A COMPARISON OF COCCIDIOSIS CONTROL METHODS AND THE EFFECTS OF MANAGEMENT PRACTICES ON BROILERS

by

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(Under the Direction of Todd J. Applegate)

ABSTRACT

One of the costliest diseases to affect the poultry industry is coccidiosis. *Eimeria* parasites (coccidia) can cause poor growth performance and increased feed conversion in broilers. A series of three experiments were conducted to evaluate the effects that management practices have on the efficacy of coccidiosis control methods commonly used in commercial broilers. Experiment 1 showed that there were no advantages to using a bioshuttle program compared to either a live coccidiosis vaccine or an ionophore program when raising broilers to 49 days. In Experiment 2, the use of dietary salinomycin and a live coccidiosis vaccine showed similar results on performance parameters and that the live coccidiosis vaccine provided better protection against *Eimeria maxima*. The effects that environmental temperature and dietary protein concentrations have on broilers given a live coccidiosis vaccine, at day of hatch, was observed in Experiment 3. In conclusion, management practices can affect coccidiosis control methods used in broilers.

INDEX WORDS: coccidiosis, bioshuttle, vaccine, broiler,

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BS, University of Georgia, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

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May 2018

ACKNOWLEDGEMENTS

Throughout the process of completing my master's degree there are many people that deserve thanks. First and foremost, I would like to thank my wonderful mother, without the many sacrifices you have made for me I would have never made it this far and would not be the man I am today. I would also like to thank my brother Matthew, many times in my life you have been the father figure I needed and have always pushed me to excel in everything I do. To Dr. Manuel Da Costa, you have been a great friend and mentor through this process and I owe it all to you. If you had not put your neck on the line and urged Dr. Pesti to take me as a student I would be riding around in a white truck as a broiler tech. I would sincerely like to thank Dr. Gene Pesti for taking me as one of his final master's students. Your love for teaching and extensive knowledge of the poultry industry and statistics will forever have an impact on my life. I hope you enjoy retirement and try to not work too hard. A special thank you goes out to my committee members, the knowledge that you all have shared with me will be of great value as I work on my next degree. I would like to thank Dr. Hector Cervantes and Dr. Ivan Alvarado, I could never thank you two enough for the knowledge and opportunities that you to have given me. I could not ask for two better mentors and look forward to our continued friendship throughout time. Last and definitely not least I would like to thank Laura McLear, without you I would be lost and constantly in trouble. Your advice and help with all that I do will never be forgotten. I look forward to the rest of our lives together and may our love only grow stronger as time endures.

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CHAPTER 1

INTRODUCTION

The prevention and treatment of coccidiosis is an ongoing battle in the poultry industry and, due to the nature of the *Eimeria* parasite, it will continue to be an area of importance to the commercial poultry industry. The use of anticoccidial drugs and coccidiosis vaccines have been the main means of prevention and treatment of coccidiosis in the poultry industry. However, due to recent consumer perception of the use of drugs and antibiotics in animal agriculture, the amount of available anticoccidials for use in poultry has been on the decline. With a decrease in the amount of anticoccidials available and no sight of any new ones coming on to the market, the importance of proper coccidiosis control methods is growing. From seeing how important coccidiosis control methods are, the objectives of this study were developed.

A series of three experiments were conducted in order to evaluate the effects of management practices and the interactions that they have on the use of coccidiosis control methods. These included:

1. A comparison of coccidiosis control programs at standard or warm environmental temperatures in commercial broilers.
2. A comparison of coccidiosis vaccine and salinomycin on the progeny of young or old breeder flocks, and the effects of reduced dietary energy levels in finisher and withdrawal feeds.
3. To evaluate the interactions of environmental temperature and dietary protein concentrations with coccidiosis vaccination.

CHAPTER 2

LITERATURE REVIEW

COCCIDIOSIS

Coccidiosis is a disease caused by an intestinal parasite known as coccidia. Coccidia are protozoan parasites that are members of the species of genus *Eimeria* (Reid, 1990; Shirley, 1992; Chapman, 2003; Chapman, 2014). *Eimeria* can be found in practically every poultry house in the United States and are very important to the production of commercial poultry. The economic losses that *Eimeria* have on the poultry industry are substantial with an estimated annual cost of 3 billion dollars worldwide (McDonald and Shirley, 2009). These expenses include loss of body weight, increased feed conversion, mortality loss, and the preventative measures that are taken to prevent coccidiosis. Due to the way modern commercial poultry are raised, in a closed house system, the disease caused by this parasite will continue to play a significant role in the production of poultry. Knowing the substantial effects this parasite has on the poultry industry, it is important to understand as much as possible about *Eimeria spp*. in order to be able to appropriately prevent and treat coccidiosis.

SPECIES

In 1891, the first species of chicken coccidia was discovered during the examination of chicken ceca by Raillet and Lucet (Raillet and Lucet, 1891a; Raillet and Lucet, 1891b; Reid, 1990; Chapman, 2003; Chapman, 2014). The species that Raillet and Lucet discovered then is known today as *Eimeria tenella* (Reid, 1990; Chapman, 2003). Almost 40 years later, Tyzzer

published work on the isolation of 3 new chicken *Eimeria* species: *E. acervulina*, *E. maxima*, and *E. mitis* (Tyzzer, 1929; Reid, 1990; Chapman, 2003; Chapman, 2014). Shortly after Tyzzer's findings, W.T. Johnson confirmed the observations of the three species that Tyzzer had found, along with describing two new *Eimeria* species, *E. necatrix* and *E. praecox* (Johnson, 1930; Reid, 1990; Chapman, 2003). *E. hagani* and *E. brunetti* were described in 1938 and 1942, respectively, by P.P. Levine (Levine, 1938; Levine, 1942.; Reid, 1990; Chapman, 2003). Though *E. hagani* has been described by Levine, no other researchers have been able to isolate it since Levine's description, therefore some question the validity of the species. The last species of *Eimeria*, affecting chickens, to be proposed is *E. mivati*, this species was discovered by Edgar and Seibold in 1964 at Auburn University (Edgar and Seibold, 1964; Reid, 1990). Since the discovery of *E. mivati*, P.L. Long has questioned if it is a different species or a variance of *E. acervulina* and M.W. Shirley has proposed that it could be a cross between *E. acervulina* and *E. mitis* (Long, 1973; Shirley, et al., 1983). Of the nine species of *Eimeria*, affecting chickes, discovered there are seven: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*, that have been officially defined and well accepted as individual *Eimeria* species, therefore in this thesis only those seven will be discussed.

CHARACTERISTICS

Eimeria are a self-limiting species that rely on host for development and replication. The replication of these parasites has been observed to be similar across all seven of the main *Eimeria* species and are host specific to chickens (*Gallus gallus domesticus*). Outside of the bird, coccidia are in the oocyst stage of their life cycle, oocysts are single cells that have a double layer outer membrane. When in the right conditions *Eimeria* oocyst will sporulate to produce

sporozoites (McDougald, et al., 2013; Chapman, 2014). There are many sources of where the birds come in contact with oocysts including drinking water, feed, litter, and feces. Due to chicken's coprophagic tendencies, the main source of oocysts comes from feces and litter (Kheysin and Todd, 2013). The life cycles of these parasites are around six to seven days long depending on the species. *Eimeria* life cycles consist of at least two, and up to four, cycles of asexual reproduction, known as schizogony, which is then followed by a single cycle of sexual reproduction, known as gameteogony (Conway and McKenzie, 2007). The beginning of infection in a bird, starts with the ingestion of sporulated oocysts that contain sporocyst. Once ingested the sporocyst containing oocysts are broken open in the gizzard and each of the four sporocyst release two sporozoites. The sporozoites then enter the mucosa cells that are in the intestinal lining where they will begin asexual reproduction. After asexual reproduction the *Eimeria* will undergo one sexual reproductive cycle. From this sexual reproductive cycle an oocyst is formed and then shed through the feces of the bird into the environment. Once in the litter and feces of the poultry house the oocyst will sporulate and become infective again if the environmental conditions are right. The first and second cycle through birds tend to be milder, but due to how fast coccidia replicate, the following cycle tends to cause more severe infections from the increased number of *Eimeria* that are being ingested.

There is no cross immunity between species of *Eimeria*, therefore infections can be observed during different ages of a flock based on which species are present (McDougald, et al., 2013). When evaluating flocks for coccidiosis there are little to no differential lesions that can be observed from the birds externally. Typical clinical signs that can be observed from birds, without conducting necropsies, include: diarrhea or feces containing blood, bleaching of the shanks and skin, mortality, and the biggest effect that coccidiosis has on poultry is reduced body

weights and poor feed efficiency. In an estimation of economic losses based on coccidiosis conducted for the UK, forty-six percent of the loss of profits were from decreases in body weight while thirty-four percent of the loss of profits were believed to be from reduced feed efficiency (Williams, 2005). These losses in body weight and feed efficiency that are observed are usually accounted for by reduced feed intake as well as poor feed absorption. It has been shown in previous research that in the presence of a coccidiosis infection an estimated seventy percent of reduced body weight that was observed was attributed to reduced feed intake while the other thirty percent was due to malabsorption (Preston-Mafham and Sykes, 1970; Williams, 2005). Other authors have evaluated different nutrients that can be effected due to malabsorption with a relatively large amount of work being based on amino-acid malabsorption (Preston-Mafham and Sykes, 1970; Ruff, 1974; Joyner, et al., 1975; Patterson, et al., 1975; Pesti and Combs, 1976; Ruff, et al., 1976; Williams, 2005; Rochell, et al., 2016b). Not only has malabsorption been observed, but there has also been work showing decreases in protein digestion during coccidiosis infection (Turk, 1972).

When defining species of *Eimeria* there are three key elements that are taken into consideration: macroscopic lesions, microscopic characteristics, and life cycle characteristics. Under macroscopic lesions the two main components that are taken into consideration are the main locations of infection within the host's intestinal tract, that the parasite manifest, and the distinct lesions that are observed in these areas during an infection. The main microscopic characteristics that are looked at include the size and the shape of the oocyst and/or the schizont stage of the parasite, along with which specific tissues the parasite targets and infiltrates. The life cycle is also taken into consideration when determining species of *Eimeria*; the two main time

frames that are of interest are the minimum prepatent period and the minimum sporulation time (McDougald, et al., 2013).

The individual species of *Eimeria* have their own distinct characteristics as well as macroscopic lesions that can be observed. These lesions are, for the most part, species specific to certain regions of the intestinal tract. *E. acervulina* and *E. praecox* generally infect the duodenum and can reach down into the upper jejunum. The main regions of the gut that *E. maxima* and *E. necatrix* infect are the jejunum and ileum, while *E. mitis* mainly infects the ileum. In *E. brunetti* infections, lesions can be seen in the ileum and large intestines while *E. tenella* are generally only found in the ceca. The locations stated above are where these species usually target and infiltrate the intestinal cells, however in severe cases lesions can be found in other areas of the intestinal tract that are not common for that species. Of the seven coccidia species, that affect poultry, *E. acervulina*, *E. maxima*, and *E. tenella* are the most common species seen in the field (De Gussem, 2007). Each of these species has distinct lesions that can be observed macroscopically and confirmed microscopically. The main *E. acervulina* lesion is characterized by white plaques that can be found in the duodenum and upper jejunum. Thickened intestinal mucosa, loss of pigmentation, wet/watery feces, and in severe cases depression in body weight and increases in feed conversion can be observed in flocks infected with *E. acervulina*.
E. maxima causes very distinct petechial hemorrhaging in the jejunum and ileum. This species can cause diarrhea, watery gut contents, decreased body weights, increases in feed conversion, pigmentation loss, and in severe cases death. With an *E. tenella* infection the main lesion that is seen is bloody ceca contents or bloody ceca cores; this occurs due to the effects that *E. tenella* has on the blood vessels in this region. In birds, infected with this species, bloody feces can be observed along with weight loss and mortality. Of the *Eimeria* species, *E. tenella* is the most

easily recognized and is considered to be the most pathogenic species to infect chickens (McDougald, et al., 2013).

As mentioned earlier, microscopic characteristics are key considerations when determining different species of *Eimeria*. The size and shape of the oocyst of *Eimeria* can help when distinguishing between the different species that are present. *E. acervulina*, *E. maxima*, and *E. tenella* oocysts are all ovoid in shape, however the sizes of the oocysts differ significantly. *E. acervulina* oocyst is the smallest of the three species and is reported to have an average length by width of 18.3x14.6 microns. In contrast, *E. maxima* has the largest oocyst of the three species, and the largest of all of the *Eimeria* species, at an average size of 30.5x18.8 microns. *E. tenella* oocysts are intermediate in size of *E. acervulina* and *E. maxima* at 22.0x19.0 microns in size. These three species of *Eimeria* have certain tissue types in the intestinal tract that they specifically target. For *E. acervulina*, the epithelial tissue is targeted by the parasite, whereas *E. maxima* and *E. tenella* target the subepithelial tissue in the intestinal tract. The key characteristics mentioned above can be used in differentiating *Eimeria* species microscopically and are useful when used in conjunction with the other characteristics to determine which species of *Eimeria* might be present in poultry populations.

The two main timeframes in the life cycle of coccidia are the minimum prepatent period and the minimum sporulation time. These two time frames are recorded in hours and can help with differentiating species when used along with macroscopic lesions and microscopic characteristics. The prepatent period is determined by the time that it takes for an oocyst to be shed in the feces from the point of ingestion. *E. maxima* has been recorded with a minimum prepatent period of 121 hours, which is the longest of the three main species of *Eimeria*. With a prepatent period of 115 hours, *E. tenella* has a slightly shorter detection time when compared to

E. maxima. Of the three main species of *Eimeria*, *E. acervulina* has the shortest prepatent period at 97 hours. Sporulation time is very important when looking at *Eimeria* because the shorter the sporulation period, the faster the oocysts become infective to the birds. *E. maxima* and *E. tenella* have relatively short minimum sporulation times, 17 and 18 hours respectively, when compared to that of *E. tenella* which has a minimum sporulation time of 30 hours.

COCCIDIOSIS CONTROL METHODS

There are two main coccidiosis control methods that are used, in the commercial poultry industry, to prevent and control coccidiosis:

1) Anticoccidial drugs

2) Coccidiosis vaccines

Anticoccidial Drugs Over the years one of the most common ways to prevent coccidiosis was through the use of anticoccidial drugs. Anticoccidial drugs are administered in the diets or drinking water of poultry at optimal inclusion rates specific to each drug (Mathis, et al., 2004). These drugs are either described as being coccidiostatic or coccidiocidal, and some may be described as being both coccidiostatic and coccidiocidal. Drugs that are described as being coccidiostatic, suppress growth of the parasite while the drug is present, but once withdrawn, the parasite is allowed to grow and reproduce freely. Those that are considered coccidiocidal cause damage and death to the parasite which makes them not viable for infection. Most sulphonamides are known as having coccidiostatic properties. Nicarbazin is an example of an anticoccidial drug that is considered to have coccidiocidal properties, while robenidine and halofuginone are considered to have coccidiostatic and coccidiocidal properties (Kant, et al.,

2013). It is important to note when using anticoccidial drugs some of the products require a pre-slaughter withdrawal period while others have no withdrawal requirements and can be administered until slaughter. These withdrawal periods are usually set based on how long residuals of the drugs can be detected at pre-determined concentrations in the meat or fat (Duquette, 2005).

Under the category of anticoccidial drugs, there are three types of products that are available, the first of which are synthetic chemotherapy drugs. These drugs are commonly referred to as "chemicals" (amprolium, diclazuril, nicarbazin, etc.). Synthetic drugs work by interfering with the synthesis of co-factors that are important for metabolism within the parasites. Of the synthetic drugs, one of the most influential has been nicarbazin. The use of nicarbazin as an anticoccidial was first shown by Cuckler (Cuckler, et al., 1955; Reid, 1990), since then, Morrison, et al. (1961) has shown the effectiveness of nicarbazin for the prevention of coccidiosis. This synthetic anticoccidial has become very popular due to its effectiveness in the commercial industry along with the reduced rate at which drug resistance is developed by the coccidia to this drug (Conway and McKenzie, 2007). Nicarbazin is best used during the winter and on birds under the age of 28 d, due to the potential of nicarbazin to make birds more susceptible to heat stress at older ages (Buys and Rasmussen, 1978; McDougald and McQuistion, 1980; Keshavarz and McDougald, 1981).

The second class of anticoccidial drugs are known as polyether ionophores antibiotics or more commonly called ionophores. These drugs are by-products from the fermentation of species of *Streptomyces* or *Actinomadura* (Conway and McKenzie, 2007). Ionophores interfere with the transport of ions, altering the membrane gradient within the parasite, which ultimately leads to the parasite's death from osmotic lysis (Shumard and Callender, 1967; Burger, et al.,

1997; Peek and Landman, 2011). All of the ionophores have the same mode of action, however the ions that they effect can be differ between the drugs. Within the ionophores there are three classes that are based on the type of ions in which they affect (1999b; Chapman, 1999a; Allen and Fetterer, 2002; Peek and Landman, 2011):

1) Divalent ionophores (lasalocid)

2) Monocarboxylic ionophores (monensin, narasin, and salinomycin)

3) Monoglycosidic ionophores (maduramicin and semduramicin)

Of the ionophores, the monocarboxylic ionophores are most commonly used; of those, monensin is one of the more popular drugs. Monensin was discovered in 1967 and is a by-product of *Streptomyces cinnamonensis* (Agtarap, et al., 1967; Haney and Hoehn, 1967). It has been one of the most used products since it has come on the market (Chapman, et al., 2010). One of the reasons why monensin has continued to do well is the ability of broilers to develop natural immunity to coccidia while being treated with monensin (Chapman, 1978). Another popular monocarboxylic ionophore that is used in the commercial poultry industry is salinomycin. Salinomycin came on the market for commercial use in the early 1980's and is a by-product from the fermentation of *Streptomyces albus.* It has been shown to be as effective as monensin and potentially even more effective for the prevention of coccidia (McDougald, 1981). Due to the effectiveness of this product, salinomycin has become one of the most popular anticoccidials used today. Along with the two ionophores mentioned above, narasin is another common monocarboxylic ionophore that is used in the commercial poultry industry. Narasin is a by-product of the fermentation of *Streptomyces aureofaciens*, and was originally described in 1977 (Berg and Hamill, 1978; Conway and McKenzie, 2007). The efficacy of narasin as an

anticoccidial was first described in 1979 and later confirmed in 1980 and 1988 (Ruff, et al., 1979; Ruff, et al., 1980; Jeffers, et al., 1988; Conway and McKenzie, 2007).

All three of the previously mentioned ionophores, when present in the diet, were shown to inhibit *E. tenella* sporozoites from entering intestinal cells when the ionophore was not present within the actual cells, these observations propose that the ionophores have more of an effect on the parasite extracellular (Smith, et al., 1981). Ionophores have become popular due to resistance not being developed as fast as that of chemicals (Jeffers, 1978a; Jeffers, 1978b; Jeffers, 1981; Chapman, 1983; Chapman, 1984a; Chapman, 1984b; Augustine, et al., 1987; Jeffers, 1989; Bafundo and Jeffers, 1990; Conway and McKenzie, 2007; Chapman, et al., 2010). Another advantage that has been observed when using ionophores, is that they do not provide complete protection from the coccidia creating what is called "leakage" cycling of low numbers of coccidia, this allows for the birds to develop immunity to the *Eimeria* species while having minimal, if any, losses to performance parameters (Jeffers, 1989; Chapman and Hacker, 1993; Chapman, 1999a; Conway and McKenzie, 2007; Peek and Landman, 2011). Compensatory growth has also been seen as an advantage when using ionophores, it has been observed that when ionophores have been withdrawn from diets, the birds have an increase in body weight gain for up to seven days, however this could be strictly due to increased feed intake when the drugs are removed (McDougald, 1980; McDougald and McQuistion, 1980; Metzler, et al., 1987a; Conway and McKenzie, 2007; Chapman, et al., 2010). There have been observations that some ionophores can also help prevent cases of necrotic enteritis due to having antibacterial properties against *Clostridium perfringens*, which is the gram-positive bacteria that causes necrotic enteritis (Shumard and Callender, 1967; Dutta and Devriese, 1984; Williams, 2005).

The last of the anticoccidial drugs is a combination of synthetic anticoccidials and ionophores. As of now there is only one product that has been approved for use in commercial broilers and it is a combination of narasin and nicarbazin. This product was developed in the 1980's and was believed to be beneficial due to having both modes of action that ionophores and chemicals have (Conway and McKenzie, 2007). Combining narasin and nicarbazin has shown to be effective in preventing coccidiosis, however there have been questions raised about whether or not the heat stress effects from the nicarbazin would still be present. There have been contradicting results comparing the effects of heat stress when using a narasin/nicarbazin blend. Long, et al. (1988), showed in their work that a 50:50 blend of narasin/nicarbazin (25 g/kg each) reduced the heat stress effects that were usually seen when using nicarbazin alone. These results differed from those of Wiernusz and Teeter (1991), in which the use of narasin mixed with nicarbazin did not alleviate the heat stress effects that can be seen when using nicarbazin.

Coccidiosis Vaccines The second coccidiosis control method that has become widely used in the poultry industry is the use of live coccidiosis vaccines. Live vaccines are made up of oocysts that have been sporulated to be infective to the birds (Peek and Landman, 2011). These vaccines are most commonly given in the hatchery in the form of a spray or gel that is ingested orally during preening. A third method of application that is used is by in-ovo application when the eggs are being transferred from the incubators to the hatchers. The first commercially available coccidiosis vaccine was developed by S. A. Edgar and put on the market in 1952 (Edgar and King, 1952); sixty-five years later coccidiosis vaccines are still being successfully used in the poultry industry and continue to be one of the most used coccidiosis control methods. *Eimeria* strains that are used in some of the live vaccines pre-date the availability of in-feed

anticoccidials; therefore they are sensitive to the anticoccidial drugs on the market, other strains that are used have been chosen for their lower pathogenicity's and sensitivities to the anticoccidial drugs. When using vaccines, birds develop immunity through multiple cycles of infection from the parasites (Reid, 1990). Due to the inability for cross protection between species, it is important for the vaccines to contain multiple species of *Eimeria* to ensure protection from the strains that might be present in the poultry houses. Previous research has shown that vaccines can provide similar protection to that of anticoccidial drugs when used properly (Danforth, et al., 1997; Danforth, 1998; Williams, et al., 1999; Williams, 2002b; Williams, 2002a; Williams and Gobbi, 2002; Conway and McKenzie, 2007). There has also been research that shows the potential for vaccines to be used to "seed" poultry houses with naïve strains of *Eimeria* in order to help restore sensitivity to drugs that have been over used (Mathis and McDougald, 1989; Chapman, 1994; Peek and Landman, 2003; Mathis and Broussard, 2006; Chapman and Jeffers, 2015).

There are two types of coccidiosis vaccines that are currently available on the market, both of which are live vaccines and are either attenuated or non-attenuated. An attenuated vaccine is one in which the coccidia have been selected for lower virulence. This procedure is usually done by inoculating birds, with the specific *Eimeria* species of interest, and collecting the oocysts from the feces with the shortest prepatent period (those with the fastest passage time) (Long, 1972; Long, 1974; Jeffers, 1975; McDougald and Jeffers, 1976; Shirley and Millard, 1986; Shirley and Bedrník, 1997; Conway and McKenzie, 2007). This provides an *Eimeria* strain that will be in the host for shorter periods of time and will cause less damage to the intestinal tissue (McDonald and Shirley, 1984; Shirley, et al., 1995; Peek and Landman, 2011). The non-attenuated vaccines are widely used and potentially used more than that of the attenuated

vaccines. Non-attenuated vaccines provide good immunity against field strains of *Eimeria* without the worry of resistance, however when the vaccine is cycling in the birds there is a slight decrease in performance parameters that can be seen between 14 and 28 days of age due to the mild infection provide from the vaccine (Chapman, et al., 2002; Lehman, et al., 2009; Rochell, et al., 2016b). One of the biggest issues that is seen when using coccidiosis vaccines is poor uniformity in vaccine administration (Chapman, et al., 2002). When the chicks do not receive a full dose of coccidiosis vaccine then they will not develop adequate immunity and will be more susceptible to future infections. These birds will ultimately have more severe reactions to coccidiosis infections at later stages of their lives. As antibiotic free and organic poultry meat production becomes more popular the use of anticoccidial drugs will decline, and the need for quality coccidiosis vaccines will continue to grow.

DRUG RESISTANCE

One of the biggest issues that accompanies the use of anticoccidial drugs is the ability of the *Eimeria* field strains to develop resistance to the drugs. Testing for drug resistance is done using anticoccidial sensitivity test (AST). The AST test evaluates the level of resistance that field strains have to anticoccidial drugs. This is done by inoculating birds with a set number of oocysts and then providing the anticoccidial of interest to the birds. After a given amount of time, the efficacy of the drug is evaluated by looking at multiple criteria which include: mortality, weight gain, feed conversion ratio, gross lesion scores, fecal scores, and oocyst production (Holdsworth, et al., 2004).

The first case of drug resistance to anticoccidials was observed in the 1950's, and since, some level of resistance has been seen to about every anticoccidial drug on the market

(Chapman, 1997; Chapman and Jeffers, 2015). Drug resistance has become very problematic since the development of new anticoccidial drugs has come to a halt and there is no promise of any future development of anticoccidials for commercial use. When providing anticoccidials in the feed the selection of drug resistance has come hand in hand with the prevention of coccidiosis, therefore optimal concentrations have been set so that protection is provided to the birds while keeping resistance to a minimal. Even though optimal drug concentrations are used, long use of single anticoccidial drugs can lead to drug resistance. Over time, coccidiosis control programs have been developed and used to help aid in preventing significant drug resistance.

COCCIDIOSIS CONTROL PROGRAMS

Throughout the years four main coccidiosis control programs have been used:

1. Straight run
2. Rotation
3. Shuttle
4. Bioshuttle

In the early years of anticoccidial drugs, straight run coccidiosis control programs were very common. In these programs companies would use one anticoccidial drug throughout multiple grow outs. When using anticoccidials in this method the field strains of *Eimeria* start to lose their sensitivity to the anticoccidial drugs and it becomes necessary to switch to other products. Most companies have moved away from this method because it allows the coccidia to develop resistance more rapidly. In previous years, vaccines were mostly used during the warmer months of the year when litter conditions were optimal; since the start of the antibiotic free

movement, some companies have actually gone to using straight run programs, but instead of using anticoccidial drugs they are using coccidiosis vaccines throughout the entire year.

A very common coccidiosis control program that is used in the commercial poultry industry is called a rotation program. In rotation programs, an anticoccidial drug will be used for a flock or two and then a new drug or vaccine will be used, rotating between products throughout the year (McDougald, et al., 2013). Rotation programs can be very beneficial because they allow companies to tailor their control program to each anticoccidial product's strength. For instance, companies might prefer to use nicarbazin in the winter, which has been shown to make birds more susceptible to heat stress during warmer months, and then in the warmer months switch to a coccidiosis vaccine (Chapman, et al., 2010). This program is beneficial because it allows producers to switch between products with different modes of action, ultimately helping keep the development of drug resistance to a minimal (Chapman and Jeffers, 2015).

Another common coccidiosis control program that is being used in the poultry industry is called a shuttle program. Since commercial broilers are given multiple diets during the grow-out phase, it has become a common practice to give either a synthetic drug (chemical) or ionophore in the starter diet and then for the following diets switch to a different synthetic drug or ionophore (Chapman, 1999a; McDougald, et al., 2013; Chapman and Jeffers, 2015). One big benefit to this method is that you can use a drug like nicarbazin in the starter feed, which should not be used past 28 days of age, and then another drug like salinomycin in the sequential feeds. These programs can be beneficial when trying to develop immunity to coccidia while preventing coccidiosis.

Bioshuttle programs are relatively new coccidiosis control programs that are being used in the commercial poultry industry. These programs are unique in the manner that an coccidiosis

vaccine is provided at day of hatch and then is followed by the use of an in-feed anticoccidial drug after the starter diet (Mathis, 2017). The main goal when using a bioshuttle program is that it allows the chickens to develop the immunity to coccidia from the vaccine, but it reduces the negative effects on performance that can be seen when the vaccine is cycling. When using this method, it is important that the anticoccidials are not put in the starter diets, to allow cycling of the vaccine strains, and to use drugs that allow "leakage" so that the vaccine strains will be able to continue to work once the drug has been added to the feed.

MANAGEMENT PRACTICES

Another important area for the prevention of coccidiosis is maintaining proper rearing practices, this includes: proper temperature, ventilation, litter conditions, bird density, maintaining equipment, lighting programs, biosecurity, and feed (Peek and Landman, 2011). One of the most basic principles when rearing poultry is maintaining the proper temperatures throughout the grow-out period. If optimal temperatures are not used, problems can occur such as too little or too much feed and water consumption. For instance, if the rearing temperatures are too warm when using an in-feed anticoccidial, the birds will consume less feed and ultimately receive a lower dose of the anticoccidial. When this occurs the birds will not receive proper protection against the coccidia and there is potential for the coccidia to cause coccidiosis (Conway and McKenzie, 2007). Ventilation comes hand in hand with proper housing temperatures and can either make or break a flock. The main goal in ventilating poultry houses is maintaining adequate gaseous exchange; this can become very important when managing birds in the colder months of the year. Using minimum ventilation allows problems such as wet litter to occur. It has been shown that increased moisture in poultry house litter can lead to improved

sporulation of oocysts, providing a friendlier environment for coccidia to thrive in, as well as being more beneficial to microorganisms such as *Clostridium perfringens* (Chapman and Johnson, 1992; Waldenstedt, et al., 2001; Williams, 2005; Conway and McKenzie, 2007). Increased litter moisture does not only occur when poor ventilation is used, but it can also be seen when there are nutritional problems, like too much salt in the diet, or when drinker lines are not maintained properly, such as leaky nipples or excessive water pressure. Higher levels of ammonia from the litter can be problematic when using coccidiosis vaccines. Ammonia has been shown to reduce oocyst survival rates; therefore, in high levels it can interfere with the efficacy of coccidiosis vaccines (Reyna, et al., 1983). Bird density is very important when trying to manage poultry flocks for the prevention of disease outbreaks and can become an area of conflict when working with integrators. In today's poultry industry, high stocking density numbers are preferred due to the ability to raise increased numbers of birds in a given area; this can become problematic when trying to prevent disease. When birds are overcrowded the number of coccidia oocysts being shed to the litter is higher and due to the coprophagic nature of poultry, the birds will eat the litter, receiving higher numbers of oocysts which can lead to outbreaks of coccidiosis (Williams, et al., 2000).

NUTRITION

Nutrition is an area in which special considerations should be taken when trying to prevent coccidiosis. Proper nutrition is part of the foundation for growing healthy and disease-free birds. Since *Eimeria* are intestinal parasites it is very important to maintain the gut health and integrity of poultry. As mentioned above, coccidiosis has an effect on the digestion and utilization of dietary amino acids. In previous work, researchers have documented the ability to

improve the development and immunity of broilers' intestinal tracts using increased levels of dietary amino acids (Tan, et al., 2014a; Tan, et al., 2014b; Gottardo, et al., 2016; Rochell, et al., 2016a; Bortoluzzi, et al., 2017). These studies show the potential to increase dietary amino acids levels to help counteract malabsorption that can occur during intestinal infections. Rochell, et al. (2016b), showed that in the presence of *E. acervulina* challenge that amino acid digestibility as well as amino acid metabolism was reduced. Studies have also shown that there is not only a decrease in amino acid digestibility, but also reduced metabolizable energy in birds infected with coccidia (Persia, et al., 2006a; Amerah and Ravindran, 2015).

Amerah and Ravindran (2015), showed that the supplementation of dietary betaine helped counteract the negative effects of coccidiosis along with improving the nutrient digestibility of other nutrients in the diet. The use of dietary supplements such as probiotics, prebiotics, essential oils, and plant extracts have become popular since the antibiotic free movement has gained ground (Peek and Landman, 2011). Some of these products have shown promise of being effective against *Eimeria*, however none of these products have shown to be as effective as the current anticoccidial drugs on the market (Habibi, et al., 2016). Other problems that arise when using these products is that there are little to no regulations on these supplements and there are potential undocumented effects that can occur in flock performance or health.

Interactions between *Eimeria* and certain feed ingredients have also been recorded in the past. It has been observed that when birds are fed diets that are wheat-based, rather than corn-based diets, *E. tenella* is more pathogenic (Williams, 2005; Conway and McKenzie, 2007). In a study conducted by Persia, et al. (2006a), looking at the interactions between dietary ingredients and *E. acervulina* infection, they showed that the inclusion of fish meal at 15% or a prebiotic supplement at 5% were able to alleviate the negative effects on growth due to coccidiosis

infection. In said study, they also showed that, in the presence of coccidiosis, a wheat-barley-pectin based diet caused losses in body weight gain, amino acid digestibility, and metabolizable energy when compared to a corn-soybean based diet. This observation is believed to be due to the amount of non-starch polysaccharides that are at higher levels in wheat when compared to that of corn. From these studies it can be concluded that diet composition can play a major role in the intestinal health of broilers in the presence of coccidiosis challenge.

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CHAPTER 3

COMPARISONS OF COCCIDIOSIS CONTROL PROGRAMS AT STANDARD OR WARM ENVIRONMENTAL TEMPERATURES IN COMMERCIAL BROILERS

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ABSTRACT

A bioshuttle program is the use of coccidiosis vaccinne at day of hatch and then the addition of an anticoccidial in the diet. Although bioshuttle programs are commonly used in the industry, there is little to no research showing their effects. The objective of this work was to evaluate the efficacy of three common bioshuttle programs compared to a straight vaccine program and a straight ionophore program at warm and standard environmental temperatures (T). The experiment was designed in a 2x6 factorial arrangement with 2 environmental T and 6 coccidiosis control methods; CM1: coccidiosis vaccine (VAC) at d of hatch and no dietary anticoccidial; CM2: VAC with dietary avilamycin (AV) 0-21 d; CM3: VAC, AV (0-21 d), and narasin (NAR) 22-41 d; CM4: no VAC and NAR from 0-41 d; CM5: VAC, AV (0-21 d), and salinomycin (SAL) 22-41 d; CM6: VAC, AV(0-21 d), and monensin (MON) 22-41 d. Day-old male chicks were distributed into 96 pens with 8 pens of 20 birds each per treatment (12 treatments). The study evaluated two T: standard (ST) (breeder guidelines) and warm (W) (3°C warmer after 6 d) (Cobb-Vantress, 2012). Data were analyzed using split plot analysis and Duncan's New Multiple Range Test for evaluating means with $P \leq 0.05$. At 14, 28, 42, and 49 d, body weight (BW) and feed conversion ratio (FCR) were measured. On 28 d, 1 bird per pen was scored for *E. acervulina* and *E. tenella* gross lesions, and *E. maxima* micro scores. Significant ($P = 0.001$) effects were observed on *E. maxima* micro scores with the warm treatment group having lower scores (0.0 vs. 0.208). Throughout the study, significant T effects were seen on BW ($P < 0.001$) and FCR ($P < 0.03$) and mortality after 13 d ($P < 0.005$). No T effects ($P > 0.05$) were observed on 0-13 d mortality, *E. acervulinum*, *E. tenella* scores, or woody breast incidence. CM4 birds showed significantly ($P < 0.05$) better 0-49 d FCR than CM1 (1.881), CM2 (1.884), and CM6 (1.861). The CM4 birds had significantly ($P < 0.05$) different white striping scores

than CM1 (1.88 vs. 2.38). The narasin only treatment provided the lowest mortality when compared to the other coccidiosis control methods. On average birds that received coccidiosis vaccine had higher white striping scores when compared to their counterparts. These results show that the effects on white striping can be marginalized when AV is in the starter and grower diets.

INTRODUCTION

Coccidiosis is one of the costliest diseases affecting the poultry industry, with an impact of over three billion dollars annually worldwide (Dalloul and Lillehoj, 2006). With such a substantial economic impact and no new anticoccidial products entering the market for use in commercial poultry, companies are resorting to non-traditional methods of controlling coccidiosis. One method that is being used in the industry today is known as a bioshuttle program. A bioshuttle program is the use of a coccidiosis vaccination (VAC) at the day of hatch, followed by supplementing an anticoccidial in the feed. Bioshuttle programs were first used in commercial broiler flocks in Mexico before being brought to the United States (Cervantes, 2017). Research has shown that there is a depression in BW of birds that are vaccinated for coccidiosis, in response to the mild infection that vaccines provide (Lehman, et al., 2009; Lee, et al., 2011). Bioshuttle programs have been proven to be successful at preventing coccidiosis and are believed to lower the adverse effects of using non-attenuated coccidiosis vaccines in commercial settings (Chapman, 1999a).

Prior to bioshuttle programs, common anticoccidial programs consisted of either using coccidiosis vaccination at day of hatch, a single synthetic drug or ionophore in the feed, or a shuttle for which either an ionophore or chemical was given in the starter feed and then a

different ionophore or chemical was administered in the subsequent diets (Chapman, 1999a; Chapman, et al., 2010; Cervantes, 2017). Bioshuttle programs have become popular from their ability to tailor the coccidiosis control program to the challenges that are presented in the field. However, some level of drug resistance has developed for anticoccidials on the market due to how long they have been used (Chapman, 1997; Chapman and Jeffers, 2015).

Previous work interactions between Nicarbazin and temperature (McDougald and McQuistion, 1980; Keshavarz and McDougald, 1981; Da Costa, et al., 2017). Da Costa, et al. (2017) showed when comparing coccidiosis gross lesion scores, birds reared at reduced temperatures had more severe lesions. From the interactions observed in previous work, it was clear that the interactions temperature had on other anticoccidials and coccidiosis vaccines needed to be investigated.

Since few anticoccidials are available, with no promise of new ones entering the market, it is important to understand what techniques can be used so that anticoccidials can continue to be effective. Though commonly used, few, if any, scientific experiments document the effects of bioshuttle programs or their benefits in poultry production. The objective of this work was to evaluate the efficacy of three common bioshuttle programs compared to a straight vaccine program and a straight ionophore program at warm and standard environmental temperatures.

MATERIALS AND METHODS

Birds and Husbandry

This experiment was conducted at the University of Georgia's Poultry Research Center and all management practices were approved by the Institutional Animal Care and Use Committee of the University of Georgia. Two windowless rooms containing 48 pens each were

used for a total of 96 pens in this experiment. A total of 1,920 one day-old male Cobb 500 chicks were raised to 49 d of age. Each pen housed 20 birds with the dimensions of 1.22 x 1.52 m, providing a stocking density of 0.093 sq. meters per bird. Each pen contained 5 nipple drinkers and one hanging feeder, while an additional feeder tray was provided during 0-3 d. Clean pine shavings were provided in each pen at a depth of 5 cm. On day 0, the birds received 24 h of daylight at 30 lux; 1 h of darkness was added per day until a total of 6 h darkness was reached and on d 6, when the lights were dimmed to 3.5 lux. There were 4 dietary phases: starter (0-13 d), grower (14-21 d), finisher (22-41 d), and withdrawal (42-49 d). The diets were formulated based on the Cobb Management Guidelines (Cobb-Vantress, 2012). Feed and water were provided *ad libitum* for the duration of the trial.

Coccidiosis Control Treatments

This study consisted of 6 coccidiosis control methods (CM; Table 3.1); CM1: coccidiosis vaccine (VAC) at d of hatch and no dietary anticoccidial; CM2: VAC with dietary avilamycin 0-21 d; CM3: VAC, avilamycin (0-21 d), and narasin 22-41 d; CM4: no VAC and narasin from 0-41 d; CM5: VAC, avilamycin (0-21 d), and salinomycin 22-41 d; CM6: VAC, avilamycin (0-21 d), and monensin 22-41 d. All dietary anticoccidials were removed during the withdrawal period (42-49 d).

Environmental Temperature Treatments

Two temperature treatments were used in this experiment: standard environmental temperature (Cobb-Vantress, 2012), which started at 34°C and the temperature changed on d 7 to 31°C, 14 d to 27°C, 21 d to 24°C, 28 d to 21°C, 35 d to 19°C, and 42 d to 18°C; warm

environmental temperature, starting at 34°C until d 7, and the temperature was set 3°C higher than the standard temperature from 7-28 d and 1.5°C higher from 28-49 d.

Data Collection

Mortality was recorded daily. On d 0, 14, 21, 28, 42, and 49 BW and feed intake were recorded by pen. BWG and FCR were calculated from d 0-14, 0-21, 0-28, 0-42, and 0-49 d. On day 28, 1 bird per pen was randomly selected and sacrificed by cervical dislocation to observe *E. maxima* micro scores and *E. acervulina* and *E. tenella* gross lesion scores using Johnson and Reid's, (1970) methodology. For *E. maxima* scores, intestinal scrapings from the jejunum located at Meckel's diverticulum were placed on microscope slides for *E. maxima* micro scoring (0-4), whereas 0= no oocysts present, 1= 1-10 oocysts per 100x field, 2= 11-20 oocysts per 100x field, 3= 21-30 oocysts per 100x field, and 4= more than 30 oocysts per 100 x field.

At the end of d 49, 4 birds per pen were randomly selected and feed withdrawn for 8 h prior to processing (d 50). After slaughter and evisceration, carcasses were chilled for 3 h and cold carcass, pectoralis major and minor weights were recorded. The pectoralis majors were scored for white striping (0-4, 4 being the most severe) and woody breast (0-1). Room temperatures were recorded twice daily from the temperature control unit. The temperatures recorded represent an average of four thermometers that were dispersed in the room.

Data Analysis

Performance and processing data were analyzed in completely randomized block design using a 2x6 factorial design: 2 environmental temperatures and 6 coccidiosis control methods. Data were analyzed using split plot analysis and Duncan's New multiple Range Test for

evaluating means with $P \leq 0.05$. Mortality data were transformed using arcsine percentage and reported *P*-values are from the transformed data. All data were analyzed using SAS 9.4 (SAS Inst. Inc., Cary, NC 2013).

RESULTS

Performance

No significant interactions were observed on BW for diet x temperature. Body weight means were significantly ($P < 0.001$) different in the birds kept in the warm environment throughout the trial, with a 334 g difference at 49 d (Table 3.2). When comparing the BW's between the different dietary treatments; CM1 had significantly different BW at 14 ($P < 0.001$) and 21 d ($P < 0.001$) of age verses CM2, CM3, CM5, and CM6. At d 28, CM1 was different ($P = 0.028$) than CM2 and CM3. At d 42 and 49, no dietary effects were seen in BW ($P > 0.05$). The treatment that received narasin and no VAC, had an intermediate BW between CM1 and the other treatments throughout the entire study.

No significant CM x temperature interactions were observed on FCR ($P > 0.05$; Table 3.3). Warmer birds had significantly ($P = 0.029$) different FCRs from 0-13 d ($P = 0.008$ and 0-21 ($P = 0.008$). FCR differences were observed for 0-28 ($P = 0.007$), 0-42 ($P = 0.034$), and 0-49 d ($P < 0.001$) in the standard temperature birds. There was an 8 point difference in FCR (1.81 vs. 1.89) seen at 49 d between temperature regimes. CM1 had the highest FCR up to d 42, while CM5 had the lowest up to d 28. At d 49, CM1 and CM2 had the highest mean FCR at 1.88, while birds only receiving narasin had the lowest mean FCR at 1.79.

There were no significant effects on mortality due to CM x temperature ($P > 0.05$) and 0-13 d mortality based on temperature treatment ($P > 0.05$; Table 3.4). From 0-21, 0-28, 0-42, and

0-49 d, significantly ($P = 0.004$, $P < 0.001$, $P < 0.001$, and $P < 0.001$) warmer birds exhibited lower mortality. The treatment group that received narasin and no VAC had the lowest average mortality throughout the trial.

There were no significant interactions for CM x temperature on gross lesion scores for *E. acervulina* or *E. tenella* ($P > 0.05$; Table 3.5). For the micro *E. maxima* scores, there were significant ($P = 0.001$) effects based on the environmental temperature treatment. No effects were observed on micro *E. maxima* scores for diet and CM x temperature interactions ($P > 0.05$).

Processing

There were significant effects ($P = 0.010$) on percent yield observed with the standard temperature treatment group having higher yields (Table 3.6). No effects were observed on percent yield for CM or CM x temperature ($P > 0.05$). Significant effects were seen on pectoralis major (pec major; $P < 0.001$) weights (693.8g vs. 566.9g), and pec major yield percentages (20.3% vs. 18.5%; $P < 0.001$) when comparing temperatures. CM1 averaged the largest pec major weights (672.9g) and percent yield (20.1%). Differences in pectoralis minor (pec minor) weights (139.2g vs. 113.3g; $P < 0.001$) and pec minor percent yield (4.1% vs. 3.7%; $P < 0.001$) were observed in the birds reared at recommended temperatures. No effects were observed on pec minor for CM ($P > 0.05$). No significant CM x temperature interactions observed on yield or parts weight ($P > 0.05$). When comparing white striping scores (WSS), warmer (smaller) birds were significantly ($P < 0.001$) less severe compared to standard temperature (1.89 vs. 2.37, respectively). The highest average WSS were seen in CM1 birds compared to CM4 with the lowest (2.38 vs. 1.88, respectively). No significant effects were observed for diet, environmental temperature, and diet x environmental temperature on WSS ($P > 0.05$).

DISCUSSION

Though it is a common industry practice, it appears that there are no scientific experiments published that analyze the effects of using a coccidiosis vaccine at day of hatch followed by administering a dietary anticoccidial during the grow out of commercial broilers. Bioshuttle programs have been discussed in interviews with Dr. Greg Mathis and Dr. Manuel Da Costa, but the use of narasin in bioshuttle program was not discussed in these interviews (Da Costa, 2017; Mathis, 2017).

An environmental temperature effect was seen on BW and FCR throughout this experiment, supporting previous results (May, et al., 1998). Birds that were in the warmer environment had lower mortality rates than birds at the standard temperatures. These observations are different from findings of prior research, in which birds housed at temperatures above 21°C from 42-49 d had higher percent mortality (May, et al., 1998). Certainly, the relative temperatures in each experiment are important, but genetics should also be considered. Modern broilers feather slower than previous generations making them less sensitive to warmer temperatures. Therefore, the temperatures for which birds become more or less stressed have changed through time and genetic selection, which has partitioned more protein to muscle growth rather than feathers.

One possible explanation for the temperature effect on *E. maxima* micro scores (Table 3.5) is an interaction with relative humidity. The increased temperature provides more moisture holding capacity in the air. This possible higher humidity and/or higher temperature could have reduced litter moisture, in turn inhibiting the sporulation of the oocyst and oocyst cycling through the birds. It is unclear whether the effects of the environmental temperature per se, or the effects of bird BW, may be the causative agent for the decreased incidence of white striping

(Table 3.6). It is important to note that in previous studies, white striping was more prevalent in birds at higher BW (Russo, et al., 2015).

A depression in BW and higher FCR up to 28 d was observed on the treatment group that received the VAC only. This is believed to have occurred as an effect of the immune response to the VAC. When birds were given VAC and dietary avilamycin, the effects on BW and FCR were not seen. Coccidiosis gross lesions were observed in the birds that only received narasin, but the use of narasin by itself proved effective at preventing adverse BW effects. Birds given narasin only averaged an FCR between 3 points to 9 points lower than other treatment groups. These results were similar to a previous trial, in which narasin improved BW and feed efficiency in broilers raised to 41 d (Brennan, et al., 2001). The four groups given avilamycin in the starter and grower diets grew better up to 28 d. When using avilamycin, average BW has been observed to increase in prior research and is seen as an effect of gut health improvements from the avilamycin (Kim, et al., 2011).

Avilamycin assisted in reducing the adverse effects on BW from coccidiosis vaccinations, however, the group that received the vaccination, after 28 d had similar BW to other groups. Lower *E. maxima* scores in the warm environment, to the author's knowledge, have not been documented before and require further research. Higher incidence in WSS seen in birds that received the VAC over the treatment group that did not could indicate the VAC may lead to a higher incidence of WSS, although more research must be conducted to draw conclusions. It is shown that narasin helped lower mortality compared to other groups and birds had similar performance to the other treatments at 49 d.

Data from this experiment show the importance of rearing temperature and the effects it has on broiler performance. Results show that when comparing: bioshuttle programs, vaccine

only programs, and ionophore only programs, similar performance was observed at 49 d without a coccidiosis challenge. In future studies, the addition of a coccidiosis challenge could show potentially different results than those found in this experiment. No coccidiosis control method by environmental temperature interaction was observed showing that the coccidiosis control programs were equally effective at different temperatures.

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Table 3.1: Coccidiosis control methods (CM) with anticoccidial inclusion rates in milligrams per kilogram (mg/kg).

	Vaccination	**Starter**	**Grower**	**Finisher**	**Withdrawal**
		0-13 d	14-21 d	22-41 d	42-49 d
CM1	+	Basal	Basal	Basal	Basal
CM2	+	Avilamycin 25 mg/kg	Avilamycin 25 mg/kg	Basal	Basal
CM3	+	Avilamycin 25 mg/kg	Avilamycin 25 mg/kg	Narasin 63 69.4 mg/kg	Basal
CM4	-	Narasin 72 79.4 mg/kg	Narasin 72 79.4 mg/kg	Narasin 63 69.4 mg/kg	Basal
CM5	+	Avilamycin 25 mg/kg	Avilamycin 25 mg/kg	Salinomycin 55.1 mg/kg	Basal
CM6	+	Avilamycin 25 mg/kg	Avilamycin 25 mg/kg	Monensin 99.2 mg/kg	Basal

Table 3.2: Effect of coccidiosis control methods and environmental temperature on body weight (BW).

Treatment			BW (g)				
Additive	Vaccine	Environmental Temperature	13 d	21 d	28 d	42 d	49 d
None	+		442.6 ± 8.7[b]	941.7 ± 19.5[b]	1517.6 ± 22.6[b]	2782.8 ± 43.2	3294.1 ± 59.6
Avilamycin/None	+		475.6 ± 7.4[a]	1003.9 ± 16.8[a]	1565.1 ± 21.3[a]	2797.8 ± 52.8	3240.4 ± 56.5
Avilamycin/Narasin	+		481.1 ± 6.3[a]	1001.0 ± 19.0[a]	1564.4 ± 19.8[a]	2806.6 ± 38.1	3281.6 ± 67.4
Narasin	-		456.6 ± 9.0[ab]	948.0 ± 18.5[ab]	1536.6 ± 21.6[ab]	2782.5 ± 46.5	3262.2 ± 60.8
Avilamycin/Salinomycin	+		470.2 ± 6.9[a]	986.1 ± 16.3[ab]	1546.5 ± 16.6[ab]	2874.4 ± 52.8	3344.8 ± 46.4
Avilamycin/Monensin	+		475.6 ± 8.3[a]	991.4 ± 17.1[ab]	1542.4 ± 22.3[ab]	2766.0 ± 45.4	3313.2 ± 64.7
		Warm	440.8 ± 3.4	917.1 ± 6.2	1479.3 ± 8.6	2656.8 ± 15.9	3122.6 ± 29.3
		Standard	493.1 ± 2.5	1040.3 ± 5.5	1611.5 ± 5.3	2946.6 ± 17.3	3456.2 ± 16.5
None	+	Warm	411.9 ± 6.2	873.9 ± 14.8	1442.0 ± 20.8	2648.8 ± 43.6	3138.4 ± 70.8
None	+	Standard	473.3 ± 4.2	1009.6 ± 9.4	1593.2 ± 10.7	2916.9 ± 30.7	3449.8 ± 57.1
Avilamycin/None	+	Warm	452.7 ± 4.7	947.0 ± 8.3	1498.7 ± 19.3	2628.2 ± 23.6	3047.7 ± 47.7
Avilamycin/None	+	Standard	498.5 ± 8.0	1060.9 ± 14.7	1631.5 ± 17.5	2967.5 ± 56.4	3433.1 ± 28.4
Avilamycin/Narasin	+	Warm	459.1 ± 4.3	938.6 ± 15.6	1507.7 ± 23.0	2685.6 ± 40.1	3112.1 ± 93.1
Avilamycin/Narasin	+	Standard	503.1 ± 3.8	1063.3 ± 13.8	1621.0 ± 15.5	2927.6 ± 20.9	3451.2 ± 50.9
Narasin	-	Warm	423.7 ± 3.1	883.2 ± 12.4	1457.3 ± 12.3	2613.4 ± 27.8	3060.1 ± 53.0
Narasin	-	Standard	489.6 ± 5.1	1012.9 ± 10.5	1615. 9± 7.4	2951.6 ± 17.8	3464.3 ± 36.5
Avilamycin/Salinomycin	+	Warm	447.8 ± 5.8	929.8 ± 9.7	1493.5 ± 15.7	2733.7 ± 27.2	3210.0 ± 52.1
Avilamycin/Salinomycin	+	Standard	492.5 ± 5.5	1042.4 ± 11.7	1599.6 ± 11.2	3015.0 ± 74.5	3479.6 ± 36.1
Avilamycin/Monensin	+	Warm	449.4 ± 9.5	930.2 ± 10.4	1476.8 ± 27.7	2631.1 ± 56.0	3167.2 ± 101.9
Avilamycin/Monensin	+	Standard	501.7 ± 2.7	1052.5 ± 8.3	1608.0 ± 11.5	2901.0 ± 22.0	3459.2 ± 38.2
Source of variation			*P*-values				
Diet (D)			<0.001	<0.001	0.028	0.080	0.444
Environmental temperature (ET)			<0.001	<0.001	<0.001	<0.001	<0.001
D *x* ET			0.193	0.927	0.502	0.727	0.779

Values are means ± SE of 8 pens per treatment combination with 20 birds per pen.
[a-d] Treatment means with the same superscript are not significantly different at $P < 0.05$, Duncan's New Multiple Range Test (1955).

Table 3.3: Effect of coccidiosis control methods and environmental temperature on feed conversion ratio (feed consumed to weight gain; FCR) adjusted for mortality.

Treatment			Adjusted FCR (g:g)				
Additive	Vaccine	Environmental Temperature	0-13 d	0-21 d	0-28 d	0-42 d	0-49 d
None	+		1.244 ± 0.008[a]	1.315 ± 0.008[a]	1.579 ± 0.013[a]	1.672 ± 0.014[a]	1.881 ± 0.024[a]
Avilamycin/None	+		1.188 ± 0.012[b]	1.279 ± 0.008[bc]	1.553 ± 0.011[ab]	1.667 ± 0.015[a]	1.884 ± 0.015[a]
Avilamycin/Narasin	+		1.179 ± 0.010[bc]	1.289 ± 0.012[abc]	1.553 ± 0.016[ab]	1.640 ± 0.011[ab]	1.841 ± 0.016[ab]
Narasin	-		1.211 ± 0.007[b]	1.320 ± 0.009[a]	1.524 ± 0.006[b]	1.597 ± 0.025[b]	1.789 ± 0.024[b]
Avilamycin/Salinomycin	+		1.152 ± 0.013[c]	1.265 ± 0.011[c]	1.543 ± 0.013[ab]	1.598 ± 0.021[b]	1.821 ± 0.016[ab]
Avilamycin/Monensin	+		1.199 ± 0.016[b]	1.301 ± 0.013[ab]	1.565 ± 0.012[a]	1.669 ± 0.032[a]	1.861 ± 0.034[a]
		Warm	1.181 ± 0.008	1.286 ± 0.005	1.568 ± 0.008	1.661 ± 0.013	1.885 ± 0.014
		Standard	1.210 ± 0.007	1.303 ± 0.007	1.538 ± 0.006	1.619 ± 0.011	1.807 ± 0.010
None	+	Warm	1.231 ± 0.007	1.298 ± 0.009	1.606 ± 0.014	1.698 ± 0.024	1.931 ±0.037
None	+	Standard	1.257 ± 0.013	1.332 ± 0.010	1.552 ± 0.017	1.646 ± 0.011	1.830 ± 0.022
Avilamycin/None	+	Warm	1.169 ± 0.013	1.272 ± 0.007	1.560 ± 0.019	1.692 ± 0.015	1.918 ± 0.022
Avilamycin/None	+	Standard	1.207 ± 0.019	1.286 ± 0.014	1.546 ± 0.013	1.641 ± 0.024	1.851 ± 0.011
Avilamycin/Narasin	+	Warm	1.168 ± 0.008	1.292 ± 0.021	1.565 ± 0.029	1.649 ± 0.021	1.878 ± 0.022
Avilamycin/Narasin	+	Standard	1.191 ± 0.017	1.287 ± 0.014	1.541 ± 0.015	1.632 ± 0.010	1.804 ± 0.016
Narasin	-	Warm	1.195 ± 0.007	1.302 ± 0.008	1.535 ± 0.006	1.593 ± 0.051	1.810 ± 0.043
Narasin	-	Standard	1.227 ± 0.010	1.338 ± 0.013	1.513 ± 0.010	1.601 ±0.010	1.768 ± 0.022
Avilamycin/Salinomycin	+	Warm	1.122 ± 0.015	1.261 ± 0.010	1.556 ± 0.022	1.618 ± 0.030	1.848 ± 0.027
Avilamycin/Salinomycin	+	Standard	1.182 ± 0.015	1.269 ± 0.020	1.530 ± 0.013	1.578 ± 0.031	1.794 ± 0.011
Avilamycin/Monensin	+	Warm	1.203 ± 0.027	1.293 ± 0.012	1.583 ± 0.019	1.719 ± 0.028	1.927 ± 0.038
Avilamycin/Monensin	+	Standard	1.196 ± 0.019	1.308 ± 0.023	1.546 ± 0.014	1.620 ± 0.054	1.795 ± 0.048
Source of variation			*P*-values				
Diet (D)			<0.001	0.003	0.044	0.017	0.012
Environmental temperature (ET)			0.008	0.029	0.007	0.034	<0.001
D *x* ET			0.409	0.738	0.876	0.560	0.668

Values are means ± SE of 8 pens per treatment combination with 20 birds per pen.
[a-d] Treatment means with the same superscript are not significantly different at $P < 0.05$, Duncan's New Multiple Range Test (1955).

Table 3.4: Effect of coccidiosis control methods and environmental temperature on percent mortality.

Treatment			Mortality (%)				
Additive	Vaccine	Environmental Temperature	0-13 d	0-21 d	0-28 d	0-42 d	0-49 d
None	+		1.89 ± 0.78	4.14 ± 1.25[ab]	6.04 ± 1.74	12.73 ± 1.60	13.67 ± 1.70[ab]
Avilamycin/None	+		4.46 ± 1.03	6.02 ± 0.71[a]	7.29 ± 0.81	14.24 ± 1.19	15.53 ± 1.47[a]
Avilamycin/Narasin	+		3.42 ± 1.18	8.40 ± 2.58[a]	8.71 ± 2.69	14.99 ± 2.63	16.60 ± 2.73[a]
Narasin	-		0.30 ± 0.30	0.92 ± 0.50[b]	2.49 ± 0.91	8.41 ± 0.88	9.66 ± 0.96[b]
Avilamycin/Salinomycin	+		1.91 ± 1.03	5.43 ± 1.38[a]	7.34 ± 1.50	14.00 ± 1.46	14.31 ± 1.44[ab]
Avilamycin/Monensin	+		2.20 ± 0.79	3.77 ± 1.33[ab]	5.35 ± 1.49	11.66 ± 1.56	12.29 ± 1.58[ab]
		Warm	2.11 ± 0.54	2.94 ± 1.07	3.89 ± 1.14	10.42 ± 1.10	11.15 ± 1.15
		Standard	1.89 ± 0.54	4.14 ± 0.54	6.04 ± 0.61	12.73 ± 0.72	13.67 ± 0.73
None	+	Warm	2.53 ± 1.35	6.38 ± 2.10	8.91 ± 2.67	15.30 ± 2.34	16.55 ± 2.46
None	+	Standard	1.25 ± 0.82	1.91 ± 0.93	3.16 ± 1.88	10.16 ± 1.90	10.79 ± 2.00
Avilamycin/None	+	Warm	4.44 ± 1.51	6.32 ± 0.86	7.57 ± 0.97	14.51 ± 1.55	16.41 ± 2.16
Avilamycin /None	+	Standard	4.47 ± 1.51	5.72 ± 1.17	7.01 ± 1.36	13.98 ± 1.91	14.6 ± 2.10
Avilamycin/Narasin	+	Warm	3.10 ± 1.31	12.42 ± 4.47	13.04 ± 4.66	18.70 ± 4.65	19.92 ± 4.73
Avilamycin/Narasin	+	Standard	3.75 ± 2.00	4.38 ± 1.99	4.38 ± 1.99	11.28 ± 2.05	11.28 ± 2.05
Narasin	-	Warm	0.60 ± 0.60	1.22 ± 0.80	3.72 ± 1.56	9.32 ± 1.47	9.94 ± 1.64
Narasin	-	Standard	0.00 ± 0.00	0.63 ± 0.63	1.25 ± 0.82	7.50 ± 0.94	9.38 ± 1.13
Avilamycin/Salinomycin	+	Warm	2.57 ± 1.68	7.73 ± 2.23	10.26 ± 2.42	16.61 ± 1.95	16.61 ± 1.95
Avilamycin/Salinomycin	+	Standard	1.25 ± 1.25	3.13 ± 1.32	4.41 ± 1.14	11.38 ± 1.85	12.01 ± 1.88
Avilamycin/Monensin	+	Warm	2.50 ± 1.34	5.63 ± 2.40	7.53 ± 2.67	15.13 ± 2.48	15.76 ± 2.56
Avilamycin/Monensin	+	Standard	1.91 ± 0.93	1.91 ± 0.93	3.16 ± 0.93	8.19 ± 0.94	8.82 ± 0.84
Source of variation			*P*-values				
Diet (D)			0.229	0.011	0.080	0.080	0.022
Environmental temperature (ET)			0.560	0.004	<0.001	<0.001	<0.001
D *x* ET			0.909	0.414	0.433	0.433	0.138

Values are means ± SE of 8 pens per treatment combination with 20 birds per pen.

[a-b] Treatment means with the same superscript are not significantly different at $P < 0.05$, Duncan's New Multiple Range Test (1955).

Mortality data were transformed using arcsine to provide the *P*-values that are presented with the true means.

Table 3.5: Effect of coccidiosis control methods and environmental temperature on coccidiosis lesion scores taken at 28 d of age.

Treatment			Lesion Score (0-4)		
Additive	Vaccine	Environmental Temperature	*E. acervulina* Gross	*E. maxima* Micro[1]	*E. tenella* Gross
None	+		0.438 ± 0.157	0.188 ± 0.136	0.125 ± 0.125
Avilamycin/None	+		0.375 ± 0.125	0.0 ± 0.0	0.0 ± 0.0
Avilamycin/Narasin	+		0.313 ± 0.120	0.063 ± 0.063	0.125 ± 0.085
Narasin	-		0.313 ± 0.120	0.063 ± 0.063	0.063 ± 0.063
Avilamycin/Salinomycin	+		0.125 ± 0.085	0.125 ± 0.085	0.063 ± 0.063
Avilamycin/Monensin	+		0.063 ± 0.063	0.188 ± 0.101	0.0 ± 0.0
		Warm	0.250 ± 0.063	0.0 ± 0.0	0.104 ± 0.054
		Standard	0.292 ± 0.073	0.208 ± 0.066	0.021 ± 0.021
None	+	Warm	0.500 ± 0.189	0.0 ± 0.0	0.250 ± 0.250
None	+	Standard	0.375 ± 0.263	0.375 ± 0.263	0.0 ± 0.0
Avilamycin/None	+	Warm	0.375 ± 0.183	0.0 ± 0.0	0.0 ± 0.0
Avilamycin/None	+	Standard	0.375 ± 0.183	0.0 ± 0.0	0.0 ± 0.0
Avilamycin/Narasin	+	Warm	0.500 ± 0.189	0.0 ± 0.0	0.250 ± 0.164
Avilamycin/Narasin	+	Standard	0.125 ± .0125	0.125 ± 0.125	0.0 ± 0.0
Narasin	-	Warm	0.125 ± 0.125	0.0 ± 0.0	0.125 ± 0.125
Narasin	-	Standard	0.500 ± 0.189	0.125 ± 0.125	0.0 ± 0.0
Avilamycin/Salinomycin	+	Warm	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Avilamycin/Salinomycin	+	Standard	0.250 ± 0.164	0.250 ± 0.164	0.125 ± 0.125
Avilamycin/Monensin	+	Warm	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Avilamycin/Monensin	+	Standard	0.125 ± 0.125	0.375 ± 0.183	0.0 ± 0.0
Source of variation				*P*-values	
Diet (D)			0.168	0.558	0.710
Environmental temperature (ET)			0.662	0.001	0.108
D *x* ET			0.243	0.558	0.381

Values are means ± SE of 8 pens per treatment combination with 1 bird per pen.
[a-b] Treatment means with the same superscript are not significantly different at $P < 0.05$, Duncan's New Multiple Range Test (1955).
[1]Micros scrapings taken from jejunum at the Meckel's diverticulum.

Table 3.6: Effect of coccidiosis control methods and environmental temperature on processing yield data, white striping scores (WSS) and woody breast scores taken at 50 d of age.

Treatment			Carcass Composition at 50 d						
Additive	Vaccine	Environmental Temperature	Yield	Pectoralis Major	Pectoralis Major	Pectoralis Minor	Pectoralis Minor	White Striping Score	Woody Breast Score
			(%)	(g)	(%)	(g)	(%)	(0-4)	(0-1)
None	+		78.3 ± 0.3	672.9 ± 23.6[a]	20.1 ± 0.4[a]	131.6 ± 3.7	3.9 ± 0.1	2.38 ± 0.12	0.44 ± 0.16
Avilamycin/None	+		78.5 ± 0.2	600.5 ± 23.2[b]	19.2 ± 0.3[ab]	120.9 ± 5.1	3.9 ± 0.1	2.00 ± 0.19	0.31 ± 0.12
Avilamycin/Narasin	+		78.5 ± 0.4	630.2 ± 28.0[ab]	19.3 ± 0.3[ab]	125.9 ± 5.4	3.9 ± 0.1	2.15 ± 0.14	0.31 ± 0.12
Narasin	-		78.4 ± 0.3	606.6 ± 21.7[b]	19.3 ± 0.3[ab]	124.6 ± 0.4	4.0 ± 0.1	1.88 ± 0.15	0.19 ± 0.10
Avilamycin/Salinomycin	+		78.4 ± 0.4	648.1 ± 24.2[ab]	19.7 ± 0.4[ab]	127.9 ± 5.0	3.9 ± 0.1	2.27 ± 0.17	0.19 ± 0.10
Avilamycin/Monensin	+		78.5 ± 0.2	623.9 ± 20.7[ab]	18.9 ± 0.3[b]	126.6 ± 4.1	3.8 ± 0.1	2.09 ± 0.16	0.19 ± 0.10
		Warm	78.1 ± 0.2	566.9 ± 11.4	18.5 ± 0.2	113.3 ± 2.1	3.7 ± 0.0	1.89 ± 0.08	0.27 ± 0.07
		Standard	78.9 ± 0.2	693.8 ± 9.0	20.3 ± 0.1	139.2 ± 1.5	4.1 ± 0.0	2.37 ± 0.09	0.27 ± 0.07
None	+	Warm	77.8 ± 0.3	601.4 ± 20.8	18.9 ± 0.2	120.3 ± 4.4	3.8 ± 0.1	2.21 ± 0.14	0.5 ± 0.19
None	+	Standard	78.9 ± 0.3	744.3 ± 22.1	21.3 ± 0.4	142.9 ± 1.7	4.1 ± 0.1	2.54 ± 0.19	0.38 ± .026
Avilamycin/None	+	Warm	78.2 ± .03	526.0 ± 21.1	18.1 ± 0.4	105.0 ± 4.7	3.6 ± 0.1	1.67 ± 0.15	0.25 ± 0.16
Avilamycin/None	+	Standard	78.9 ± 0.3	675.0 ± 16.7	20.2 ± 0.2	136.7 ± 4.4	4.1 ± 0.1	2.33 ± 0.30	0.38 ± 0.18
Avilamycin/Narasin	+	Warm	78.7 ± 0.7	554.1 ± 35.4	18.5 ± 0.4	110.9 ± 7.1	3.7 ± 0.1	1.92 ± 0.19	0.25 ± 0.16
Avilamycin/Narasin	+	Standard	78.4 ± 0.5	706.3 ± 21.1	20.0 ± 0.2	140.8 ± 3.4	4.0 ± 0.1	2.38 ± 0.18	0.38 ± 0.18
Narasin	-	Warm	78.0 ± 0.2	554.4 ± 25.1	18.6 ± 0.4	113.5 ± 3.3	3.8 ± 0.1	1.67 ± 0.21	0.25 ± 0.16
Narasin	-	Standard	78.9 ± 0.6	658.9 ± 24.5	20.1 ± 0.4	135.8 ± 4.8	4.2 ± 0.1	2.08 ± 0.19	0.13 ± 0.13
Avilamycin/Salinomycin	+	Warm	77.7 ± 0.7	587.3 ± 34.4	18.7 ± 0.6	113.9 ± 6.0	3.6 ± 0.1	2.09 ± 0.30	0.25 ± 0.16
Avilamycin/Salinomycin	+	Standard	79.1 ± 0.3	709.0 ± 16.2	20.6 ± 0.2	142.0 ± 3.7	4.1 ± 0.1	2.46 ± 0.17	0.13 ± 0.13
Avilamycin/Monensin	+	Warm	78.0 ± 0.3	578.4 ± 28.2	18.1 ± 0.3	116.3 ± 5.1	3.7 ± 0.1	1.77 ± 0.14	0.13 ± 0.13
Avilamycin/Monensin	+	Standard	79.0 ± 0.3	669.4 ± 21.3	19.6 ± 0.4	137.0 ± 3.7	4.0 ± 0.1	2.42 ± 0.25	0.25 ± 0.16
Source of variation			*P*-values						
Diet (D)			0.989	0.015	0.020	0.260	0.363	0.220	0.684
Environmental temperature (ET)			0.010	<0.001	<0.001	<0.001	<0.001	<0.001	1.000
D *x* ET			0.404	0.641	0.732	0.736	0.660	0.953	0.923

Values are means ± SE of 8 pens per treatment combination with 20 birds per pen.
[a-b] Treatment means with the same superscript are not significantly different at $P < 0.05$, Duncan's New Multiple Range Test (1955).

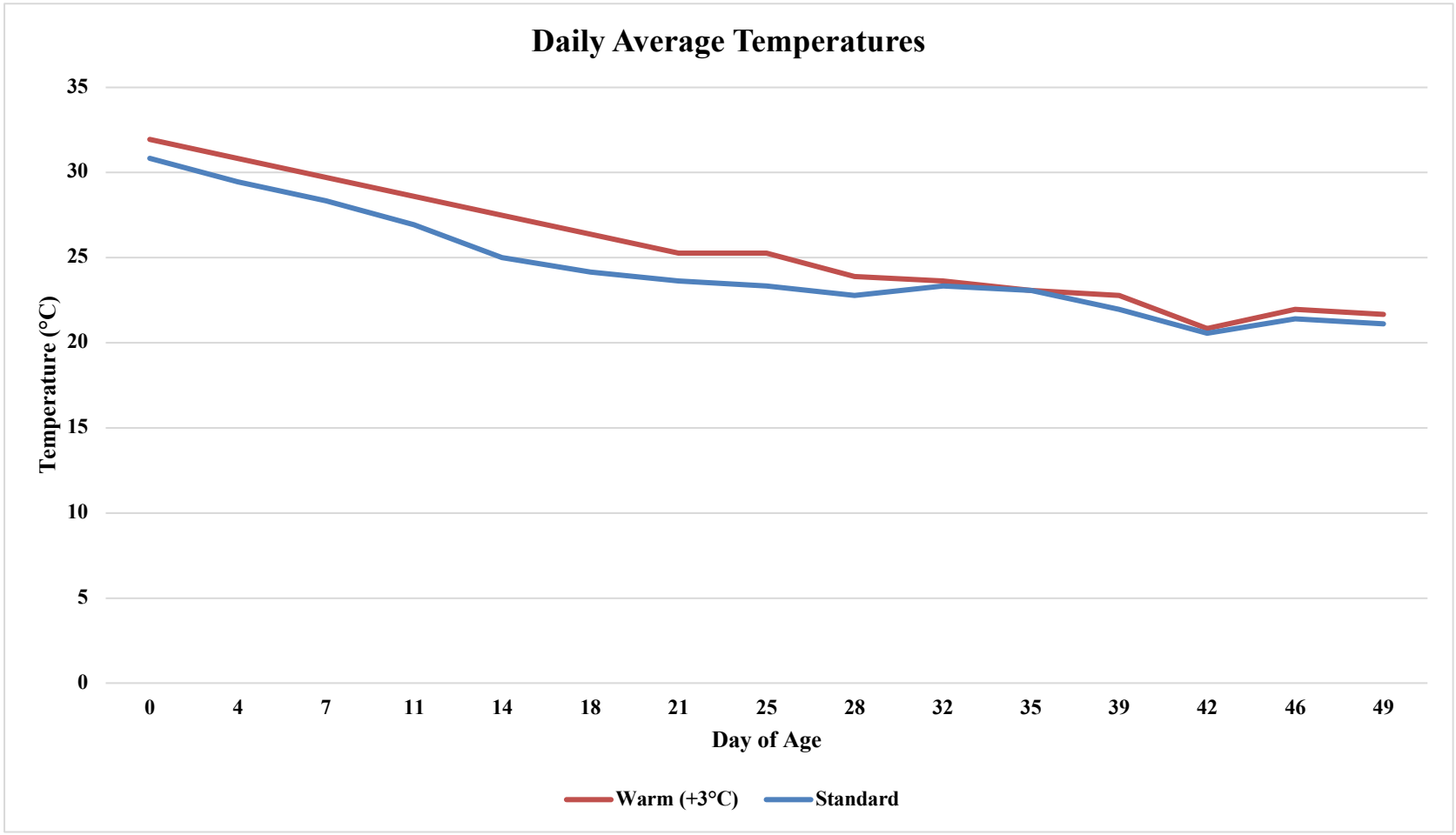

Figure 3.1: Daily average temperatures for the standard (breeder guidelines) and warm (+3°C from 0-28 d, +1.5°C 28-49 d) temperature treatments.
Standard temperature was based on the breeder guidelines.
Daily averages were an average of four thermometers per temperature.

CHAPTER 4

COMPARISON OF COCCIDIOSIS VACCINE VERSUS SALINOMYCIN ON THE PROGENY OF YOUNG OR OLD BREEDER FLOCKS, FED STANDARD OR REDUCED DIETARY CALORIC DENSITY IN FINISHER AND WITHDRAWAL FEEDS

[1]J.H. Oxford, I.R. Alvarado, M.J. Da Costa, and G.M. Pesti. To be submitted to *Poultry Science*.

ABSTRACT

With the current consumer preference for antibiotic free meat, the use of coccidiosis vaccines is becoming increasingly important. The objectives of this research were 1) to compare a live coccidiosis vaccine (COV) and salinomycin (SAL) on the progeny of young or old breeder flocks, and 2) to compare the use of standard and reduced dietary caloric density in finisher and withdrawal feeds on broiler performance. Twelve hundred forty-eight Ross 708 chicks were used for this experiment; Half (624) of the chicks were the progeny from an old (50 weeks of age) breeder flock (OBF) and the other half were from a young (30 weeks of age) breeder flock (YBF). Data were analyzed as a 2x2x2 factorial arrangement of treatments with: 1) COV at d of hatch or dietary SAL 0-16 d and 28-48 d of age; 2) standard or reduced dietary caloric densities (-50 kcal finisher and -80 kcal withdrawal) and 3) coccidial challenge or not, on d 22. There were 4 dietary phases: starter (0-16 d), grower (16-28 d), finisher (28-34 d), and withdrawal (34-48 d). Body weight (BW), feed intake and feed conversion ratio (FCR) were measured at 16, 28, 34, and 48 d of age. No interactions between breeder age and treatments were observed therefore breeder age was considered a block. There were significant differences in 0-28 d FCR ($P = 0.008$). The challenged birds had higher mean FCRs (1.564 vs. 1.505). At 28 d no significant differences observed in BW, gain, or feed intake. By 34 d the effect of challenge was still significant (1.612 vs. 1.558, $P = 0.019$). At 48 d, the effect of coccidiosis control method had small effects on BW (3775 vs. 3817g, $P = 0.344$) and FCR (1.748 vs. 1.745, $P = 0.870$). Reduced dietary caloric density in finisher and withdrawal feeds increased mean FCR at 48 d (1.762 vs. 1.731, $P = 0.130$). There were no significant effects of dietary caloric density on any of the performance parameters at 48 d ($0.843 > P > 0.406$). The results show that coccidiosis

vaccination provided similar protection to salinomycin under challenge conditions and helped reduce microscopic *E. maxima* scores.

INTRODUCTION

Of the many diseases that affect the poultry industry, coccidiosis is one of the most impactful. Globally, there is an estimated annual economic impact of over three billion dollars due to coccidiosis and the prevention of it (Dalloul and Lillehoj, 2006), and there have only been increases since then. Coccidiosis can cause decreases in BW, increases in FCR, pigmentation loss, intestinal damage, and in severe cases can cause mortality (Collins, et al., 1955; McDougald, et al., 2013). Another problem in the poultry industry that has been associated with coccidiosis infections is increased incidence of necrotic enteritis. Coccidiosis is considered to be one of the biggest precursors of necrotic enteritis (Williams, et al., 2003; Li, et al., 2010; Lee, et al., 2013)

With the movement to antibiotic free (ABF) production there has been an increase in the incidence of necrotic enteritis with an estimated daily mortality rate of up to one percent in infected flocks (Williams, 2005; Van Immerseel, et al., 2009; Cervantes 2017). One class of anticoccidials that has been shown to be very effective are the ionophores (Conway and McKenzie, 2007). Of the ionophores, one of the most commonly used is salinomycin (Chapman, 1999). Since ionophore anticoccidials are considered antibiotics, companies are moving away from the use of them; this has left many producers only able to use synthetic "chemical" anticoccidials for in-feed use. It has been almost 20 years since the release of any new anticoccidials and resistance has developed to every anticoccidial approved by the USDA (Chapman, 1997; Chapman and Jeffers, 2015). Synthetic anticoccidials can be very effective at

reducing the incidence of coccidiosis, the issue with their use is that drug resistance is developed at a faster rate compared to ionophores (Chapman, et al., 2010). Since there is a movement away from the use of ionophores and there are a limited number of synthetic anticoccidials available, the use of coccidiosis vaccines has become increasingly more important to the poultry industry.

The first coccidiosis vaccine came on to the market in 1952 and was developed by S. A. Edgar (Williams, 2002; McDonald and Shirley, 2009; Peek and Landman, 2011). Vaccines are widely used and can be very beneficial for developing immunity to coccidiosis. The strains that are used in the vaccines today all predate the anticoccidials available on the market. Since these *Eimeria* strains are sensitive to any anticoccidials, they have been used to help "seed" houses to help reduce the number of "wild" coccidia that have resistance to anticoccidials. Previous research has shown that live vaccines can be used to restore sensitivity to anticoccidials when administered after a drug program has been over used (Mathis and Broussard, 2006; Jenkins, et al., 2010; Lee, et al., 2013; Chapman and Jeffers, 2015). Another benefit of using live vaccines is the ability to use them all year and no concern with tissue residues. Some anticoccidials have been shown to have adverse effects like heat stress toxicity therefore they should only be used during the winter months of the year (Da Costa, et al., 2017). In previous work live coccidiosis vaccines have been shown to perform better than salinomycin, along with providing improved BW (Lee, et al., 2013).

The popularity of coccidiosis vaccines has grown since the start of the antibiotic-free movement and will most likely continue to grow (Cervantes, 2017). Therefore, it is increasingly important to understand if there are interactions between live vaccines and dietary nutrients and growth rates. The goal of this research was to compare a live coccidiosis vaccine and

salinomycin on the progeny of young or old breeder flocks, fed standard or reduced dietary caloric densities in finisher and withdrawal feeds on broiler performance.

MATERIALS AND METHODS

Birds and Husbandry

All management practices were approved by the University of Georgia, Institutional Animal Care and Use Committee. The experiment was conducted in a windowless room, equipped with evaporative cool cells and tunnel ventilation. The room contained 48, 1.22 x 1.52 m floor pens, each with one hanging feeder and 5 drinking nipples with 5 cm clean pine shavings litter. Half (624) of the Ross 708 chicks were the progeny of an old (50 weeks of age) breeder flock (OBF) and the other half were from a young (30 weeks of age) breeder flock (YBF) with the same genetics verified by the breeding company. Each pen was allocated 26 chicks, (stocking density = 0.071 sq. m/ bird). From placement to d 3, each pen had an additional feeder tray. On d 0 the photoperiod was 24:0 L:D, each following day 1 h of darkness was added until 18:6 for the rest of the experiment. Birds received 30 lux d 0 to 6 and 3.5 lux thereafter.

Coccidiosis Treatment

The two coccidiosis control method treatments were coccidiosis vaccine (COV, Coccivac B52®) or salinomycin (SAL). On d 0, COV was applied using a spray cabinet. Salinomycin (SAL) was added to the feed from 0-16 d (starter diet) and 28-48 d (finisher and withdrawal diet). SAL was not administered in the grower diet.

Eimeria Challenge

On d 22 half of the pens in each treatment group were fasted for 2 h and then provided 1 kg of feed mixed with a 20 x dose of coccidiosis vaccine per bird to induce a coccidiosis infection. *Ad libitum* feeding re-commenced when challenge feeds were consumed.

Dietary Caloric Density

There were 4 diet phases: starter (0-16 d), grower (16-28 d), finisher (28-34 d), and withdrawal (34-48 d). The starter and grower diets were formulated based on the breeder recommendations and were the same for all of the birds in this experiment. There were two caloric density treatments for each of the finisher and withdrawal diet phases, standard dietary caloric density and reduced dietary caloric density. Both diets were based on the breeder guidelines, but the reduced caloric density diets had 50 Kcal/kg less in the finisher and 80 Kcal/kg less in the withdrawal feeds (Table 4.1). Feed and water was provided *ad libitum*.

Data Collection

On d 0, 16, 28, 34, and 48, group BWs and feed intakes were assessed per pen and used to calculate the FCR. Mortality was recorded daily. At 6 d post challenge (day 28), 2 birds were randomly selected from each pen and necropsied and lesion scored for coccidiosis including *E. acervulinum, E. maxima, and E. tenella* gross scores (Johnson and Reid, 1970). Intestinal scrapings from the jejunum located around Meckel's diverticulum were used for *E. maxima* microscopic scores (0-4) using McDougald, et al., (2013) methodologies, whereas 0= no oocysts present, 1= 1-10 oocysts per 100x field, 2= 11-20 oocysts per 100x field, 3= 21-30 oocysts per 100x field, and 4= more than 30 oocysts per 100 x field. On days 34 and 48 four birds per pen

were randomly selected and fasted before processing (35 and 49 d). Four birds per pen were randomly selected, tagged, and individually weighed for processing. Following slaughter and evisceration the carcass were chilled for 3 h; cold carcass, pectoralis major, pectoralis minor, wings, saddle, and shell weights were recorded to calculate yield percentages.

Data Analysis

Performance and processing data were analyzed using a completely randomized block design with a factorial arrangement of: 2 coccidia treatments, 2 challenge status, and 2 caloric densities. Individual pens were considered the experimental units. The breeder flock age was considered as a block, no significant interactions were observed for breeder flock age and treatment groups. Mortality data were arc sine transformed which provided the *P*-values that are presented with the true means. For *P*-values less than or equal to 0.05, the means were separated using Tukey and Kramer tests. For the coccidiosis lesion scores data, the individual birds sampled were considered the experimental unit. Categorical data was analyzed using a Chi-Square test. Data were analyzed using JMP Pro 11 (SAS Inst. Inc., Cary NC) software.

RESULTS

Performance

There were no significant effects ($P > 0.301$) of coccidiosis control methods observed in performance parameters at 16 d (Table 4.2). At day of hatch there were significant differences ($P < 0.001$) in chick BW, with the OBF progeny being over 7 grams heavier (47.03 vs. 39.57 g). Differences in BW at 16 d ($P < 0.001$) reached an average of 50 grams with the OBF offspring continuing to have higher BWs. This difference between breeder flock progeny was also

observed in BWG at 16 d ($P < 0.001$, 558 vs. 515 g). A higher feed intake (764 vs. 702 g) was observed in the OBF progeny from 0 to16 d. No effects ($P > 0.501$) were seen in FCR or percent mortality from 0-16 d based on breeder age.

No effects ($P > 0.173$) were seen in performance parameters from coccidiosis control method or interactions between coccidiosis control method and challenge status at 28 d (Table 4.3). Significant differences were seen in FCR based on challenge status at 0-28 d ($P = 0.008$, 1.564 vs. 1.505) as well as FCR from 16-28 d ($P = 0.023$, 1.664 vs. 1.588). There were significant effects ($P < 0.001$) observed in BW and BWG at 28 d and BWG 16-28 d based on breeder age. Effects of breeder age were still observed in feed intake at 28 d with the OBF progeny having almost 100 g more feed intake than that of the YBF ($P = 0.003$, 2395 vs. 2297 g).

At 6 d post challenge (28 d), significant effects were seen in *E. maxima* microscopic scores based on coccidiosis control method with the group that received the coccidiosis vaccine having significantly lower microscopic scores ($P = 0.001$, 0.59 vs. 1.27, Table 4.4). Significant differences between the challenged and non-challenged groups were observed in *E. acervulina* gross lesion scores ($P < 0.001$, 1.09 vs. 0.28) and *E. maxima* microscopic scores ($P < 0.001$, 1.57 vs. 0.29). There were significant differences observed in *E. acervulina* gross lesion scores ($P < 0.001$) and *E. maxima* microscopic scores ($P = 0.002$) seen from coccidiosis control method and challenge status interactions with the challenged vaccine group having better scores than the challenged salinomycin group (0.46 vs. 1.71). No gross lesion scores were observed for *E. maxima*. There were no significant effects in *E. tenella* gross lesion scores ($P > 0.156$) based on any parameter. No significant effects observed for gross lesion scores and microscopic scores from breeder age block ($P > 0.114$).

There were significant effects seen at 34 d in BW ($P < 0.001$), BWG ($P = 0.001$), and feed intake ($P < 0.001$) based on breeder age block with OBF progeny continuing to have higher BW (2298 vs. 2183 g), BWG (2251 vs. 2144 g), and feed intake (3552 vs. 3404 g, Table 4.5). No significant effects ($P > 0.260$) were observed in BW and BWG based on the other parameters. Differences in FCR based on challenge status observed ($P = 0.019$) with the non-challenged group having a 5 point better FCR (1.558 vs. 1.612). Other than challenge status, there were no effects ($P > 0.299$) observed in FCR based on any other parameter.

At 48 d, neither FCR nor mortality were influenced by any independent variable (Table 4.6). Significant differences ($P < 0.035$) observed in BW, BWG, and feed intake based on breeder age. Effects of dietary caloric density were observed in feed intake from 34-48 d with the low caloric density diets consuming more feed ($P = 0.046$, 3121 vs. 3020 g). From 28-48 d, there were significant interactions based on breeder age in feed intake ($P = 0.017$) and FCR ($P = 0.048$). No other significant interactions ($P > 0.050$) were observed in performance parameters from 28-48 and 34-48 d (Table 4.7).

Processing

No significant interactions ($P > 0.065$) from any treatment observed in processing live weights, cold carcass weights, and all cut up weights from the 35 d (Table 4.8). Significant differences were observed in 35 d processing yields based on coccidiosis control method with the SAL group having higher mean yields ($P = 0.047$, 10.04 vs. 9.56 %, Table 4.9). There were significant interactions ($P = 0.037$) observed in cold carcass yield based on coccidiosis control method and challenge status. Significant effects ($P < 0.049$) were seen in leg yield percentage looking at the interactions between challenge status x dietary caloric density. OBF progeny had

significantly different shell weights versus the YBF progeny ($P = 0.015$, 20.43 vs. 19.03). No significant effects in processing yields were observed at 35 d based on challenge status ($P > 0.071$), dietary caloric density ($P > 0.553$), coccidiosis control method x dietary caloric density ($P > 0.363$), and coccidiosis control method x challenge status x dietary caloric density ($P > 0.372$).

For the 49 d processing results, significant effects ($P = 0.007$) were observed in pectoralis minor weights based on the interactions of coccidiosis control method x challenge status x diet caloric density (Table 4.9). Significant differences were seen in live weights ($P = 0.038$, 3720 vs. 3602 g) and leg weights ($P = 0.038$, 1104 vs. 1057 g) based on breeder age. No significant effects were observed, based on coccidiosis control method ($P > 0.191$), challenge status ($P > 0.306$), or dietary caloric density ($P > 0.430$). Differences observed in pectoralis minor yields from coccidiosis control treatments with the vaccine group having higher mean yields ($P = 0.024$, 5.66 vs. 5.42 %, Table 4.11). Significant effects ($P = 0.005$) were also seen in pectoralis minor yields based on the interactions of coccidiosis control method x challenge status x dietary caloric density. There were no significant effects seen in percentage yields of cold carcass ($P > 0.269$), pectoralis major ($P > 0.060$), wings ($P > 0.237$), legs ($P > 0.091$), and shell ($P > 0.118$).

DISCUSSION

The daily average temperatures can be seen in Figure 4.1, The desired differences in room temperatures were not reached. However, due to the differences in BW and FCR between the two temperature treatments it is believed that the effects of temperature were observed in the birds

Coccidiosis vaccines and dietary salinomycin have been compared before with contradicting results. Lehman, et al. (2009), showed that in birds raised to 8 weeks of age, the birds that were treated with salinomycin had improved BWG compared to birds given coccidiosis vaccine while having similar amounts of feed intake. Lee, et al. (2013), showed higher BW at 28 and 42 d in birds treated with coccidiosis vaccine verses those that received dietary salinomycin.

The coccidiosis vaccine provided better protection against multiple species of *Eimeria* and similar BWs to that of the salinomycin group (Table 4.6). These BW results do not agree with the work done by either Lehman, et al. (2009) or Lee, et al. (2013). Salinomycin provided adequate protection against *E. acervulina* and *E. tenella* however did not provide similar protection to *E. maxima*. With the coccidiosis challenge simulating a coccidiosis infection, nutrient absorption and utilization may have been inhibited causing an increase in FCR (Persia, et al., 2006). With feed cost of 70% of production it is very important for poultry companies to make sure that they are using their coccidiosis control methods and housing management techniques properly to try and minimize the effects that coccidiosis has on bird performance (Eits, et al., 2005; Dozier, et al., 2006a; Dozier, et al., 2006b; Aftab, 2012; Trevisan, et al., 2014; Basurco, et al., 2015).

The increases in feed intake at 34 d in the salinomycin group, were expected. McDougald and McQuistion (1980), showed that when salinomycin has been withdrawn from the feed there are increases in feed intake. The effects on feed intake are not seen after 34 d, these results agree with the results of other trials showing a compensatory feed intake due to the withdrawal of ionophores which levels out after a week or more (McDougald and McQuistion, 1980; Metzler, et al., 1987).

The differences in chick weights at d of hatch were expected, it is known that older breeder hens lay larger eggs than that of younger breeder hens and egg size is correlated with chick weight (Wyatt, et al., 1985; Christensen, et al., 2002; Lourens, et al., 2006; Iqbal, et al., 2016). At 16 d, the OBF progeny had larger BW, it was shown in previous work that chick weight effects the size of BW later on in life (Wyatt, et al., 1985; Iqbal, et al., 2016). It is impossible to know if the larger BW were due to the birds consuming more feed or if the birds consumed more feed due to their larger BW.

The 100g difference in feed intake seen from comparing diet caloric densities from 34-48 d shows how important the levels of caloric density are in broilers diets. The difference in caloric densities in the diets may seem relatively small but as shown in this study there can be big effects on feed consumed. It is important for integrators to compare cost on whether it is more beneficial to pay for the addition of extra energy or pay for more feed for the birds to consume. These differences in feed intake are believed to be from the birds needing to consume a larger amount of feed to receive a similar amount of energy as those of their counterparts (Leeson, et al., 1996; Perween, et al., 2016).

The 35 d processing data show no effects on part weight based on any independent variable. The weight of the OBF offspring shell was not significantly heavier with a 19g difference in weight, however there was a higher % yield seen in this group. This higher shell % yield may be due to the OBF offspring having more fat deposition/skin or larger skeletal frames. The breeder age influenced the progeny weight on the 49 d processing and showed to have an effect on saddle weights as well. These results are similar to those of prior research (Wyatt, et al., 1985; Iqbal, et al., 2016).

Although differences were seen on live weight and saddle weights at 49 d, these differences were not seen in percentage yields. In return, there were significant differences in pec minor percentage yields but there were no differences in the weights.

It is apparent from this research that breeder age has significant effects on the progeny throughout the entirety of their lives. It is important for integrators to know how breeder age affects their progeny so that they can tailor their management programs to the birds that they are using. This could be important when deciding when to process birds so that the company can provide a uniform bird size to help prevent plant condemnations.

The results show that coccidiosis vaccination provided similar protection to salinomycin under challenge conditions and helped reduce microscopic *E. maxima* scores. The results were similar for progeny of young and old breeder flocks and different finisher diet caloric densities showing no interactions between coccidiosis control method and other management inputs.

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Table 4.1: Ingredient composition (%) and formulated caloric densities of the finisher and withdrawal diets with differing caloric densities.

	Finisher Diet		Withdrawal Diet	
Diet Blend	Standard Caloric Density	Reduced Caloric Density	Standard Caloric Density	Reduced Caloric Density
ME, kcal/kg	3,100	3,050	3,180	3,100
	~~ % ~~~~~~~~~~~~~~~~~~~~			
Corn	66.91	68.16	68.01	70.2
Soybean Meal, 48%	28.69	28.49	26.47	26.12
Poultry fat	1.38	0.27	2.62	0.78
Defluorinated phosphorus, 18%	1.22	1.22	1.13	1.12
Limestone	0.69	0.69	0.68	0.69
DL-Methionine	0.24	0.24	0.21	0.21
L-Lysine-HCL, 78%	0.12	0.12	0.12	0.12
L-Threonine, 98.5%	0.03	0.03	0.02	0.02
Salt (NaCl)	0.30	0.35	0.30	0.30
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.08	0.08	0.08	0.08
BMD[3]	0.05	0.05	0.05	0.05
Phytase	0.02	0.02	0.02	0.02

[1] Vitamin mix provided the following (per kilogram of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0g; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-reinyl acetate, 2,500 IU; all-rac-tocopherol acetate, 11 IU; ethoxyquin, 150 mg.

[2] Trace mineral mix provides the following (per kilogram of diet): manganese (MnSO4.H2O), 101 mg; iron (FeSO4.7H2O), 20 mg; zinc (Zn)), 80 mg; copper (CuSO4.5H2O), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3] BMD (Bacitracin Methylene Disalicylate - Type A) provides (per kilogram of diet): feed grade bacitracin methylene disalicylate equivalent to 50 mg bacitracin.

Table 4.2: Effect of coccidiosis control method and parent stock age on body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio (FCR), and mortality of Ross 708 broilers at hatch and 16 d.

Coccidiosis control method	Breeder age	Body weight		BWG	Feed intake	FCR	Mortality
		Hatch	16 d	0 to 16 d			
		---------- g ----------				-- g:g --	%
Salinomycin		43.11 ± 0.82	579 ± 7	536 ± 6	734 ± 10	1.371 ± 0.011	2.06 ± 0.16
Vaccine		43.50 ± 0.83	580 ± 7	537 ± 7	731 ± 9	1.363 ± 0.011	1.44 ± 0.12
Breeder age (block)	Old	47.03 ± 0.34	605 ± 5	558 ± 5	764 ± 5	1.370 ± 0.011	1.58 ± 0.15
	Young	39.57 ± 0.16	554 ± 4	515 ±4	702 ± 8	1.364 ± 0.011	1.92 ± 0.13
Source of variation		---------- *P*-value ----------					
Coccidiosis control (CC)		0.302	0.846	0.862	0.761	0.623	0.571
Breeder age (Block)		<0.001	<0.001	<0.001	<0.001	0.691	0.502

Values are means ± SE of 24 pens per treatment with 26 birds per pen.
[1]Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

Table 4.3: Effect of coccidiosis control method and *Eimeria* challenge (22 d of age) on body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio (FCR), and mortality of Ross 708 broilers at 28 d and from 16 to 28 d.

Coccidiosis control method	Challenge status[1]	BW	BWG	Feed intake	FCR	Mortality	BWG	Feed intake	FCR
		28 d					16 to 28 d		
		---------- g ----------			-- g:g --	-- % --	----- g -----		-- g:g --
Salinomycin		1570	1527	2356	1.547	2.70	991	1622	1.642
Vaccine		1578	1535	2336	1.523	2.25	998	1604	1.609
	+	1561	1517	2371	1.564	2.57	984	1634	1.664
	-	1587	1544	2321	1.505	2.38	1005	1592	1.588
Salinomycin	+	1549 ± 24	1506 ± 23	2392 ± 31	1.591 ± 0.023	2.89 ± 0.84	975 ± 15	1656 ± 30	1.703 ± 0.036
	-	1591 ± 25	1548 ± 24	2320 ± 27	1.502 ± 0.025	2.52 ± 0.96	1007 ± 18	1587 ± 27	1.582 ± 0.040
Vaccine	+	1573 ± 22	1529 ± 21	2350 ± 30	1.538 ± 0.016	2.25 ± 0.74	993 ± 14	1613 ± 28	1.626 ± 0.024
	-	1584 ± 24	1540 ± 23	2322 ± 45	1.508 ± 0.023	2.25 ± 0.74	1003 ± 15	1596 ± 35	1.593 ± 0.031
Breeder age (block)	Old	1627 ± 13	1580 ± 13	2395 ± 24	1.516 ± 0.011	2.22 ± 0.55	1023 ± 9	1632 ± 24	1.596 ± 0.018
	Young	1520 ± 12	1481 ± 12	2297 ± 19	1.554 ± 0.020	2.73 ± 0.59	966 ± 10	1594 ± 18	1.656 ± 0.029
Source of variation				---------- *P*-value ----------					
Coccidiosis control (CC)		0.635	0.656	0.519	0.275	0.773	0.598	0.567	0.312
Challenge status (CS)		0.138	0.140	0.111	0.008	0.699	0.121	0.160	0.023
CC *x* CS		0.387	0.386	0.476	0.174	0.699	0.426	0.384	0.184
Breeder age (Block)		<0.001	<0.001	0.003	0.082	0.557	<0.001	0.218	0.069

Values are means ± SE of 12 pens per treatment combination with 26 birds per pen.
[1]Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

Table 4.4 Effect of coccidiosis control method and *Eimeria* challenge 22 d of age on *E. acervulina*, *E. maxima* and *E. tenella* gross lesion scores, and *E. maxima* microscopic scores at 28 d (6 d post-challenge).

Coccidiosis control method	Challenge status[1]	*E. acervulina*	*E. maxima* gross lesion	*E. tenella*	*E. maxima* micro[2]
		average score (0 – 4)			
Salinomycin		0.92	0	0.26	1.27[a]
Vaccine		0.44	0	0.40	0.59[b]
	+	1.09	0	0.44	1.57
	-	0.28	0	0.21	0.29
Salinomycin	+	1.71	0	0.38	2.25
	-	0.13	0	0.13	0.29
Vaccine	+	0.46	0	0.50	0.88
	-	0.42	0	0.29	0.29
Source of variation		---------- Chi Square *P*–values ----------			
Coccidiosis control (CC)		0.290	-	0.488	0.001
Challenge status (CS)		<0.001	-	0.157	<0.001
CC *x* CS		<0.001	-	0.296	0.002
Breeder age (Block)		0.249	-	0.372	0.115

Values are means ± SE of 12 pens per treatment combination with 2 birds per pen.
[1]Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.
[2]Micros scrapings taken from jejunum at the Meckel's diverticulum.

Table 4.5: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher diet caloric density levels on body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio (FCR), and mortality of Ross 708 broilers at 34 d and from 28 to 34 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	BW	BWG	Feed intake	FCR	Mortality
			34 d				
			---------- g ----------			-- g:g --	-- % --
Salinomycin			2251	2208	3519	1.597	3.66
Vaccine			2230	2187	3437	1.573	3.21
	+		2224	2181	3512	1.612	3.21
	-		2257	2213	3444	1.558	3.66
		Low	2236	2193	3479	1.590	3.34
		High	2245	2202	3477	1.580	3.52
Salinomycin	+		2224	2181	3544	1.627	3.53
	-		2279	2235	3494	1.566	3.79
Vaccine	+		2225	2182	3481	1.597	2.89
	-		2235	2192	3393	1.550	3.53
Salinomycin		Low	2243	2200	3503	1.597	3.48
		High	2260	2216	3534	1.596	3.84
Vaccine		Low	2230	2186	3455	1.584	3.21
		High	2230	2187	3419	1.563	3.21
	+	Low	2216	2173	3498	1.613	2.89
		High	2233	2190	3527	1.611	3.53
	-	Low	2257	2213	3461	1.568	3.80
		High	2257	2213	3426	1.548	3.51
Salinomycin	+	Low	2212±59	2170±58	3508±55	1.622±0.042	3.21±1.54
		High	2237±41	2193±39	3579±54	1.633±0.022	3.85±0.99
	-	Low	2274±48	2231±47	3498±30	1.572±0.034	3.76±1.95
		High	2283±58	2240±57	3490±80	1.560±0.022	3.82±1.40
Vaccine	+	Low	2220±49	2176±48	3487±70	1.604±0.027	2.57±0.81
		High	2230±18	2187±16	3475±33	1.589±0.020	3.21±1.18
	-	Low	2240±53	2196±52	3424±49	1.563±0.044	3.85±0.99
		High	2231±39	2187±38	3363±96	1.536±0.026	3.21±1.84
Breeder age	Old		2298±23	2251±22	3552±28	1.580±0.014	3.34±0.57
	Young		2183±15	2144±15	3404±27	1.590±0.017	3.53±0.73
Source of variation			---------- *P*-value ----------				
Coccidiosis control (CC)			0.461	0.451	0.036	0.300	0.889
Challenge status (CS)			0.261	0.267	0.077	0.019	0.921
CC *x* CS			0.442	0.444	0.613	0.736	0.810
Dietary caloric density (DE)			0.759	0.762	0.942	0.640	0.816
CC *x* DE			0.777	0.789	0.374	0.646	0.431
CS *x* DE			0.755	0.769	0.405	0.687	0.507
CC *x* CS *x* DE			0.976	0.969	0.845	0.908	0.785
Breeder age (Block)			<0.001	0.001	<0.001	0.671	0.953

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.6: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher and withdrawal dietary caloric density levels on body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio (FCR), and mortality of Ross 708 broilers at 48 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	BW	BWG	Feed intake	FCR	Mortality
					48 d		
			---------- g ----------			-- g:g --	-- % --
Salinomycin			3817	3774	6578	1.745	6.70
Vaccine			3775	3732	6519	1.748	6.41
	+		3753	3710	6545	1.766	5.61
	-		3839	3796	6552	1.727	7.50
		Low	3793	3750	6601	1.762	7.19
		High	3800	3756	6496	1.731	5.92
Salinomycin	+		3760	3717	6548	1.764	5.77
	-		3875	3832	6608	1.726	7.62
Vaccine	+		3747	3703	6541	1.769	5.45
	-		3804	3760	6496	1.728	7.37
Salinomycin		Low	3809	3766	6607	1.757	7.00
		High	3826	3782	6550	1.733	6.39
Vaccine		Low	3777	3733	6595	1.768	7.37
		High	3773	3730	6442	1.728	5.45
	+	Low	3745	3702	6571	1.777	6.41
		High	3761	3717	6519	1.755	4.81
	-	Low	3841	3797	6631	1.748	7.97
		High	3838	3795	6474	1.706	7.03
Salinomycin	+	Low	3755±77	3713±77	6559±88	1.770±0.037	7.05±2.88
		High	3764±68	3720±67	6537±106	1.758±0.020	4.49±1.54
	-	Low	3863±65	3819±64	6654±53	1.744±0.027	6.96±2.08
		High	3887±78	3844±77	6563±151	1.707±0.018	8.29±1.55
Vaccine	+	Low	3735±74	3692±74	6582±104	1.785±0.034	5.77±1.65
		High	3758±64	3715±64	6501±53	1.752±0.029	5.13±1.28
	-	Low	3819±49	3775±48	6608±128	1.751±0.034	8.98±2.92
		High	3789±35	3745±34	6384±117	1.705±0.029	5.77±1.31
Breeder age	Old		3848±23	3801±33	6665±47	1.755±0.013	6.70±1.01
	Young		3744±27	3705±27	6432±44	1.738±0.016	6.41±0.94
Source of variation			---------- *P*-value ----------				
Coccidiosis control (CC)			0.344	0.338	0.364	0.870	0.884
Challenge status (CS)			0.056	0.056	0.913	0.063	0.117
CC *x* CS			0.511	0.513	0.423	0.946	0.909
Dietary caloric density (DE)			0.880	0.887	0.118	0.130	0.745
CC *x* DE			0.817	0.824	0.466	0.712	0.546
CS *x* DE			0.832	0.843	0.422	0.638	0.827
CC *x* CS *x* DE			0.700	0.692	0.775	0.892	0.406
Breeder age (Block)			0.023	0.034	0.001	0.412	0.882

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.7: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher and withdrawal dietary caloric density levels on body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio (FCR), and mortality of Ross 708 broilers 34 to 48 d, and from 28 to 48 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	BWG	Feed intake	FCR	BWG	Feed intake	FCR
			34 to 48 d			28 to 48 d		
			---------- g ----------		-- g:g --	----- g -----		-- g:g --
Salinomycin			1566	3060	1.962	2248	4223	1.882
Vaccine			1545	3082	2.007	2197	4183	1.909
	+		1529	3033	2.004	2193	4174	1.912
	-		1583	3109	1.965	2252	4231	1.879
		Low	1557	3121	2.020	2226	4256	1.918
		High	1554	3020	1.949	2219	4149	1.873
Salinomycin	+		1536	3005	1.971	2211	4157	1.886
	-		1596	3114	1.953	2284	4289	1.878
Vaccine	+		1522	3060	2.037	2174	4192	1.937
	-		1569	3103	1.978	2220	4174	1.880
Salinomycin		Low	1566	3103	1.992	2251	4276	1.904
		High	1566	3016	1.932	2244	4169	1.860
Vaccine		Low	1547	3139	2.048	2200	4236	1.931
		High	1543	3024	1.966	2194	4130	1.886
	+	Low	1530	3073	2.038	2195	4223	1.933
		High	1528	2993	1.970	2190	4125	1.890
	-	Low	1584	3170	2.003	2256	4289	1.902
		High	1581	3047	1.928	2248	4174	1.856
Salinomycin	+	Low	1544±71	3051±51	1.994±0.079	2218±64	4198±58	1.899±0.050
		High	1528±52	2959±55	1.948±0.071	2204±58	4115±71	1.873±0.050
	-	Low	1589±47	3156±74	1.990±0.041	2284±40	4355±54	1.909±0.028
		High	1604±51	3073±118	1.916±0.047	2284±53	4222±147	1.847±0.039
Vaccine	+	Low	1515±88	3095±42	2.081±0.138	2172±78	4249±66	1.968±0.074
		High	1528±58	3026±41	1.992±0.064	2176±57	4135±27	1.907±0.050
	-	Low	1579±25	3184±86	2.015±0.031	2229±6	4223±96	1.895±0.044
		High	1558±20	3021±68	1.940±0.046	2212±16	4125±69	1.865±0.030
Breeder age	Old		1550±30	3113±29	2.024±0.040	2221±28	4270±36	1.928±0.025
	Young		1561±23	3029±40	1.945±0.028	2224±23	4136±40	1.862±0.020
Source of variation			---------- *P*-value ----------					
Coccidiosis control (CC)			0.602	0.659	0.371	0.183	0.464	0.413
Challenge status (CS)			0.182	0.132	0.445	0.117	0.294	0.321
CC *x* CS			0.863	0.501	0.687	0.719	0.170	0.453
Dietary caloric density (DE)			0.955	0.046	0.164	0.856	0.054	0.176
CC *x* DE			0.958	0.776	0.832	0.994	0.983	0.980
CS *x* DE			0.992	0.669	0.943	0.969	0.876	0.979
CC *x* CS *x* DE			0.686	0.597	0.839	0.812	0.757	0.608
Breeder age (Block)			0.780	0.095	0.125	0.927	0.017	0.048

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.8: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher diet caloric density on carcass cut-up weights of Ross 708 at 35 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	Live	Cold Carcass	Pectoralis major	Pectoralis minor	Wings	Legs (saddles)	Shell
					---------- g ----------				
Salinomycin			2181	1655	420	89	165	663	330
Vaccine			2203	1693	425	90	161	663	327
	+		2199	1685	431	90	165	674	334
	-		2185	1663	414	89	161	652	323
		Low	2179	1674	422	89	164	664	326
		High	2205	1674	423	90	162	662	331
Salinomycin	+		2189	1663	430	90	168	672	330
	-		2174	1647	410	88	162	653	329
Vaccine	+		2210	1707	431	90	162	676	338
	-		2197	1680	419	89	159	651	317
Salinomycin		Low	2171	1640	415	89	166	654	328
		High	2191	1669	425	89	164	671	331
Vaccine		Low	2188	1708	428	89	162	674	324
		High	2219	1679	422	90	160	652	331
	+	Low	2218	1715	436	91	167	674	333
		High	2181	1655	425	89	164	674	334
	-	Low	2141	1633	407	87	161	655	319
		High	2230	1694	422	90	160	649	327
Salinomycin	+	Low	2210±58	1684±43	434±12	92±3	170±6	668±22	332±16
		High	2167±41	1642±43	425±19	88±3	166±7	677±11	328±15
	-	Low	2132±52	1597±59	397±13	86±4	161±6	641±20	325±14
		High	2215±59	1697±51	424±14	90±2	162±4	665±18	334±20
Vaccine	+	Low	2226±34	1746±38	437±12	90±1	164±5	680±13	335±7
		High	2194±48	1667±41	425±18	90±3	161±5	671±19	341±14
	-	Low	2149±74	1670±47	418±14	88±4	160±5	669±14	314±19
		High	2245±38	1690±27	419±13	90±3	159±2	633±15	320±16
Breeder age	Old		2185±26	1667±26	427±8	90±1	165±2	660±9	338±7
	Young		2199±25	1681±19	418±6	89±1	161±3	666±8	319±8
Source of variation					---------- *P*-value ----------				
Coccidiosis control (CC)			0.554	0.233	0.653	0.738	0.258	0.968	0.843
Challenge status (CS)			0.712	0.502	0.128	0.510	0.220	0.081	0.328
CC *x* CS			0.974	0.871	0.732	0.909	0.651	0.819	0.352
Dietary caloric density (DE)			0.491	0.999	0.859	0.762	0.663	0.818	0.679
CC *x* DE			0.874	0.367	0.476	0.884	0.952	0.116	0.863
CS *x* DE			0.097	0.066	0.226	0.263	0.690	0.821	0.760
CC *x* CS *x* DE			0.985	0.744	0.592	0.506	0.818	0.403	0.761
Breeder age (Block)			0.706	0.657	0.407	0.905	0.409	0.594	0.077

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.9 Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher diet caloric density levels on carcass yield of Ross 708 at 35 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	Cold Carcass	Pectoralis major	Pectoralis minor	Wings	Legs (saddles)	Shell
			----------% ----------					
Salinomycin			76.22	25.55	5.42	10.04	40.29	19.99
Vaccine			76.26	25.30	5.34	9.56	39.56	19.47
	+		76.67	25.90	5.42	9.92	40.56	20.03
	-		75.81	24.95	5.34	9.68	39.29	19.43
		Low	76.34	25.30	5.35	9.82	39.90	19.57
		High	76.14	25.55	5.41	9.78	39.95	19.89
Salinomycin	+		76.14	26.20	5.47	10.23	40.95	20.02
	-		76.29	24.90	5.37	9.85	39.64	19.96
Vaccine	+		77.19	25.60	5.36	9.62	40.17	20.03
	-		75.34	25.01	5.32	9.51	38.94	18.91
Salinomycin		Low	76.10	25.47	5.45	10.14	40.07	20.07
		High	76.33	25.63	5.39	9.94	40.51	19.92
Vaccine		Low	76.57	25.13	5.25	9.51	39.74	19.07
		High	75.96	25.48	5.43	9.62	39.38	19.87
	+	Low	77.05	25.60	5.35	9.79	39.62	19.53
		High	76.28	26.21	5.48	10.06	41.51	20.52
	-	Low	75.62	25.01	5.35	9.86	40.19	19.60
		High	76.01	24.90	5.34	9.50	38.39	19.27
Salinomycin	+	Low	76.53±0.29	26.08±0.87	5.50±0.26	10.19±0.21	40.05±1.39	19.81±0.65
		High	75.76±0.88	26.33±1.81	5.45±0.27	10.28±0.63	41.85±1.87	20.24±1.08
	-	Low	75.68±0.50	24.87±0.79	5.39±0.17	10.10±0.24	40.10±0.70	20.33±0.72
		High	76.90±0.50	24.93±0.56	5.34±0.24	9.60±0.38	39.18±1.12	19.60±1.07
Vaccine	+	Low	77.58±0.82	25.12±0.58	5.21±0.07	9.39±0.29	39.18±0.68	19.26±0.44
		High	76.80±0.25	26.09±1.19	5.52±0.24	9.84±0.23	41.16±1.58	20.81±0.81
	-	Low	75.56±0.79	25.15±0.59	5.30±0.17	9.63±0.21	40.29±0.92	18.87±0.95
		High	75.11±0.77	24.86±0.78	5.35±0.21	9.40±0.15	37.60±1.38	18.94±0.62
Breeder age	Old		76.24±0.38	25.79±0.55	5.41±0.10	9.95±0.18	39.92±0.69	20.43±0.37
	Young		76.24±0.30	25.07±0.37	5.35±0.10	9.65±0.15	39.93±0.58	19.03±0.40

Source of variation	*P*-value					
Coccidiosis control (CC)	0.917	0.726	0.619	0.047	0.423	0.347
Challenge status (CS)	0.072	0.183	0.631	0.303	0.170	0.287
CC *x* CS	0.037	0.620	0.818	0.554	0.966	0.339
Dietary caloric density (DE)	0.676	0.725	0.689	0.851	0.964	0.554
CC *x* DE	0.364	0.896	0.450	0.501	0.664	0.389
CS *x* DE	0.214	0.611	0.667	0.178	0.049	0.235
CC *x* CS *x* DE	0.373	0.710	0.656	0.911	0.594	0.883
Breeder age (Block)	0.991	0.314	0.682	0.199	0.991	0.015

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.10: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher and withdrawal dietary caloric density levels on carcass cut-up weights of Ross 708 at 49 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	Live	Cold Carcass	Pectoralis major	Pectoralis minor	Wings	Legs (saddles)	Shell
					---------- g ----------				
Salinomycin			3693	2902	835	157	274	1086	525
Vaccine			3629	2855	811	162	272	1075	515
	+		3654	2863	814	161	271	1083	515
	-		3668	2894	833	158	275	1078	524
		Low	3656	2871	819	159	273	1072	522
		High	3666	2887	828	160	273	1089	517
Salinomycin	+		3678	2865	818	159	271	1087	512
	-		3707	2939	852	156	278	1086	537
Vaccine	+		3631	2862	810	163	272	1079	518
	-		3628	2849	813	160	272	1071	511
Salinomycin		Low	3719	2919	845	159	276	1085	527
		High	3667	2885	826	156	272	1088	523
Vaccine		Low	3594	2822	793	160	270	1059	518
		High	3665	2888	830	164	274	1091	511
	+	Low	3616	2837	802	160	271	1072	514
		High	3693	2890	826	162	271	1094	517
	-	Low	3697	2904	836	158	274	1071	531
		High	3639	2883	830	157	275	1085	517
Salinomycin	+	Low	3696±49	2889±45	837±20	166±5	274±6	1085±21	510±12
		High	3659±82	2842±47	800±16	153±3	268±6	1090±28	515±11
	-	Low	3741±63	2949±59	853±20	152±8	278±5	1084±37	543±15
		High	3674±123	2928±107	851±39	159±7	277±10	1087±50	531±21
Vaccine	+	Low	3535±76	2785±64	767±27	155±5	269±6	1059±27	517±16
		High	3726±105	2938±96	852±31	172±9	275±9	1098±39	520±20
	-	Low	3653±63	2859±55	819±18	164±1	271±5	1058±33	520±13
		High	3604±63	2839±50	808±24	156±3	273±7	1083±15	503±12
Breeder age	Old		3720±39	2918±35	831±14	162±3	276±3	1104±15	528±7
	Young		3602±38	2840±31	816±12	157±3	270±3	1057±14	511±8
Source of variation					---------- *P*-value ----------				
Coccidiosis control (CC)			0.258	0.335	0.192	0.296	0.607	0.600	0.355
Challenge status (CS)			0.809	0.528	0.307	0.400	0.475	0.822	0.410
CC *x* CS			0.774	0.373	0.404	0.912	0.480	0.887	0.146
Dietary caloric density (DE)			0.862	0.739	0.612	0.880	0.964	0.431	0.612
CC *x* DE			0.272	0.301	0.126	0.377	0.420	0.529	0.891
CS *x* DE			0.229	0.444	0.401	0.718	0.921	0.854	0.406
CC *x* CS *x* DE			0.346	0.301	0.075	0.007	0.647	0.884	0.961
Breeder age (Block)			0.038	0.111	0.389	0.151	0.199	0.038	0.112

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

Table 4.11: Effect of coccidiosis control method, *Eimeria* challenge 22 d of age, and finisher and withdrawal dietary caloric density levels on carcass yield of Ross 708 at 49 d.

Coccidiosis control method	Challenge status[1]	Diet caloric density[2]	Cold Carcass	Pectoralis major	Pectoralis minor	Wings	Legs (saddles)	Shell
			----------% ----------					
Salinomycin			78.64	28.77	5.42	9.48	37.38	18.11
Vaccine			78.62	28.35	5.66	9.55	37.65	18.06
	+		78.39	28.38	5.62	9.50	37.81	18.03
	-		78.88	28.75	5.46	9.53	37.22	18.14
		Low	78.52	28.47	5.55	9.54	37.34	18.22
		High	78.75	28.65	5.54	9.49	37.69	17.96
Salinomycin	+		78.02	28.58	5.56	9.48	37.87	17.93
	-		79.27	28.97	5.29	9.48	36.89	18.30
Vaccine	+		78.76	28.17	5.69	9.53	37.75	18.13
	-		78.49	28.52	5.63	9.57	37.54	17.99
Salinomycin		Low	78.54	28.93	5.44	9.48	37.14	18.04
		High	78.75	28.62	5.41	9.47	37.62	18.19
Vaccine		Low	78.50	28.01	5.66	9.59	37.54	18.40
		High	78.75	28.69	5.66	9.51	37.75	17.72
	+	Low	78.50	28.17	5.64	9.59	37.83	18.14
		High	78.28	28.58	5.60	9.42	37.79	17.92
	-	Low	78.54	28.77	5.45	9.49	36.85	18.30
		High	79.22	28.73	5.47	9.57	37.58	17.99
Salinomycin	+	Low	78.25±0.74	28.94±0.45	5.74±0.16	9.49±0.16	37.57±0.37	17.66±0.34
		High	77.80±1.12	28.22±0.51	5.38±0.09	9.46±0.14	38.17±0.46	18.19±0.21
	-	Low	78.83±0.34	28.92±0.45	5.13±0.24	9.48±0.21	36.71±0.74	18.41±0.29
		High	79.70±0.43	29.02±0.67	5.44±0.11	9.49±0.10	37.07±0.50	18.18±0.61
Vaccine	+	Low	78.75±0.19	27.40±0.50	5.55±0.04	9.68±0.11	38.09±0.39	18.62±0.37
		High	78.77±0.40	28.95±0.27	5.83±0.16	9.38±0.10	37.41±0.53	17.65±0.32
	-	Low	78.25±0.42	28.62±0.32	5.77±0.09	9.50±0.16	36.98±0.71	18.18±0.46
		High	78.73±0.54	28.43±0.48	5.49±0.12	9.64±0.16	38.10±0.34	17.80±0.22
Breeder age	Old		78.41±0.35	28.42±0.25	5.57±0.07	9.51±0.07	37.83±0.24	18.15±0.20
	Young		78.85±0.22	28.70±0.24	5.51±0.09	9.52±0.07	37.19±0.28	18.02±0.17

Source of variation	*P*-value					
Coccidiosis control (CC)	0.860	0.215	0.024	0.508	0.462	0.898
Challenge status (CS)	0.270	0.270	0.101	0.812	0.102	0.768
CC *x* CS	0.082	0.964	0.233	0.879	0.292	0.379
Dietary caloric density (DE)	0.524	0.583	0.989	0.688	0.334	0.342
CC *x* DE	0.987	0.147	0.959	0.753	0.716	0.119
CS *x* DE	0.276	0.487	0.699	0.238	0.278	0.902
CC *x* CS *x* DE	0.608	0.061	0.005	0.369	0.176	0.215
Breeder age (Block)	0.294	0.397	0.520	0.880	0.092	0.630

Values are means ± SE of 6 pens per treatment combination with 26 birds per pen.

[1] Birds were challenged with a 20 x dose of Coccivac B52® at 22 d of age.

[2] Dietary treatments of low and high caloric densities were introduced at 28 d of age when finisher phase started.

CHAPTER 5

INTERACTIONS OF ENVIRONMENTAL TEMPERATURE AND DIETARY PROTEIN CONCENTRATIONS IN BROILERS WITH COCCIDIOSIS VACCINATION

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ABSTRACT

The objectives of this study were to investigate the effects of environmental temperature and dietary protein concentrations on commercial broilers given coccidiosis vaccine at d of hatch. Two thousand one hundred and twelve, male d-old chicks were randomly assigned to 24 pens of 22 chicks each in a 2x2 factorial arraignment consisting of 2 environment temperatures (ET), standard (S) or reduced (R; 3℃ cooler after 3 d of age), and 2 dietary protein concentrations, high (HP) or low (LP). Two trials were conducted with the room treatments alternated between trials to account for room effects, Trial 1 (T1) and Trial 2 (T2) respectively. There were two dietary phases: starter (0-16 d, HP 23% CP, LP 18% CP) and grower (16-29 d, HP 23% CP, LP 19% CP). Two birds per pen were randomly selected for cloacal temperature measurements at 8, 15, 22, and 29 d of age. BW was recorded at 0, 16, and 29 d and FCR at 16 and 29 d. Significant effects ($P < 0.036$) on BW were observed based on ET at 16 and 29 d for T1 with the warmer birds having higher BW, however in T2 the cool birds had higher BW at 16 and 29 d ($P < 0.008$). In both trials the HP treatment birds were heavier at 16 d ($P < 0.039$). In T1 this observation was also seen at 29 d ($P < 0.001$). In T1 the warmer birds had lower FCR from 0-16 d and 0-29 ($P < 0.001$), this observation was not seen in T2 ($P > 0.546$). Feeding the HP diet resulted in lower FCR in both trials from 0-16 d, 0-29 d, and 16-29 d ($P < 0.024$). In T1 the cooler birds had higher mortality throughout the study ($P < 0.001$), however this was not observed in T2 ($P > 0.292$). Higher mortality was observed in the LP fed birds in T2 (16.67%vs. 11.55%, $P = 0.012$). Dietary protein level did not affect mortality in either trial ($P > 0.501$). In T1, cloacal temperatures at 15 d were lower in the reduced temperature room ($P = 0.035$); in T2 they were lower at 8 ($P = 0.022$) and 15 d ($P = 0.003$). No interactions between ET and protein level were observed for BW, BWG, FCR, or mortality. Most of the mortalities observed during

the study showed lesions typical of necrotic enteritis. In T1 there was much more mortality in the reduced environmental temperature room due to necrotic enteritis (NE) suggesting an interaction between temperature and infection rate. In T2, NE was equally distributed in the two rooms. Therefore, diet and temperature results were confounded by disease. These observations show when additional variables such as disease stressors are added to trials it becomes difficult to understand the results.

INTRODUCTION

Since the development of the first coccidiosis vaccine by Edgar at Auburn University, the popularity of coccidiosis vaccines has grown substantially (Edgar and King, 1952). From consumer pressure, there has been a push to move away from the use of antibiotics in animal agriculture, including ionophore anticoccidial drugs (Cervantes, 2015). Due to these changes in consumer preferences the use of coccidiosis vaccines have become more important to the sustainability of coccidiosis prevention in poultry. In previous years these vaccines were most commonly used during the warmer months of the year, however some poultry integrators have moved to using the vaccines all year long. Research has shown that birds reared in cooler temperatures are more susceptible to other enteric diseases, like necrotic enteritis (Tsiouris, et al., 2015; Tsiouris, 2016). Regnier and Kelley (1981), demonstrated that when birds were cold stressed their immune responses were suppressed.

Two of the most common issues that arise during a coccidiosis infection in broilers are decreased body weights and increased feed conversion ratios. For instance, a study was conducted on the economic losses that occur in the U.K. poultry industry from coccidiosis, an estimated 46% was due to decreased body weights and 34% was from reduced feed efficiency

(Williams, 2005). Reductions in performance parameters have been shown to occur during subclinical infections similar to that of coccidiosis vaccination cycling (Danforth, 1998; Williams and Gobbi, 2002). These decreases in performance parameters have been shown to be in part due to reductions in the digestibility of amino acids and metabolizable energy in the bird's diets (Persia, et al., 2006; Amerah and Ravindran, 2015; Rochell, et al., 2016b). In work done by Lee, et al. (2011), birds given reduced crude protein starter diets while being vaccinated for coccidiosis had reduced body weights compared to those on standard crude protein diets. Supplemental amino acids have the ability to improve the development and immunity of the intestinal tracts of broilers (Tan, et al., 2014a; Tan, et al., 2014b; Gottardo, et al., 2016; Rochell, et al., 2016a; Bortoluzzi, et al., 2017). Since live vaccines develop immunity by presenting mild infections in the host, the effects of these mild infections could reduce protein digestion or absorption through intestinal damage.

While the effects of heat stress and dietary protein have been studied, most nutritional research on reduced environmental temperatures has been on consumption and not specifically protein concentrations. Therefore, the objective of this study was to evaluate the interactions of environmental temperature and dietary protein concentrations in birds given coccidiosis vaccination at d of hatch.

Materials and Methods

Birds and Husbandry

This study was conducted in two rooms with separate temperature controllers. To replicate the effects of temperature, dietary treatments were identical in each room and the standard temperature regime was in one room at time = 1 (Trial 1, T1) and the other at time = 2

(Trial 2, T2). A total of 1,056 day-old male Cobb 500 chicks were raised to 30 d of age for each trial. Each trial consisted of 2 environmental temperatures and 2 dietary protein concentrations. Within the 2 trials, each treatment combination was allocated 12 pen replicates consisting of 22 birds per pen, for a stocking density of 0.084 sq. m per bird, for a total of 264 birds per treatment. All birds were vaccinated using a spray cabinet at day of hatch with a live coccidiosis vaccine.

There were 2 windowless rooms used for each trial. These rooms had 24, 1.22 x 1.52 m floor pens, supplemented with a hanging feeder, 5 drinking nipples, and 5 cm of new pine shaving litter. Cardboard feeder trays were provided for each pen from the d of placement to d 3. Light was provided for 24 h on the d of placement, thereafter 1 h of darkness was added each day until 6 h of darkness was reached on d 6, from d 6 to 30 the birds received 18 h of light and 6 h of darkness. From 0 to 6 d, light was supplied at 30 lux and thereafter at 3.5 lux.

Dietary Treatments

The birds were fed a 2-phase feeding program with a starter diet, 0 to 16 d, and a grower diet, 16 to 30 d. The starter diets were fed as a crumble while the grower diets were in pellet form. There were 2 dietary treatments: high protein (HP), with roughly 23% CP in the starter and grower, low protein (LP), which had roughly18% CP in the starter diet and 19% CP in the grower diet (Table 5.1). The diets were corn-soybean meal based and were formulated according to the breeder recommendations. All diets contained sub-therapeutic levels of bacitracin methylene disalicylate at 50 mg/kg. Feed and water were consumed *ad libitum*.

Environmental Temperature Treatments

There were 2 environmental temperature treatments, standard and reduced environmental temperatures. The standard temperature treatment was based on the breeder guidelines with the initial temperature set at 34℃, then decreasing daily to meet target temperatures of 31℃ (d 7), 27℃ (d 14), 24℃ (d 21), and 21℃ (d 28). The reduced temperature treatment started at 34℃, and then decreased daily for to reach target temperatures of 27℃ (d 7), 24℃ (d 14), 23℃ (d 21), and 18℃ (d 28). For each trial, 2 identical rooms were used, one per environmental temperature treatment respectively. Temperature treatment assignments were switched between the 2 rooms for the two trials in order to negate any possible room effects. Temperatures were recorded twice daily, representing an average of four thermometers that were dispersed in the room, and used for calculation of daily temperature averages.

Data Collection

Pen group BW and feed intake were recorded at d of placement and again at 16 and 29 d of age. FCR was calculated from 0 to 16, 0 to 29, and 16 to 29 d of age. Mortality was recorded twice daily, and necropsies were conducted on mortality that was still warm. On 8, 15, 22, and 29 d of age cloacal temperatures from 2 randomly selected birds per pen were measured using a digital thermometer. Intestinal tissue samples were collected in Trial 1 for histological examination. For both trials *C. perfringens* samples were collected and isolated from the jejunum of 5 sample birds. Minimum inhibitory concentration (MIC) test were conducted for bacitracin methylene disalicylate sensitivity in the *C. perfringens* isolates using agar the dilution method. Four birds per pen were randomly selected, tagged, and fasted on d 29 for processing. On d 30 the birds were slaughtered, eviscerated, and then chilled for 3 h. Live weights, cold carcass,

pectoralis major, pectoralis minor, white striping scores (0-4), wings, saddle, and shell weights were recorded and used to calculate yield percentages.

Data Analysis

Trial by treatment interactions were observed therefore the trials were analyzed individually. Performance and processing data were analyzed in a completely randomized block design with a factorial arrangement of 2 environmental temperatures and 2 dietary protein concentrations. Pens were considered as the trial unit for both performance and processing data. Mortality and processing yield percentages were arc sine transformed, the true means for these parameters are presented with the *P*-values from the transformed data. Means were separated using Duncan's new multiple range test when *P*-values were equal or less than 0.05. All data were analyzed using SAS 9.4.

RESULTS

Unexpected Outcomes

There was an unexpected outcome with an excess of mortality for both trials. In the first trial, mortality peaked on d 7 and then again on d 13 (1.89% each day). The histological reports confirmed that the lesions observed during necropsies were from necrotic enteritis. Due to the excessive mortality from necrotic enteritis penicillin was provided in the drinker lines for 5 d at 1,500,000 units of penicillin per 3.8 L. In Trial 2, high mortality was also observed, the first peak occurred on d 9 in which the reduced temperature room had 1.80% mortality for the day and the standard temperature room had 1.14%. A second peak in mortality occurred on d 24 for the reduced temperature room at 0.76% and d 25 for the standard temperature with 1.42% for the

day. Penicillin was administered in the drinker lines for 5 d at 1,500,000 units of penicillin per 3.8 L starting on d 9 and again on d 25 to help alleviate mortality. Results from the MIC test showed that the C. perfringens isolates were severely resistant to bacitracin methylene disalicylate (> 64 μg/mL).

Trial 1

There were significant differences in 16 d BW based on temperature treatment ($P <$ 0.001) and dietary protein level ($P < 0.001$, Table 5.2). These differences were still apparent in 29 d BW with the standard temperature room averaging 4.3% larger BW ($P = 0.035$), as well as the high protein treatment averaging 9.1% larger BW ($P < 0.001$) than the low protein treatment. The 16-29 d BWG difference reflected the 29 d BW differences based on protein level ($P =$ 0.002), however no temperature effect on 16-29 d BWG was observed ($P = 0.433$). The birds reared at the standard temperature and fed the high protein diet had the highest BW at 16 d ($P =$ 0.018). Birds reared at the standard temperature had lower ($P < 0.001$) FCR from 0-16 and 0-29 d, the 16-29 d FCR was close to significant at $P = 0.051$ (Table 5.4). High protein diet birds had improved FCR from 0-16 d ($P < 0.001$), 0-29 d ($P < 0.001$), and 16-29 d ($P = 0.023$). The cool birds had over 20% higher mortality ($P < 0.001$) than their counter parts from 0-16 and 0-29 d. There were no dietary protein effects ($P > 0.501$) observed on percent mortality. At 15 d, birds reared in the reduced temperature treatment had lower cloacal temperatures ($P = 0.035$), no other significant results were observed at any other time point for all parameters (Table 5.6). There were no other main effect interactions observed in performance parameters or percent mortality for Trial 1 ($P > 0.221$).

The standard temperature birds had higher cold carcass weights ($P = 0.019$) and yields ($P < 0.001$, Table 5.8). Although these differences were not translated in pectoralis major and minor weights or yield ($P > 0.053$), they were seen in wing weights ($P = 0.004$) and yields ($P = 0.014$) along with saddle weights ($P = 0.026$) and yields ($P = 0.040$, Table 5.9). Higher white striping scores were observed in the standard environmental temperature birds (1.32 vs. 0.75, $P < 0.001$ respectively). The birds fed the higher protein diet had higher cold carcass weights (1275.9 vs 1197.7, $P < 0.001$ respectively), no differences were seen in total yields between the two groups ($P = 0.946$). Increased weights ($P < 0.018$) were seen for pectoralis major, pectoralis minor, wing, and saddle weights in the high protein birds, these differences were also seen in the yields for the previous cut-up pieces ($P < 0.001$) except for wings ($P = 0.792$). No diet by environmental temperature interactions ($P > 0.092$) were seen for all parameters except live weight ($P =0.025$), cold carcass weight ($P = 0.013$), and saddle weight ($P = 0.008$). There were no significant differences ($P > 0.108$) observed for white striping scores based on dietary protein level or diet by environmental temperature.

Trial 2

A temperature effect was ($P < 0.008$) observed in 16 and 29 d BW, with the reduced temperature treatments having heavier BW, this was reflected in 16-29 d BWG ($P < 0.005$, Table 5.3). The high protein birds had heavier BW at 16 d ($P = 0.038$), however this was not detectable at 29 d ($P = 0.225$) or for 16-29 d BWG ($P = 0.456$). No temperature effects were observed in FCR ($P > 0.425$) or mortality ($P > 0.292$) at any time point (Table 5.5). The high protein birds showed higher FCR from 0-16 d ($P < 0.001$), 0-29 d ($P = 0.001$), and 16-29 d BWG ($P = 0.023$). The low protein group had over 5% higher mortality ($P = 0.012$), from 0-16 d, however

differences were not seen in 0-29 d mortality ($P = 0.666$). No main effect interactions were observed in Trial 2 for any performance parameters or percent mortality time points ($P > 0.172$). The birds reared in the reduced environmental temperature room had lower cloacal temperatures at 8 and 15 d of age ($P = 0.022$, $P = 0.003$ respectively, Table 5.7), these differences were not seen for the remainder of the trial ($P > 0.062$). There were no significant effects from diet or diet by environmental temperature seen in cloacal temperatures for any recorded time point ($P > 0.134$).

The reduced temperature birds had higher cold carcass weights ($P = 0.008$, Table 5.10) and pectoralis weights ($P = 0.028$), however these differences were not reflected in total carcass yield ($P = 0.274$) or pectoralis major yield ($P = 0.554$, Table 5.11). No differences ($P > 0.300$) were observed in pectoralis minor weights and yields or wing weights, the standard temperature birds however did present higher wing yields (7.94 vs. 7.64, $P = 0.025$). Higher saddle weights were observed in the reduced temperature treatment group (513.1 vs 492.6, $P = 0.013$), these differences were not seen in saddle yields ($P = 0.487$). Cold carcass weights in Trial 2 were similar to Trial 1, with the high protein diet birds having larger cold carcass weights ($P = 0.001$), no differences were seen in yields for this treatment ($P = 0.131$). The high protein birds had larger weights ($P < 0.001$) and yields ($P < 0.032$) for pectoralis major, pectoralis minor, and wings. Around a 15 g larger saddle was seen in the high protein birds, however this difference was unable to be shown ($P = 0.060$). There were no protein effects ($P = 0.181$) on saddle yields. The birds reared in the reduced environmental temperature and fed the reduced protein diet had the highest weights ($P = 0.020$) and yields ($P = 0.010$). No other diet by environmental temperature interactions ($P > 0.211$) were observed for any of the other processing parameters

recorded. No significant ($P > 0.307$) observations were observed for either main effects or their interactions for white striping scores.

DISCUSSION

The Daily average temperatures for Trials 1 and 2 can be seen in Figures 5.1 and 5.2, the desired differences in temperature were not reached in either trial. When comparing BW and FCR between the two treatments groups, it appears that the temperature differences did impact the birds in the reduced environmental temperature.

Of the research that has been done most, if not all, of the studies have included coccidiosis vaccine and protein concentrations but have not evaluated if environmental temperatures played a role in these interactions. Trials 1 and 2 had contradicting performance parameters results. Due to the severe necrotic enteritis mortality that was observed in the cooler room in Trial 1 and the standard room in Trial 2, it is hypothesized that the rooms were not adequately disinfected between the two trials. The spore forming bacteria, *C. perfringens*, can be difficult to eradicate when in the spore phase. These spores are very durable and have been shown to remain viable after being treated with disinfectants, broad ranges of temperature, high pressure, and radiation (Talukdar, et al., 2015). It is believed that the reduced BW, increased FCR, and higher percent mortality observed in the second trial in the standard temperature birds was a result of excessive C. perfringens challenge in the room mentioned above. Even though the reduced temperature room was plagued with necrotic enteritis in the first trial, the birds in trial one had body weights that were over 50 grams heavier than the breeder guideline predicts for 29 d however their FCR were higher. This observation shows that even though these birds were infected with necrotic enteritis they were still able to grow exceptionally well even with poor

FCR. Protein level has a large effect when the birds were closer to growing at their genetic potential, shown in Trial 1, and a significant protein and temperature interaction could be seen at 16 d, with a 63 g difference between the two protein concentrations in the standard temperature room and a 30 g difference in the reduced temperature room. It is not clear if the decreases in growth in the reduced temperature room were due to temperature or necrotic enteritis. When the genetic potential of birds in both rooms was suppressed by necrotic enteritis, similar to Trial 2, the effect of protein level was not as apparent at 16 d, with a difference of 22 g between the two protein concentrations in the standard temperature room and 5 g in the reduced temperature room. The difference seen at 29 d were larger than those at 16 d, however even with relatively small standard errors the differences were not able to be shown at $P < 0.005$.

Birds fed the high protein diet had larger BW and improved FCR in both trials, previous researchers have shown similar results (Pesti and Fletcher, 1983; Roush, 1983; Pesti and Fletcher, 1984; Pesti and Smith, 1984; Cabel and Waldroup, 1991). The differences in BW at 29 d between the protein treatments that were able to be shown in Trial 1 were not able to be shown in Trial 2, this may be due to the interactions that have been documented between dietary protein concentrations and necrotic enteritis. It has been documented in previous research that higher concentrations of crude protein can predispose birds to necrotic enteritis (M'Sadeq, et al., 2015). Timbermont, et al. (2011), hypothesized that when diets are rich in protein they provide more nutrients for the bacteria to thrive on which ultimately leaves the birds more susceptible to necrotic enteritis.

Reduced cloacal temperatures seen at 15 d in the first trial and d 8 and 15 in the second trial are similar to results reported by Da Costa, et al. (2017), however in their study the birds reared at a reduced temperature had reduced cloacal temperatures throughout the grow out. One

explanation for the differences in our results and Da Costa's, was the high incidence of necrotic enteritis that was seen in our study. The higher part weights and yields that were observed for the high protein treatment were expected, in research conducted by Widyaratne and Drew (2011), it was shown that high protein diets provided higher white meat yields and weights compared to low protein diets.

In Trial 1 increased mortality in the reduced environmental temperature room due to necrotic enteritis (NE) suggested an interaction between temperature and infection rate. However, in Trial 2 NE was distributed equally in the two rooms, therefore, diet and temperature results were confounded by disease. This study shows when additional stressors, like disease, are unexpectedly added to studies, the interpretation of the results and the expected outcomes can be interfered. Understanding factorial design trials can be difficult and the addition of external factors (necrotic enteritis) can really make these studies particularly difficult to interpret.

Decreased production costs from reduced management practices are negated when there is an increase in mortality from poor management practices, mortality losses can ultimately lead to large profit loss for integrators. The effect of varying protein concentrations can have a significant effect on performance, it is very important to run cost analysis to see if the benefits of additional protein outweigh the cost. Overall this study shows how unexpected disease stressors, such as necrotic enteritis, can influence trial results. This was seen with the birds having improved body weights when fed higher dietary protein while in the cool environment in Trial 1, but in Trial 2 these results were obscured due to the effects of necrotic enteritis in both rooms.

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Table 5.1: Ingredient composition (%) and formulated nutrient contents of the starter and grower diets with differing concentrations of crude protein.

Diet Blend	Starter		Grower	
	Low Protein	High Protein	Low Protein	High Protein
% Crude Protein	18.02	23.00	19.17	23.30
	%			
Corn	69.61	55.14	64.24	51.74
Soybean Meal, 48%	25.80	38.12	29.93	40.46
Poultry fat	0.50	2.51	2.55	4.57
Dicalcium phosphorus, 19%	1.38	1.31	1.20	1.14
Limestone	1.29	1.23	1.20	1.15
DL-Methionine	0.26	0.42	0.08	0.13
L-Lysine-HCL, 78%	0.28	0.33	0.00	0.00
L-Threonine, 98.5%	0.08	0.13	0.00	0.00
Salt (NaCl)	0.40	0.40	0.40	0.40
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.09	0.09	0.09	0.09
BMD[3]	0.05	0.05	0.05	0.05
Phytase	0.02	0.02	0.02	0.02
Total:	100.00	100.00	100.00	100.00
Calculated Nutrient Composition				
ME, kcal/g	3.05	3.04	3.11	3.11
CP, %	18.02	23.00	19.17	23.30
Calcium, %	0.90	0.90	0.84	0.84
Total Phosphorus, %	0.64	0.67	0.62	0.65
Avail. Phosphorus< %	0.45	0.45	0.42	0.42
Sodium, %	0.18	0.18	0.18	0.18
Chloride, %	0.27	0.27	0.27	0.27
Choline, mg/kg	1.72	1.91	1.78	1.94
dLys, %	1.07	1.41	0.96	1.21
dMet, %	0.54	0.75	0.38	0.47
dTSAA, %	0.80	1.05	0.65	0.79
dThr, %	0.69	0.91	0.67	0.82
dTrp, %	0.20	0.27	0.22	0.28
dArg%	1.12	1.48	1.24	1.55
dVal%	0.88	1.12	0.96	1.16

[1] Vitamin mix provided the following (per kilogram of diet): thiamin-mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0g; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-reinyl acetate, 2,500 IU; all-rac-tocopherol acetate, 11 IU; ethoxyquin, 150 mg.

[2] Trace mineral mix provides the following (per kilogram of diet): manganese (MnSO4.H2O), 101 mg; iron (FeSO4.7H2O), 20 mg; zinc (Zn)), 80 mg; copper (CuSO4.5H2O), 3 mg; iodine (ethylene diamine dihydroiodide), 0.75 mg; magnesium (MgO), 20 mg; selenium (sodium selenite), 0.3 mg.

[3] BMD (Bacitracin Methylene Disalicylate - Type A) provides (per kilogram of diet): feed grade bacitracin methylene disalicylate equivalent to 50 mg bacitracin.

Table 5.2: Body weight (BW) and body weight gain (BWG) for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 1).

Treatment		BW (g)			BWG (g)
Environmental temperature	Protein concentration	0 d	16 d	29 d	16-29 d
Standard		43.0±0.2	630.1±7.8	1798.3±24.7	1168.1±18.8
Reduced		42.5±0.1	580.8±5.8	1723.7±32.1	1142.9±29.6
	High	42.7±0.2	628.5±8.0	1837.6±21.1	1209.0±19.4
	Low	42.8±0.2	582.4±6.0	1684.4±28.4	1102.0±24.8
Standard	High	42.8±0.2	661.4[a]±6.4	1884.8±17.1	1223.5±15.5
Standard	Low	43.2±0.3	598.9[b]±6.0	1711.6±29.9	1112.8±26.1
Reduced	High	42.5±0.2	595.7[b]±5.8	1790.3±34.1	1194.5±36.0
Reduced	Low	42.5±0.3	565.9[c]±8.2	1657.1±48.3	1091.2±43.3
Source of variation		*P*-values			
Environmental temperature		0.045	<0.001	0.035	0.433
Protein concentration		0.501	<0.001	<0.001	0.002
PC *x* ET		0.501	0.018	0.562	0.909

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature
[a-c] Treatment means with the same superscript are not significantly at $P < 0.05$, Duncan's New Multiple Range Test (1955).

Table 5.3: Body weight (BW) and body weight gain (BWG) for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 2).

Treatment		BW (g)			BWG (g)
Environmental temperature	Protein concentration	0 d	16 d	29 d	16-29 d
Standard		43.3±0.2	549.0±3.9	1594.9±18.1	1045.9±16.3
Reduced		43.3±0.1	566.2±5.0	1672.6±13.7	1106.3±11.0
	High	43.3±0.1	564.1±3.9	1647.7±18.6	1083.6±16.6
	Low	43.3±0.1	551.1±5.3	1619.7±16.8	1068.6±13.6
Standard	High	43.3±0.2	559.7±4.2	1613.0±26.9	1053.3±25.9
Standard	Low	43.2±0.2	538.3±4.9	1576.8±24.1	1038.5±20.8
Reduced	High	43.3±0.2	568.5±6.4	1682.5±22.4	1114.0±17.8
Reduced	Low	43.3±0.2	563.9±8.0	1662.6±16.4	1098.7±13.2
Source of variation		*P*-values			
Environmental temperature		0.925	0.007	0.001	0.004
Protein concentration		0.862	0.038	0.225	0.456
PC *x* ET		0.661	0.173	0.723	0.990

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature

Table 5.4: Feed conversion ratio (FCR) and percent mortality for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 1).

Treatment		FCR (g:g)			Mortality (%)	
Environmental temperature	Protein concentration	0-16 d	0-29 d	16-29 d	0-16 d	0-29 d
Standard		1.278±0.014	1.432±0.015	1.510±0.017	4.18±1.55	11.15±2.38
Reduced		1.415±0.018	1.508±0.020	1.554±0.025	26.99±2.47	36.12±2.77
	High	1.282±0.017	1.402±0.017	1.463±0.016	16.20±3.04	24.28±3.37
	Low	1.411±0.017	1.537±0.014	1.601±0.016	14.97±3.25	22.99±3.94
Standard	High	1.216±0.010	1.368±0.013	1.445±0.016	5.70±2.91	13.85±4.24
Standard	Low	1.339±0.005	1.496±0.009	1.575±0.013	2.65±1.04	8.44±2.11
Reduced	High	1.347±0.018	1.436±0.018	1.481±0.027	26.69±3.19	34.71±3.13
Reduced	Low	1.483±0.017	1.579±0.022	1.628±0.028	27.29±3.92	37.54±4.69
Source of variation		*P*-values				
Environmental temperature		<0.001	<0.001	0.051	<0.001	<0.001
Protein concentration		<0.001	<0.001	<0.001	0.511	0.502
PC *x* ET		0.630	0.635	0.697	0.434	0.222

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature

Table 5.5: Feed conversion ratio (FCR) and percent mortality for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 2).

Treatment		Adjusted FCR (g:g)			Mortality (%)	
Environmental temperature	Protein concentration	0-16 d	0-29 d	16-29 d	0-16 d	0-29 d
Standard		1.386±0.023	1.593±0.023	1.695±0.031	14.21±1.65	25.61±2.40
Reduced		1.394±0.016	1.575±0.023	1.662±0.029	14.02±1.22	21.95±1.89
	High	1.337±0.010	1.534±0.024	1.631±0.036	11.55±1.28	23.29±2.29
	Low	1.443±0.022	1.635±0.015	1.727±0.018	16.67±1.41	22.27±2.08
Standard	High	1.331±0.014	1.550±0.034	1.662±0.056	10.99±2.20	24.42±3.37
Standard	Low	1.442±0.039	1.636±0.026	1.729±0.025	17.42±2.16	26.79±3.52
Reduced	High	1.343±0.014	1.517±0.034	1.600±0.047	12.12±1.41	22.15±2.21
Reduced	Low	1.483±0.017	1.579±0.022	1.628±0.028	15.910±1.90	21.75±2.13
Source of variation		*P*-values				
Environmental temperature		0.757	0.547	0.426	0.792	0.293
Protein concentration		<0.001	0.001	0.023	0.012	0.666
PC *x* ET		0.838	0.586	0.476	0.377	0.707

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature

Table 5.6: Cloacal temperatures for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 1).

Treatment		Cloacal Temperature (°C)			
Environmental temperature	Protein concentration	8 d	15 d	22 d	29 d
Standard		41.43±0.03	41.17±0.03	41.05±0.03	41.18±0.04
Reduced		41.35±0.04	41.03±0.06	41.14±0.05	41.23±0.03
	High	41.36±0.04	41.07±0.05	41.12±0.05	41.18±0.03
	Low	41.42±0.03	41.13±0.05	41.07±0.04	41.23±0.04
Standard	High	41.41±0.04	41.13±0.04	41.08±0.03	41.17±0.05
Standard	Low	41.45±0.04	41.22±0.05	41.03±0.05	41.20±0.06
Reduced	High	41.31±0.05	41.01±0.09	41.17±0.09	41.20±0.04
Reduced	Low	41.39±0.06	41.05±0.07	41.11±0.06	41.25±0.03
Source of variation		*P*-values			
Environmental temperature		0.088	0.035	0.142	0.370
Protein concentration		0.200	0.325	0.400	0.370
PC *x* ET		0.732	0.679	0.888	0.765

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature

Table 5.7: Cloacal temperatures for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 2).

Treatment		Cloacal Temperature (°C)			
Environmental temperature	Protein concentration	8 d	15 d	22 d	29 d
Standard		41.33±0.04	41.49±0.03	41.27±0.05	41.22±0.04
Reduced		41.18±0.05	41.36±0.03	41.13±0.06	41.31±0.03
	High	41.25±0.05	41.44±0.04	41.20±0.05	41.25±0.03
	Low	41.26±0.05	41.41±0.02	41.20±0.06	41.28±0.04
Standard	High	41.23±0.08	41.38±0.05	41.11±0.07	41.29±0.03
Standard	Low	41.13±0.06	41.35±0.03	41.15±0.10	41.33±0.06
Reduced	High	41.28±0.05	41.50±0.05	41.30±0.07	41.22±0.05
Reduced	Low	41.38±0.07	41.47±0.03	41.25±0.06	41.22±0.05
Source of variation		*P*-values			
Environmental temperature		0.022	0.003	0.063	0.078
Protein concentration		0.924	0.416	0.956	0.673
PC *x* ET		0.135	1.000	0.545	0.673

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature

Table 5.8: Processing weights, yields, and white striping scores for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 1).

Treatment		Carcass Composition at 30 d			
Environmental temperature	Protein concentration	Live Weight	Cold Weight	Yield	White Striping Score
		g	g	(%)	(0-4)
Standard		1765.6±27.3	1261.6±20.9	71.41±0.18	1.32±0.09
Reduced		1720.6±17.3	1212.1±12.3	70.41±0.21	0.75±0.06
	High	1798.1±21.9	1275.9±17.9	70.90±0.24	1.13±0.11
	Low	1688.1±18.6	1197.7±13.7	70.92±0.20	0.95±0.09
Standard	High	1851.9±30.7[a]	1327.2±24.6[a]	71.61±0.21	1.46±0.13
Standard	Low	1679.2±28.7[c]	1196.0±20.9[b]	71.21±0.28	1.19±0.11
Reduced	High	1744.4±23.1[b]	1224.7±15.9[b]	70.20±0.31	0.79±0.10
Reduced	Low	1696.9±24.7[bc]	1199.5±18.7[b]	70.63±0.28	0.71±0.09
Source of variation		*P*-values			
Environmental temperature		0.103	0.019	<0.001	<0.001
Protein concentration		<0.001	<0.001	0.946	0.109
PC *x* ET		0.025	0.013	0.136	0.392

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.

PC – Protein concentration

ET – Environmental Temperature

[a-c] Treatment means with the same superscript are not significantly at $p < 0.05$, Duncan's New Multiple Range Test (1955).

Table 5.9: Processing cut-up weights and yields for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 1).

Treatment		Carcass Composition at 30 d							
Environmental temperature	Protein concentration	Pectoralis Major		Pectoralis Minor		Wings		Saddle	
		(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Standard		248.0±5.8	13.99±0.15	54.1±1.4	3.06±0.04	146.5±2.4	8.30±0.05	531.8±8.1	30.13±0.14
Reduced		237.5±3.8	13.76±0.16	52.6±1.1	3.05±0.05	138.4±1.8	8.06±0.08	512.1±5.2	29.76±0.15
	High	257.7±4.4	14.30±0.12	57.4±1.1	3.19±0.04	147.3±2.1	8.19±0.06	532.6±7.5	29.62±0.14
	Low	227.7±3.4	13.46±0.14	49.3±0.9	2.92±0.03	137.6±2.0	8.17±0.08	511.4±5.9	30.28±0.13
Standard	High	267.3±7.1	14.39±0.18	58.8±1.7	3.17±0.05	153.6±2.7	8.30±0.06	554.3±9.9[a]	29.94±0.21
Standard	Low	228.7±4.8	13.60±0.19	49.4±1.4	2.94±0.05	139.4±2.9	8.31±0.09	509.3±9.2[b]	30.34±0.18
Reduced	High	248.2±4.0	14.20±0.15	56.0±1.4	3.21±0.06	141.0±1.9	8.09±0.11	510.9±7.0[b]	29.30±0.14
Reduced	Low	226.8±4.9	13.32±0.21	49.2±1.0	2.89±0.04	135.8±2.9	8.03±0.12	513.4±7.9[b]	30.23±0.18
Source of variation		*P*-values							
Environmental temperature		0.054	0.218	0.287	0.861	0.004	0.014	0.026	0.040
Protein concentration		<0.001	<0.001	<0.001	<0.001	<0.001	0.792	0.017	<0.001
PC *x* ET		0.115	0.773	0.379	0.417	0.093	0.725	0.008	0.144

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature
[a-c] Treatment means with the same superscript are not significantly at $p < 0.05$, Duncan's New Multiple Range Test (1955).

Table 5.10: Processing weights, yields, and white striping scores for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 2).

Treatment		Carcass Composition at 30 d			
Environmental temperature	Protein concentration	Live Weight	Cold Weight	Yield	White Striping Score
		g	g	(%)	(0-4)
Standard		1651.8±16.2	1207.5±12.7	73.08±0.17	0.60±0.06
Reduced		1710.7±17.8	1255.1±14.2	73.35±0.18	0.59±0.07
	High	1717.2±15.3	1260.7±11.8	73.41±0.17	0.65±0.06
	Low	1645.3±17.5	1201.9±14.0	73.03±0.18	0.55±0.06
Standard	High	1699.3±17.9	1246.2±12.9	73.33±0.26	0.65±0.10
Standard	Low	1604.4±19.2	1168.9±15.3	72.83±0.20	0.56±0.08
Reduced	High	1735.1±24.6	1275.3±19.4	73.84±0.22	0.65±0.08
Reduced	Low	1686.3±24.7	1234.9±19.7	73.22±0.29	0.54±0.10
Source of variation		*P*-values			
Environmental temperature		0.001	0.008	0.274	0.909
Protein concentration		0.002	0.001	0.131	0.308
PC *x* ET		0.296	0.286	0.635	0.909

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein concentration
ET – Environmental Temperature
[a-b] Treatment means with the same superscript are not significantly at $p < 0.05$, Duncan's New Multiple Range Test (1955).

Table 5.11: Processing cut-up weights and yields for birds fed high and low protein diets and reared at standard or reduced environmental temperatures (Trial 2).

Treatment		Carcass Composition at 30 d							
Environmental temperature	Protein concentration	Pectoralis Major		Pectoralis Minor		Wings		Saddle	
		(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Standard		253.7±4.8	15.34±0.20	49.8±1.7	3.01±0.09	131.1±2.0	7.94±0.09	492.6±5.8	29.82±0.16
Reduced		265.1±4.8	15.47±0.18	52.0±1.7	3.02±0.09	130.4±2.2	7.63±0.11	513.1±6.3	30.00±0.24
	High	274.2±3.6	15.96±0.16	55.2±1.6	3.21±0.09	136.1±1.5	7.94±0.10	510.5±5.3	29.73±0.17
	Low	244.6±3.8	14.84±0.13	46.6±1.3	2.82±0.07	125.4±2.0	7.63±0.10	495.3±7.0	30.09±0.23
Standard	High	271.7±5.5	15.99±0.26	54.7±2.3	3.22±0.13	136.7±2.1	8.05±0.10	509.8±6.3[a]	29.99±0.26[ab]
Standard	Low	235.8±2.7	14.69±0.15	44.9±1.7	2.80±0.11	125.5±2.6	7.84±0.14	475.5±6.7[b]	29.64±0.20[b]
Reduced	High	276.7±4.9	15.94±0.20	55.7±2.5	3.20±1.33	135.5±2.3	7.83±0.18	511.2±8.8[a]	29.46±0.19[b]
Reduced	Low	253.4±6.2	14.99±0.22	48.3±1.9	2.85±0.09	125.3±3.1	7.42±0.12	515.1±9.4[a]	30.55±0.38[a]
Source of variation		*P*-values							
Environmental temperature		0.028	0.554	0.301	0.987	0.787	0.025	0.013	0.487
Protein concentration		<0.001	<0.001	<0.001	0.002	<0.001	0.031	0.06	0.181
PC *x* ET		0.212	0.436	0.553	0.818	0.851	0.501	0.02	0.01

Values are means ± SE of 12 pens per treatment combination with 22 birds per pen.
PC – Protein Level
ET – Environmental Temperature
[a-b] Treatment means with the same superscript are not significantly at $p < 0.05$, Duncan's New Multiple Range Test (1955).

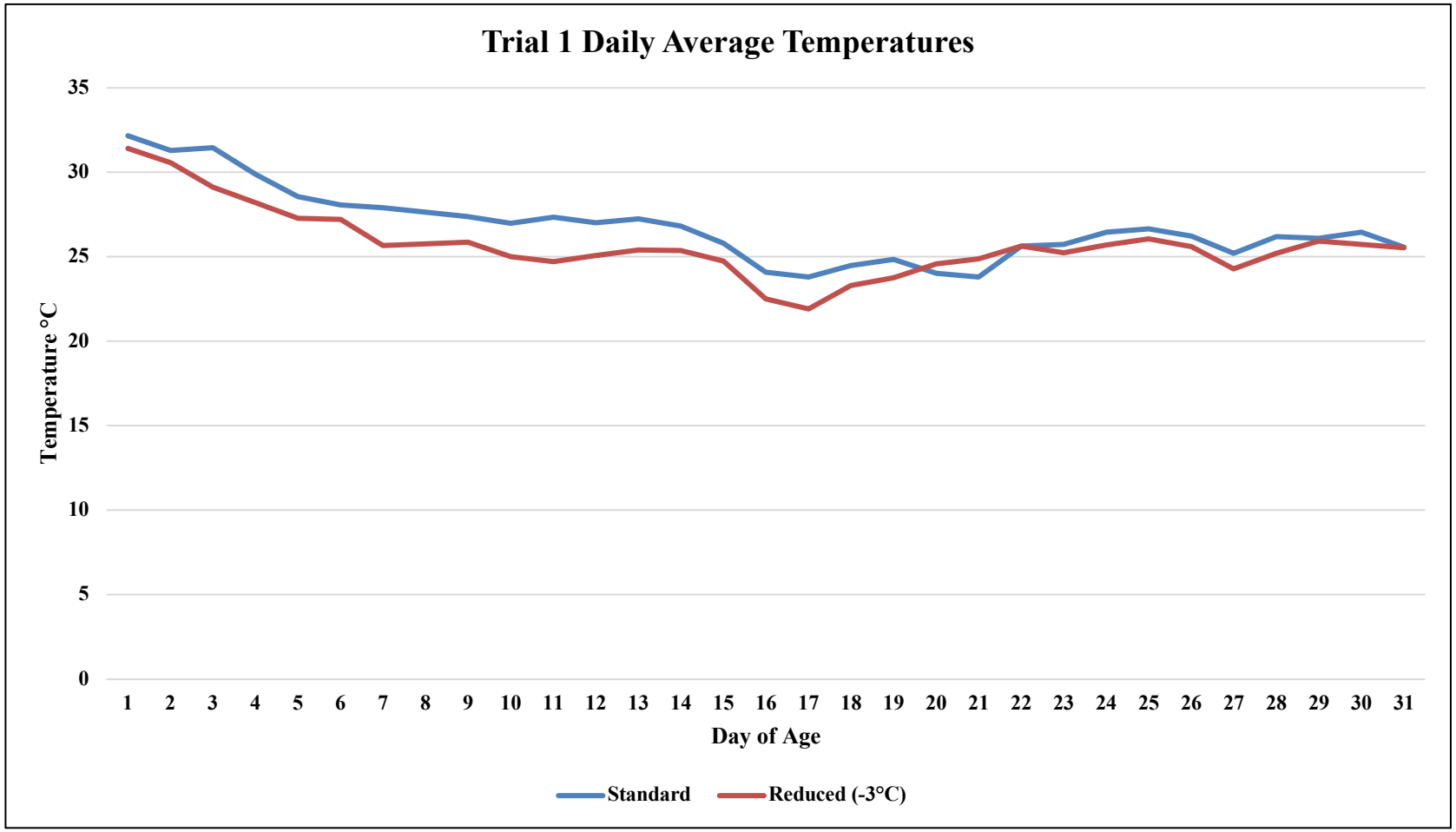

Figure 5.1: Daily average temperatures for the standard (breeder guidelines) and reduced (-3°C) temperature treatments (Trial 1).
Standard temperature was based on the breeder guidelines.
Daily averages were an average of four thermometers per temperature.

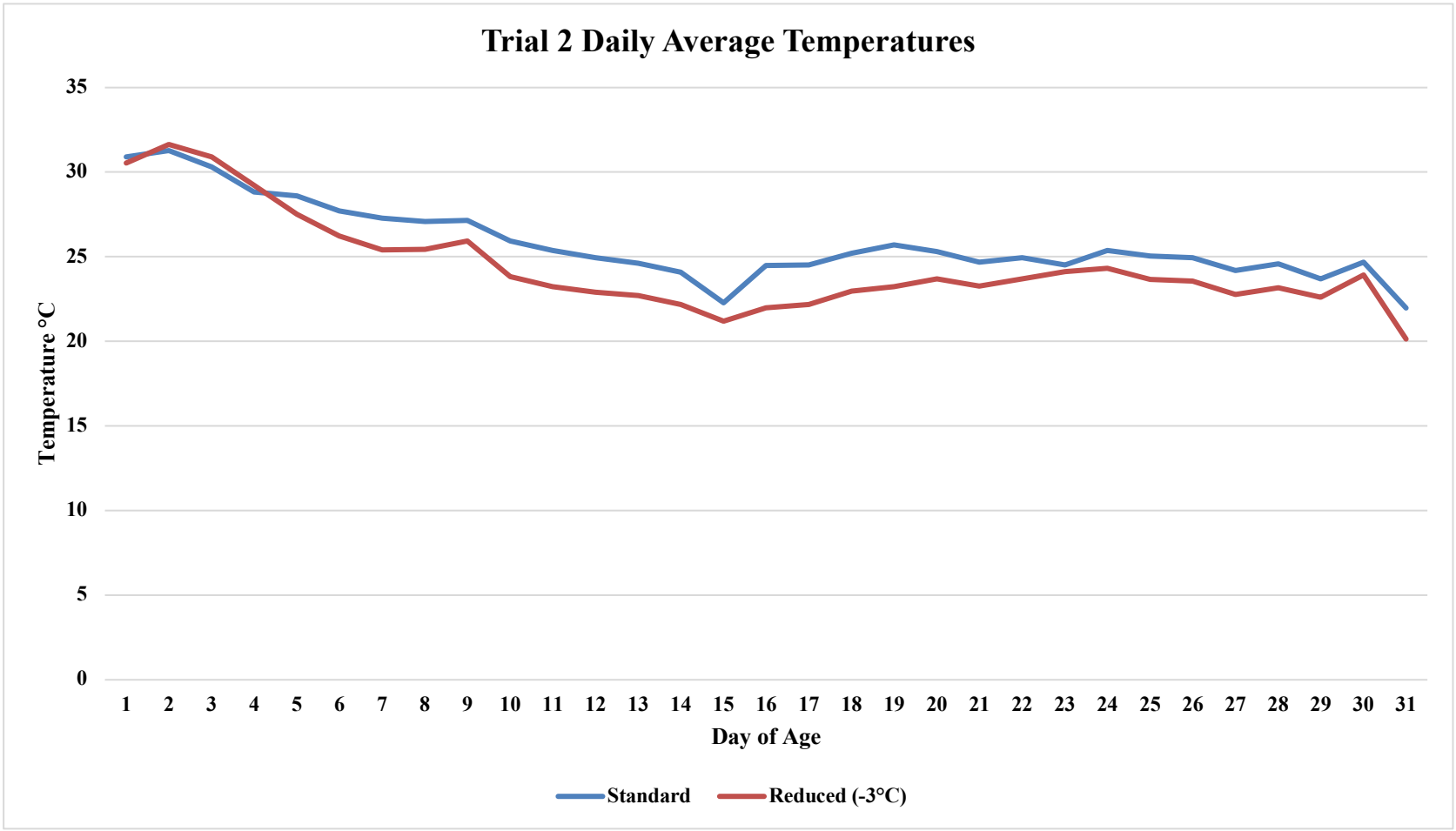

Figure 5.2: Daily average temperatures for the standard (breeder guidelines) and reduced (-3°C) temperature treatments (Trial 2).
Standard temperature was based on the breeder guidelines.
Daily averages were an average of four thermometers per temperature.

CHAPTER 6

CONCLUSIONS

The state of Georgia is the number one producer of poultry meat in the U.S., which is the number one producer of poultry meat globally. Due to the efficiency of the poultry industry, poultry meat has become one of the cheapest and highest consumed sources of animal protein. In poultry production, nutrition is regarded the most expensive production cost, making up roughly 70% of the total cost of production. Therefore, any variables added to the production system that may affect nutrient absorption or utilization can play a substantial role in the cost of production. Coccidiosis is an intestinal tract disease that causes poor feed digestion and absorption, which leads to decreases in feed efficiency and reduced body weights in commercial broilers. Due to the large economic impact that this disease has on the poultry industry, it is very important to understand coccidiosis control methods. Currently the poultry industry has a limited number of anticoccidials available on the market. Consumer pressure for antibiotic free production threatens the ionophore class of anticoccidials making the already limited list of anticoccidials smaller. With a shrinking list of usable anticoccidial drugs and no new advances in coccidiosis vaccines, it is increasingly more important to understand our coccidiosis control methods and how management practices can affect them.

In this work one can see the importance of proper management practices. When birds are heat stressed there are significant losses in body weight, 9% or more, and feed conversion ratios, over 4% higher, and even higher incidences of mortality. Heat and cold stress depresses growth and can also make birds more susceptible to other stressors such as diseases like necrotic

enteritis. When disinfectant procedures are not adequate enough to lower the bacterial challenge within the poultry houses, sequential flocks will have to overcome the bacterial challenge and potentially end up with clinical or subclinical infections. The disinfection of these bacteria can be imperative when using live coccidiosis vaccines, which can be a source of stress on the bird, as a coccidiosis control program. Special care in housing sanitation and litter management should be given when using live coccidiosis vaccines in order to reduce the potential for secondary infections.

It can be concluded that when there is no coccidiosis challenge, commercial broilers raised to 49 d will have similar performance when given bioshuttle programs, a coccidiosis vaccine only program, or an ionophore only program. When birds are challenged with coccidiosis, immunity developed from a live coccidiosis vaccine can provide equivalent bird performance and coccidiosis protection compared to salinomycin. There were no dietary and coccidiosis control method interactions, however the reduced dietary caloric density levels in the finisher and withdrawal diets that were used in this work were not adequate enough to cause differences in performance.