The impact of increasing dietary amino acid density and feed allocation during the first three weeks of life for broilers vaccinated against coccidiosis

by

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ABSTRACT

The objective of this research was to ameliorate growth depression and meat yield reductions associated with coccidiosis vaccination. Coccidiosis vaccination induces an enteric challenge to develop immunity from *Eimeria* infection. To achieve the research objective, 2 experiments were conducted providing diets with increased digestible (**d**) amino acid (**AA**) density during the starter period. The first experiment evaluated effects of starter diets formulated to either 1.15% dLys density or 1.25% dLys density in 3 different starter feed allotments to vaccinated broilers from 1 to 21 d of age on growth performance and meat yield during a 6 wk production period. The second experiment compared growth performance and meat yield responses of vaccinated broilers provided 6 dietary treatments resulting from combinations of pre-starter and starter diets from 1 to 19 d of age during a 6 wk production period. Combinations consisted of 3 pre-starter diets formulated to 1.35 % dLys density, 1.25% dLys density or 1.15% dLys density and 2 starter diets formulated to 1.25% dLys density or 1.15% dLys density. In experiment 1, vaccinated broilers fed the 1.25% dLys AA diet consumed 5.4 g more cumulative dAA, which translated to a 38 g increase in total breast meat weight (*P* = 0.002) compared with those given the 1.15% dLys AA diet. Additionally, increasing the starter feed allocation beyond the 0.45 kg/bird allotment reduced cumulative feed conversion ratios by 6.5 points and increased carcass weights by 98 g. In experiment 2, feeding the 1.35% dLys AA density pre-starter diet increased cumulative dAA intake by 11.9 g, translating to a 40 g increase in total breast weight (*P* = 0.016) compared to broilers provided the 1.15% dLys AA density pre-starter diet. Growth performance and meat yield responses of broilers fed the 1.35% dLys density pre-starter diet were similar to those observed when feeding the 1.25% dLys diet from 1 to 19 d, which as likely due to the similar dAA intake between the 2 treatments. In both experiments, broilers fed the 1.15% dLys AA density displayed growth depression compared with non-vaccinated broilers fed the same dAA density. These results provide strategies for integrators utilizing coccidiosis vaccination to ameliorate growth performance and meat yield reductions.

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This thesis represents the culmination of the past two years of my life. It is a document that is not only a reporting of results but a commentary on the research conducted, memories made, and conclusions drawn that will forever serve as a reminder to me of this time. It would be remiss of me to claim to have to completed this undertaking alone. Therefore, I would like to acknowledge some of the people who have supported me over the past two years.

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TABLE OF CONTENTS

LIST OF TABLES

I. INTRODUCTION

Vaccination against coccidiosis has become more prevalent in the broiler industry as consumer demand increases for meat products originating from broilers raised in antibiotic free production. In 2017, approximately 8 billion broilers were raised for retail (USDA, 2018), of which approximately 50% were raised without antibiotics. Live coccidiosis vaccines induce an enteric challenge that depresses the growth and breast muscle development of broilers that is not completely recovered for birds marketed up to 2.8 kg (Lehman et al., 2009). This has economic ramifications, particularly for broilers utilized for the tray-pack market from reduced breast meat yield.

Previous research attempting to ameliorate the negative effects of the vaccination on broiler growth performance and meat yield has concluded that intervention may be needed to achieve performance similar to non-vaccinated broilers (Chapman et al., 2002). Manipulating the dietary amino acid (**AA**) density during the starter period is a potential strategy. Previous research has established that increases in dietary AA density will facilitate increased AA intake of broilers resulting in increased body weight and meat yield (Dozier et al., 2007). Additionally, studies have reported that increases in dietary crude protein enhanced growth performance of vaccinated broilers (Lehman et al., 2009; Lee et al., 2011). There is a dearth of studies applying manipulation of dietary AA density to vaccinated broilers, particularly early in development. Increasing AA density in diets fed during the starter period may enhance growth performance prior to the initiation of the vaccine infection. Previous research has also reported that the cumulative

BW and meat yield of broilers is influenced by the AA density of the diet fed from 1 to 14 d of age (Kidd et al., 1998). Additionally, increasing feed allocation during the starter period may increase the AA intake of vaccinated broilers translating to improved growth performance. Research investigating starter period interventions to improve vaccinated broiler growth performance and meat yield are sparse.

Two experiments were conducted to evaluate the impacts of manipulating dietary AA density during the starter period on ameliorating the negative effects of coccidiosis vaccination. Experiment 1 provided vaccinated broilers starter diets formulated to either 1.15% dig Lys density or 1.25% dig Lys density at 3 different starter feed allotments (0.45 kg/bird, 0.73 kg/bird, or 1.00 kg/bird) based on commercial practices. Two positive control (PC) treatments were utilized where the broilers were not vaccinated and received medicated diets to minimize coccidiosis infections. These PC broilers were fed at the same dietary AA densities as the vaccinated broilers. Experiment 2 provided vaccinated broilers pre-starter (1 to 9 d of age) and starter (10 to 19 d of age) diets varying in AA density. Six dietary treatments were based on the combination of 3 pre-starter diet AA densities: 1.15% dig Lys, 1.25% dig Lys, and 1.35% dig Lys; and 2 starter diet AA densities: 1.15% dig Lys and 1.25% dig Lys. One PC treatment was utilized where broilers were not vaccinated and fed a medicated version of the 1.15% dig Lys density to determine the vaccination response. These data should enhance understanding of vaccinated broiler growth responses to dietary AA density increases early in development.

II. LITERATURE REVIEW

COCCIDIOSIS IN ANTIBIOTIC FREE PRODUCTION

In the United States, antibiotic free (**ABF**) production accounts for approximately 40% of the broilers marketed (O'Keefe, 2017). One of the major reasons for this rise in ABF production has been due to food companies committing to serve ABF products in response to consumer demands. Consumers purchasing ABF products are often concerned about the safety of their food in regard to antibiotic residues despite a lack of scientific evidence (Cervantes, 2015). Within the past 10 years, several companies have made announcements that they are moving towards sourcing 100% of their meat from ABF production. Due to these announcements, some integrated broiler companies have also committed to eliminating antibiotic usage in production (Halloran and Bohne, 2017). These announcements commit the broiler industry to the production of ABF birds for the near future and many producers will need to transition to ABF production in order to meet the food service commitments.

One of the major challenges for integrators transitioning to ABF production is controlling enteric challenges such as coccidiosis and necrotic enteritis. These 2 diseases are recognized as the most prevalent and costly diseases of broiler chickens (Cervantes, 2015). Coccidiosis is a parasitic disease caused by the ampicomplexan protozoa *Eimeria* that is estimated to cost the poultry industry $3 billion annually for the prevention, treatment, and lost production by infected birds (Blake and Tomley, 2014). *Eimeria* invade the intestinal epithelium and undergo multiple stages of replication resulting in an increase in parasite number (Blake and Tomley, 2014). Multiple cells are infected at once causing tissue destruction and leading to the symptoms of coccidiosis. The symptoms can be described as mortality, malabsorption, diarrhea, bloody feces, and weight loss (Williams, 2002). In cases of a milder infection, birds will only experience decreased feed intake and decreased weight gain. Subclinical coccidiosis, while not as detrimental to the bird, is of great concern to integrators as infected birds lose productivity resulting in reduced profits.

Coccidiosis vaccination, especially using wild-type strains, is designed to cause subclinical coccidiosis in order to hasten the development of immunity. The infection retards the growth of broilers beyond the point of recovering before slaughter, especially for smaller bird programs (Danforth, 1998; Williams, 1998; Chapman, 2000). Vaccination occurs at hatch with live oocysts, which will induce protective immunity by causing infection before the bird is exposed to wild oocysts in the litter. Vaccine oocysts are ingested by the bird following spray application, in-ovo administration, or from gel cubes. The sporozoites contained within the vaccine oocysts invade the intestinal enterocytes and multiply. Following multiplication, sexual reproduction occurs producing a new oocyst, which ruptures from the cell into the intestinal tract and is excreted into the litter. Once in the litter, it will sporulate and become infective to repeat the cycle. Coccidiosis vaccines rely on this repeated infection cycle to develop immunity. It takes 3 cycles to develop full immunity in the bird and each cycle has less effect on the bird (Williams, 2002). Each cycle spans about 7 d to complete whereas immunity develops around 21 d of age (Chapman et al., 2002).

IMPACT OF COCCIDIOSIS ON NUTRIENT ABSORPTION AND IMMUNITY

Coccidiosis vaccination hinders normal intestinal development (Williams, 1998). The vaccine associated infection causes cell lysis and tissue destruction that increases epithelial cell turnover resulting in the shrinking of available absorptive surface area (Williams, 2002). Healthy enterocytes live approximately 4 d, which is equivalent to the time required for an enterocyte to migrate from the crypt (Sklan, 2001; Moran, 2016). This developmental correlation enables the villi functioning to allow optimal nutrient absorption. Damage to villi cells increases the rate of cell turnover and leads to blunting of the villi resulting in impaired nutrient absorption. *Eimeria* infection effects expression of nutrient transporters located in the brush border of gastrointestinal tract (**GIT**) epithelia due to the tissue damage caused by parasites exiting host cells residing in the epithelial layer (Miska and Fetterer, 2018). The reduced expression of nutrient transporters is a result of the downregulation of liver expressed antimicrobial peptide 2 (**LEAP2**), following the invasion of intestinal cells (Su et al., 2014). Infected cells inhibit replication of *Eimeria* by altering expression of nutrient transporters to reduce the available energy sources for the invading parasite (Su et al., 2014). Decreased weight gain is the net result of the decreased nutrient flow into intestinal enterocyte (Su et al., 2014; Miska and Fetterer, 2018).

In addition to the intestine specific damage caused by the parasite, its invasion activates the bird’s immune response to fight infection. The immune response causes inflammation of the intestine and increased mucus production (Tan et al., 2014; Zhang et al., 2017). Inflammation is the host’s mechanism to kill and starve out an invading pathogen. It is characterized by vasodilation and increased permeability of surrounding tissue, which allows for the accumulation of immune cells (Parham, 2014). The accumulation of these cells facilitates direct action and containment of the infection. Inflammation is an effective response to infection that enables infected cells to be rapidly killed (Parham, 2014). However, destroying infected cells causes the release of cytotoxic contents, which can lead to further tissue damage. This can prolong tissue recovery after eliminating the pathogen and can disrupt the balance of the intestinal microbiota potentially leading to future infections, such as necrotic enteritis (Parham, 2014; Broom and Kogut, 2018). Additionally, activation of the immune system causes nutrients to be diverted away from growth and maintenance towards supporting the activated immune cells suppressing the infection (Selvaraj, 2012). Activation of the immune cells and the accompanying inflammation also suppress appetite leading to decreased feed and water intake, which compromises growth and can potentially exacerbate the severity of the infection (Parham, 2014).

POST-HATCH GASTROINTESTINAL TRACT DEVELOPMENT

The first 3 weeks of life are critical for the bird's gastrointestinal development. During this time, the bird transitions from using the yolk as the sole nutritional source to utilizing feed. The GIT lengthens and develops mature enterocytes and villi-crypt structures, which enhances the functional surface area. Chicks prioritize the development of their GIT by directing nutrients from feed and the yolk (Sklan, 2001). Prior to hatching, the embryo ingests the albumen-amniotic fluid mixture within the egg and internalizes the yolk sac through the navel (Moran, 2007). The utilization of albumen and yolk proteins stimulates enzyme secretion facilitating digestion of exogenous feed. Immediately following hatch, intestinal crypts begin developing, which are the site of proliferating mature enterocytes (Sklan, 2001). Mature enterocytes migrate up the villi towards the lumen for access to nutrients (Moran, 2016). For several days post-hatch, the intestine consists of both mature and embryonic enterocytes to ensure successful utilization of feed and the residual yolk (Moran, 2007). The presence of feed and absorption of exogenous nutrients initiates functional development of the intestine that continues over the next few days. Within 2 weeks, the entire intestinal surface has progressed from embryonic structures to mature cells comparable with adult birds (Sklan, 2001; Moran, 2007). During this period the bird also begins to increase its body weight (**BW**). Broiler chicks have the potential to achieve a 10-fold increase in its BW in approximately 3 weeks.

ROLE OF THR, SER, GLY, AND GLN ON INTESTINAL HEALTH AND MUCIN FORMATION

The GIT is considered the first line of mucosal defense against intestinal pathogens to, which the body is continuously exposed (Ruth and Field, 2013). The GIT comprises approximately 5% of the body weight but consumes 15 to 30% of the O_2, protein, and energy due to its rapid and continuous cellular turnover (Bortoluzzi et al., 2018). The intestine is responsible for extensive catabolism of dietary amino acids during first pass metabolism (Wu et al., 2014). A majority of Gln, Glu, and Asp are utilized by the intestine for adenosine triphosphate (**ATP**) synthesis and maintaining the integrity and functionality of the GIT (Wu, 2010). The catabolism of AA by the GIT is responsible for regulating non-essential AA (**NEAA**) synthesis and modulating inter-organ AA flow to extra-intestinal tissue (Wu, 2010). Inter-organ transport of AA is influenced by nitric oxide (**NO**) regulation of blood flow across tissues. In addition to being an energy source, Gln serves as a growth signal by regulating protein synthesis and is a major shuttle for carbon and nitrogen throughout the body for use in anabolic reactions. Enterocytes are the only cell type to contain Glu transporters that can rapidly transport and metabolize Glu to ATP, providing energy for cellular functions and proliferation (Li et al., 2007) and requiring other cell types to utilize Gln for Glu synthesis (Ruth and Field, 2013). Inter-organ AA metabolism prioritizes Gln availability in plasma, and therefore it is the most abundant AA in plasma. However, the high priority of Gln constrains other tissues to rely on synthesizing Gln in order to meet their needs (Calder and Yaqoob, 1999; Li et al., 2007). Branched chain AA catabolized from the skeletal muscle provide the α-amino groups for biosynthesis of Gln in extra-intestinal tissue.

The GIT must maintain a precarious balance of barrier integrity, immunological preparedness, and a favorable microbiome (Moran, 2017). The mucosal barrier consists of a single layer of cells lining the intestine and tight junctions between them (Wang et al., 2015). Protecting this single layer is the unstirred water layer (**USWL**) consisting of membrane-associated mucin spread along the villous surface (Moran, 2016). Mucins, secreted by goblet cells along the intestinal surface, are glycoproteins that adhere to microvilli to create a protective layer (Moran, 2017). This is only possible due to the unique structure of mucin: “Bottle-brush” oligosaccharide structures formed from sequences containing Thr, Pro, Gly, and Ser protruding from the core and cross-linked with other mucins to build the protective net (Moran, 2017). Cystine is responsible for bridging the composite glycoproteins into the net-like configuration and is essential to the secretory mechanism within goblet cells (Akinde, 2014b; Moran, 2016). The USWL acts as a sieve entangling large polypeptides providing enzymatic access thereby enhancing digestibility of nutrients, while allowing small molecules to pass through for absorption (Moran, 2016; Moran, 2017). Degradation of the USWL is a regular occurrence requiring continuous mucin secretion to ensure adequate protection (Moran, 2017). This continuous turnover contributes to the high maintenance requirement for Thr as it constitutes 30 to 40% of the total AA composition in mucin (Horn et al., 2009; Zhang et al., 2017; Bortoluzzi et al., 2018). During enteric infections, the USWL may erode beyond the replacement of mucin allowing the microvillous surface of the intestine to be directly accessible to pathogens.

The intestinal epithelium acts a selective barrier allowing absorption of nutrients by enterocytes from the intestinal lumen into circulation (Wang et al., 2015). Following penetration of the USWL, invaders can attach and proliferate along the epithelial surface. Lymphoid cells distributed along the GIT recognize the invasion and activate the gut-associated lymphoid tissue (**GALT**) immune response. The major response is inflammation, which is essential for survival of the bird (Bortoluzzi et al., 2018). The initiation of inflammation and enterocyte damage from colonizing bacteria contribute to the dysfunction of the barrier integrity producing a dissolving effect on the tight junctions between cells and allowing for pathogens to enter circulation (Rhoads and Wu, 2009). Glutamine is essential to the barrier function of the intestine and supplementation has been shown to reduce intestinal atrophy through inhibition of mechanistic target of rapamycin (**mTOR**) pathways and stimulation of heat shock proteins, which result in decreased intestinal permeability (Rhoads and Wu, 2009; Wang et al., 2015; Bortoluzzi et al., 2018).

Amino acids are also involved in the recovery process following successful immune clearance of the pathogen. Inflammation commonly results in tissue damage (Parham, 2014; Broom and Kogut, 2018). Glutamine enhances cell proliferation through a variety of mechanisms: oxidation to ATP within cells, serving as precursor to synthesis of purine and pyrimidine nucleotides, upregulating polyamine synthesis, and enhancing gene expression of signaling pathways (Wang et al., 2015). Polyamines are considered essential to gastrointestinal recovery by promoting anabolic functions, such as nucleotide synthesis and uptake of AA and proteins (Khajali and Wideman, 2010; Rhoads and Wu, 2009). Arginine and Pro are involved in polyamine synthesis (Rhoads and Wu, 2009; Wu et al., 2011). Proline and Gly are incorporated into collagen synthesis, which is essential for tissue repair (Akinde, 2014a; Wu et al., 2011). Supplying adequate amounts of dietary AA should facilitate concurrent immune functionality and growth during times of infection and enhance recovery following the immune response.

ROLE OF AMINO ACIDS IN MUSCLE DEVELOPMENT

Breast muscles of broilers comprise approximately 30% of the total edible meat and 60% of the edible protein from the carcass (Mehri et al., 2012). In the last 5 years, breast meat yields of broilers grown to 2.27 kg have increased by 0.5% per year (Gous, 2010). Muscle growth occurs through enlargement and elongation of myofibers driven by an increase in cytoplasmic volume surrounding each myonucleus (Scheuermann et al., 2003; Nierobisz et al., 2007). Accumulation of muscle protein is the result of the difference between the rates of protein synthesis and degradation (Kang et al., 1985). The accretion of breast muscle requires increased dietary AA concentrations to support the rate of muscle deposition (Scheuermann et al., 2003; Vieira and Angel, 2012).

Dietary AA composition must meet the needs for maintenance and muscle development to ensure optimal white meat accretion (Vieira and Angel, 2012). Amino acids induce lean tissue deposition by stimulating protein synthesis and inhibiting proteolysis (Tesseraud et al., 2010). Protein synthesis is controlled through Leu activation of mTOR, and consequently activates the initiation phase of mRNA translation by enhancing the binding of mRNA and initiator methionyl-tRNA (met-tRNA) to the 40S ribosomal subunit (Suryawan and Davis, 2012). Methionine is central for protein synthesis due to its ability to enhance gene expression through DNA methylation and translational regulation (Hocquette et al., 2007; Tesseraud et al., 2009). Methionine is also the first limiting AA in poultry diets, and when Met deficient diets are fed whole body protein synthesis is decreased (Hocquette et al., 2007; Tesseraund et al., 2010). Lysine exerts specific stimulatory effects on protein synthesis through its stimulation of insulin-like growth factor 1 (**IGF-1**) (Liao et al., 2015). Insulin-like growth factor-1 stimulates protein synthesis through mTOR activation similar to Leu (Surwayan and Davis, 2012). Insulin-like growth factor-1 also regulates both differentiation and proliferation of muscle satellite cells and suppresses proteolysis (Florini et al., 1996). Type 1 IGF receptor activation mediates AA and glucose uptake by skeletal muscle cells (Dodson et al., 1996; Florini et al., 1996). Lysine is also involved in post-translation modification, which alters gene expression, cell signaling, and regulates protein synthesis (Liao et al., 2015). Additionally, the accumulation of Lys in skeletal muscles allows it to be mobilized during deficiency states to ensure necessary protein synthesis occurs (Tesseraund et al., 2010).

Protein synthesis can be influenced to the greatest extent early in life, and dietary AA have been shown to regulate synthesis (Vieira and Angel, 2012). This period is also marked by breast muscle accretion, which is controlled by major satellite cell mitotic activity during the first week of life (Kang et al., 1985; Scheuermann et al., 2003; Nierobisz et al., 2007). Research has shown that the concentration of Lys in the starter diet affects final growth responses of broilers irrespective of the grower and finisher dietary Lys concentration (Kidd et al., 1998). Increasing concentrations of dietary AA fed during early development have been observed to stimulate muscle development (Gous, 2010). Additionally, the first week post-hatch has been shown to influence cumulative BW and breast muscle weight by enhancing satellite cell activity (Powell et al., 2014). Nutrient restrictions have been shown to impair satellite cell mitotic activity leading to a decrease in nuclei number, which reduces the muscle growth potential (Nierobisz et al., 2007; Powell et al., 2013, 2014). Essential AA reductions have been reported to limit the muscle growth potential and increase muscular apoptosis (Powell et al., 2013, 2014). Satellite cells contribute to post-hatch hypertrophic muscle growth by fusing with and donating their nuclei to existing muscle fiber that leads to myonuclear accretion and the potential for later fiber enlargement and elongation (Powell et al., 2014). After the first week post-hatch, satellite cell mitotic activity declines and the remaining muscle growth that occurs is through mass accumulation of fiber enlargement and elongation (Sheuermann et al., 2003; Nierobisz et al., 2007).

Lysine comprises approximately 9% of the CP of breast meat (*pectoralis major and minor muscles*) (USDA Nutrient Data Laboratory, 2018). Other AA consist of only 4% of the CP of breast meat (USDA Nutrient Data Laboratory, 2018). However, other essential AA work in concert with Lys to maximize breast meat yield (Kidd et al., 1998; Vieira and Angel, 2012). Threonine, Gly, Ser, and Pro comprise the major AA composition of thighs and drums (Hamm, 1981). This difference in AA composition is likely due to the difference in functions. Leg muscles contain mixed red and white fiber types (Ono et al., 1993) and a greater proportion of connective tissue than breast muscles (*pectoralis major and minor muscles*) (Moran and Stilborn, 1996). Additionally, these muscles undergo a greater growth rate prior to hatch compared with breast muscles (*pectoralis major and minor muscles*) to ensure functionality at hatch and continuously afterwards (Ono et al., 1993; Tesseraud et al., 1996). Therefore, leg muscles are less sensitive to Lys deficiencies as protein synthesis within mixed fiber tissues favor NEAA and AA involved in collagen synthesis (Moran and Stilborn, 1996; Tesseraud et al., 1996).

EFFECTS OF AMINO ACID DENSITY IN THE PRE-STARTER AND STARTER DIETS ON SUBSEQUENT GROWTH PERFORMANCE AND MEAT YIELD

Providing broilers diets containing increased concentrations of AA throughout the growing period enhances BW, feed conversion ratio (**FCR**), and breast meat accretion (Kidd et al., 2004; Corzo et al., 2005; Kidd et al., 2005). Research has reported that feeding diets relatively higher in AA density early in development is important for growth performance and meat yield of broilers and the responses observed in the starter period influence subsequent growth periods (Kidd et al., 1998; Kidd et al., 2004; Dozier et al., 2007). Broilers have been reported to adjust feed intake to increase AA intake when diets have reduced dietary AA density (Dozier et al., 2007). This compensatory response may not allow adequate AA intake to obtain BW gain and breast meat yield values of broilers fed diets containing moderate or high increased AA density (Kidd et al., 1998; Kidd et al., 2004; Dozier et al., 2007). Kidd et al. (2004) reported broilers provided a starter diet formulated to 1.38% Lys (high) had a 35 d BW of 1.66 kg, which was 94 g heavier than those fed the 1.28% Lys (moderate) starter diet and 118 g heavier than the 1.15% Lys (low) starter diet. Furthermore, broilers fed the high starter diet followed by the low diets (1.00% Lys grower and 0.83% Lys finisher) in subsequent periods had a 172 g greater BW than those fed the low (1.15% Lys starter, 1.00% Lys grower, and 0.83% Lys finisher) diets throughout the study. Broilers given the high (1.38% Lys) starter and the high (1.20% Lys) grower diets regardless of the finisher diet provided had greater 0 to 35 d BW than the other treatments. Additionally, broilers fed the high diets for the (1.38% Lys) starter and (1.20% Lys) grower periods followed by the medium (0.98% Lys) or high (1.08% Lys) finisher diets had increased 35 d total breast meat yield by 0.98% points compared with those fed the low (0.83% Lys) finisher diet. These responses highlight the importance of the first 28 d for establishing muscle mass of the broiler. Moreover Kidd et al. (1998) reported broilers provided 1.25% total Lys in the starter diet had a 1 to 49 d BW increase of 160 g and a breast weight increase of 42 g over those given the 1.04% Lys starter diet, irrespective of the diets provided in subsequent periods. These results imply that the starter dietary AA density is critical to final BW gain, and may affect breast meat responses more than grower and finisher diets.

Dietary AA concentrations should be increased in the first 10 d post-hatch to meet the demands of broiler's growth potential (Gous, 2010). This can be achieved through a pre-starter diet fed for the first week to compensate for physiological limitations of the bird post-hatch (Leeson, 2008; Willemsen et al., 2010; Shariatmadari, 2012). Essential

AA restrictions during the first week post-hatch have been shown to impair satellite cell mitotic activity resulting in a decrease in nuclei number, which reduces the muscle growth potential (Nierobisz et al., 2007; Powell et al., 2013, 2014). Kidd et al. (2005) utilized a pre-starter diet for the first 5 d and observed a 49 g difference in BW at 35 d between the 2 pre-starter dietary AA densities formulated with AA ratios to 1.38% total Lys (high) or 1.28% Lys (moderate). Broilers provided the high (1.38% total Lys) pre-starter diet and then a high (1.36% total Lys) starter diet had an increased BW of 78 g over broilers provided the moderate (1.28% total Lys) pre-starter and moderate (1.24% total Lys) starter diets. However, broilers provided the high (1.38% total Lys) pre-starter and then the moderate (1.24% total Lys) starter had lower 35 d BW than broilers provided the high (1.38% total Lys) pre-starter and (1.36% total Lys) starter diets but similar BW to that of broilers fed moderate (1.28% total Lys) pre-starter and (1.24% total Lys) starter diets. These results indicate that the early increase in dietary AA density resulted in enhanced growth performance responses.

EFFECTS OF STARTER FEED ALLOCATION ON SUBSEQUENT GROWTH PEROFRMANCE AND MEAT YIELD

Recommendations for the duration of the starter period ranges from 1 to 7 or up to 1 to 21 d of age (Saleh et al., 1997; Leeson, 2008; Willemsen et al., 2010; Shariatmadari, 2012). Research to determine optimal growth performance and carcass characteristics when broilers were fed starter diets from 1 to 7 or up to 1 to 17 d of age found that feeding starter diets to 7 d of age produced optimal growth performance responses (Waldroup et al., 1992; Watkins et al., 1993; Saleh et al., 1997). However, researchers indicated that broilers reared for different market weights have different optimal starter durations (Saleh et al., 1996, 1997a, 1997b). Watkins et al. (1993) recommended a starter feed allotment of 0.11 kg to last approximately 7 d of age for optimal performance of broilers raised to 2.2 kg. Currently, starter diets are fed until 14 to 19 d of age depending upon market weights (Shariatmadari, 2012). Previous research evaluating extending the starter duration beyond 14 d found no growth performance benefits to broilers (Waldroup et al., 1992). However, adoption of live coccidiosis vaccinations has developed interest in feeding for a longer starter duration. Lee et al. (2011) investigated this by providing starter diets to vaccinated broilers until 13, 17, and 21 d of age. This study found that the duration of the starter phase did not affect BW gain, however, broilers changed to the grower diets at 13 d of age had a higher FCR from 1 to 21 d of age than the broilers changed at 17 or 21 d of age. This study did not raise broilers throughout a 6 week production period, but the early period results prompts questioning into the cumulative impact of starter feed allocation on vaccinated broiler growth performance and meat yield.

KNOWLEDGE GAPS IN THE LITERATURE

Previous research has investigated the impact of dietary protein concentrations and coccidiosis infection. High crude protein (**CP**) concentrations (24%) have been shown to minimize weight loss during clinical infection (Sharma et al., 1973). Increased dietary CP has also been shown to enhance growth performance and meat yield in broilers that received the coccidiosis vaccination (Lehman et al., 2009; Lee et al., 2011). Increasing dietary AA density during the starter period for broilers vaccinated against coccidiosis may similarly mitigate the negative effects from the vaccine associated infection by increasing the amount of AA available for immune activity and should allow adequate growth to be maintained during the infection. Research is limited on assessing dietary AA density responses of vaccinated broilers. Increasing AA intake before the initiation of the vaccine induced infection may minimize the impact of the infection on growth performance. Increased dietary CP before and during coccidial infection has been shown to minimize weight loss (Sharma et al., 1973; Lehman et al., 2009; Lee et al., 2011). Authors from previous studies attribute the protective effect observed to increased AA intake and the resulting increased protein synthesis prior to the onset of damage from the infection. Additionally, increasing dietary AA density during the starter period has a large influence on the final BW and meat yield irrespective of the grower and finisher dietary AA density (Kidd et al., 1998). An alternative intervention to increase early AA intake could be the utilization of a pre-starter diet containing increased AA density (1.35% digestible Lys) for the first 10 d of age followed by an starter diet containing moderate (1.15% digestible Lys) or high (1.25% digestible Lys) AA density. Utilization of a pre-starter diet could facilitate increased AA intake before the vaccine infection results in depressed feed intake and partitioning of AA between the immune system and muscle accretion. Therefore, it is possible that growth responses from increasing the dietary AA density only during the pre-starter and starter periods would stimulate muscle cell development and ameliorate breast meat yield reductions caused by the coccidiosis vaccination, however, no research has yet investigated this hypothesis.

Another strategy to increase AA intake of broilers is to manipulate starter feed allocation. Providing increased amounts of starter feed to broilers vaccinated against coccidiosis could extend the starter period through most of the challenge period. This would support feed intake adjustments by broilers in order to compensate for increased

AA demands during immune activation. However, it is unknown if vaccinated broilers provided an increased AA intake through extending the starter feed allotment would ameliorate breast meat yield reductions during a 6 week production period.

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III. EFFECTS OF DIETARY AMINO ACID DENSITY AND FEED ALLOCATION DURING THE STARTER PERIOD ON 41 DAYS OF AGE GROWTH PERFORMANCE AND PROCESSING CHARACTERISTICS OF BROILER CHICKENS GIVEN COCCIDIOSIS VACCIATION AT HATCH

ABSTRACT

A study was conducted to determine if increasing digestible (**dig**) amino acid density or feed allocation of diets fed to broilers receiving coccidiosis vaccination during the starter period can ameliorate poor cumulative growth performance and reduce meat yield. A total of 1,600 Yield Plus Ross × Ross 708 male broilers was assigned to a 2 × 3 factorial arrangement of dig AA density [Moderate (1.15% dig Lys) and High (1.25% dig Lys)] and feed allotment (0.45, 0.73, and 1.00 kg/bird) with 2 positive control diets during the starter period. Diets were formulated to essential AA ratios relative to dig Lys. Vaccinated birds received a 1x dosage of Coccivac$^{®}$- B52 prior to placement, whereas non-vaccinated birds in the positive control groups were fed diets containing Diclazuril. Following consumption of the starter diets, birds were provided common grower and finisher diets. Broilers fed the High AA density diet during the starter period had higher cumulative BW gain and lower cumulative feed conversion ratios ($P < 0.05$) than those fed the Moderate AA density diet. Broilers fed the High AA density diet had heavier ($P \leq 0.005$) carcass and total breast weights than birds fed the Moderate AA density diet. Broilers fed a starter allotment of 1.00 kg/bird produced heavier carcass weights ($P \leq$

0.006) than did birds provided lower allotments. Additionally, broilers fed the High AA density diet had a greater percentage of 0 scores (*P* = 0.005) for the upper intestinal region during scoring. Results from this study indicated that feeding the High AA density diet at higher feed allotments during the starter period resulted in increased AA intake, which supported the bird through the vaccine challenge and enhanced the cumulative growth and meat yield.

INTRODUCTION

In recent years, the food service industry has placed an increased demand on poultry products originating from ABF production. Many ABF programs, however, restrict the use of ionophores and other coccidiostats to suppress coccidiosis infection. One alternative is the use of live coccidial vaccinations, which is associated with growth depression that is often not recovered by slaughter (Williams, 2002; Lehman et al., 2009). This is a concern for integrators raising broilers for the tray-pack market stemming from the short production time of these programs as their time to market is not enough to fully recover the breast meat yield reduction from the vaccine challenge.

Dietary crude protein modulation has been investigated as a strategy to ameliorate the negative effects of coccidiosis vaccination (Lehman et al., 2009; Lee et al., 2011). The enhanced growth performance from increased CP are likely due to the increase in dietary AA density, but formulating on a digestible amino acid ratio basis could be a more effective strategy to meet AA needs of the birds while being more cost effective. The vaccine-associated infection activates the immune system requiring the bird to divert AA from muscle accretion (Selvaraj, 2012). Supplying increased concentrations of dietary AA should facilitate immune activities and support continued growth during the infection. Increasing AA intake of broilers during the starter period has been shown to influence cumulative BW and breast meat yield (Kidd et al., 1998, 2004). Increasing dietary AA density is costly, but formulating to higher AA specifications during the starter period would not be as costly relative to grower and finisher periods due to the lower feed intake of the young broiler (Vieira and Angel, 2012).

Manipulating starter feed allocation given to broilers is a management strategy to increase AA intake without increasing dietary AA density. Previous research has evaluated the effects of manipulating starter feed allocation for vaccinated broilers and reported few significant differences for response criteria measured at 21 d of age (Lee et al., 2011). However, this study did not investigate the cumulative effects of starter duration on subsequent growth performance and meat yield. Research evaluating the impact of increasing dietary AA density and starter feed allotment on ameliorating growth depression and meat yield reductions of broilers receiving coccidiosis vaccination is sparse. Therefore, the current study was conducted to determine the effects of increasing dietary amino acid density and feed allotment of diets fed during the starter period to broiler chickens receiving coccidial vaccination at hatch on growth performance and processing characteristics.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2017-3076).

Dietary Treatments

Dietary treatments were arranged as a 2 × 3 factorial with 2 positive controls to evaluate the effects of dietary AA density and feed allotment during the starter period on broilers provided coccidiosis vaccination. Diets consisting of 2 dig AA densities were formulated to reflect nutritional programs in commercial practice during the starter period (Table 3.1). The Moderate AA density diet was chosen to reflect a moderate AA density used in commercial practice while the High AA density diet was designed to represent an aggressive AA feeding program. The Moderate AA density diet was formulated to a 1.15% dig Lys, which was equivalent to 90% of the Aviagen Ross 708 AA recommendations. The High AA density diet was formulated to 1.25% dig Lys, equivalent to 98% of the Aviagen Ross 708 AA recommendations. Both diets were isocaloric and were formulated to dig ratios of TSAA (0.74), Thr (0.67), Val (0.75), and Ile (0.67) to dig Lys. Starter diets were fed as crumbles and subsequent diets provided as whole pellets. Starter diets were analyzed for total AA concentrations in triplicate by the University of Missouri Agricultural Experiment Station Chemical Laboratory using AOAC procedures (method 982.30 E (a,b,c), AOAC, 2006). Performic acid oxidation (method 985.28; AOAC, 2006) was conducted before acid hydrolysis for the determination of Met and Cys, whereas all other AA were determined after acid hydrolysis.

Three starter feed allotments were provided to broilers: 0.45 kg/bird, 0.73 kg/bird, and 1.00 kg/bird. These 3 treatment allotments were based upon a 0.73 kg/bird allotment, which several integrators producing broilers to approximately 2.8 kg in the United States use during the starter period. The 0.45 kg/bird and 1.00 kg/bird starter feed allotments were then established above or below the 0.73 kg/bird allotment, representing lower and higher portions of the 0.73 kg/bird allotment. Broilers fed the 2 positive control (**PC**) treatments received Moderate and High AA density diets that contained a coccidiostat, Diclazuril. Both were fed at a 0.73 kg/bird starter feed allotment.

Bird Husbandry

One thousand and six hundred Yield Plus Ross × Ross 708 (Aviagen Inc., Huntsville, AL) male broilers were randomly allocated to 64 floor pens (0.08 m^2/bird; 25 birds/pen) based on their vaccination status. Prior to placement, 1,200 broilers received a 1x dosage with Coccivac$^{®}$-B52 (Merck Animal Health, Summit, NJ) via spray cabinet at a commercial hatchery. The remaining 400 broilers did not receive coccidiosis vaccination and were given diets containing Diclazuril to minimize infection with coccidiosis. All broilers received vaccinations for Marek’s disease, Newcastle disease, and infectious bronchitis. The experimental facility was a solid-sided house containing floor pens with used litter equipped with a hanging feeder and nipple water line (5 to 6 nipples/pen). The experimental facility contained a negative-pressure ventilation system equipped with electronic controller, vent boards, exhaust fans, and evaporative cooling pads. Ambient temperature was set at 33°C at placement and decreased gradually with advancing bird age to 21°C by the end of the study. A 23L:1D photoperiod was set at placement and decreased to 20L:4D at 7 d of age for the remainder of the study. Light intensity was set at 30 lux at placement and decreased to 10 and 5 lux at 7 and 12 d of age, respectively. Light intensity settings were verified at bird height following each adjustment using a photometric sensor calibrated with the National Institute of Standards and Technology- traceable calibration (403125, Extech Instruments, Waltham, MA).

Measurements

Broilers were given the 3 feed allotments of the experimental starter diets at placement and the grower diets were added to feeders upon completion of the starter diets. The day at which grower diets (Table 3.2) were added to each pen was based on the starter feed allotment placed in the pen. The change occurred at 11 d of age for the 0.45 kg/bird, 14 d of age for the 0.73 kg/bird, and 21 d of age for the 1.00 kg/bird allotment. At 28 d of age, all pens were provided a common finisher diet for the remainder of the study (Table 3.2). Birds and feed were weighed to determine body weight gain, feed intake, and feed conversion ratio at 1, 21, 28, and 40 d of age. Incidences of mortality were recorded daily. Feed conversion ratios were adjusted to account for mortality. Digestible AA intakes were calculated by multiplying the feed intake with the dig AA content of the diet for the 7 most limiting AA: TSAA, Lys, Thr, Val, Ile, Arg, and Trp.

At 21 d of age, 4 birds per pen were selected for intestinal lesion scoring to assess vaccine efficacy. The percentage of each score per pen was calculated based on the lesion scores of each bird for statistical analysis. Gross lesion scoring was conducted based on the Johnson and Reid (1970) method using intestinal regions outlined by Conway and Mckenzie (2007) for scoring mixed infections. Intestinal regions were defined as: duodenal and proximal jejunal intestine as the upper intestinal region, distal jejunal and proximal ileal intestine as the middle intestinal region, distal ileal and rectum as the lower intestinal region, and the ceca alone as the cecal intestinal region (Conway and Mckenzie, 2007). Intestinal regions for lesion scoring were used because the vaccine contained *Eimeria acervulina*, *E. maxima*, *E. maxima* mixed field strain precocious (MFP), *E.*

mivati, and *E. tenella* strains that would infect different segments of the intestinal simultaneously.

On 41 d of age, 12 birds per pen were selected for processing. Birds were processed in a pilot processing facility at the Auburn University Poultry Research Unit following a 12 h feed withdrawal period. Broilers were electrically stunned, exsanguinated, scalded, mechanically picked, mechanically eviscerated, and then placed on ice. Carcasses were chilled in slush ice-water for 3 h and then drained of excess water for 3 min. Abdominal fat was removed from carcasses and weighed separate from the whole carcass. Carcasses were deboned the following day to obtain breast fillets (*pectoralis major muscles*), tenders (*pectoralis minor muscles*), wings, drums, and boneless thigh meat by experienced personnel using stationary cones. Meat yield percentages were based on the live bird weight at 40 d of age. Tenders and breast fillets weights were combined for the determination of total breast meat.

Statistical Analysis

This study was designed as a 2 × 3 factorial with 2 positive control treatments represented by 8 replicate pens as a randomized complete block design. Pen location was considered as the blocking factor. Analysis of variance was performed for interactive and main effects of the factorial using PROC Mixed (SAS 9.4) by the following mixed effect model:

$$Y_{ijk} = \mu_{\ldots} + \tau_i + \beta_j + (\tau\beta)_{ij} + \rho_k + \varepsilon_{ijk}$$

where $\mu_{\ldots}$ is the overall mean; the τ_i are the fixed factor level effects corresponding to the i^{th} dietary AA density of the starter diet such that $\sum \tau_i = 0$; the β_j are the fixed factor level effects corresponding to the j^{th} starter feed allotment such that

$\sum \beta_j = 0$; the $(\tau\beta)_{ij}$ are the interaction level effects corresponding to either to the i^{th} dietary AA density and the j^{th} starter feed allotment such that $\sum_i (\tau\beta)_{ij} = 0$ and $\sum_j (\tau\beta)_{ij} = 0$; the ρ_k are identically and independently normally distributed random block effects with mean 0 and variance σ_ρ^2; and the random error ε_{ijk} are identically and independently normally distributed with mean 0 and variance σ^2. All percentage data were transformed using square root arcsine to meet normality requirements before conducting analyses. Mean separation was conducted using Tukey's Honestly Significant Difference test (Tukey, 1953) when significant ANOVA results were observed. Statistical significance was established at $P \leq 0.05$.

Pre-planned orthogonal contrasts were analyzed using PROC Mixed (SAS 9.4) by the following mixed model, which included the 2 PC treatments:

$$Y_{ij} = \mu_{..} + \tau_j + \rho_i + \varepsilon_{ij}$$

where $\mu_{..}$ is the overall mean; the τ_i are fixed factor level effects corresponding to the i^{th} treatment such that $\sum \tau_i = 0$; the ρ_j are identically and independently normally distributed random block effects with mean 0 and variance σ_ρ^2; and the random error ε_{ij} are identically and independently normally distributed with mean 0 and variance σ^2. Pre-planned orthogonal contrasts were conducted between PC treatments and those receiving the 0.73 kg/bird allotment as an evaluation of the effect of vaccination. Statistical significance was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

Diet Analysis

Amino acid composition of the starter diets was similar to calculated values (Table 3.3). The 2 AA densities were formulated to vary by 0.10% points dig Lys.

Analysis determined that the total Lys concentration between the Moderate AA density diet and the High AA density diet varied by 0.06% points. Positive control diets had similar AA composition to their calculated values. Moderate diets had the largest variation in amino acid composition between the PC and the test diet. The difference in total Lys was 0.07% between them, whereas there was only a difference of 0.02% points total Lys between the High AA density diet and the High AA density PC diet.

Growth Performance

There were no significant interactions ($P \geq 0.15$) of dietary AA density and feed allotment of the starter diets observed for growth performance. Due to this lack of significance, only the main effects of dietary AA density and feed allotment of the starter diets are discussed in the following sections. Interaction data are included in Tables 3.4, 3.5, and 3.6 for the purpose of facilitating discussion of the pre-planned orthogonal contrasts.

1 to 21 days of age. Broilers that received the High AA density diet had increased BW gain ($P < 0.001$) and decreased FCR ($P < 0.001$) compared with birds fed the Moderate AA density diet (Table 3.4). Broilers receiving the High AA density diet were 59 g heavier than their counterparts receiving the Moderate AA density diet. An increase in mortality was observed with the Moderate AA density diet ($P = 0.04$) however, the mortality was below 3% and occurred mainly during the first week without any specific cause. There was no effect ($P > 0.05$) of starter feed allotment during this period on BW gain, feed intake, or FCR. Additionally, there was no difference ($P > 0.05$) for any response criteria between vaccinated and non-vaccinated broilers during this period.

Lee et al. (2011) reported vaccinated broilers provided starter diets containing 22% dietary CP had similar BW from 1 to 21 d of age compared with non-vaccinated broilers, but BW differences were noted between vaccinated and non-vaccinated broilers provided 20% or 24% CP starter diets. In contrast, Lehman et al. (2009) reported no difference in BW gain between broilers vaccinated with Coccivac®- D fed the low (22%) and high (24%) CP diets from 0 to 3 weeks of age. However, these authors did observe an increase in BW gain in broilers that received diets with increased concentrations of Pro and Gly + Ser, which was achieved through dietary inclusion of gelatin. This study also noted significant differences in growth performance between vaccinated and medicated broilers, whereas the current study did not observe any vaccination effect from 1 to 21 d of age. In the current study, it is likely that the increased BW gain is related to the increase in dig AA intake that accompanied the BW gain increase for the broilers consuming the High AA density diet. Lehman et al. (2009) used diets that only had a total Lys variation of 0.05% as compared with the difference of 0.10% in the current study. Additionally, Lehman et al. (2009) formulated diets on a CP basis whereas the current study formulated on a dig AA ratio to Lys basis. Therefore, the small variation of AA concentration between the high and low CP diets likely led to a smaller difference in dig AA intake between their diets than was observed in the current study. This may explain the lack of BW differences reported by Lehman et al. (2009) as digestible AA intake is related to muscle synthesis and breast muscle accretion (Kidd et al., 1998).

The lack of a significant effect of starter feed allotment on growth performance criteria during the first 3 weeks of age was consistent with previous research. Lee et al. (2011) fed a 22% CP starter diet to vaccinated and non-vaccinated broilers for 3 durations: 13, 17, and 21 d of age. The authors only observed a FCR difference at 21 d of age between the 3 starter durations, where the 13 d duration had the highest FCR compared with the longer durations. No significant differences in BW were reported between the 3 starter durations; however, a 30 g numerical variation in BW at 21 d was noted between the 13 d duration and the 2 longer starter durations. These authors did report BW differences between the vaccinated and non-vaccinated broilers at 17 and 21 d of age. Vaccine infection typically cause symptoms of growth depression that may have contributed to the variation in BW and higher FCR for the 13 d duration of starter feed allotment. The current study did not observe any growth performance differences between vaccinated and non-vaccinated broilers from 1 to 21 d of age.

1 to 28 days of age. Broilers provided the High AA density diet had increased (*P* < 0.0001) BW gain and decreased (*P* = 0.002) FCR as compared with the those given the Moderate AA density diet (Table 3.5). Broilers receiving the High AA density diet consumed more feed (*P* = 0.01) than those fed the Moderate AA density diet. There were no observed effects of starter feed allotment on BW gain, feed intake, or FCR during this period (*P* > 0.05). The lack of responses to the starter feed allocation were likely due to the bird's ability to compensate through numerically increasing feed intake from 21 to 28 d of age (data not shown). Growth performance differences were observed between vaccinated and non-vaccinated broilers that received the Moderate AA density diet. Vaccinated broilers fed the Moderate AA density diet had decreased BW gain (*P* = 0.02) and feed intake (*P* = 0.04) compared with their non-vaccinated counterparts fed the Moderate AA density PC diet. There were no differences observed between vaccinated and non-vaccinated broilers given the High AA density diet (*P* ≥ 0.61). This supports the concept that the additional AA supplied in the High AA density diet provide a protective effect to the bird during the challenge likely by providing enough AA for intestinal repair while maintaining an adequate growth rate. Moreover, broilers fed the Moderate AA density diet consumed 66 g less feed from 1 to 28 d of age than those on the High AA density diet. Decreased feed consumption is a hallmark sign of subclinical coccidiosis and vaccine infections implying that broilers fed the Moderate AA density diet were experiencing greater infection symptoms than those given the High AA density diet (Williams, 2002). However, no differences in mortality were observed during this period.

1 to 41 days of age. Broilers that received the High AA density diet had increased BW gain ($P = 0.04$) and decreased FCR ($P = 0.0003$) as compared with those given the Moderate AA density diet (Table 3.6). Broilers fed the High AA density diet were 79 g heavier than those fed the Moderate AA density diet. Differences from starter feed allocations were noted between feed intake ($P = 0.04$) and FCR ($P = 0.01$) but not BW gain ($P = 0.49$). Broilers allocated 0.73 kg/bird consumed less feed and had 6.5 points lower FCR than those given ($P \leq 0.038$) 0.45 kg/bird. The 1.00 kg/bird feed allotment had similar feed intake and FCR compared with the 0.45 kg/bird and 0.73 kg/bird allotments. There were no differences observed in cumulative mortality among the treatments. Additionally, there were no growth performance differences between vaccinated and non-vaccinated broilers ($P < 0.05$).

Immunity from coccidiosis vaccination is achieved around 21 d of age (Williams, 2002). Following the development of immunity, mucin production decreases and the immune response disappears allowing AA to be redirected towards the growth and repair of damaged intestinal villi (Selvaraj, 2012). After tissue repairs are completed, broiler growth increases to compensate for the previous depression as dietary AA are able to prioritize protein synthesis for muscle growth (Lehman et al., 2009; Selvaraj, 2012). In the current study, increased dietary AA density supported recovery from the vaccine during this period by providing adequate AA for intestinal repair and protein synthesis.

Cumulative feed intake was affected by starter feed allocation with broilers provided 0.45 kg/bird feed allotment indicating that these birds were consuming more to meet their nutritional needs. The dig AA concentration decreased to 1.04% dig Lys in the grower diet from 1.15% or 1.25% dig Lys in the starter diets. For the 0.45 kg/bird allotment group that started consuming the grower diet at 11 d of age, it is likely that broilers adjusted their feed intake to compensate for the reduced AA concentration. The impact of lowering the dig AA density in the grower diet was likely less for the 1.00 kg/bird and 0.73 kg/bird allotment group. The 0.45 kg/bird allotment consumed 27 g and 118 g more feed than the 1.00 kg/bird and 0.73 kg/bird allotments, respectively. This increased feed intake allowed a similar BW gain compared with the 2 larger starter allotments but at the expense of feed conversion. Dozier et al. (2007) reported a similar response of increased feed intake in broilers given a reduced dietary AA density diet.

Intestinal Lesion Scores and Vaccine Efficacy

Intestinal lesion scoring revealed that vaccinated broilers had greater ($P = 0.003$) percentages of lesions than the non-vaccinated broilers indicating a successful vaccine administration (Table 3.7). There was not a significant interaction between dietary AA density and starter feed allotment for any intestinal regions ($P \geq 0.33$). Broilers fed the High AA density diet had greater percentage ($P = 0.005$) of scores of 0 compared with broilers receiving the Moderate AA density diet for the upper intestinal region, and numerical increases for all other intestinal regions. Conversely, broilers fed the Moderate AA density diet had a greater percentage ($P = 0.007$) of scores of 1 for the upper intestinal region compared with the broilers provided the High AA density diet. This implies that the High AA density diet supported the birds through the challenge period better than the Moderate AA density diet. This implication is supported by the results of the contrasts between the non-vaccinated broilers fed the PC diets vs. the vaccinated birds. From the High AA density diet contrast, it was determined that there was no difference ($P \geq 0.16$) between the percentage of scores of 0. Alternatively, there was approximately 20% points more scores of 0 for the upper and middle intestinal regions ($P \leq 0.008$) for the non-vaccinated broilers given the Moderate AA density PC diet compared with their vaccinated counterparts. In the cecal intestinal region, broilers given 0.73 kg/bird starter allotment had a greater percentage of scores of 0 ($P = 0.009$) compared with the 1.00 kg/bird allotment. There were few scores of 2 observed across all intestinal regions and no differences were found between them ($P > 0.05$; data not shown). There were no lesion scores above 2, which is consistent with published reports from vaccinated broilers (Williams, 2003).

Processing Characteristics

There were no significant interactions ($P \geq 0.06$) of dietary AA density and feed allotment during the starter period on processing characteristics (Table 3.8). However, providing the High AA density diet resulted in heavier ($P \leq 0.009$) carcass and total breast weights as compared with those given the Moderate AA density diet. Broilers that received the High AA density diet had increased total breast yield ($P = 0.0005$) and decreased thigh meat yield ($P = 0.017$) as compared with those given the Moderate AA density diet. Broilers provided starter allotments of 1.00 kg/bird and 0.73 kg/bird had higher carcass, drum, and wing weights ($P \leq 0.002$) than the 0.45 kg/bird allotment. Broilers provided the 1.00 kg/bird starter allotment produced a 98 g heavier carcass (P = 0.006) and 10 g heavier drum (P = 0.013) and wing (P = 0.002) weights than those given the 0.45 kg/bird allotment. Carcass and parts weights produced by broilers fed the 0.73 kg/bird allotment were similar to those of the 1.00 kg/bird allotment. Total breast weights varied numerically between the starter feed allotments, where the broilers receiving the 1.00 kg/bird allotment had a 33 g increase weight over the 0.45 kg/bird allotment, but this was not significant (P = 0.08). There were no meat yield differences between birds fed varying the starter allotments in this study. Broilers fed the High AA density diet in the starter period had 38 g increased (P = 0.002) total breast weight compared with those on the Moderate AA density diet. Vaccinated birds given the Moderate AA density diet had decreased drum (P = 0.01) and thigh meat yields (P = 0.03) compared with the Moderate AA PC broilers.

Cumulative AA intakes of dig Val, dig Ile, dig Arg, and dig Trp were increased ($P <$ 0.05) for broilers fed the High AA density diet (Table 3.9). There was an average cumulative AA intake increase of 5.4 g between the High AA density and Moderate AA density diet ($P < 0.05$). This difference in AA intake resulted in a 38 g increase in total breast meat weight for broilers given the High AA density diet compared with those given the Moderate AA density diet. Digestible AA intake differences supported the increase in breast meat weights (Dozier et al., 2008; Vieira and Angel, 2012). Lysine has the highest concentration relative to CP compared with other AA comprising approximately 9% of breast muscle CP, while other AA constitute only 4% of the breast muscle CP (Nutrient Data Laboratory, 2018). In the current study, dig Lys intake was increased ($P < 0.0001$) in the starter and grower periods (data not shown) but not cumulatively ($P = 0.06$) for broilers given the High AA density diet. Previous studies have established that increasing AA density in the starter period enhanced cumulative growth performance and meat yield (Kidd et al., 1998, 2004; Dozier et al., 2008).

Providing increased AA early in life enables early protein accretion and muscle development (Dozier et al., 2008; Vieira and Angel, 2012). Vaccine-associated infections partition AA towards gastrointestinal tract defense and repair away from muscle accretion, which limits the growth related benefits of increasing AA density in the starter period (Williams, 2002; Selvaraj, 2012). Lehman et al. (2009) observed decreased carcass weights for vaccinated broilers and suggested that the result was from vaccinated birds failing to recover from the earlier growth depression. The current study only observed growth performance differences between vaccinated and non-vaccinated broilers provided the Moderate AA density diet from 1 to 28 d of age. These differences did not result in carcass or total breast weight or yield differences ($P > 0.09$). Although, drum and thigh meat yields of vaccinated broilers were decreased compared with their non-vaccinated counterparts ($P \leq 0.02$) when fed Moderate AA density diets. This difference may be due to the distinct AA composition of those parts as compared with the breast muscle, which displayed no vaccination effect. Hamm (1981) found that thigh meat contains greater concentrations of Gly, Pro, Thr, and Ser than breast meat. These AA are also important to intestinal development and repair (Lehman et al., 2009).

In conclusion, results from this study indicated that feeding diets varying in increased dig AA density and feed allotment during the starter period support the bird through the challenge period of the vaccine without compromising performance and meat yield. Increasing dig AA density during the starter period resulted in cumulative dig AA intake increases of 5.4 g for broilers fed the High AA diet and coalesced in a 38 g increase in total breast weight. Cumulative FCR, wing, drum, and carcass weights were enhanced due to the starter feed allocation. These results indicate that providing a higher AA density diet during the starter period to broilers vaccinated for coccidiosis will ameliorate growth performance and meat yield depression.

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Table 3.1. Ingredient and nutrient composition of experimental starter diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52

Ingredient, % "as-fed"	Moderate[1] Diet	Moderate[1] PC[2]	High[1] Diet	High[1] PC[2]
Corn	59.45	59.45	53.76	53.76
Soybean meal (48%)	33.22	33.22	38.01	38.01
DDGS	3.00	3.00	3.00	3.00
Soy oil	0.76	0.76	1.72	1.72
Dicalcium Phosphate	1.18	1.18	1.13	1.13
Calcium Carbonate	1.12	1.12	1.11	1.11
DL-Methionine	0.28	0.28	0.32	0.32
Vitamin Premix[3]	0.10	0.10	0.10	0.10
Mineral Premix[4]	0.10	0.10	0.10	0.10
NaCl	0.36	0.36	0.36	0.36
L-Lys·HCl	0.17	0.17	0.15	0.15
Choline	0.08	0.08	0.06	0.06
L-Threonine	0.10	0.10	0.10	0.10
TBCC[5]	0.02	0.02	0.02	0.02
Sand	0.05	---	0.05	---
Clinacox®[2]	---	0.05	---	0.05
Phytase[6]	0.01	0.01	0.01	0.01
Xylanase[7]	0.01	0.01	0.01	0.01
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg	3,000	3,000	3,000	3,000
Crude Protein	21.18	21.18	23.02	23.02
Digestible Lys	1.15	1.15	1.25	1.25
Digestible Met	0.57	0.57	0.62	0.62
Digestible TSAA	0.86	0.86	0.93	0.93
Digestible Thr	0.78	0.78	0.84	0.84
Digestible Val	0.86	0.86	0.94	0.94
Digestible Arg	1.26	1.26	1.39	1.39
Ca	0.96	0.96	0.96	0.96
Non-phytate P	0.48	0.48	0.48	0.48
Na	0.20	0.20	0.20	0.20

[1] Dietary density formulated based off 90% of Ross 708 amino acid Nutrition Specifications for moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for high density diets.

[2] Clinacox® (Huvepharma, Inc., Peachtree City, GA) provides 0.02% diclazuril to the diet. Positive control diets contained Clinacox® at 0.02% at the expense of sand.

[3] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[4] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.

[5] TBCC = tri-basic copper chloride (IntelliBond C; Micronutrients, Inc., Indianapolis, IN)

[6] Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[7] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per ton inclusion.

Table 3.2. Ingredient and nutrient composition of grower and finisher diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52

Ingredient, % "as-fed"	Grower[1]	Grower[1] PC[2]	Finisher[1]
Corn	63.51	63.51	66.90
Soybean meal (48%)	27.40	27.40	21.81
DDGS	5.00	5.00	7.00
Soy oil	0.87	0.87	1.47
Dicalcium Phosphate	0.94	0.94	0.70
Calcium Carbonate	1.07	1.07	1.01
DL-Methionine	0.24	0.24	0.21
Vitamin Premix[3]	0.10	0.10	0.10
Mineral Premix[4]	0.10	0.10	0.10
NaCl	0.35	0.35	0.34
L-Lys·HCl	0.19	0.19	0.20
Choline	0.09	0.09	0.09
L-Threonine	0.08	0.08	0.07
TBCC[5]	0.02	0.02	0.02
Sand	0.05	---	---
Clinacox®[2]	---	0.05	---
Phytase[6]	0.01	0.01	0.01
Xylanase[7]	0.01	0.01	0.01
Calculated Nutrient Content (%, unless otherwise indicated)			
AME_n, kcal/kg	3,100	3,100	3,185
Crude Protein	19.28	19.28	17.45
Digestible Lys	1.04	1.04	0.92
Digestible Met	0.51	0.51	0.47
Digestible TSAA	0.78	0.78	0.72
Digestible Thr	0.69	0.69	0.61
Digestible Val	0.78	0.78	0.70
Digestible Arg	1.11	1.11	0.96
Ca	0.87	0.87	0.78
Non-phytate P	0.44	0.44	0.39
Na	0.20	0.20	0.20

[1] Dietary density formulated based off 90% of Ross 708 amino acid Nutrition Specifications.

[2] Positive control diets contained Clinacox® (Huvepharma, Inc., Peachtree City, GA) which provides 0.02% diclazuril to the diet at the expense of sand.

[3] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[4] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.

[5] TBCC = tri-basic copper chloride (IntelliBond C; Micronutrients, Inc., Indianapolis, IN)

[6] Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[7] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per ton inclusion.

Table 3.3. Diet analysis results of experimental starter diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52

Calculated Nutrient Content (%)	Moderate[1]	Moderate[1] PC[2]	High[1]	High[1] PC[2]
Crude Protein	21.18	21.18	23.02	23.02
Total Lys	1.27	1.27	1.38	1.38
Total Met	0.59	0.59	0.65	0.65
Total TSAA	0.94	0.94	1.02	1.02
Total Thr	0.88	0.88	0.95	0.95
Total Val	0.96	0.96	1.04	1.04
Total Arg	1.37	1.37	1.51	1.51
Analyzed Nutrient Content[3] (%)				
Crude Protein	21.80	20.79	23.22	23.28
Total Lys	1.35	1.28	1.41	1.43
Total Met	0.54	0.57	0.63	0.61
Total TSAA	0.89	0.90	1.00	0.97
Total Thr	0.89	0.86	0.95	0.96
Total Val	1.08	1.03	1.12	1.15
Total Arg	1.43	1.34	1.51	1.52

[1] Dietary density formulated based off 90% of Ross 708 amino acid Nutrition Specifications for moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for high density diets.

[2] Positive control diets contained Clinacox® (Huvepharma, Inc., Peachtree City, GA) which provides 0.02% diclazuril to the diet.

[3] Diet analysis conducted at University of Missouri Agricultural Experiment Station Chemical Laboratory using AOAC procedures (method 982.30 E (a,b,c), AOAC, 2006).

Table 3.4. Growth performance of Yield Plus × Ross 708 male broilers fed diets formulated varying in amino acid (**AA**) density provided 3 starter feed allocation amounts from 1 to 21 d of age that were vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bird)	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
Moderate	0.45	0.948	1.302	1.376	0.04
	0.73	0.952	1.290	1.356	0.14
	1.00	0.955	1.300	1.361	0.10
	PC[3]	0.972	1.318	1.357	0.16
High	0.45	1.004	1.325	1.318	0.03
	0.73	1.012	1.343	1.328	0.04
	1.00	1.016	1.293	1.271	0.03
	PC[3]	1.006	1.337	1.329	0.00
SEM[4]		0.010	0.019	0.018	0.033
Dietary AA Density[2] Main Effect					
Moderate		0.952	1.297	1.364	0.09
High		1.011	1.320	1.306	0.03
SEM[4]		0.006	0.011	0.012	0.019
Starter Allocation Main Effect					
	0.45	0.976	1.313	1.347	0.04
	0.73	0.982	1.317	1.342	0.09
	1.00	0.985	1.296	1.316	0.06
SEM[4]		0.011	0.019	0.014	0.025
Analysis of Variance		***Probabilities***			
Dietary AA Density × Starter Amount		0.95	0.31	0.16	0.46
Dietary AA Density		0.0001	0.16	0.0001	0.036
Starter Feed Amount		0.64	0.54	0.14	0.31
High AA PC[3] vs. High AA 0.73 kg		0.80	0.76	0.87	0.27
Moderate AA PC[3] vs. Moderate AA 0.73 kg		0.12	0.27	0.98	0.58

[1] Each value represents the least-square means of 8 replicate pens with each pen having 25 chicks at placement.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for Moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for High density diets.

[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.

[4] SEM= pooled standard error

Table 3.5. Growth performance of Yield Plus × Ross 708 male broilers fed diets formulated varying in amino acid (**AA**) density provided 3 starter feed allocation amounts from 1 to 28 d of age that were vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bird)	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
Moderate	0.45	1.538	2.248	1.463	0.04
	0.73	1.564	2.247	1.437	0.15
	1.00	1.581	2.294	1.453	0.11
	PC[3]	1.628	2.324	1.428	0.16
High	0.45	1.650	2.307	1.399	0.07
	0.73	1.657	2.367	1.430	0.08
	1.00	1.669	2.312	1.387	0.07
	PC[3]	1.655	2.325	1.419	0.05
SEM[4]		0.025	0.034	0.019	0.039
Dietary AA Density[2] Main Effect					
Moderate		1.561	2.263	1.451	0.10
High		1.659	2.329	1.405	0.07
SEM[4]		0.013	0.018	0.010	0.023
Starter Allocation Main Effect					
	0.45	1.594	2.278	1.431	0.06
	0.73	1.610	2.307	1.433	0.11
	1.00	1.625	2.303	1.420	0.07
SEM[4]		0.016	0.022	0.012	0.028
Analysis of Variance		***Probabilities***			
Dietary AA Density × Starter Amount		0.86	0.22	0.15	0.46
Dietary AA Density		0.0001	0.010	0.002	0.31
Starter Feed Amount		0.39	0.58	0.68	0.36
High AA PC[3] vs. High AA 0.73 kg		0.95	0.61	0.64	0.64
Moderate AA PC[3] vs. Moderate AA 0.73 kg		0.022	0.043	0.69	0.82

[1] Each value represents the least-square means of 8 replicate pens with each pen having 25 chicks at placement.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for Moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for High density diets.

[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.

[4] SEM = pooled standard error

Table 3.6. Growth performance of Yield Plus × Ross 708 male broilers fed diets formulated varying in amino acid (**AA**) density provided 3 starter feed allocation amounts from 1 to 41 d of age that were vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bird)	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
Moderate	0.45	2.753	4.835	1.758	0.07
	0.73	2.779	4.668	1.680	0.18
	1.00	2.937	4.801	1.694	0.16
	PC[3]	2.878	4.717	1.640	0.18
High	0.45	2.846	4.740	1.668	0.10
	0.73	2.888	4.671	1.617	0.11
	1.00	2.870	4.718	1.649	0.14
	PC[3]	2.842	4.643	1.636	0.18
SEM[4]		0.053	0.057	0.023	0.052
Dietary AA Density[2] Main Effect					
Moderate		2.790	4.768	1.711	0.13
High		2.869	4.710	1.645	0.12
SEM[4]		0.029	0.033	0.012	0.027
Starter Allocation Main Effect					
	0.45	2.800	4.787^{a}	1.713^{a}	0.08
	0.73	2.834	4.669^{b}	1.648^{b}	0.14
	1.00	2.870	4.760^{ab}	1.672^{ab}	0.15
SEM[4]		0.043	0.039	0.015	0.034
Analysis of Variance		***Probabilities***			
Dietary AA Density × Starter Amount		0.66	0.52	0.57	0.59
Dietary AA Density		0.038	0.14	0.0003	0.67
Starter Feed Amount		0.49	0.041	0.011	0.28
High AA PC[3] vs. High AA 0.73 kg		0.45	0.72	0.49	0.85
Moderate AA PC[3] vs. Moderate AA 0.73 kg		0.12	0.49	0.15	1.00

[1] Each value represents the least-square means of 8 replicate pens with each pen having 25 chicks at placement.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for high density diets.

[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.

[4] SEM = pooled standard error of the mean

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey’s Honestly Significant Difference test.

Table 3.7. Gross lesion score percentages of Yield Plus × Ross 708 male broilers at 21d of age fed diets formulated varying in amino acid (**AA**) density provided 3 starter feed allocation amounts from 1 to 21 d of age that vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bird)	Upper Intestine 0 %	Upper Intestine 1 %	Middle Intestine 0 %	Middle Intestine 1 %	Lower Intestine 0 %	Lower Intestine 1 %	Ceca 0 %	Ceca 1 %
Moderate	0.45	68.75	31.25	75.00	21.88	65.25	0.00	75.00	6.25
	0.73	76.47	21.93	55.52	33.83	61.59	7.35	81.05	1.28
	1.00	65.63	28.13	50.00	18.75	56.25	3.13	56.25	6.25
	PC[3]	93.75	6.25	78.13	18.75	68.75	6.25	81.25	0.00
High	0.45	90.63	9.38	75.00	15.65	53.13	6.25	84.38	3.13
	0.73	90.00	10.15	70.41	19.99	75.20	3.33	89.82	1.88
	1.00	84.38	12.50	78.13	18.75	65.63	3.13	68.75	6.25
	PC[3]	93.75	6.25	71.88	12.50	65.63	6.25	87.50	0.00
SEM[4]		7.49	7.49	10.42	8.21	10.68	3.76	8.19	3.53
Dietary AA Density[2] Main Effect									
Moderate		70.29	27.10	60.17	24.82	58.03	3.49	70.77	4.59
High		88.33	10.67	74.51	18.12	64.65	4.24	80.98	3.75
SEM[4]		3.97	4.08	6.14	4.38	7.13	2.00	4.97	2.03
Starter Allocation Main Effect									
	0.45	78.69	20.31	75.00	18.75	60.94	3.13	79.69^{ab}	4.69
	0.73	83.23	16.04	62.96	26.91	68.39	5.34	85.43^{a}	1.58
	1.00	75.00	20.31	64.06	18.75	54.65	3.13	62.50^{b}	6.25
SEM[4]		5.29	5.35	7.70	5.82	8.37	2.66	6.13	2.58
Analysis of Variance					***Probabilities***				
AA Density × Starter Amount		0.85	0.68	0.33	0.85	0.48	0.30	0.92	0.78
Dietary AA Density		0.005	0.007	0.08	0.23	0.46	0.69	0.07	0.53

Starter Amount	0.57	0.89	0.41	0.43	0.20	0.76	0.009	0.29
High AA PC[3] vs. High AA	0.25	0.18	1.00	0.16	0.22	0.21	0.64	0.49
Moderate PC[3] vs. Moderate AA	0.003	0.007	0.008	0.005	0.23	0.78	0.89	0.49

[1] Each value represents the least-square mean score percentages of 8 replicate pens with each pen having 4 birds scored.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for Moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications for High density diets.

[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.

[4] SEM=pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.8. Carcass and yield characteristics of Yield Plus × Ross 708 male broilers fed diets formulated varying in amino acid (**AA**) density provided 3 starter feed allocation amounts from 1 to 41 d of age that vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bd)	Carcass Wt (g)	Carcass Yield (%)	Abdominal Fat Wt (g)	Abdominal Fat Yield (%)	Drums Wt (g)	Drums Yield (%)	Wings Wt (g)	Wings Yield (%)	Total Breast Wt (g)	Total Breast Yield (%)	Thighs Wt (g)	Thighs Yield (%)
Moderate	0.45	2,054	70.72	32	1.10	247	8.69	221	7.79	740	25.05	264	9.29
	0.73	2,111	72.70	31	1.08	256	8.95	229	7.89	716	24.96	269	9.39
	1.00	2,180	72.73	29	1.00	260	8.68	233	7.78	752	25.40	279	9.30
	PC[3]	2,184	71.76	31	1.01	262	8.50	238	7.77	749	25.24	275	8.90
High	0.45	2,137	73.70	32	1.10	256	8.82	226	7.77	747	25.61	272	9.11
	0.73	2,193	73.55	34	1.13	258	8.66	237	7.89	774	25.89	275	9.15
	1.00	2,208	71.83	33	1.09	262	8.64	234	7.86	765	25.84	270	8.82
	PC[3]	2,177	73.54	31	1.04	256	8.53	232	7.74	759	25.64	273	8.99
SEM[4]		29	0.99	2	0.06	3	0.11	4	0.13	15	0.27	5	0.18
Dietary AA Density[2] Main Effect													
Moderate		2,115	72.05	31	1.06	255	8.77	227	7.83	724	25.14	271	9.33
High		2,179	73.03	33	1.11	259	8.71	232	7.83	762	25.78	272	9.03
SEM[4]		17.17	0.55	0.94	0.03	1.92	0.09	1.94	0.08	8.68	0.18	3.60	0.10
Starter Allocation Main Effect													
	0.45	2,096^{b}	72.21	32	1.10	251^{b}	8.76	223^{b}	7.78	725	25.33	268	9.20
	0.73	2,152^{ab}	73.12	33	1.10	257^{ab}	8.80	233^{a}	7.89	745	25.43	272	9.27
	1.00	2,194^{a}	72.29	32	1.04	261^{a}	8.68	233^{a}	7.82	758	25.62	274	9.06
SEM[4]		20.63	0.66	1.13	0.04	2.30	0.10	2.34	0.09	10.18	0.20	4.00	0.12
Analysis of Variance						***Probabilities***							
AA Density × Starter Amount		0.20	0.13	0.52	0.56	0.54	0.06	0.97	0.87	0.25	0.45	0.12	0.40
AA Density		0.009	0.21	0.13	0.31	0.13	0.34	0.06	0.83	0.002	< 0.001	0.67	0.017
Starter Amount		0.006	0.56	0.80	0.68	0.013	0.22	0.002	0.53	0.08	0.39	0.31	0.20
High PC[3] vs. High AA		0.72	0.99	0.14	0.22	0.56	0.46	0.53	0.46	0.55	0.42	0.75	0.29

Mod PC[3] vs. Mod AA	0.09	0.54	0.77	0.37	0.23	0.011	0.045	0.52	0.09	0.32	0.43	0.028

[1] Each value represents the least-square means of 8 replicate pens with each pen providing 12 carcasses at 42 d of age.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for Moderate (Mod) density diets and 98% of Ross 708 amino acid Nutrition Specifications for High density diets.

[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.

[4] SEM = pooled standard error of the mean

Wt = Weight

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant difference test.

Table 3.9. Cumulative digestible (**d**) amino acid (**AA**) intake of Yield Plus × Ross 708 male broilers fed starter diets formulated varying in AA density provided 3 starter feed amounts from 1 to 41d vaccinated at hatch with Coccivac® B52[1]

Dietary AA Density[2]	Starter Amount (kg/bd)	dTSAA Intake (g)	dLys Intake (g)	dThr Intake (g)	dVal Intake (g)	dIle Intake (g)	dArg Intake (g)	dTrp Intake (g)
Moderate	0.45	36.4	47.5	31.6	35.9	32.5	50.1	8.8
	0.73	35.7	46.7	31.1	35.2	32.0	49.3	8.6
	1.00	36.1	47.2	31.5	35.6	32.4	50.0	8.8
	PC[3]	36.2	47.4	31.6	35.8	32.6	50.2	8.8
High	0.45	36.4	47.5	31.6	35.9	32.6	50.3	8.8
	0.73	36.8	48.2	32.1	35.2	33.2	51.2	8.9
	1.00	37.0	48.5	32.3	36.6	33.4	51.6	9.0
	PC[3]	37.0	48.4	32.2	36.6	33.3	51.4	9.0
SEM[4]		0.47	0.60	0.40	0.46	0.41	0.63	0.11
Dietary AA Density[2] Main Effect								
Moderate		36.7	47.1	31.4	35.6	32.0	49.8	8.7
High		36.1	48.1	32.0	36.3	33.1	51.0	8.9
SEM[4]		0.27	0.35	0.23	0.26	0.24	0.36	0.06
Starter Allocation Main Effect								
	0.45	36.4	47.5	31.6	35.9	32.6	50.2	8.8
	0.73	36.3	47.4	31.6	35.8	32.6	50.3	8.8
	1.00	36.5	47.8	31.9	36.1	32.9	50.8	8.9
SEM[4]		0.33	0.43	0.28	0.32	0.29	0.45	0.07
Analysis of Variance				***Probabilities***				
AA Density × Starter Amount		0.43	0.41	0.43	0.40	0.38	0.37	0.40
Dietary AA Density		0.08	0.06	0.07	0.048	0.033	0.024	0.044
Starter Allocation		0.84	0.79	0.70	0.80	0.66	0.54	0.53
High PC[3] vs. High AA		0.78	0.79	0.79	0.79	0.79	0.80	0.79
Moderate PC[3] vs. Moderate AA		0.34	0.32	0.32	0.33	0.33	0.32	0.33

[1] Each value represents the least-square means from 10 replicate pens with each pen having 25 chicks at placement.

[2] Amino acid specifications formulated based off 90% of Ross 708 amino acid Nutrition Specifications for Moderate density

diets and 98% of Ross 708 amino acid Nutrition Specifications for High density diets.
[3] PC = Positive control birds were fed at the 0.73 kg/bd starter allotment and not vaccinated against coccidiosis.
[4] SEM = pooled standard error of the mean

IV. EFFECTS OF PRE-STARTER DIETS VARYING IN AMINO ACID DENSITY GIVEN TO BROILERS THAT RECEIEVED COCCIDIOSIS VACCIATION AT HATCH

ABSTRACT

An experiment was conducted to evaluate broilers vaccinated against coccidiosis fed combinations of pre-starter and starter diets varying in digestible amino acid (**dAA**) density from 1 to 19 d of age on subsequent growth performance and meat yield. One thousand eight hundred Yield Plus Ross × Ross 708 male broilers were allocated to 60 floor pens and assigned to 1 of 6 treatments. Four pre-starter diets varying in dAA density [1.15% dLys, 1.25% dLys, 1.35% dLys, and Positive Control (1.15% dLys + Diclazuril)] were fed until 9 d of age. Then, 3 starter diets varying in dAA density [1.15% dLys, 1.25% dLys, and PC (1.15% dLys + Diclazuril)] were given from 10 to 19 d of age. All diets were formulated to similar dAA ratios to dLys, hence resulting in increasing AA density among the dietary treatments. Birds were given common grower and finisher diets for the remainder of the experiment. At 19, 27, and 40 d of age, broilers that received the 1.35% and 1.25% dLys density pre-starter and 1.25% dLys density starter diets had increased BW gain ($P \leq 0.006$) compared with broilers fed the 1.15% dLys density diets. Broilers that received the 1.15% dLys density pre-starter diet had a lower cumulative BW gain ($P = 0.007$) than those fed the 1.35% and 1.25% dLys density pre-starter diets. Broilers that received the 1.35% and 1.25% dLys density pre-starter and

1.25% dLys density starter diets had the heaviest ($P \leq 0.008$) total breast weights. Increasing the pre-starter dAA density enhanced ($P \leq 0.03$) total breast weight by 28 g (1.25% dLys density) and 51 g (1.35% dLys density). Results from this study indicated that increasing dAA density from 1 to 19 d of age enabled a 1 to 19 d dAA intake increase of 4.8 g, which enhanced cumulative growth and meat yield of broilers vaccinated against coccidiosis.

INTRODUCTION

The demand for meat products originating from antibiotic free production in the United States has induced broiler companies to vaccinate against coccidiosis instead of using coccidiostats for coccidiosis control. This vaccination elicits an enteric infection, which depresses growth and reduces meat yield. Reduction in breast meat yield has major economic ramifications for integrators producing broilers for tray-pack markets. In recent years, studies have focused on ameliorating these negative effects through dietary CP modulation (Lehman et al., 2009; Lee et al., 2011). Another potential strategy investigated has been to increase the dietary AA density in the starter period by providing adequate AA to support immune functions and muscle accretion (Cloft et al., 2018). This should allow for optimum growth to continue while undergoing the vaccine challenge.

Previous work from our laboratory reported that increasing dietary AA density during the starter period improved cumulative growth performance and 41 d total breast meat weight of broilers (Cloft et al., 2018). These responses were largely attributed to a dietary AA density difference characterized by 0.10% dLys increase in the starter diet, which resulted in increased cumulative AA intake (dTSAA, dLys, dThr, dVal, dIle, dArg, dTrp) of 5.6 g. Pre-starter diets may be utilized to provide higher dietary AA density to broilers early in development instead of the entire starter period as a strategy to reduce cost (Leeson, 2008). Utilization of a pre-starter diet could facilitate increased AA intake before the vaccine infection results in growth depression. Therefore, the objective of this experiment was to determine the impact of feeding vaccinated broilers pre-starter and starter diets varying in AA density until 19 d on subsequent growth performance and meat yield during a 6 wk production period.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2017-3171).

Dietary Treatments

Six dietary treatments (**TRT**) consisting of combinations of pre-starter and starter diets were fed in the current study. Diets were formulated to dAA ratios to Lys to achieve varying dietary AA densities. Three pre-starter dietary AA densities were chosen: 1.15, 1.25, and 1.35% dLys. Two starter dietary AA densities were used: 1.15 and 1.25% dLys. Non-vaccinated broilers served as the positive control and received a medicated version of the 1.15% dLys density pre-starter and starter diets. The first 5 TRT received coccidiosis vaccination and TRT 6 was designated as the PC. Treatment 1 received 1.15% dLys density diets during the pre-starter and starter periods. Treatment 3 received 1.25% dLys density diets during the pre-starter and starter periods. The 1.15% dLys density was chosen as it is used in commercial starter diets by integrators to reduce feed cost and the 1.25% dLys density was designed to optimize growth performance. Treatment 2 consisted of 1.25% dLys density pre-starter and 1.15% dLys density starter diets. Treatment 4 and TRT 5 were assigned to the 1.35% dLys density pre-starter diet and then the 1.15% dLys density and 1.25% dLys density starter diet, respectively. The 1.35% dLys density was chosen as it reflects the AA requirements of broilers during the first week of life, which is ideal for a pre-starter diet but may be higher than what is needed during the later portion of the starter phase.

Pre-starter diets were fed to 9 d of age and starter diets from 10 to 19 d (Tables 4.1 and 4.2). Following the starter period, broilers were provided a common grower diet (Table 4.3). Non-vaccinated broilers received a medicated version of the grower diet. At 27 d of age, all broilers were provided a common non-medicated finisher diet for the remainder of the study (Table 4.3). Corn, soybean meal, and corn DDGS were used in diet formulation as the primary ingredients. All diets within a specific growth period were formulated to be isocaloric and to contain similar ratios of dTSAA (0.74), dThr (0.67), dVal (0.75), and dIle (0.67) to dLys. Pre-starter and starter diets were fed as crumbles and subsequent diets provided as whole pellets. Experimental diets were analyzed for total AA concentrations in triplicate by the University of Missouri Agricultural Experiment Station Chemical Laboratory using AOAC procedures (method 982.30 E (a,b,c), AOAC, 2006). Performic acid oxidation (method 985.28; AOAC, 2006) was conducted before acid hydrolysis for the determination of Met and Cys, whereas all other AA were determined after acid hydrolysis.

Bird Husbandry

One thousand and eight hundred Yield Plus Ross × Ross 708 (Aviagen Inc., Huntsville, AL) male broilers were randomly allocated to 60 floor pens (0.08 m^2/bird; 30 birds/pen) based on their vaccination status. Prior to placement, 1,500 broilers received a 1x dosage of Coccivac®-B52 (Merck Animal Health, Summit, NJ) via spray cabinet at a commercial hatchery. The remaining 300 broilers did not receive a coccidiosis vaccination and were provided diets containing Diclazuril to prevent infection with coccidiosis. All broilers received vaccinations for Marek's disease, Newcastle disease, and infectious bronchitis. The experimental facility was a solid-sided house containing floor pens with used litter equipped with a hanging feeder and nipple water line (5 to 6 nipples/pen). The experimental facility contained a negative-pressure ventilation system equipped with electronic controller, vent boards, stir fans, exhaust fans, and evaporative cooling pads. Ambient temperature was set at 33°C at placement and decreased gradually with advancing bird age to 20°C by the end of the study. A 23L:1D photoperiod was set at placement and decreased to 20L:4D at 7 d of age for the remainder of the study. Light intensity was set at 30 lux at placement and decreased to 10 and 5 lux at 7 and 12 d of age, respectively. Light intensity settings were verified at bird height following each adjustment using a photometric sensor calibrated with the National Institute of Standards and Technology- traceable calibration (403125, Extech Instruments, Waltham, MA).

Measurements

Birds and feed were weighed to determine BW gain, feed intake, and feed conversion ratio at 1, 9, 19, 27, and 40 d of age. Incidences of mortality were recorded daily. Feed conversion ratios were adjusted to account for mortalities. Digestible AA intakes were calculated by multiplying the feed intake with the dAA content of the diet for the 7 most limiting AA: TSAA, Lys, Thr, Val, Ile, Arg, and Trp.

At 9 d of age, all birds per pen were scored for pododermatitis lesions according to the scoring system described by Nagaraj et al. (2007) (0 = none; 1 = mild lesions < 1.5 cm ; 2 = severe lesions > 1.5 cm). The percentage of each pododermatitis score per pen was calculated based on scores of each bird for statistical analysis. Then, 8 birds per pen were selected for collection of plasma for the determination of plasma carotenoid concentrations. Selected birds were euthanized via CO_2 asphyxiation. Immediately following euthanasia, blood was collected via cardiac puncture into tubes containing lithium heparin, then placed on ice and centrifuged for 20 min at 1,250 × g and 4°C. Plasma was pooled by pen and stored at -80°C until further analysis. Blood processing and carotenoid assessment were conducted under yellow light (560 to 590 nm). Plasma carotenoid concentrations were determined by spectrophotometry as described by Allen et al. (1996). Briefly, plasma was diluted to 1:10 with absolute ethanol, measured at 474 to 530 nm, and then multiplied by extinction coefficient of 81 to determine lutein equivalents per mL of plasma.

At 19 d of age, 4 birds per pen were selected for intestinal lesion scoring and plasma collection to assess vaccine efficacy. The percentage of each score per pen was calculated based on scores of each bird for statistical analysis. Gross lesion scoring was conducted based on the Johnson and Reid (1970) (0 = none; 1= scattered and small petechiae ; 2 = numerous petechiae ; 3 = intestinal thickening and blood tinged exudate ; 4 = hemorrhage, intestinal distention and necrosis) method using intestinal regions outlined by Conway and Mckenzie (2007) for scoring mixed infections. Intestinal regions were defined as: duodenal and proximal jejunal intestine as the upper region, distal jejunal and proximal ileal intestine as the middle intestinal region, distal ileal and rectum as the lower intestinal region, and the ceca alone as the cecal region (Conway and Mckenzie, 2007). Intestinal regions for lesion scoring were used because the vaccine contained *Eimeria acervulina*, *E. maxima*, *E. maxima* mixed field strain precocious (MFP), *E. mivati*, and *E. tenella* strains that would infect the intestine simultaneously.

At 41 d of age, all birds per pen were scored for incidence of pododermatitis lesions according to the system described by Nagaraj et al. (2007) (0 = none; 1 = mild lesions < 1.5 cm ; 2 = severe lesions > 1.5 cm). The percentage of each pododermatitis score per pen was calculated based on scores of each bird for statistical analysis. Then, 14 birds per pen were selected for processing. Birds were processed in a pilot processing facility at the Auburn University Poultry Research Unit following a 12 h feed withdrawal period. Broilers were electrically stunned, exsanguinated, scalded, mechanically picked, mechanically eviscerated, and then placed on ice. Carcasses were chilled in slush ice-water for 3 h and then drained of excess water for 3 min. Abdominal fat was removed from carcasses and weighed separate from the whole carcass. Carcasses were then split and the front halves were deboned the following day to obtain fillets (*pectoralis major muscles*) and tenders (*pectoralis minor muscles*) by experienced personnel using stationary cones. Meat yield percentages were based on the live bird weight at 40 d of age. Tender and fillet weights were combined for the determination of total breast meat. Fillets were scored on a 4 point scale (0 = none; 1 = mild ; 2 = moderate ; 3 = severe) for wooden breast and white striping using visual and tactile assessment. All fillets were scored by the same evaluator. Wooden breast and white striping scoring systems were based on the descriptions in Tijare et al. (2016). Wooden breast and white striping incidences are reported as the proportion of each score observed for each pen.

Statistical Analysis

The experimental design consisted of 6 dietary TRT with each TRT represented by 10 replicate pens. Dietary TRT were applied in a randomized complete block design with pen location as the blocking factor. Pen was considered as the experimental unit. Prior to analysis all percentage data were transformed using square-root arcsine. Performance, processing and subjective score data were analyzed using a one-way ANOVA using PROC Mixed (SAS 9.4) and treatment differences were elucidated through pre-planned orthogonal contrasts using the following mixed model:

$$Y_{ij} = \mu_{..} + \tau_j + \rho_i + \varepsilon_{ij}$$

where $\mu_{..}$ is the overall mean; the τ_i are fixed factor level effects corresponding to the i^{th} TRT such that $\sum \tau_i = 0$; the ρ_j are identically and independently normally distributed random block effects with mean 0 and variance σ_ρ^2; and the random error ε_{ij} are identically and independently normally distributed with mean 0 and variance σ^2. Tukey’s Honestly Significant Difference was conducted for mean separation when significant ANOVA results were observed. Statistical significance was established at $P \leq 0.05$.

Pre-planned orthogonal contrasts were conducted to assess 3 research questions in addition to the ANOVA. First, 2 contrasts were conducted to assess the benefit of providing increased dietary AA density during the pre-starter period by comparing TRT 1 with TRT 4 and TRT 1 with TRT 5. Second, 2 contrasts were conducted to compare the effect of providing an increased pre-starter dietary AA density with an increased starter dietary AA density. This was achieved by comparing TRT 3 and TRT 4 and TRT 3 with

TRT 5. Third, to assess the impact of the coccidiosis vaccination in this study, by comparing TRT 1 and TRT 6 (PC). Statistical significance was established at $P \leq 0.05$.

The effect of pre-starter dietary AA density was analyzed separately using a one-way ANOVA based on the mixed-model described above where the τ_i represented the different pre-starter diets instead of TRT. Mean separation conducted using Tukey's Honestly Significant Difference when significant ANOVA results were observed (Tukey, 1953). Pre-starter dietary AA density effect analysis was unable to isolate the pre-starter diet effect from the starter diet effect, but was conducted to understand the impact of the pre-starter dietary AA density on growth performance and meat yield responses. Data collected during the pre-starter period (1 to 9 d of age) were only analyzed for pre-starter effect with the exception of plasma carotenoids. Both 9 and 19 d collections of plasma carotenoid were subjected to paired t-tests between diet and d. Statistical significance was established at $P \leq 0.05$.

RESULTS

Diet Analysis

Pre-starter and starter diets were analyzed for total AA concentrations. Total AA concentrations of the pre-starter diets determined that analyzed values were within 0.05% points of the calculated values (Table 4.1). Analyzed total AA concentrations for the 1.15% dLys density pre-starter diet and the PC pre-starter diet were similar (1.31% total Lys and 1.32% total Lys). The 1.15% dLys density pre-starter and PC pre-starter diets had slightly higher Lys values than calculated total values (1.27% Lys). Additionally, the 1.25% dLys density and 1.35% dLys density pre-starter diets had slightly lower total AA values (1.36% Lys and 1.40% Lys) than calculated (1.38% Lys and 1.49% Lys). This resulted in a smaller actual difference of 0.05% points instead of 0.10% points Lys between the different pre-starter diets.

Starter diet analysis revealed a difference of 0.10% points between analyzed total AA concentrations and calculated total AA concentrations of the experimental diets (Table 4.2). The 1.15% dLys density starter diet and the 1.15% dLys density-PC starter diet AA (1.36% Lys and 1.39% Lys) concentrations were in good agreement. The analyzed values for the 1.15% dLys density and 1.25% dLys density starter diets were 1.36% and 1.49% total Lys, respectively. While the concentrations were higher than calculated values (1.27% total Lys for the 1.15% dLys density starter diets and 1.38% total Lys for the 1.25% dLys density starter diets), the difference between the 2 diets remained similar.

Growth Performance

1 to 9 d of age. During the pre-starter period, FCR was lowest (*P* = 0.002) for broilers receiving the 1.35% dLys density pre-starter diet compared with the other pre-starter diets (Table 4.4). Broilers fed the 1.35% dLys density pre-starter diet had 6 points lower (*P* = 0.002) FCR than the 1.25% dLys density pre-starter diet. The difference in FCR is likely a result of the broilers provided the 1.35% dLys density pre-starter diet having a numerically decreased feed intake of 9 g but similar BW gain as the birds given the 1.25% dLys density pre-starter diet. Scoring of pododermatitis lesions at 9 d of age revealed few visible lesions with 90% of the birds having scores of 0 (*P* > 0.05; data not shown).

1 to 19 d of age. Broilers fed TRT 5 (1.35% dLys pre-starter (**PS**) | 1.25% dLys starter (**S**)) had similar BW gain to TRT 3 (1.25% dLys PS | 1.25% dLys S) (*P* = 0.49)

(Table 4.5). Birds given TRT 3 (1.25% dLys PS | 1.25% dLys S) had greater (*P* = 0.036) BW gain than those fed TRT 4 (1.35% dLys PS | 1.15% dLys S). Broilers provided TRT 5 (1.35% dLys PS | 1.25% dLys S), TRT 4 (1.35% dLys PS | 1.15% dLys S), and TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S) had greater ($P \geq 0.03$) BW gain than those fed TRT 1 (1.15% dLys PS | 1.15% dLys S). Broilers fed TRT 5 (1.35% dLys PS | 1.25% dLys S) and TRT 4 (1.35% dLys PS | 1.15% dLys S) had 6 points and 3 points lower ($P \geq 0.008$) FCR, respectively, than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed birds. Additionally, broilers provided TRT 1 (1.15% dLys PS | 1.15% dLys S) had a 29 g lower (*P* = 0.027) feed intake than birds given TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S). The pre-starter diet effect influenced BW gain (*P* = 0.039) and FCR (*P* = 0.003) of broilers from 1 to 19 d of age. Broilers fed the 1.15% dLys density pre-starter had the lowest (*P* = 0.04) BW gain compared with those given the 1.35% dLys density pre-starter, 1.25% dLys density and 1.15% dLys density-PC pre-starter diets. Broilers receiving the 1.35% dLys density pre-starter diet had the lowest FCR (*P* = 0.003) compared with broilers receiving any other pre-starter diets.

1 to 27 d of age. Broilers fed TRT 3 (1.25% dLys PS | 1.25% dLys S) and TRT 5 (1.35% dLys PS | 1.25% dLys S) had similar (*P* = 0.96) BW gain (Table 4.6). Birds given TRT 3 (1.25% dLys PS | 1.25% dLys S) had greater (*P* = 0.01) BW gain than those given TRT 4 (1.35% dLys PS | 1.15% dLys S). Birds provided TRT 4 (1.35% dLys PS | 1.15% dLys S), TRT 5 (1.35% dLys PS | 1.25% dLys S), and TRT 6 (1.15%-PC dLys PS | 1.15%-PC dLys S) had greater ($P \geq 0.047$) BW gain than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers. Additionally, broilers fed TRT 4 (1.35% dLys PS | 1.15% dLys S) consumed more (*P* = 0.03) feed than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers. No other feed intake or FCR differences ($P > 0.05$) were observed. Broilers fed the 1.15% dLys density-PC, 1.25% dLys density, and 1.35% dLys density pre-starter diets had a higher ($P = 0.009$) BW gain than the 1.15% dLys density pre-starter diet. No other differences ($P \geq 0.23$) were observed due to the pre-starter diet effect through 4 weeks of age.

1 to 40 d of age. Broilers fed TRT 5 (1.35% dLys PS | 1.25% dLys S) and TRT 3 (1.25% dLys PS | 1.25% dLys S) had similar BW gain ($P = 0.32$) but had greater ($P = 0.006$) growth rate than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed birds (Table 4.7). Birds fed TRT 4 (1.35% dLys PS | 1.15% dLys S) and TRT 6 (1.15%-PC dLys PS | 1.15%-PC dLys S) also had increased ($P \leq 0.02$) BW gain compared with TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers. Additionally, broilers fed TRT 4 (1.35% dLys PS | 1.15% dLys S) consumed more ($P = 0.02$) feed than those fed TRT 1 (1.15% dLys PS | 1.15% dLys S). Broilers given the 1.15% dLys density pre-starter diet resulted in the lowest ($P = 0.007$) BW gain compared with the other pre-starter diets. Scoring of pododermatitis lesions at 40 d of age revealed few visible lesions with 86% of the birds having scores of 0 ($P > 0.05$; data not shown).

There were cumulative dAA intake differences for the first 7 limiting AA, wherein an increase ($P \leq 0.005$) of 11.9 g dAA intake for broilers fed the 1.35% dLys density pre-starter diet was observed above those fed the 1.15% dLys density or the 1.15% dLys density-PC pre-starter diets. Additionally, there was a cumulative difference ($P \leq 0.009$) of 11.3 g dAA intake between TRT 1 (1.15% dLys PS | 1.15% dLys S) vs. TRT 4 (1.35% dLys PS | 1.15% dLys S) fed broilers and 12.6 g dAA intake for broilers fed TRT 1 (1.15% dLys PS | 1.15% dLys S) vs. TRT 5 (1.35% dLys PS | 1.25% dLys S), where TRT 1 had the lowest dAA intake. A difference (*P* < 0.05) was also detected between TRT 1 (1.15% dLys PS | 1.15% dLys S) vs. TRT 4 (1.35% dLys PS | 1.15% dLys S) fed broilers and broilers fed TRT 1 (1.15% dLys PS | 1.15% dLys S) vs. TRT 5 (1.35% dLys PS | 1.25% dLys S) from 1 to 19 d of age for dLys, dVal, dIle, dArg, and dTrp (data not shown), where TRT 1 fed broilers again had the lowest dAA intake. Broilers fed the increased pre-starter diets (1.25% and 1.35% dLys density pre-starter diets) consumed 4.8 g more ($P \leq 0.05$) dAA than those fed the 1.15% dLys density pre-starter diet (data not shown). Birds provided TRT 3 (1.25% dLys PS | 1.25% dLys S), TRT 4 (1.35% dLys PS | 1.15% dLys S), and TRT 5 (1.35% dLys PS | 1.25% dLys S) had similar ($P \geq 0.49$) 1 to 19 d and cumulative dAA intakes.

Vaccine Efficacy Assessments

Broilers not receiving coccidiosis vaccination had a greater percentage of scores of 0 (*P* = 0.017) than those given the 1.35% dLys density pre-starter and 1.25% dLys density pre-starter diets in the upper intestinal region (Table 4.9). Consequently, PC fed broilers had a lower percentage of scores of 1 (*P* = 0.031) than those given the 1.35% dLys density pre-starter and 1.25% dLys density pre-starter diets for the upper intestinal region. No differences ($P \leq 0.07$) were observed in the other intestinal regions. Lesion scores obtained in this study ranged from 0 to 2, which is consistent with published reports of vaccinated broilers (Williams, 2003). The elevated incidence of scores for broilers that received coccidiosis vaccination at hatch is indicative of vaccination success. Plasma carotenoid analysis revealed no difference ($P \geq 0.69$) between the 1.15% dLys density pre-starter diet and the 1.15% dLys density-PC pre-starter diet at either 9 or 19 d of age (data not shown). Additionally, no difference in plasma carotenoid concentrations were observed at 9 and 19 d of age for either the 1.15% dLys density pre-starter diet or the 1.15% dLys density-PC pre-starter diet analyzed ($P \geq 0.59$). Plasma carotenoids have been used as indirect indicator of *Eimeria* infection severity in chickens as it reflects the disruption of intestinal mucosa (Rochell et al., 2017).

Processing Characteristics

Broilers fed TRT 5 (1.35% dLys PS | 1.25% dLys S) and TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S) had heavier ($P \leq 0.008$) carcass weights than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers (Table 4.10). Broilers fed TRT 1 (1.15% dLys PS | 1.15% dLys S) also had lighter total breast weights than ($P \leq 0.03$) those given TRT 4 (1.35% dLys PS | 1.15% dLys S), TRT 5 (1.35% dLys PS | 1.25% dLys S), and TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S). Broilers fed the 1.15% dLys density pre-starter diet had the lowest total breast weights ($P = 0.016$), with 26 g less than the 1.25% dLys density pre-starter diet, 40 g less than the 1.35% dLys density pre-starter diet, and 32 g less than the PC pre-starter diet. Additionally, broilers fed the 1.15% dLys density pre-starter had lower breast fillet ($P = 0.032$) and tender ($P = 0.008$) weights than the those given the 1.35% dLys density pre-starter diet. No differences were observed for yields of carcasses, breast fillets, tenders, total breast, abdominal fat pad percentage or weights between the pre-starter diets or dietary TRT ($P \geq 0.07$). Breast fillet (*pectoralis major* muscle) scoring of white striping and wooden breast only revealed that birds given TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S) had a 5.29% greater ($P = 0.045$) percentage of wooden breast scores of 3 than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers (data not shown).

DISCUSSION

Providing the combination of increased dAA density in the pre-starter and starter diets enhanced growth and meat yield of broilers vaccinated against coccidiosis by increasing AA intake. In agreement, Kidd et al. (2005) reported broilers fed a moderate AA density starter diet after a high AA density pre-starter diet had lower BW than broilers provided high AA density pre-starter and starter diets. The decreased growth performance by broilers provided the moderate AA density starter diet implies that changing to a reduced AA density starter diet did not support optimal growth. In the current study, broilers provided TRT 4 (1.35% dLys PS | 1.15% dLys S) and TRT 5 (1.35% dLys PS | 1.25% dLys S) did not have enhanced cumulative growth performance beyond that of TRT 3 (1.25% dLys PS | 1.25% dLys S) fed birds. This is likely due to the similar AA intake between broilers fed TRT 3 (1.25% dLys PS | 1.25% dLys S), TRT 4 (1.35% dLys PS | 1.15% dLys S), and TRT 5 (1.35% dLys PS | 1.25% dLys S). The actual total Lys concentration difference between the 1.25% and 1.35% dLys density pre-starter diets was 0.05% points instead of 0.10% points Lys. This smaller difference may have limited the response of the 1.35% dLys density pre-starter diet fed birds resulting in similarities in growth performance and carcass characteristics.

Dietary AA intake during the first week post-hatch optimizes the growth of broilers through regulating satellite cell mitotic activity (Barekatain and Swick, 2016). Broilers fed diets containing increased AA density during the first week of life stimulates increased growth through activation of ribosomal S6 kinase involved in regulation of protein synthesis (Everaert et al., 2010; Barekatain and Swick, 2016). In the research reported herein from 1 to 19 d of age, birds given TRT 4 (1.35% dLys PS | 1.15% dLys S) and TRT 5 (1.35% dLys PS | 1.25% dLys S) had similar feed intakes as those provided TRT 1 (1.15% dLys PS | 1.15% dLys S) but consumed a greater amount of AA than TRT 1 (1.15% dLys PS | 1.15% dLys S) fed broilers. The increased AA intake supported enhanced BW gain and lower FCR. The dAA intake differences likely contributed to the observed BW gain and carcass differences between broilers provided TRT 1 (1.15% dLys PS | 1.15% dLys S), TRT 4 (1.35% dLys PS | 1.15% dLys S), and TRT 5 (1.35% dLys PS | 1.25% dLys S). Previous studies have reported breast meat weight increases when providing broilers adequate dietary AA density diets during the pre-starter and starter periods (Kidd et al., 2004; Kidd et al., 2005). Diets adequate in AA density stimulates protein synthesis resulting in greater muscle accretion by increasing the amount of AA needed for protein deposition through regulating gene expression and the mechanistic target of rapamycin (mTOR) pathway (Surawayan and Davis, 2012). The results of the current study indicate that providing high AA density diets early in development enabled increased AA intake throughout the growing period, which facilitated increased muscle accretion leading to pronounced differences in total breast meat.

Feed intake differences were observed between broilers fed TRT 1 (1.15% d Lys PS | 1.15% dLys S) and TRT 4 (1.35% dLys PS | 1.15% dLys S) of 113 g from 1 to 27 d of age and 174 g from 1 to 40 d of age. These differences may be due to 2 complementary responses. First, broilers provided TRT 1 (1.15% dLys PS | 1.15% dLys S) likely experienced depressed feed intake as a result of the coccidiosis infection caused by the vaccination. Second, birds fed TRT 4 (1.35% dLys PS | 1.15% dLys S) could have increased feed intake to compensate for the starter diet having a lower dietary AA density than the previous pre-starter diet. This intake adjustment has been reported in older broilers attempting to meet their AA requirements while fed a diet low in dietary AA content (Dozier et al., 2007). Collectively, these responses may have contributed to large feed intake differences between TRT 1 (1.15% dLys PS | 1.15% dLys S) and TRT 4 (1.35% dLys PS | 1.15% dLys S) observed in this study.

Depressed feed intake is a common symptom of mild coccidiosis infection (Williams, 2002; Miska and Fetterer, 2018). Feed intake is decreased due to the inflammation response caused by the parasite invasion, whereas anorexia is induced as a by-product of immune signaling through pro-inflammatory cytokines, IFN-γ and TNF-α (Lillehoj and Trout, 1996). Miska and Fetterer (2018) observed a feed intake depression leading up to and coinciding with peak infection at 7 d post-infection. Feed intakes returned to a similar amount as the non-infected birds by 11 d post-infection. However, Miska and Fetterer (2018) housed broilers in battery cages that prevented parasite recycling, which is an essential mechanism for the development of immunity against coccidiosis (Williams, 2002). Therefore, it would be expected to have feed intake depression in vaccinated broilers occurring through multiple infection cycles prior to development of full immunity. Accompanying this anorexia is a downregulation of many intestinal nutrient transporters (Miska and Fetterer, 2018), which further reduces available nutrients exaggerating the growth performance differences between broilers provided TRT 1 (1.15% PS | 1.15% S) and TRT 6 (1.15% dLys-PC PS | 1.15% dLys-PC S).

From 1 to 27 and 1 to 40 d of age, broilers provided the PC pre-starter diet had greater BW gain than those fed the 1.15% dLys density pre-starter diet but similar to that of broilers provided 1.25% and 1.35% dLys density pre-starter diets. The reduced BW gain of broilers given the 1.15% dLys density pre-starter diet is likely due to the combination of growth depression caused by the coccidiosis vaccination infection and lack of AA to support concurrent recovery from the infection and adequate growth. Coccidial infection is characterized by parasite invasion of enterocytes resulting in intestinal tissue damage from cell lysis (Williams, 2002). These symptoms stimulate enhanced mucogenesis to repair intestinal damage, which increases the demand for AA associated with mucin and intestinal barrier function, such as Thr, Gly, Ser, and Cys (Moran, 2017). Dietary inclusion of such AA in greater concentrations should provide relief from endogenous synthesis and allow other AA be incorporated into muscle development. In the current study, the 1.25% and 1.35% dLys density pre-starter diets were able to support the broilers during the vaccine challenge based on their similarity of BW gain to the non-vaccinated broilers provided the PC pre-starter diet BW gain. The difference in Gly+Ser, Thr, and Cys concentrations in the 1.15% dLys density pre-starter diet were 0.04% points lower than the 1.25% dLys density pre-starter diet and 0.16% points lower than the 1.35% dLys density pre-starter diet, indicating that broilers fed the 1.15% dLys density pre-starter diet may not have been provided sufficient amounts of these AA to meet the recovery demands. Similarly, Lehman et al. (2009) reported vaccinated broilers fed high CP diets had increased BW gain from 3 to 6 wk of age compared with those fed low CP diets. These authors attributed this difference to compensatory gain following recovery from the Coccivac®-D vaccine infection enabled by adequate dietary AA concentrations of Gly+Ser, Thr, and Cys in the high CP diet, which were 0.09% points greater than the low CP diet.

Coccidiosis vaccination also contributed to the differences observed in breast fillets, tenders, and total breast weights between the pre-starter diets. The decreased meat accretion by the 1.15% dLys density pre-starter diet fed broilers may be due to the combination of vaccine depression and lack of AA to support recovery and growth simultaneously. Lehman et al. (2009) noted broilers fed low CP diets failed to fully recover from the vaccine infection by the end of experimentation, which resulted in lower carcass and breast fillet weights. In the current research, vaccinated broilers provided the 1.25% and 1.35% dLys density pre-starter diets had similar total breast weights as the non-vaccinated broilers given the 1.15% dLys density-PC pre-starter diet. Without the vaccine depression it would be expected that broiler fed pre-starter diets containing increased dietary AA density may produce greater total breast weights than the broilers provided the PC pre-starter diet.

In summary, vaccinated broilers fed pre-starter diets formulated to 1.35% dLys density, regardless of the dAA density during the starter period, did not improve cumulative growth performance or total breast meat beyond broilers given 1.25% dLys density diets for the pre-starter and starter periods. Broilers fed diets with increasing dAA density in a pre-starter diet beyond 1.15% dLys density enhanced broiler BW gain and total breast weights. Broilers receiving coccidiosis vaccination fed the 1.15% dLys density pre-starter diet displayed lower cumulative BW gain and total breast weight compared with the non-vaccinated PC broilers. These results indicate that providing diets with increased dAA density until 19 d of age enabled a 1 to 19 d dAA intake increase of 4.8 g to vaccinated broilers should enhance growth performance and breast meat yield.

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Table 4.1. Ingredient and nutrient composition of experimental pre-starter diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52 from 1 to 9 d of age.[1]

Ingredient, % "as-fed"	1.15% dLys	1.25% dLys	1.35% dLys	PC[2]
Corn	58.35	52.65	47.31	58.35
Soybean meal (48%)	33.39	38.18	42.67	33.39
Corn DDGS[3]	3.00	3.00	3.00	3.00
Soy oil	1.16	2.11	3.00	1.16
Dicalcium Phosphate	1.18	1.13	1.09	1.18
Calcium Carbonate	1.12	1.11	1.10	1.12
DL-Methionine	0.28	0.32	0.35	0.28
Vitamin Premix[4]	0.10	0.10	0.10	0.10
Mineral Premix[5]	0.10	0.10	0.10	0.10
NaCl	0.36	0.36	0.36	0.36
L-Lys·HCl	0.20	0.15	0.18	0.20
Choline	0.08	0.06	0.05	0.08
L-Threonine	0.10	0.10	0.11	0.10
TBCC[6]	0.02	0.02	0.02	0.02
Sand	0.05	0.05	0.05	---
Clinacox®[2]	---	---	---	0.05
Titanium Dioxide[7]	0.50	0.50	0.50	0.50
Phytase[8]	0.01	0.01	0.01	0.01
Xylanase[9]	0.01	0.01	0.01	0.01
Calculated Nutrient Content[10] (%, unless otherwise indicated)				
AME$_n$, kcal/kg	3,000	3,000	3,000	3,000
Crude Protein	21.21	23.05	24.78	21.21
Digestible Lys	1.15	1.25	1.35	1.15
Digestible Met	0.57	0.62	0.68	0.57
Digestible TSAA	0.86	0.93	1.00	0.86
Digestible Thr	0.78	0.84	0.91	0.78
Digestible Val	0.86	0.94	1.01	0.86
Digestible Ile	0.80	0.88	0.96	0.80
Digestible Arg	1.26	1.39	1.52	1.26
Digestible Trp	0.22	0.24	0.27	0.22
Ca	0.96	0.96	0.96	0.96
Non-phytate P	0.48	0.48	0.48	0.48
Na	0.20	0.20	0.20	0.20

[1] Dietary density formulated based from 90% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014), 98% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014) and 105% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014).

[2] Positive control diets contained Clinacox® (Huvepharma, Inc., Peachtree City, GA) provides 0.02% diclazuril to the diet. at 0.02% at the expense of sand.

[3] Corn Dried Distillers Grains with Solubles

[4] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[5] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.

[6] TBCC = tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN)

[7] Titanium Dioxide included as indigestible marker for digestibility analysis.

[8] Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[9] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per ton inclusion.

[10] Analyzed Nutrient Content (% Total): 1.15% dLys diet- 21.08% CP, 1.31% Lys, 0.55% Met, 0.89% TSAA, 0.85% Thr, 1.00% Val, 0.91% Ile, 1.32% Arg, 0.25% Trp; 1.25% dLys diet- 21.68% CP, 1.36% Lys, 0.60% Met, 0.94% TSAA, 0.88% Thr, 1.03% Val, 0.95% Ile, 1.36% Arg, 0.27% Trp; 1.35% dLys diet - 23.85% CP, 1.40% Lys, 0.64% Met, 0.99% TSAA, 0.91% Thr, 1.06% Val, 0.99% Ile, 1.42% Arg, 0.27% Trp; PC diet –21.53% CP, 1.33% Lys, 0.52% Met, 0.86% TSAA, 0.86% Thr, 1.02% Val, 0.94% Ile, 1.35% Arg, 0.25% Trp.

Table 4.2. Ingredient and nutrient composition of experimental starter diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52 from 10 to 19 d of age.[1]

Ingredient, % "as-fed"	1.15% dLys	1.25% dLys	PC[2]
Corn	58.35	52.65	58.35
Soybean meal (48%)	33.39	38.18	33.39
Corn DDGS[3]	3.00	3.00	3.00
Soy oil	1.16	2.11	1.16
Dicalcium Phosphate	1.18	1.13	1.18
Calcium Carbonate	1.12	1.11	1.12
DL-Methionine	0.28	0.32	0.28
Vitamin Premix[4]	0.10	0.10	0.10
Mineral Premix[5]	0.10	0.10	0.10
NaCl	0.36	0.36	0.36
L-Lys·HCl	0.20	0.15	0.20
Choline	0.08	0.06	0.08
L-Threonine	0.10	0.10	0.10
TBCC[6]	0.02	0.02	0.02
Sand	0.05	0.05	---
Clinacox®[2]	---	---	0.05
Phytase[7]	0.01	0.01	0.01
Xylanase[8]	0.01	0.01	0.01
Calculated Nutrient Content[9] (%, unless otherwise indicated)			
AME_n, kcal/kg	3,000	3,000	3,000
Crude Protein	21.21	23.05	21.21
Digestible Lys	1.15	1.25	1.15
Digestible Met	0.57	0.62	0.57
Digestible TSAA	0.86	0.93	0.86
Digestible Thr	0.78	0.84	0.78
Digestible Val	0.86	0.94	0.86
Digestible Ile	0.80	0.88	0.80
Digestible Arg	1.26	1.39	1.26
Digestible Trp	0.22	0.24	0.22
Ca	0.96	0.96	0.96
Non-phytate P	0.48	0.48	0.48
Na	0.20	0.20	0.20

[1] Dietary density formulated based from 90% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014) for moderate density diets and 98% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014) for high density diets.

[2] Positive control diets contained Clinacox® (Huvepharma, Inc., Peachtree City, GA) provides 0.02% diclazuril to the diet. at 0.02% at the expense of sand.

[3] Corn Dried Distillers Grains with Solubles

[4] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[5] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.

[6] TBCC = tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN)

[7] Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[8] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per ton inclusion.

[9] Analyzed Nutrient Content (% Total): 1.15% dLys diet- 21.60% CP, 1.36% Lys, 0.56% Met, 0.91% TSAA, 0.88% Thr, 1.04% Val, 0.95% Ile, 1.36% Arg, 0.27% Trp; 1.25% dLys diet- 23.57% CP, 1.49% Lys, 0.64% Met, 1.02% TSAA, 0.96% Thr, 1.13% Val, 1.04% Ile, 1.51% Arg, 0.29% Trp; PC diet- 21.66% CP, 1.39% Lys, 0.57% Met, 0.92% TSAA, 0.90% Thr, 1.05% Val, 0.96% Ile, 1.38% Arg, 0.27% Trp.

Table 4.3. Ingredient and nutrient composition of grower and finisher diets fed to Yield Plus × Ross 708 male broilers that were vaccinated with Coccivac® B52 from 20 to 40 d of age.[1]

Ingredient, % "as-fed"	Grower	Grower PC[2]	Finisher
Corn	63.47	63.47	66.87
Soybean meal (48%)	27.40	27.40	21.82
Corn DDGS[3]	5.00	5.00	7.00
Soy oil	0.87	0.87	1.47
Dicalcium Phosphate	0.94	0.94	0.70
Calcium Carbonate	1.07	1.07	1.01
DL-Methionine	0.24	0.24	0.21
Vitamin Premix[4]	0.08	0.08	0.05
Mineral Premix[5]	0.10	0.10	0.10
NaCl	0.35	0.35	0.34
L-Lys·HCl	0.21	0.21	0.22
Choline	0.09	0.09	0.09
L-Threonine	0.08	0.08	0.07
TBCC[6]	0.02	0.02	0.02
Sand	0.05	---	---
Clinacox®[2]	---	0.05	---
Phytase[7]	0.01	0.01	0.01
Xylanase[8]	0.01	0.01	0.01
Calculated Nutrient Content (%, unless otherwise indicated)			
AME_n, kcal/kg	3,100	3,100	3,185
Crude Protein	19.30	19.30	17.47
Digestible Lys	1.04	1.04	0.92
Digestible Met	0.51	0.51	0.47
Digestible TSAA	0.78	0.78	0.72
Digestible Thr	0.69	0.69	0.61
Digestible Val	0.78	0.78	0.70
Digestible Ile	0.71	0.71	0.63
Digestible Arg	1.11	1.11	0.96
Digestible Trp	0.19	0.19	0.17
Ca	0.87	0.87	0.78
Non-phytate P	0.44	0.44	0.39
Na	0.20	0.20	0.20

[1] Dietary density formulated based from 90% of Ross 708 amino acid Nutrition Specifications (Aviagen, 2014).

[2] Positive control diets contained Clinacox® (Huvepharma, Inc., Peachtree City, GA) provides 0.02% diclazuril to the diet, at 0.02% at the expense of sand.

[3] Corn Dried Distillers Grains with Solubles

[4] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-

pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[5] Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.

[6] TBCC = tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN)

[7] Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[8] Econase® XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per ton inclusion.

Table 4.4. Growth performance of Yield Plus × Ross 708 male broilers fed pre-starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 9 d of age that were vaccinated at hatch with Coccivac® B52[1]

Pre-Starter Dietary AA Density[2]	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
1.15% dLys	0.156	0.190	1.22^{a}	0.05
1.25% dLys	0.161	0.195	1.21^{a}	0.04
1.35% dLys	0.163	0.186	1.15^{b}	0.05
PC[3]	0.163	0.197	1.21^{a}	0.07
SEM[4]	0.003	0.004	0.019	0.029
Analysis of Variance	***Probabilities***			
Pre-Starter Dietary AA Density	0.37	0.11	0.002	0.87

[1] Each value represents the least-square means of 10 replicate pens with each pen having 30 chicks at placement.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM = pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.5. Growth performance of Yield Plus × Ross 708 male broilers fed pre-starter and starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 19 d of age that were vaccinated at hatch with Coccivac® B52[1]

	Dietary Treatments[2] Pre-Starter Dietary AA Density	Starter Dietary AA Density	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
1	1.15% dLys	1.15% dLys	0.717^{b}	0.896	1.25^{a}	0.08
2	1.25% dLys	1.15% dLys	0.719^{b}	0.894	1.24^{a}	0.06
3	1.25% dLys	1.25% dLys	0.764^{a}	0.918	1.20^{b}	0.04
4	1.35% dLys	1.15% dLys	0.741^{ab}	0.900	1.22^{ab}	0.04
5	1.35% dLys	1.25% dLys	0.757^{a}	0.899	1.19^{b}	0.07
6	PC[3]	PC[3]	0.741^{ab}	0.925	1.25^{a}	0.09
SEM[4]			0.008	0.009	0.009	0.030
Pre-Starter Dietary AA Density						
1.15% dLys			0.717^{b}	0.896	1.25^{a}	0.08
1.25% dLys			0.740^{a}	0.906	1.22^{a}	0.05
1.35% dLys			0.749^{a}	0.900	1.20^{b}	0.05
PC[3]			0.741^{a}	0.925	1.25^{a}	0.09
SEM[4]			0.009	0.009	0.010	0.029
Analysis of Variance			***Probabilities***			
Dietary Treatments			0.0002	0.09	0.0001	0.72
Pre-Starter Dietary AA Density			0.039	0.10	0.003	0.61
Treatment 1 vs. Treatment 6			0.029	0.027	0.89	0.85
Treatment 3 vs. Treatment 4			0.036	0.18	0.26	1.00
Treatment 3 vs. Treatment 5			0.49	0.15	0.32	0.39
Treatment 1 vs. Treatment 4			0.030	0.75	0.008	0.29
Treatment 1 vs. Treatment 5			0.0006	0.83	0.0001	0.85

[1] Each value represents the least-square means of 10 replicate pens with each pen having 30 chicks at placement.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM = pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey’s Honestly Significant Difference test.

Table 4.6. Growth performance of Yield Plus × Ross 708 male broilers fed pre-starter and starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 27 d of age that were vaccinated at hatch with Coccivac® B52[1]

Dietary Treatments[2]						
	Pre-Starter Dietary AA Density	Starter Dietary AA Density	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
1	1.15% dLys	1.15% dLys	1.429^{b}	1.949	1.37	0.10
2	1.25% dLys	1.15% dLys	1.440^{b}	1.961	1.36	0.09
3	1.25% dLys	1.25% dLys	1.515^{a}	2.029	1.34	0.04
4	1.35% dLys	1.15% dLys	1.467^{ab}	2.062	1.41	0.04
5	1.35% dLys	1.25% dLys	1.516^{a}	2.017	1.33	0.07
6	PC[3]	PC[3]	1.499^{a}	2.018	1.35	0.09
SEM[4]			0.014	0.036	0.003	0.032
Pre-Starter Dietary AA Density						
1.15% dLys			1.429^{b}	1.949	1.37	0.10
1.25% dLys			1.477^{ab}	1.995	1.35	0.06
1.35% dLys			1.491^{ab}	2.040	1.37	0.06
PC[3]			1.499^{a}	2.018	1.35	0.09
SEM[4]			0.016	0.036	0.027	0.032
Analysis of Variance				***Probabilities***		
Dietary Treatments			0.0001	0.24	0.34	0.69
Pre-Starter Dietary AA Density			0.009	0.23	0.86	0.68
Treatment 1 vs. Treatment 6			0.0004	0.18	0.62	0.83
Treatment 3 vs. Treatment 4			0.01	0.52	0.06	0.86
Treatment 3 vs. Treatment 5			0.96	0.81	0.80	0.43
Treatment 1 vs. Treatment 4			0.047	0.031	0.23	0.24
Treatment 1 vs. Treatment 5			0.0001	0.19	0.35	0.57

[1] Each value represents the least-square means of 10 replicate pens with each pen having 30 chicks at placement.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM = pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.7. Growth performance of Yield Plus × Ross 708 male broilers fed pre-starter and starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 40 d of age that were vaccinated at hatch with Coccivac® B52[1]

	Dietary Treatments[2]					
	Pre-Starter Dietary AA Density	Starter Dietary AA Density	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
1	1.15% dLys	1.15% dLys	2.915^{b}	4.572	1.57	0.16
2	1.25% dLys	1.15% dLys	2.972^{ab}	4.667	1.57	0.14
3	1.25% dLys	1.25% dLys	3.018^{a}	4.753	1.58	0.11
4	1.35% dLys	1.15% dLys	2.995^{ab}	4.746	1.59	0.09
5	1.35% dLys	1.25% dLys	3.052^{a}	4.710	1.54	0.13
6	PC[3]	PC[3]	3.008^{ab}	4.672	1.55	0.11
SEM[4]			0.024	0.053	0.019	0.037
Pre-Starter Dietary AA Density						
1.15% dLys			2.915^{b}	4.572	1.57	0.16
1.25% dLys			2.995^{a}	4.710	1.57	0.13
1.35% dLys			3.024^{a}	4.728	1.56	0.11
PC[3]			3.008^{a}	4.672	1.55	0.11
SEM[4]			0.025	0.053	0.020	0.036
Analysis of Variance				***Probabilities***		
Dietary Treatments			0.006	0.16	0.62	0.76
Pre-Starter Dietary AA Density			0.007	0.09	0.84	0.70
Treatment 1 vs. Treatment 6			0.009	0.18	0.55	0.35
Treatment 3 vs. Treatment 4			0.50	0.91	0.75	0.64
Treatment 3 vs. Treatment 5			0.32	0.56	0.20	0.67
Treatment 1 vs. Treatment 4			0.022	0.021	0.54	0.16
Treatment 1 vs. Treatment 5			0.0002	0.06	0.32	0.61

[1] Each value represents the least-square means of 10 replicate pens with each pen having 30 chicks at placement.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM = pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.8. Cumulative amino acid (**AA**) intake of Yield Plus × Ross 708 male broilers fed pre-starter and starter diets and formulated varying in digestible (**d**) AA density from 1 to 40 d of age that were vaccinated at hatch with Coccivac® B52[1]

Dietary Treatments[2]									
	Dietary AA Density	Starter Dietary AA Density	dTSAA Intake (g)	dLys Intake (g)	dThr Intake (g)	dVal Intake (g)	dIle Intake (g)	dArg Intake (g)	dTrp Intake (g)
1	1.15% dLys	1.15% dLys	34.8^{b}	45.4^{b}	30.3^{b}	34.3^{b}	31.2^{b}	48.1^{b}	8.4^{c}
2	1.25% dLys	1.15% dLys	35.6^{ab}	46.5^{ab}	31.0^{ab}	35.1^{ab}	31.9^{ab}	49.2^{ab}	8.6^{abc}
3	1.25% dLys	1.25% dLys	36.8^{a}	48.1^{a}	32.0^{a}	36.4^{a}	33.1^{a}	51.1^{a}	8.9^{a}
4	1.35% dLys	1.15% dLys	36.5^{ab}	47.6^{ab}	31.7^{ab}	35.9^{ab}	32.7^{ab}	50.5^{ab}	8.9^{abc}
5	1.35% dLys	1.25% dLys	36.6^{ab}	47.8^{ab}	31.8^{ab}	36.2^{ab}	32.9^{ab}	50.9^{a}	8.9^{ab}
6	PC[3]	PC[3]	34.9^{b}	45.5^{b}	30.3^{ab}	34.4^{b}	31.2^{b}	48.2^{b}	8.5^{b}
SEM[4]			0.51	0.66	0.44	0.50	0.46	0.70	0.12
Pre-Starter Dietary AA Density									
1.15% dLys			34.8^{b}	45.4^{b}	30.3^{b}	34.3^{b}	31.2^{b}	48.1^{c}	8.4^{b}
1.25% dLys			36.2^{ab}	47.3^{ab}	31.5^{ab}	35.7^{ab}	32.5^{ab}	50.2^{ab}	8.8^{ab}
1.35% dLys			36.5^{a}	47.7^{a}	31.8^{a}	36.0^{a}	32.8^{a}	50.7^{a}	8.9^{a}
PC[3]			34.9^{b}	45.5^{b}	30.3^{b}	34.4^{b}	31.2^{b}	48.2^{b}	8.5^{b}
SEM[4]			0.51	0.67	0.45	0.51	0.46	0.71	0.13
Analysis of Variance					***Probabilities***				
Dietary Treatments			0.005	0.007	0.007	0.004	0.003	0.002	0.003
Pre-Starter Dietary AA Density			0.004	0.005	0.005	0.003	0.003	0.002	0.002
Treatment 1 vs. Treatment 6			0.90	0.93	0.90	0.91	0.90	0.88	0.90
Treatment 3 vs. Treatment 4			0.56	0.58	0.64	0.51	0.50	0.49	0.64
Treatment 3 vs. Treatment 5			0.76	0.74	0.77	0.74	0.71	0.79	0.86
Treatment 1 vs. Treatment 4			0.013	0.015	0.013	0.013	0.011	0.010	0.009
Treatment 1 vs. Treatment 5			0.006	0.009	0.009	0.006	0.005	0.003	0.004

[1] Each value represents the least-square means from 10 replicate pens with each pen having 30 chicks at placement.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM=pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.9. Gross lesion scores of Yield Plus × Ross 708 male broilers at 19 d of age fed pre-starter and starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 19 d that were vaccinated at hatch with Coccivac® B52[1]

Dietary Treatments[2]										
	Dietary AA Density	Starter Dietary AA Density	Upper Intestine 0 %	Upper Intestine 1 %	Middle Intestine 0 %	Middle Intestine 1 %	Lower Intestine 0 %	Lower Intestine 1 %	Ceca 0 %	Ceca 1 %
1	1.15% dLys	1.15% dLys	65.00	25.00	82.50	17.50	95.00	5.00	100.00	0.00
2	1.25% dLys	1.15% dLys	60.00	27.50	75.00	22.50	95.00	5.00	92.50	7.50
3	1.25% dLys	1.25% dLys	55.83	33.33	74.17	23.33	95.00	5.00	90.00	10.00
4	1.35% dLys	1.15% dLys	62.50	25.00	90.00	10.00	100.00	0.00	95.00	5.00
5	1.35% dLys	1.25% dLys	55.00	35.00	87.50	12.50	100.00	0.00	95.00	5.00
6	PC[3]	PC[3]	90.00	7.50	92.50	7.50	100.00	0.00	97.50	2.50
SEM[4]			9.01	7.41	6.57	6.55	2.36	2.36	3.50	3.50
Pre-Starter Dietary AA Density										
1.15% dLys			65.00^{ab}	25.00^{ab}	82.50	17.50	95.00	5.00	100.00	0.00
1.25% dLys			57.92^{b}	30.42^{a}	74.58	22.92	95.00	5.00	91.25	8.75
1.35% dLys			58.75^{b}	30.00^{a}	88.75	11.25	100.00	0.00	95.00	5.00
PC[3]			90.00^{a}	7.50^{b}	92.50	7.50	100.00	0.00	97.50	2.50
SEM[4]			8.92	7.37	6.46	6.45	2.73	2.32	3.45	3.45
Analysis of Variance							***Probabilities***			
Dietary Treatments			0.07	0.09	0.22	0.40	0.23	0.23	0.42	0.44
Pre-Starter Dietary AA Density			0.017	0.031	0.07	0.17	0.07	0.07	0.17	0.18
Treatment 1 vs. Treatment 6			0.056	0.08	0.30	0.29	0.13	0.13	0.59	0.59
Treatment 3 vs. Treatment 4			0.59	0.76	0.11	0.13	0.13	0.13	0.39	0.43
Treatment 3 vs. Treatment 5			0.84	0.75	0.16	0.18	0.13	0.13	0.39	0.43
Treatment 1 vs. Treatment 4			0.81	0.66	0.47	0.49	0.13	0.13	0.28	0.27
Treatment 1 vs. Treatment 5			0.33	0.29	0.59	0.60	0.13	0.13	0.28	0.27

[1] Each value represents the least-square means of the percentage of scores from 10 replicate pens with each pen having 4 birds

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scored at 19 d of age.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM=pooled standard error

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey’s Honestly Significant Difference test.

Table 4.10. Carcass and yield characteristics of Yield Plus × Ross 708 male broilers fed pre-starter and starter diets formulated varying in digestible (**d**) amino acid (**AA**) density from 1 to 40 d of age that vaccinated at hatch with Coccivac® B52[1]

Dietary Treatments[2]			Carcass		Abdominal Fat		Fillets		Tenders		Total Breast	
	Dietary AA Density	Starter Dietary AA Density	Wt (g)	Yield (%)	Wt (g)	Yield (%)	Wt (g)	Yield (%)	Wt (g)	Yield (%)	Wt (g)	Yield (%)
1	1.15% dLys	1.15% dLys	2,215^{b}	74.29	28	0.93	670^{b}	22.45	128^{b}	4.28	797^{b}	26.74
2	1.25% dLys	1.15% dLys	2,237^{ab}	74.24	27	0.90	677^{ab}	22.49	130^{b}	4.36	808^{b}	27.04
3	1.25% dLys	1.25% dLys	2,286^{ab}	73.45	27	0.86	705^{a}	22.73	133^{ab}	4.30	838^{a}	26.81
4	1.35% dLys	1.15% dLys	2,247^{ab}	73.66	26	0.85	693^{ab}	22.73	132^{ab}	4.33	825^{a}	27.06
5	1.35% dLys	1.25% dLys	2,314^{a}	74.04	28	0.90	710^{a}	22.71	138^{a}	4.41	848^{a}	27.12
6	PC[3]	PC[3]	2,271^{ab}	74.09	27	0.88	696^{ab}	22.72	133^{ab}	4.33	829^{a}	27.05
SEM[4]			20	0.34	1	0.03	8	0.20	2	0.04	9	0.21
Pre-Starter Dietary AA Density												
1.15% dLys			2,215	74.29	28	0.94	670^{b}	22.47	128^{b}	5.76	797^{b}	26.74
1.25% dLys			2,262	74.00	27	0.88	691^{ab}	22.62	132^{ab}	5.83	823^{a}	26.93
1.35% dLys			2,280	73.85	27	0.88	702^{a}	22.72	135^{a}	5.93	837^{a}	27.09
PC[3]			2,271	74.09	27	0.88	696^{ab}	22.72	133^{ab}	4.33	829^{a}	27.05
SEM[4]			21	0.33	1	0.03	8	0.23	2	0.05	9	0.21
Analysis of Variance							***Probabilities***					
Dietary Treatments			0.008	0.50	0.42	0.18	0.006	0.80	0.002	0.28	0.003	0.72
Pre-Starter Dietary AA Density			0.08	0.61	0.64	0.18	0.032	0.33	0.008	0.07	0.016	0.53
Treatment 1 vs. Treatment 6			0.04	0.61	0.35	0.10	0.025	0.35	0.028	0.39	0.017	0.29
Treatment 3 vs. Treatment 4			0.14	0.81	0.61	0.91	0.30	0.96	0.63	0.57	0.31	0.95
Treatment 3 vs. Treatment 5			0.31	0.47	0.21	0.25	0.69	0.89	0.043	0.047	0.47	0.78
Treatment 1 vs. Treatment 4			0.24	0.11	0.08	0.019	0.054	0.33	0.052	0.39	0.034	0.28
Treatment 1 vs. Treatment 5			0.006	0.50	1.00	0.001	0.001	0.37	0.001	0.024	0.003	0.20

[1] Each value represents the least-square means of 10 replicate pens with each pen providing 14 carcasses at 40 d of age.

[2] Diets were formulated on a digestible amino acid ratio basis.

[3] PC = Positive control diets supplemented with Clinacox® formulated at 1.15% dLys AA density fed to non-vaccinated birds.

[4] SEM = pooled standard error of the mean

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

V. CONCLUSIONS

Experiment 1 evaluated the effects of increasing the dietary amino acid density and feed allocation of diets fed during the starter period to broilers vaccinated against coccidiosis on cumulative growth performance and meat yield during a 6 wk production period. Broilers fed the 1.25% dig Lys density diet consumed 5.4 g more dig AA, which translated to a 38 g increase in total breast meat weight compared with those given the 1.15% dig Lys density diet. Broilers provided the 1.25% dig Lys density diet had a lower percentage of lesion scores of 1 in the upper intestinal region. Additionally, it was observed that vaccinated broilers given the 1.15% dig Lys density diet had decreased BW gain compared with their non-vaccinated counterparts during the 1 to 28 d period. Cumulative feed conversion ratio and carcass weights were enhanced due to increasing the starter feed allotment by 6.5 points and 98 g, respectively. These findings indicated that broilers fed the 1.25% dig Lys density diet at the larger starter feed allotments had improved growth performance and meat yield responses.

Experiment 2 examined the effects of vaccinated broilers provided diets varying in AA density from 1 to 19 d of age on growth performance and meat yield responses during a 6 wk production period. Broilers that received the 1.35% dig Lys density pre-starter diet, regardless of their starter diet dig AA density, had increased cumulative BW gain and total breast meat weight compared with the broilers given the 1.15% dig Lys density diet during both the pre-starter and starter periods. Broilers provided TRT 5

(1.35% dLys PS | 1.25% dLys S) consumed 12.6 g dig AA more than broilers fed TRT 1 (1.15% dLys PS | 1.15% dLys S), which resulted in 51 g increase in total breast weight over TRT 1. In contrast, the 1.35% dig Lys density pre-starter diet did not improve cumulative growth performance or total breast weight beyond that of broilers given the 1.25% dig Lys density diet during both the pre-starter and starter periods. As in the previous experiment, the non-vaccinated broilers had increased cumulative growth performance and muscle accretion compared with the vaccinated broilers.

The feed allocation response in experiment 1 was only significant in the cumulative growth performance responses of broilers. The cumulative growth performance responses were likely due to compensatory feed intake responses of broilers given the 0.45 kg/bird allotment that were switched to the grower diet earlier than the other feed allotments. Bird and feed weights for the starter period were only collected on d 21. If bird weights had been measured on the d that the grower diet was placed for each allotment, a feed intake response might have been detected from 1 to 21 d of age. There was also a large difference in total breast weights between the starter feed allocations that was nearly significant. The lack of significance was likely due to the larger standard error of the mean associated with the starter feed allocation main effect than the dietary AA density main effect. There is not a readily available explanation for the variability.

During the vaccine-associated infection, AA are partitioned between protein synthesis and intestinal recovery. Increasing AA intake before the initiation of the vaccine-associated infection may minimize the impact of the infection on growth performance. In experiment 2, the similarity in performance and meat yields between the 1.25% and 1.35% dig Lys density pre-starter treatments may be due to the smaller actual difference between the dietary AA concentrations. If the calculated differences in AA concentrations were achieved in the actual 1.25% and 1.35% dig Lys density pre-starter diets, the responses could have been different. These data indicated that increasing the AA intake allowed broilers to maintain adequate growth while recovering from the effects of the coccidiosis vaccination. It would be beneficial to compare growth performance and meat yield responses of vaccinated and non-vaccinated broilers fed the increased dig AA density pre-starter diets. The similarity in BW gain and breast meat yield of broilers provided the 1.25% and 1.35% dig Lys density pre-starter diets compared with the 1.15% dig Lys density positive control treatment implies that the growth potential of the broilers was limited by the vaccination. Similarly, the vaccine utilized in the current research was notably mild, and vaccination with a heavier dosage or through oral gavage might elicit a stronger infection, which the dietary treatments may not have ameliorated.

Achieving adequate dig AA intake utilized in these experiments could also be accomplished through the inclusion of highly digestible ingredients while potentially formulating to lower dig AA concentration. Previous research including highly digestible ingredients in pre-starter diets have reported improved growth performance related to the ability of the young chick to utilize more of the nutrients provided in the diet before the gastrointestinal tract matures (Barekatain and Swick, 2016). Additionally, other substrates involved in protein synthesis, such as nucleic acids could have been included in the diet to improve growth performance and intestinal recovery. Nucleic acids have been considered as a pre-starter supplement due to their ability to be absorbed intact by multiplying cells sparing their synthesis, especially important in chicks developing their gastrointestinal tract and undergoing an infectious challenge.

Manipulation of the starter period duration by using both pre-starter diets and increased starter feed allotments may further enhance broiler growth performance and meat yield. The use of increased dig AA density diets during the starter period supported increased growth rates of vaccinated broilers, which may predispose broilers with accelerated growth rates early in development to metabolic syndromes. Experiment 2 did evaluate the incidence of wooden breast and white striping, but no differences were observed between treatments. The incidence of muscle myopathies increase with broiler age warrant further research into the use of increase dig AA density diets being fed during the first 3 weeks of life. An additional concern in broilers provided increased dig AA density diets is necrotic enteritis. Necrotic enteritis is a bacterial disease caused by the over proliferation of *Clostridium perfringens* from the lower intestinal tract and is the second most costly disease of poultry. This disease can be observed in broilers vaccinated against coccidiosis because *Clostridia* is an opportunistic pathogen. The current research utilizes 2 predisposing factors for necrotic enteritis: coccidiosis vaccination and high concentrations of dietary AA. Additional research is warranted to ensure that feeding increased dig AA density starter diets does not result in a higher incidence of necrotic enteritis when feeding high AA density pre-starter diets.