally, phenols, such as thymol, eugenol, and carvacrol, are known to be spasmolytic, anesthetic, irritant, and immune stimulating (Pengelly, 2004; Ghelardini et al., 2001; De Sousa et al., 2008; 2011). Another antibacterial, antiviral, and antifungal compound is known to be cinnamaldehyde, which can act as an insecticide too (Cheng et al., 2004; Geng et al., 2011). Aldehydes, such as cinnamaldehyde, are also spasmolytic, as well as hypotensive and calming (Dorman & Deans, 2000).

Due to their complex chemical composition, essential oil molecular pathway of action is difficult to identify. Most likely, each component of essential oils has its own mechanism of action. A possible antimicrobial activity is generation of non-reversible damage to the membrane of bacterial cells, which causes cytoplasmic loss, leakage of ions, and loss of energy substrate (glucose, ATP). Carvacrol and thymol both disintegrated the membrane of bacteria, leading to the release of membrane-associated material from the cells to the external medium. On the other hand, cinnamaldehyde failed to affect the membrane, but exhibited antibacterial activity, indicating that it has a different mechanism for antibacterial activity (Helander et al., 1998). This disruption of bacterial equilibrium causes cytolysis (bacterial death). Another proposed mechanism of action is inhibition of amylase and protease production, which stops toxin

production, electron flow, and causes cell content coagulation (Bakkali et al., 2008; Burt, 2004). Antifungal modes of action are similar to antimicrobial ones. The mechanism of antifungal action of cinnamaldehyde has also been investigated (Kurita et al., 1979) and it was proposed that it takes place through the reaction with sulfhydryl groups, which are irreplaceable for fungal growth, and that the formation of charge transfer complexes with electron donors in the fungus cell could lead to inhibition of cell division and thus interferes with cell metabolism. Cinnamaldehyde also inhibits the fungal-cell-wall synthesizing enzymes (Bang et al, 2000). However, yeast inhibition also happens through the establishment of a pH gradient across the cytoplasmic membrane and the blocking of energy production. A complex mixture of EOs exhibits higher antiviral activity than individual compounds, most likely due to a synergistic effect. Studies suggest that EOs prevent cell-to-cell virus diffusion by interfering with the virus envelope through inhibition of specific processes in the viral replication cycle or by masking viral components, which are vital for entry into host cells (Djilani and Dicko, 2012).

### *7.4.2 Effects of Dietary Essential Oils in Broiler Nutrition*

Essential oils can stimulate appetite and endogenous digestive enzyme secretions or exert antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals (Chowdhury et al., 2018). Commonly used dietary essential oils in broiler nutrition research include oregano, garlic, thyme, rosemary, and sage (Horosova et al., 2006). Inclusion levels are lower for essential oils than for aromatic plant parts added to feed (Lee et al., 2004). There is still a lack of evidence of the underlying mechanisms by which dietary essential oils affect growth performance, however, dietary supplementation of essential oils has a beneficial effect on intestinal microflora and digestive enzymes (Khattak et al., 2014). Essential oils enhance intestinal activities of trypsin,

lipase, and amylase in broilers (Lee et al., 2004). In broilers, essential oils have also been shown to enhance the secretions of jejunal chyme and reduce the adherence of pathogens to the intestinal wall. Essential oils have positive effects on the morphology of the small intestine, which include longer villi, decreased crypt depth, and increased goblet cell counts. All of the described beneficial effects of essential oils are thought to improve nutrient digestibility and growth performance of broilers. Essential oils are already being used as feed supplements to improve growth performance under intensive management systems (Jang et al., 2007).

Because of the complex chemical compositions of essential oils, conducted broiler studies report a variety of findings. Lee et al., 2004 showed no differences in apparent ileal digestibilities of crude protein and starch in addition to total tract fat digestibility in 21 and 40 days old female Cobb broilers fed a corn-soybean meal basal diet supplemented with thyme, cinnamaldehyde, or a commercial essential oil at 100 mg/kg diet. In a wheat-corn-soybean meal diet supplemented with two three-component essential oil products fed to Ross male broilers, improvements in ileal digestibility of dry matter and starch and the total tract apparent digestibility of dry matter, crude protein, and fat were seen when compared to the un-supplemented control (Hernandez et al., 2004). Additionally, male Hubbard broilers did not have a significant increase in the apparent ileal digestibility of nutrients when their corn-soybean meal or wheat-barley-soybean meal diets were supplemented with carvacrol, cinnamaldehyde, and capsicum oleoresin (Cross et al., 2007). On the other hand, Cobb broilers fed a corn-soybean meal diet supplemented with EOs from oregano, anise, and citrus showed an increased ileal apparent fat digestibility (Theron and Lues, 2007; Stanaćev et al., 2011b).

Dietary intake of essential oils, such as rosemary and sage extracts (Lopez-Bote et al., 1998; Govaris et al., 2007; Spernakova et al., 2007) and oregano oil (Young et al., 2003), has shown beneficial effects on stored meat quality by reducing or delaying lipid oxidation. Food microbiological safety and quality of meat stored in raw or cooked stages is known to improve with the addition of essential oils in diets because of their antimicrobial and antioxidant functions (Soltan et al., 2008). In order to achieve higher microbiological safety and quality of meat, essential oils can either reduce pathogens in the gut, promoting a healthy gut environment, or they can inhibit the growth of spoilage or pathogenic bacteria (Puvača et al., 2013). A healthy gut environment may lead to a reduction of carcass contamination at slaughter. No beneficial effects of essential oils are reported on carcass yield.

### *7.5 Summary*

Because antimicrobial growth promoters, or AGPs, are being removed from poultry diets, there is a need for alternatives that act in the same way that AGPs do. These should increase growth while improving feed efficiency and mortality due to subclinical necrotic enteritis. One of the alternatives that are being actively researched are essential oils, or the bioactive and aromatic oils extracted from various plants or spices. EOs can either be eaten, inhaled, or spread over skin, however, the most common use of essential oils in broilers is dietary supplementation. Because of differences in essential oil chemical compositions, they have a wide variety of physiological effects. Some EOs have been shown to possess antimicrobial, antifungal, and antiviral properties, while others have been shown to be anesthetic and to increase appetite. Studies have found that dietary supplementation of EOs has a beneficial effect on broiler

intestinal microflora and digestive enzymes. Dietary essential oils have also been found to increase stored broiler meat quality through the reduction or delay of lipid oxidation.

# Chapter 8

## Statement of Purpose

Male chicks produced by broiler breeders are raised commercially as broilers to be sold for their meat. Because of rising antibiotic resistance that may impact both animal and human health, antibiotic growth promoter usage has slowed and new ways of growing broilers are being sought after. One of these ways that is currently being researched is essential oil feed additives. Various essential oil feed additives have been studied as a substitute for antibiotic growth promoters, however, not many scientific papers explore the physiological effects of essential oil feed additives. This research investigates the various impacts that essential oil feed additives may have on broiler meat: growth via weights at different ages, meat make-up via proximate analysis, and oxidative stress damage via the TBARS assay.

# Chapter 9

## Materials and Methods

### *9.1 Animal Rearing*

A total of 1100 one-day-old Cobb 500 male byproduct chicks were brought to the University of Georgia Poultry Research Center on September 2, 2019. Twelve replicate pens (1.524 X 1.22 $m_2$; 23 chicks) were randomly allocated to four treatments (276 birds per treatment): control diet (PC), PC + Essential Oil X (40 ppm), PC + Xtract (100 ppm), and PC + BioStrong (150 ppm). The control diet was a positive control, containing no growth promotants, no prebiotics, nor any essential oils. Essential Oil X contained three essential oils: thymol (thyme), eugenol (cinnamon), and piperine (black pepper). Xtract (Pancosma SA) contained carvacrol (thyme), cinnamaldehyde (cinnamon), and capsicum oleoresin (chilli peppers). BioStrong (Delacon Biotechnik Ges.m.b.H.) had the most diverse makeup of essential oils out of all the treatment groups; it contained thymol (thyme), anethole (star anise), as well as different herbs and spices.

Allocation of chicks was based on equalizing initial body weights across pens. Each pen contained litter from two previous flocks, which was not top-dressed before the start of the experiment and was allowed to air dry for over two months. During the time of allocation, chicks within alternating pens were sprayed with a different color non-toxic dye to quickly identify and

allow correction of any chick migration between pens. One pan feeder (base circumference of 104 cm) was placed in each pen, and additional accessory feed trays were provided for more feeder space during the first three days of brooding. Water was supplied *ad libitum* by five nipple drinkers in each pen. The feeding program consisted of three phases: starter diet from 0 to 14 days, grower diet from 15 to 28 days, and finisher diet from 29 to 42 days. The feed was presented *ad libitum* as a crumble during the starter phase and as pellets for the remaining experimental period. Diet composition can be seen in **Figure 3.1.** The level of each feeder was monitored daily to ensure free access to feed**.** The facility where birds were raised was equipped with an evaporative cooling system, two gas fired furnaces and 6 forty-six cm ceiling circulation fans with a computerized environmental controller. Target temperature slowly decreased from 33.89 to 22.78°C**.** Lighting was decreased from 20 lux to 2 lux over the course of the experiment.

The chicks were raised to 42 days. Temperature, humidity, and morbidity and mortality were recorded twice daily (in the morning and the afternoon). Throughout the experiment, animals showing health deficiency or that were unable to access feed or water were euthanized via cervical dislocation. Body weight was measured four times during the course of the experiment: on Day 0, 14, 28, and 42. On Day 42, a pen-by-pen evaluation was made for footpad score based on a 3-point visual ranking scale (Bilgili et al., 2006). The same individual conducted this evaluation across all pens to avoid error due to evaluator.

*9.2 Processing & Further Processing*

At the end of the finisher period (Day 42), all birds were weighed by pen. Mean body weight for each replicate pen was calculated, then eight birds, weighing within plus or minus 300g of the pen mean body weight, were selected from each pen. The selected birds were labeled

with numbered and color-treatment specific leg bands and placed in coops on the floor in front of each pen for an overnight feed withdrawal. The next morning (Day 43), after an approximate 12 to 15 h feed withdrawal, the coops were moved to the University of Georgia Processing Plant. Each bird was weighed before it was hung on the processing line (fasted live weight). Birds were stunned, exsanguinated by severing the carotid artery, and scalded at approximately 134$^{o}$C for approximately two minutes prior to mechanical picking. After feather removal but before the legs/feet were cut off, the band was moved above the hock joint. The neck of each carcass was removed 5/8 inches above the shoulder before each carcass was eviscerated both mechanically and manually. The hot carcass of each bird was weighed, then placed in chilled ice water and a 4$^{o}$C cooler for about four hours. After cooling, carcasses were drained for deboning, which was done in an assembly line manner so the same cuts were made by the same individual to minimize sampling error. The following yield data for each bird was collected as part of this study: WOG hot carcass) and chilled: *Pectoralis majors*, *Pectoralis minors,* wings, and leg quarters (thigh and drum stick). Yield data/results were determined on a pen basis.

*9.3 Proximate Analysis*

Four breast meat halves from each pen were placed in Ziploc bags, which were stored at -80$^{o}$C until sample preparation. There were 12 repetitions per each treatment. For sample preparation, samples were allowed to thaw in a cold room enough to be cut up and placed in a food processor. Between each of the samples, all instruments used were washed with hot water to ensure no meat was left behind to contaminate the next sample. Once all samples were processed, 0.20g of each sample was weighed out three times into three small pieces of aluminum foil. Samples were kept in order by labeled Styrofoam microcentrifuge tube holders

until protein analysis was conducted via a LECO Corporation (St. Joseph, MI) nitrogen analyzer. Two repetitions of each pen were analyzed in a Leco machine, then relative standard deviation (RSD) was calculated in the Leco software. If RSD was less than five for the two repetitions, then the 3rd repetition was not run. If RSD was more than five for the two repetitions, the 3rd repetition was analyzed and the two repetitions of the three which had an RSD of less than five were used in data analysis. All pens had two repetitions of protein analysis conducted in the end.

For moisture and lipid content, a small portion of each sample was frozen in liquid nitrogen, then blended to obtain a fine powder-like consistency. This was done because ether was able to penetrate the powder-like consistency more easily than large particle samples. Samples were stored in -80$^{o}$C. Two repetitions of each pen, labeled as Pen # a and Pen # b, were run. Bag weight was recorded, the scale was tared, and 1.1g of sample was weighed out. Labeled bags with sample were put into an oven at 100$^{o}$C overnight. After samples were dried overnight, they were put into a desiccator for about 10 mins, then weight after first dry (g) was recorded. From this weigh data, % moisture for each repetition was calculated, as well as average % moisture, % error, standard deviation, and CV for each pen. Samples were then run through the ANKOM Technology (Macedon, NY) ether extraction machine, put into the oven for about 10 mins, put into the desiccator for about 10 mins, and weighed to obtain weight after fat extraction (g). From the data obtained after ether extraction, % fat for each repetition was calculated, as well as average % fat, % error, standard deviation, and CV for each pen. Any sample not used was stored in -80$^{o}$C.

*9.4 Thiobarbituric Acid Reactive Substances (TBARS)*

The thiobarbituric acid reactive substances (TBARS) assay was used to determine oxidative damage to breast meat. A standard curve for the TBARS assay was prepared as per Cayman Chemical TBARS assay kit instructions. The same samples used for ether extraction were used for the TBARS assay and 0.10XXg of each sample was weighed out, in duplicate, into labeled microcentrifuge tubes. Once weighed out, 500 ul of RIPA lysis buffer was added into the tubes, then samples were sonicated on ice to ensure homogenization. Samples were centrifuged at 16,000 x g for 10 mins at 4$_{o}$C. From the microcentrifuge tubes, 0.1 mL of the supernatant was pipetted into labeled 3 ml vials. Following that, 0.1 mL of SDS solution was added to each vial, which were swirled to mix the liquids. Color Reagent (1 mL) was also added into the vials, which were placed in a 100$_{o}$C water bath for 1 hour to allow color reaction to take place. Vials were immediately placed in an ice bath for 10 mins to stop the reaction. Vials were then centrifuged at 16,000 x g for 10 mins at 4$_{o}$C. Cloudiness of each sample cleared upon warming to room temperature. Each sample (150 ul) was pipetted into a Cayman Chemicals TBARS assay clear plate well and the absorbance was read at 532 nm using a SpectraMax plate reader.

*9.5 Statistics*

Carcass characteristics data were subjected to ANOVA according to the General Linear Model (GLM). Tukey's multiple-comparison procedure was used to detect significant differences among dietary treatments. The statistical model included treatment and ventilation block as factors. Proximate analysis and TBARS data were subjected to ANOVA and analyzed by Tukey-Kramer HSD means comparisons for all pairs. These statistical procedures were

completed with the JMP Pro statistical software package (Release 14, Cary, NC). Differences were considered to be significant when $P<0.05$.

**Figure 11.** Composition of the experimental diets.

| Ingredient | Diets[1] | | |
|---|---|---|---|
| | Starter | Grower | Finisher |
| | | % | |
| Corn | 55.2057 | 59.9789 | 63.5560 |
| Soybean Meal | 31.4122 | 25.1945 | 22.2656 |
| Soybean Oil | 2.9121 | 3.3840 | 3.7548 |
| Corn DDGS | 4.0000 | 5.0000 | 5.0000 |
| MBM | 3.0000 | 3.2817 | 2.3642 |
| Limestone | 0.7464 | 0.7580 | 0.7614 |
| Defluorinated P. | 0.3291 | 0.0000 | 0.0000 |
| Sodium carbonate | 0.2219 | 0.2746 | 0.2674 |
| Salt | 0.2626 | 0.2315 | 0.2236 |
| L-Lysine, HCl 78.8% | 0.2406 | 0.2499 | 0.2266 |
| DL-Methionine 99% | 0.3270 | 0.3017 | 0.2612 |
| L-Threonine 98% | 0.0955 | 0.0914 | 0.0706 |
| Choline Cl 60% | 0.0306 | 0.0374 | 0.0322 |
| Vitamin mix[2] | 0.5670 | 0.5670 | 0.5670 |
| Minaral mix[3] | 0.0793 | 0.0794 | 0.0794 |
| Sand[4] | 0.5000 | 0.5000 | 0.5000 |
| Diclazuril | 0.0500 | 0.0500 | 0.0000 |
| Phytase 5,000 FTU/g[5] | 0.0200 | 0.0200 | 0.0200 |
| | | | |
| Calculated analysis | | | |
| AME (kCal/kg) | 3020 | 3108 | 3164 |
| Crude protein (%) | 21.85 | 19.78 | 18.13 |
| Calcium (%) | 0.95 | 0.85 | 0.75 |
| Available P (%) | 0.48 | 0.43 | 0.38 |
| Digestible Met (%) | 0.62 | 0.57 | 0.51 |
| Digestible TSAA (%) | 0.88 | 0.81 | 0.74 |
| Digestible Lys (%) | 1.15 | 1.02 | 0.92 |
| Digestible Thr (%) | 0.77 | 0.69 | 0.62 |
| Digestible Ile (%) | 0.79 | 0.69 | 0.64 |
| Digestible Val (%) | 0.87 | 0.78 | 0.72 |
| Digestible Trp (%) | 0.23 | 0.2 | 0.18 |

[1]The starter diet was fed from day 0 to day 14. The grower diet was fed from day 15 to day 28, and the finisher diet was fed from day 29 to day 42.

[2]Vitamin mix provided the following per 100g of diet: vitamin A, 551IU; vitamin $D_3$, 110IU; vitamin E, 1.1IU; vitamin $B_{12}$, 0.001mg; riboflavin, 0.44mg; niacin, 4.41mg; d-panthotenic acid, 1.12mg; choline, 19.13mg; menadione sodium bisulfate; 0.33mg; folic acid, 0.55mg; pyridoxine HCl, 0.47mg; thiamin, 0.22mg; d-biotin, 0.011mg; and ethoxyquin, 12.5mg.

[3]Mineral mix provided the following in mg per 100g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.

[4]The test products were added at the expense of sand.

[5]HiPhos 5000 GT added in premix at 0.40 lbs/2,000 lbs feed to replace 0.15% P & 0.12% Ca.

# Chapter 10

## Results

Body weight and body weight gain were significantly ($P<0.05$) lower in BioStrong than Essential Oil X from 0 to 14 days of age, however, they did not differ significantly from 14 to 28 days of age, 0 to 28 days of age, 28 to 42 days of age, and 0 to 42 days of age. Feed to gain, feed intake, and mortality were not significantly different for all time points. Additionally, foot pad score, fasted weight, and loss of weight did not significantly differ between the four treatments from 0 to 42 days of age. Live weight was significantly ($P<0.05$) lower in the BioStrong treatment than the Xtract treatment on Day 43. The Control and Essential Oil X treatment body weight did not differ significantly from each other nor from Xtract and BioStrong. Hot carcass and chilled carcass weights from 43 day old broilers were significantly ($P<0.05$) higher in the Xtract treatment than in the BioStrong treatment group. Again, hot carcass and chilled carcass weights from the Control and Essential Oil X treatment groups were not significantly different from each other nor from the other two treatment groups.

Following this same trend, *pectoralis major* and total white meat (*pectoralis major* plus *pectoralis minor*) weights were significantly ($P<0.05$) higher in the Xtract treatment than in the BioStrong treatment group. Control and Essential Oil X groups were not significantly different from any of the treatments. Pectoralis minor and leg quarters weights were not significantly different between all treatment groups. However, wings as a percent of live fasted weight was

significantly ($P<0.05$) higher in the Essential Oil X treatment than in the Xtract treatment. Control and BioStrong wings as a percent of live fasted weight did not significantly differ from any of the treatment groups. Color, percent moisture, percent protein, percent fat, and malondialdehyde concentration were not significantly different between each of the four treatment groups.

**Table 1**. Body weight, body weight gain and feed efficiency of broilers fed different dietary essential oils from 0 to 14 days of age[1].

| Dietary treatment | Body weight | Body weight gain | Feed to gain | Feed Intake | Mortality/culls |
|---|---|---|---|---|---|
| | | g/bird | | | % (#) |
| Control | 504 ± 5[ab] | 462 ± 5[ab] | 1.227 ± 0.013 | 562 ± 5 | 2.174 (6) |
| Essential Oil X | 511 ± 4[a] | 469 ± 4[a] | 1.234 ± 0.011 | 572 ± 3 | 2.536 (7) |
| Xtract | 504 ± 4[ab] | 461 ± 4[ab] | 1.237 ± 0.014 | 564 ± 6 | 2.536 (7) |
| BioStrong | 492 ± 4[b] | 450 ± 4[b] | 1.251 ± 0.013 | 561 ± 4 | 0.725 (2) |

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

**Table 2**. Body weight, body weight gain and feed efficiency of broilers fed different dietary essential oils from 14 to 28 days of age[1].

| Dietary treatment | Body weight | Body weight gain | Feed to gain | Feed Intake | Mortality/culls |
|---|---|---|---|---|---|
| | | g/bird | | | % (#) |
| Control | 1770 ± 9 | 1267 ± 5 | 1.453 ± 0.005 | 1819 ± 11 | 2.174 (6) |
| Essential Oil X | 1766 ± 19 | 1255 ± 16 | 1.451 ± 0.008 | 1821 ± 17 | 0.000 (0) |
| Xtract | 1776 ± 15 | 1273 ± 13 | 1.447 ± 0.006 | 1826 ± 16 | 3.261 (9) |
| BioStrong | 1736 ± 11 | 1244 ± 10 | 1.461 ± 0.004 | 1801 ± 13 | 2.174 (6) |

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments.

**Table 3**. Body weight, body weight gain and feed efficiency of broilers fed different dietary essential oils from 0 to 28 days of age[1].

| Dietary treatment | Body weight | Body weight gain | Feed to gain | Feed Intake | Mortality/culls |
|---|---|---|---|---|---|
| | | g/bird | | | % (#) |
| Control | 1770 ± 9 | 1727 ± 9 | 1.391 ± 0.005 | 2367 ± 13 | 4.348 (12) |
| Essential Oil X | 1766 ± 19 | 1724 ± 19 | 1.392 ± 0.007 | 2383 ± 16 | 2.536 (7) |
| Xtract | 1776 ± 15 | 1734 ± 15 | 1.390 ± 0.003 | 2375 ± 19 | 5.797 (16) |
| BioStrong | 1736 ± 11 | 1694 ± 11 | 1.404 ± 0.004 | 2355 ± 15 | 2.899 (8) |

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments.

**Table 4**. Body weight, body weight gain and feed efficiency of broilers fed different dietary essential oils from 28 to 42 days of age[1].

| Dietary treatment | Body weight | Body weight gain | Feed to gain | Feed Intake | Mortality/culls |
|---|---|---|---|---|---|
| | | g/bird | | | % (#) |
| Control | 3252 ± 36 | 1494 ± 31 | 1.803 ± 0.016 | 2564 ± 47 | 0.725 (2) |
| Essential Oil X | 3167 ± 49 | 1401 ± 35 | 1.852 ± 0.025 | 2505 ± 43 | 0.725 (2) |
| Xtract | 3265 ± 36 | 1488 ± 25 | 1.807 ± 0.011 | 2583 ± 45 | 0.725 (2) |
| BioStrong | 3132 ± 32 | 1400 ± 28 | 1.850 ± 0.010 | 2440 ± 40 | 1.449 (4) |

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments.

**Table 5**. Body weight, body weight gain and feed efficiency of broilers fed different dietary essential oils from 0 to 42 days of age[1].

| Dietary treatment | Body weight | Body weight gain | Feed to gain | Feed Intake | Mortality/culls | Foot pad score |
|---|---|---|---|---|---|---|
| | | g/bird | | | % (#) | |
| Control | 3252 ± 36 | 3210 ± 36 | 1.577 ± 0.006 | 4845 ± 69 | 5.072 (14) | 1.10 ± 0.08 |
| Essential Oil X | 3167 ± 49 | 3125 ± 49 | 1.593 ± 0.010 | 4863 ± 60 | 3.261 (9) | 1.18 ± 0.08 |
| Xtract | 3265 ± 36 | 3222 ± 36 | 1.578 ± 0.005 | 4859 ± 73 | 6.522 (18) | 1.18 ± 0.09 |
| BioStrong | 3132 ± 32 | 3090 ± 32 | 1.600 ± 0.004 | 4702 ± 67 | 4.348 (12) | 0.94 ± 0.10 |

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments.

**Table 6**. Processing yields from 43 day old broilers fed dietary essential oils from 0 to 42 days of age[1].

| Dietary treatments | Live weight | Fasted weight (day 43 of age) | Loss of weight |
|---|---|---|---|
| | g | g | %[2] |
| Control | 3252 ± 35[ab] | 3090 ± 33 | 5.00 ± 0.15 |
| Essential Oil X | 3168 ± 41[ab] | 3014 ± 39 | 4.83 ± 0.17 |
| Xtract | 3269 ± 35[a] | 3104 ± 33 | 5.04 ± 0.20 |
| BioStrong | 3129 ± 31[b] | 2986 ± 29 | 4.63 ± 0.16 |

[1]The values are means ± SEM, n = 12 replicate pens with 8 birds per pen selected per pen for processing.. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]As a percent of live weight.

**Table 7**. Processing yields from 43 day old broilers fed dietary essential oils from 0 to 42 days of age[1].

| Dietary treatments | Hot carcass | | Chilled carcass | | Frame | |
|---|---|---|---|---|---|---|
| | g | %[2] | g | %[2] | g | %[2] |
| Control | 2355 ± 23[ab] | 76.25 ± 0.11 | 2382 ± 24[ab] | 77.06 ± 0.10 | 603 ± 6 | 19.51 ± 0.16 |
| Essential Oil X | 2290 ± 30[ab] | 76.02 ± 0.18 | 2321 ± 31[ab] | 77.04 ± 0.18 | 598 ± 7 | 19.87 ± 0.17 |
| Xtract | 2366 ± 28[a] | 76.20 ± 0.22 | 2390 ± 29[a] | 76.98 ± 0.21 | 614 ± 8 | 19.77 ± 0.13 |
| BioStrong | 2265 ± 20[b] | 75.99 ± 0.17 | 2291 ± 22[b] | 76.83 ± 0.18 | 590 ± 6 | 19.77 ± 0.10 |

[1]The values are means ± SEM, n = 12 replicate pens with 8 birds per pen selected for processing. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]As a percent of live fasted weight.

**Table 8**. Processing yields from 43 day old broilers fed dietary essential oils from 0 to 42 days of age[1].

| Dietary treatments | Pectoralis major | | Pectoralis minor | | Total white meat[2] | | Wings | | Leg quarters | |
|---|---|---|---|---|---|---|---|---|---|---|
| | g | %[3] | g | %[3] | g | %[3] | g | %[3] | g | %[3] |
| Control | 658 ± 12[ab] | 21.1 ± 0.3 | 121 ± 2 | 3.93 ± 0.05 | 779 ± 13[ab] | 23.93 ± 0.2 | 247 ± 2 | 8.00 ± 0.05[ab] | 742 ± 8 | 24.03 ± 0.16 |
| Essential Oil X | 630 ± 14[ab] | 20.9 ± 0.2 | 119 ± 2 | 3.96 ± 0.03 | 749 ± 15[ab] | 23.61 ± 0.2 | 244 ± 3 | 8.11 ± 0.05[a] | 723 ± 10 | 24.02 ± 0.18 |
| Xtract | 669 ± 12[a] | 21.5 ± 0.2 | 122 ± 2 | 3.94 ± 0.04 | 792 ± 14[a] | 24.17 ± 0.2 | 246 ± 3 | 7.92 ± 0.05[b] | 730 ± 9 | 23.53 ± 0.15 |
| BioStrong | 622 ± 11[b] | 20.8 ± 0.2 | 117 ± 2 | 3.91 ± 0.05 | 739 ± 12[b] | 23.58 ± 0.3 | 240 ± 2 | 8.04 ± 0.04[ab] | 718 ± 6 | 24.11 ± 0.19 |

[1]The values are means ± SEM, n = 12 replicate pens with 8 birds per pen selected for processing. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]Pectoralis major plus pectoralis minor
[3]As a percent of live fasted weight.

**Table 9**. Pectoralis major color, protein content, fat content, moisture content and malondialdehyde (MDA) content, from 43 day old broilers fed dietary essential oils from 0 to 42 days of age[1].

| Dietary treatments | Color | | | Moisture | Protein | Fat | MDA |
|---|---|---|---|---|---|---|---|
| | L*[2] | a*[3] | b*[4] | % | % | %g | uM |
| Control | 62.43 ± 0.44 | 0.39 ± 0.09 | 11.47 ± 0.24 | 74.17 ± 0.32 | 22.20 ± 0.26 | 2.92 ± 0.21 | 31.03 ± 3.94 |
| Essential Oil X | 62.28 ± 0.31 | 0.25 ± 0.10 | 10.81 ± 0.33 | 73.85 ± 0.34 | 22.20 ± 0.34 | 3.25 ± 0.20 | 30.94 ± 4.05 |
| Xtract | 62.00 ± 0.30 | 0.40 ± 0.11 | 11.47 ± 0.30 | 73.79 ± 0.34 | 22.33 ± 0.20 | 3.16 ± 0.22 | 32.59 ± 5.35 |
| BioStrong | 61.93 ± 0.36 | 0.46 ± 0.11 | 11.29 ± 0.30 | 73.30 ± 0.30 | 22.58 ± 0.22 | 3.26 ± 0.19 | 31.04 ± 4.64 |

[1]The values are means ± SEM, n = 12 replicate pens with 8 birds per pen selected for processing.
[2]L* is a measure of the difference in lightness and darkness with lightness registering as a more positive number.
[3]a* is a measure of the difference in red and green, with redness registering as a more positive number.
[4]b* is a measure of the difference in yellow and blue, with yellowness registering as a more positive number.

# Chapter 11

## Discussion

Because of the wide variety of essential oils being used as dietary additives in broiler research, contradicting evidence exists in regard to dietary essential oil impact on carcass characteristics. Some research suggests that essential oils increase dressing %, front part %, breast meat %, carcass meat %, gizzard weight % and intestinal diameter, as well as decrease abdominal fat % (Awaad et al., 2014). Other findings have shown that supplementation of a combination of two herb extracts had no effect on carcass characteristics (Hernandez et al., 2004; Sarica et al., 2005), and even others suggest an improvement in chicken carcass but no effect on abdominal fat percentage (Alçiçek et al., 2003). Broilers raised in this study were performing above the Cobb 500 growth curve (Cobb-Vantress, 2013) and were raised in optimal environmental conditions, which included fresh litter. BioStrong had significantly lower body weight and body weight gain than Essential Oil X from 0 to 14 days of age most likely due to immune-stimulating effects of essential oils found in BioStrong. Because the broilers used in this study were raised in an ideal environment, these immune-stimulating effects may have caused a decreased response in the BioStrong treatment group because of energy being redirected from growth to the immune system in the BioStrong treatment group in the first two weeks of growth.

The immune-stimulating effects of BioStrong caused a decreased growth response in broilers found in that treatment group under ideal environmental conditions. However, other

studies have shown that the specific combination of essential oils in BioStrong has no effect on body weight and feed intake in broilers (Amad et al., 2013; Jang et al., 2007; Erdogan et al., 2010). They may have not seen an effect of BioStrong due to the environmental conditions used in the studies. In less ideal litter conditions, which are not uncommonly found in commercial broiler houses, BioStrong's immune stimulation may be beneficial to fight off any infections. Furthermore, the use of BioStrong's specific essential oils could be used to slow the growth rate during the initial phase under optimal conditions, which would decrease the incidence of muscle myopathies that occurs as a result of fast growth. This conclusion can be supported by the fact that *pectoralis major* and total white meat from the BioStrong treatment group also had significantly lower weights on Day 43.

The specific combination of essential oils in Xtract has been shown to have a positive impact on poultry production due to its antioxidant activity (Botsoglou et al., 2002; 2004), which enhances digestibility by stimulating endogenous enzyme activity and facilitating nitrogen absorption (Gill, 2001), and/or due to its anti-microbial activities (Kollanoor-Johny et al., 2012; Azaz et al., 2002; Dorman and Deans, 2000). It has also been reported that broilers fed with an herb extract had higher breast weight when compared to the control group (Jamroz and Kamel, 2002). These previously reported findings, in addition to BioStrong's immune-stimulating effects, may be the reason that live weight and hot, and chilled, carcass weights were significantly lowered in BioStrong when compared to Xtract on Day 43. Since Xtract produced higher live, chilled carcass, and hot carcass weights, it is reasonable that it also produced a higher *pectoralis major* and total white meat weight. Moreover, the findings from the above studies support the significantly higher *pectoralis major* and total white meat weights in the Xtract treatment group.

Wings as a percent of live fasted weight was significantly higher in the Essential Oil X group than in the Xtract group. Since these two treatments had two similar essential oils added to their diet, thymol and carvacrol (both from thyme) and eugenol and cinnamaldehyde (both from cinnamon), this difference can most likely be attributed to the presence of piperine in Essential Oil X and/or the presence of capsicum oleoresin in Xtract. Color, percent moisture, percent protein, percent fat, and malondialdehyde concentration were assessed to determine if any of the essential oil treatments had negative impacts on breast meat. These parameters were not significantly different between the four treatments in this study. Malondialdehyde concentration was not significantly different in any of the treatments most likely due to the low amount of essential oils added to the broiler diet because essential oils have been previously shown to delay oxidation in stored meat (Lopez-Bote et al., 1998; Govaris et al., 2007; Spernakova et al., 2007; Young et al., 2003). Additionally, it has been shown that adding 200 ppm of essential oils from thyme and cinnamon to broiler diets significantly improved BW gain and FCR during a 6-week growth period (Al-Kassie, 2009). Jamroz et al. (2005), has also consistently reported improved FCR due to the presence of cinnamaldehyde, carvacrol, and capsaicin at 40,000 ppm, which is a much higher level than the 100 ppm added during this research. Varying combinations of essential oils must be studied to find the one that has the most positive impact on broiler production. Once this specific combination of essential oils is determined, the broiler industry will be one step closer to finding a viable and cost-effective alternative to antibiotic growth promoters.

## Chapter 12

## References


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