THE EFFECT OF GELATIN AND DIETARY CRUDE PROTEIN LEVEL ON

BROILERS VACCINATED FOR COCCIDIOSIS

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THE EFFECT OF GELATIN AND DIETARY CRUDE PROTEIN LEVEL ON BROILERS VACCINATED FOR COCCIDIOSIS

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VITA

Regina N. Lehman, daughter of Carl and Mabel Lehman, was born on July 6, 1984 in Mechanicsburg, Pennsylvania. She graduated from Cumberland Valley High School in June 2002. She then attended The Pennsylvania State University where she received a Bachelor of Science degree with a major in Animal Sciences and minors in Spanish and Poultry and Avian Sciences. She graduated in May 2006 and entered into Graduate School at Auburn University in August 2006 under the direction of Dr. Edwin T. Moran, Jr.

THESIS ABSTRACT

THE EFFECT OF GELATIN AND DIETARY CRUDE PROTEIN LEVEL ON BROILERS VACCINATED FOR COCCIDIOSIS

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Vaccinating for coccidiosis has been shown to adversely affect live performance of broilers compared to those given a dietary anticoccidial. Feeding diets with higher crude protein alleviates negative response to vaccination, presumably by providing the bird with the amino acids essential for recovery. Alternate means of providing nonessential amino acids for vaccination recovery have yet to be explored.

Experiments were conducted to evaluate whether gelatin, as a source of proline and glycine, aided in recovery of vaccinated birds compared to coccidiostat-protected birds fed low and high crude protein diets; and to estimate a crude protein level where use of gelatin is optimized in diets of vaccinated broilers.

In the first experiment, male and female broiler chicks were fed either a low or high crude protein (CP) diet from 0-8 weeks of age with limiting EAA levels meeting or

exceeding NRC (1994) recommendations. Gelatin was included in half of the diets at 2% to increase proline and glycine. Half of the birds were vaccinated and the rest were protected with a dietary coccidiostat. Vaccination adversely affected performance during the first 3 weeks. Addition of gelatin reduced the early negative response of vaccinated birds, but by 8 weeks, vaccinated birds still had reduced gain and carcass weight compared to coccidiostat-protected birds. Addition of gelatin to low CP diets improved feed conversion to that of birds fed the high CP diets; however carcass yield of low CP diets was not improved with addition of gelatin.

The second experiment was performed to estimate the optimal levels of crude protein and NEAA of vaccinated male broilers. Diets with graded levels of crude protein: 20, 21, 22, or 23% from 0-3 weeks; 19, 20, 21, or 22% from 3-6 weeks; and 18, 19, 20, or 21% from 6-8 weeks, respectively, were administered from 0-8 weeks of age, half of which contained 2% gelatin to increase proline and glycine. Increasing CP improved body weight gain while gelatin inclusion generally improved feed conversion. Carcass yield was increased and abdominal fat decreased with CP, but gelatin resulted in greater abdominal fat without affecting meat yield of the carcass. Additional NEAA, provided by gelatin and CP, generally improve performance of vaccinated birds, although specific estimates could not be determined.

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I. INTRODUCTION

Coccidiosis is the most economically important disease of poultry. Current management involves the use of dietary anticoccidials; however, due to recent concerns about bacteria developing antibiotic resistance, vaccination as an alternative to coccidiostats is being explored. Though effective at producing immunity, vaccination with live oocysts has led to decreased performance that may not be fully recovered by the time the birds are marketed. Attempts to alleviate the negative effects and accelerate recovery of vaccinated birds have successfully included increasing crude protein (CP) in the diet.

The following literature review is intended to provide a description of coccidiosis and how vaccination and infection affect the chick intestine. It is important to comprehend the mechanism of infection to understand how best to improve recovery through dietary alterations.

II. LITERATURE REVIEW

2.1 Coccidiosis

Coccidiosis is one of the most commonly reported and costly diseases of commercial poultry, costing the world's industry more than $1.5 billion annually for anticoccidial medications, treatment of disease if medication fails, and losses due to poor performance and mortality (Williams, 1998, 1999; Dalloul and Lillehoj, 2005). By adversely affecting the performance of poultry, coccidiosis greatly increases the cost of production.

Coccidiosis is caused by intracellular protozoan parasites of the genus *Eimeria.* There are seven species of *Eimeria* that are known to affect chickens: *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox,* and *Eimeria tenella.* Each species is host specific, causes infection in different areas of the intestine, and causes varying degrees of damage. For example, *E. acervulina* infects the duodenum, *E. necatrix* infects the jejunum, and *E. brunetti* infects the ileum, cecum, and colon. The loss of production associated with *Eimeria* infections is a result of the intestinal damage that is caused by the parasites.

SIGNS & LESIONS

Birds that are infected with coccidiosis become pale, anemic, depressed, huddled, and may consume less feed and water. A drop in egg production may be seen along with watery and/or bloody droppings. Cecal coccidiosis tends to produce more mortality than the intestinal version, which is chronic in nature. Postmortem lesions of the infected bird depend on the species of coccidia, the severity of infection, and the stage of the disease. The infected intestine may contain necrotic and/or hemorrhagic foci, undigested food and gas, and be enlarged with ballooning. In its later stages, cecal coccidiosis may produce a plug of clotted blood and necrotic mucosa. These differences suggest that mechanisms involved in cecal and intestinal coccidiosis may be different (Gabriel et al., 2003; McDougald, 2003).

TRANSMISSION AND INFECTION

Transmission of coccidiosis is caused by consumption of sporulated oocysts present in the fecal material. Oocysts are zygotes that are shed in the feces after infection and can be spread easily by contaminated clothing, wild birds, equipment, insects and rodents. In order to infect another bird, they must undergo sporulation, or sporogony, outside of the host, which requires moisture and oxygen and normally takes around 48 hours. After sporulation, oocysts must be ingested and excyst in the intestine, which is less efficient in very young chicks compared to older birds. Sporulated oocysts contain four sporocysts, each containing two sporozoites. Once ingested, the gizzard crushes the oocysts, and the enzymes of the small intestine release the sporozoites, which later attach

to the epithelial cells of the gut surface. The sporozoites undergo two rounds of asexual reproduction, or schizogony, by binary fission that result in the release of many schizonts. These are later released as merozoites that cause cell rupture and tissue damage that maximizes by day 6 of the infection (Turk, 1972). A round of sexual reproduction, gametogony, follows where the zygote matures into an oocyst that is released from the intestinal mucosa and shed in the feces. Shed oocysts may live in poultry litter for a period of up to three weeks if not ingested by another chicken. Oocysts that pass through the host without completing excystation and infection have been shown to potentially infect other birds later if ingested (Chapman et al., 2002; Williams, 1995). The process from oocyst ingestion to shedding takes an average of four to seven days, after which the bird begins to develop immunity to coccidiosis. However, stress caused by overcrowding, overheating, disturbance, beak trimming, feed restriction, and dietary deficiencies may limit the immune response to infection. After infection, a flock may develop resistance to one particular species, but may still be infected by any of the other species. As birds achieve immunity, oocyst excretion decreases until immunity begins to wane and oocyst recycling increases. Host immunity to *Eimeria* can be compromised by other viruses such as infectious bursal disease, chick anemia and Marek's disease, so immunization against these viruses is advisable (Chapman et al, 2002; Williams, 1998).

FACTORS THAT INFLUENCE REPRODUCTION OF *EIMERIA*

Two things that are crucial to the sporulation of oocysts are moisture and oxygen, but there are many other factors that can influence the successful reproduction of *Eimeria*

in poultry houses. Initially, high numbers of oocysts present in the litter before placement can result in a larger accumulation of oocysts at a later period. Built-up feces from high chick density and properly disbursed birds give oocysts an environment in which they can thrive. However, too great a chick density can cause high ammonia and decreased oxygen levels that negatively affect the chick as well as the survival of the oocyst (Williams, 1998). Though moisture is needed for sporulation, even in dry litter conditions, the high moisture content of the droppings is sufficient for oocyst survival (Chapman et al., 2002). Wet litter caused by chick overcrowding can reduce oocyst survival (Graat et al., 1994; Williams, 1998), while temperature and relative humidity seem to have no influence on sporulation (Graat et al., 1994).

Although seemingly beneficial, high fecundity of parasites results in crowding and lowers reproductive potential of *Eimeria.* A high number of parasites in the intestine limits access to epithelial cells, which are needed for development. Because of this need, attenuated vaccines may develop better in the gut because they cause minimal cell damage compared to non-attenuated vaccines (Williams, 2001). In addition, cell-mediated immunity may contribute to the crowding effect. Interactions between species have been shown to lower reproductive potential as well. Finally, a higher immunogenicity of the bird leads to lower oocyst numbers while a greater susceptibility increases the amount of oocysts. (Chapman et al., 2002).

EFFECT ON DIGESTION

As mentioned earlier, the loss of production associated with coccidiosis is great, making it the most costly disease of the poultry industry. *Eimeria* causes tissue damage that interferes with digestion and absorption of nutrients. It can also cause dehydration, blood loss and increased susceptibility to other diseases (Idris et al., 1997). The specific nutrient, area of the intestine attacked by parasites, and stage of infection all have an impact on the amount of nutrient absorbed from an infected intestine (Turk, 1972).

Coccidial infections are known to damage intestinal mucosa, increase passage rates which affect amino acid absorption, and increase sloughing of mucosa and blood cells (Apajalahti, 2004); however, the most probable reason for poor performance during infection is shortened intestinal villi. Studies done on the deleterious effects of coccidiosis have shown that *E. maxima* shortened the villi heights in the duodenum in infected birds by 20 to 70% below the controls. Villi heights of the jejunum were shortened to 50%. This shortening of the villi, caused by atrophy and mucosal sloughing, greatly reduced the surface area for nutrient digestion and absorption as was reflected by lower growth rates of infected chicks (Idris et al., 1997). It has been demonstrated that some compensatory mucosal hyperplasia of the small intestine may be seen as a response to infection (Williamson and Malt, 1980), however for the most part, high doses of *E. mitis, E. praecox,* and *E. acervulina* adversely affect weight gain and feed conversion ratio (FCR) (Williams, 1998). In addition to intestinal damage, *E. maxima* infection may cause a change in the microbial communities in the gut and the pattern of fermentation in the ileum and ceca (Apajalahti et al., 2004). Intestinal digesta viscosity is also lower in

Eimeria-inoculated birds than in non-inoculated birds (Waldenstedt et al., 2000), which may be caused by osmotic and absorptive changes in the intestinal tract (Crompton, 1976).

Protein is a specific nutrient whose digestion and absorption are affected by coccidiosis infection. Normal digestion of protein is due to many proteolytic enzymes: pepsin is located in the stomach; trypsin, chymotrypsin, elastase I and II, and carboxypeptidase A and B are located in the pancreatic juice in the lumen of the upper small intestine; and peptidases are located in the brush boarder membrane of mucosal epithelial cells in the small intestine. Final digestion of peptides to single amino acids occurs at the brush boarder membrane and in the cytoplasma (Reutersward, 1984).

Protein digestion and absorption in the three areas of the chick intestine are affected differently by the various species of *Eimeria.* Parker et al. (2007) has found that chickens infected with mixed coccidia have reduced ileal digestibility of amino acids. A possible reason for the reduced amino acid digestibility may be decreased absorption during infection (Preston-Mafham and Sykes, 1970; Ruff and Wilkins, 1980). *E. necatrix* infections, which occur in the jejunum, decrease protein digestion and absorption during the acute phase of infection, suggesting that the jejunum is critical for these functions. It is more likely that protein absorption is affected rather than digestion because most of the proteolytic enzymes are secreted into the duodenum, where initial digestion occurs. Protein absorption was not found to be affected by *E. acervulina* or *E. brunetti* (Turk, 1972).

MANAGEMENT

Currently, coccidiosis is managed with dietary anticoccidials or coccidiostat medication. However, with the recent emergence of multiple antibiotic resistant bacteria, several countries have become wary of antimicrobial use in poultry feed. To combat the incidence of glycopeptide-resistant Enterococci (GRE) and other bacteria, Norway and Denmark were the first countries to ban the use of Avoparcin as a growth promoter. Germany followed suit the next year along with all European Union countries in 1997 (M. Sørum, 2006). Other antibiotics have since been banned in these countries. The food animal industries in Denmark have voluntarily halted the use of all antimicrobial agents for growth promotion, and the European Union requires application for approval at fixed intervals to use growth promoters and other microbial feed additives (Frank Moller Aarestrup, 2001). Unlike the EU and other countries, the United States continues to allow the use of antimicrobials, including coccidiostat medication, in poultry feed. However, with pressure from concerned citizens and organizations, producers may be forced to employ other alternatives.

2.2 Vaccinating for Coccidiosis

Chemotherapy, between-flock sanitation, selective breeding for immune lines and vaccination are alternatives to current coccidiostat medication; however, vaccination is most likely the only practical method (Williams, 1998). Live vaccines enhance the natural immunity of the chick through exposure to low numbers of oocysts (Chapman et al., 2002; Williams, 1998) and eliminate the need for in-feed medication. A live vaccine,

Coccivac®, has been available in the US since 1952 (Edgar and King, 1952). The other available virulent vaccine is Immucox®, and the two attenuated vaccines are Paracox® and Livacox®. Many of the live vaccines include three or more species of *Eimeria*, such as *E. acervulina, E. maxima, and E. tenella* (Lillehoj and Lillehoj, 2000, Dalloul and Lillehol, 2005). Because of various problems which will be discussed later, vaccines have not been used much for broilers, but rather have been reserved for use in broiler breeders and layers (Chapman et al., 2002). Current developments in administration techniques at the hatchery, and the development of attenuated vaccines have made it more practical to vaccinate broilers, which help boost immunity to *Eimeria* after subsequent exposure to infection. (Chapman et al., 2002).

VACCINE PRODUCTION

There are currently three methods available to produce a live vaccine for coccidiosis. The first method is by passing coccidia through an embryonated chicken egg. This method has drawbacks, as only a few species may be passed through eggs. Those that can have a loss of immunogenicity which may not produce a stable attenuation (Shirley, 1993). Gamma irradiation of coccidia is another method which is unlikely to lead to a commercial vaccine due to the instability of the final product. The final method of attenuation is by selection for precocity. These selected lines of *Eimeria* have a reduced prepatent time, an attenuation of virulence from reduced reproductive potential, maintained immunogenicity, and genetically controlled stability (Williams, 1998).

VACCINE ADMINISTRATION

There are many different methods to administer vaccines. One method of vaccination is the use of a colored edible gel provided to the chicks at one day of age either at the hatchery or the poultry house (Chapman et al., 2002). As the chicks eat the gel, they also ingest oocysts that provide them with protection from severe infection. Another way to vaccinate is through the drinking water, but this method has proved less than satisfactory. Spraying vaccines directly on the feed results in a more uniform exposure to oocysts than the drinking water application (Chapman et al., 2002). Oral inoculation of each bird guarantees uniform exposure but is impractical in commercial operations because of time involvement and high labor costs. Another way to vaccinate is by spraying an oocyst suspension into the eye. The oocysts are believed to travel through the lacrimal duct to the nasal cavity and finally reach the intestinal tract through the oropharynx (Chapman, 2001). This method is also not frequently used due to impracticality. Vaccination by spray-cabinet, where the vaccine in an aqueous suspension is sprayed directly on the birds, is becoming more common. To ensure uniform inoculation, the vaccine contains a dye that identifies which birds had contact with the spray. During normal preening, the chicks ingest the oocysts and become infected. A final method of vaccination involves inoculating an embryonated egg. This method, although still under development, is thought to work by introduction of oocysts into the amniotic fluid of 18-day-old chick embryos, which then move to the gizzard and gut within 72 hours (Weber et al., 2001). After hatching, the oocysts are shed and capable of excysting to actively infect the chick. As mentioned earlier, excystation in very young

chicks is not as efficient as in older birds, but this is not known to affect the efficiency of day-old vaccination (Rose, 1967). Because this technology, known as Inovoject® is currently used for Marek's disease vaccination, successful *Eimeria* vaccination via this method would help save time and reduce vaccination costs.

DRAWBACKS TO VACCINATION

Despite the consumer acceptability of vaccinating for coccidiosis, there are disadvantages that make this option less than appealing for producers. One drawback is that vaccination has been shown to lead to further enteritis (Chapman et al., 2002; Wages and Kenneth, 2003; Williams, 2005). In addition, weight gain and feed conversion of vaccinated broilers is a concern for producers using this method of control rather than anticoccidial medication (Waldenstedt et al., 1999). Birds given a non-attenuated vaccine via spray-cabinet had poorer weight gain and feed conversion at 21 days of age as compared to birds given an anticoccidial medication (Mathis, 1999). Williams (1998) found that this decrease in weight gain due to virulent vaccines was so severe it could not be compensated for prior to slaughter. On the other hand, Mathis (1999) showed that the poor weight gain and feed conversion seen in the first three weeks of vaccinated birds is reversed between 28 and 42 days of age, suggesting that compensatory growth may be responsible for the reverse in performance. Despite this finding, with coccidiosis already being the most costly disease, producers are reluctant to use vaccines without seeing a definite advantage.

VACCINATION AND DIETARY CRUDE PROTEIN

Variable levels of crude protein (CP) have been found to affect the response of coccidiosis-vaccinated broilers (Sharma et al., 1973; Richter and Wiesner, 1988). Parker et al. (2007) discovered that broilers fed a high CP level (23%) and vaccinated for coccidiosis had better BWG and FCR when compared to birds that received feeds with lower CP. In addition, an initial low-CP diet leads to a higher incidence of infection and oocyst shedding in coccidiosis-vaccinated birds.

High protein diets may provide an advantage to vaccinated birds, but lower protein diets are frequently used regardless of benefits that may be realized with a high CP diet. Accessibility of synthetic amino acids has made formulation for lower CP diets common as the birds' need for amino acids is met through supplemental amino acids rather than crude protein. Additionally, birds given low protein diets supplemented with crystalline amino acids utilize more protein and have reduced nitrogen excretion, which minimizes pollution of soil and water (DeSchepper and DeGroote, 1995; Ferguson et al., 1998; Bregendahl et al., 2002; Si et al., 2004a,b; Sohail et al., 2003; Bregendahl et al., 2002). In broilers, when dietary CP is reduced by 2.5%, there is a subsequent reduction in N excretion of 21% (Jacob et al., 1994).

Another benefit of low CP diets is improved air quality in the poultry house. Control of NH_3 in poultry houses has been attempted by changing the diet, ventilation and litter management, and adding chemicals to the litter. Adding chemicals to the litter either to neutralize NH_3 or reduce microbial fermentation adds an additional cost. Ventilation is also associated with a higher energy cost, especially in winter (Gates et al.,

1996; Hartung and Phillips, 1994; Xin et al., 1996). The seemingly cheapest method of controlling NH_3 production would be to limit the amount of dietary CP by using synthetic amino acids or by reducing CP in diets as the birds age (Ferguson et al., 1998). Lowering the crude protein will lead to a drier litter with increased acidity that may inhibit the bacteria that hydrolyze uric acid and urea to NH_3 and is correlated with decreased NH_3 emissions. Reducing CP can cause equilibrium NH_3 gas concentration and litter N to decrease by 31 and 16.5%, respectively (Ferguson et al., 1998). This improves air quality and may reduce heating costs in winter associated with increased ventilation.

2.3 The Chick Intestine

Understanding the intestinal structure of the chick is important to be able to comprehend how dietary crude protein level may influence chick recovery from coccidiosis vaccination. The intestinal tract of chickens is covered with projections known as villi that increase the surface area for nutrient digestion and absorption. Enterocytes and goblet cells cover the surface of the villi. On the surface of the enterocytes are smaller microvilli that also aid in digestion. Small filaments, called membrane-associated mucin filaments, are attached to the apical surface of microvilli and compose the glycocalyx layer, which is involved in final digestion of proteins and sugars.

Mucus, which is a blend of secretions and exfoliated epithelial cells, helps to lubricate the intestinal surface. The main component of mucus is high molecular weight glycoproteins, called mucins (Forstner and Forstner, 1994). The mucins form a crucial barrier between the intestinal mucosa and the lumen. In addition to being a barrier to

nutrients, drugs, ions, toxins, and macromolecules, mucins also lubricate the epithelial surfaces; bind bacteria, viruses, and parasites; bind heavy metals for detoxification; protect mucosa against proteases; interact with the immune surveillance system, and interact with microfilaments, like actin (Forstner and Forstner, 1994).

The secretory mucin that protects and lubricates the mucosal surface of the small intestine is produced by the goblet cells. Goblet cells originate in the intestinal crypts from pluripotential stem cells or from poorly differentiated cells known as oligomucous cells (Cheng and Leblond, 1974; Cheng, 1974). They take approximately three days to ascend the villi. As they ascend, they become specialized and develop an array of microtubules and intermediate filaments called the "theca" (Specian and Neutra, 1984). The granular zone of the goblet cells stores mucin granules separate from the rest of the cytoplasma and allows for continual production and secretion. The outer limits of the zone are defined by the theca that gives goblet cells a characteristic shape (Forstner and Forstner, 1994).Similar to enterocytes, goblet cells possess apical microvilli, but fewer numbers.

STRUCTURE OF MUCINS

Mucins contain a core peptide that is 1,500 to over 4,500 amino acids in length. Each core peptide contains a major domain and a minor domain. The major domain is densely glycosylated, making it resistant to proteases, and contains plenty of serine, threonine, and proline. The minor portion is weakly or non-glycosylated, susceptible to proteases, and cysteine rich (Neutra and Forstner, 1987). Attached to the serine or

threonine of the core peptides are hundreds of O-linked oligosaccharide branches, which provide 50 to 80% of the dry weight of the molecule (Forstner and Forstner, 1994). The filamentous mucin is composed of about 75% carbohydrates which help to maintain structure and rigidity while secretory mucins only contain about 50% carbohydrates.

EXPULSION OF MUCINS

Mucins are packaged in the secretory granules of goblet cells and are expelled through holes in the plasma membrane via exocytosis, which may be accelerated by a response known as compound exocytosis. There is a continual baseline secretion of mucin that replenishes the mucus coating on the surface of the enterocytes. When cells are stimulated, all of the stored mucin can be released for immediate use; however, not all cells respond uniformly to stimuli, resulting in varying degrees of emptying. After exocytosis, it takes about 60 to 120 minutes for the goblet cell to completely refill (Specian and Oliver, 1991). Once the mucins reach the epithelial surface, they lose the cationic charge shielding they originally had, become hydrated, expand, and transform into a gel-like material (Neutra and Forstner, 1987; Verdugo, 1990). This mucin becomes entangled in the glycocalyx later to create the unstirred water layer.

MUCIN ROLE IN INFECTION

Mucins may play an important role in intestinal infection and disease recovery. The mucus layer in the intestine has been shown to serve as a place for pathogenic organism colonization (Rozee et al., 1982). Mucins on the cell surface are considered

hydrophilic, but may utilize a hydrophobic surface for bacterial interactions (Forstner and Forstner, 1994), especially for the binding of gram-positive organisms (Evans and Evans, 1990). Several pathogens attach to mucin fibers and use that to "track" toward the epithelium in order to colonize (Conway et al., 1990; Drumm et al., 1988). The process of binding is influenced by mucin composition, quality and quantity (Tse and Chadee, 1991), gut motility and luminal fluid flow (Conway et al., 1990).

On the contrary, mucins may aid in parasite defense and disease recovery. As a defense, the binding sites on mucins compete with microorganisms for receptors on epithelial cells reducing pathogen access to the mucosal layer (Tse and Chadee, 1991). As a means to recovery, studies have shown that when the intestinal tract is infected, the number of goblet cells increases (Miller et al., 1983). The hyperplasia is T-cell dependent and when expulsion occurs, a great amount of mucus and exfoliated cells are released into the lumen of the intestine (Miller and Narva, 1979). The subsequent increase in mucin production may be to flush out the pathogens and protect the epithelial cells from further parasitism.

ADVANTAGES OF NEAA

After a coccidiosis infection, birds increase secretory mucin production and need to regenerate the mucin filaments on the surface of the damaged enterocytes. Mucin is composed of a high percentage of nonessential amino acids, and although the bird is able to synthesize these, *de novo* synthesis may not be rapid enough to satisfy high demands after infection or vaccination. Poultry require a specific quantity and balance of dietary

essential amino acids and nitrogen that can be used to synthesize nonessential amino acids (Aftab et al., 2006), but providing the bird directly with nonessential amino acids may hasten the recovery process.

In the past, addition of nonessential amino acids to broiler feed has helped to improve performance (Parr and Summers, 1991; Aftab et al., 2006). Although it is unclear precisely which amino acids provide the benefit, proline and glycine are two amino acids that are extremely important to the growing chick. When amino acids were deleted from a crystalline amino acid diet, only proline and glycine-serine were synthesized *in vivo* at a significant rate to maintain chick performance (Baker et al., 1968; Graber and Baker, 1973). However, the synthesis of these two amino acids may prove to be difficult for the bird under stressful conditions. Glycine is readily synthesized by the chick, but this synthesis may not be rapid enough to satisfy the needs for fast tissue growth (Jiang et al., 2001; Corzo et al., 2004; Dean et al., 2006), especially after tissue damage caused by infection. In a study by Baker and Sugahara (1970), when chicks were given glycine deficient diets with added glyoxylate, there was a depression in growth. This result suggests that the chick cannot aminate glyoxyate to glycine at a physiologically significant rate.

The bird's ability to synthesize glycine and proline when given low crude protein diets may also be affected. Aftab et al. (2006) has found that birds given low CP diets respond favorably to glycine supplementation over the NRC suggested levels. Also, addition of glycine to an AA-supplemented 16% CP diet led to similar growth and feed efficiency as broilers fed a 22% CP diet. Studies have also suggested that the requirement

for glycine is higher in low CP diets (Dean et al., 2006). These findings may contradict the traditional view of glycine as a nonessential AA, especially during low CP or other stressful conditions. In addition, Almquist and Grau (1944) demonstrated that proline is a dispensable amino acid for growing chicks, but later work revealed that proline is essential in diets of growing chicks (Klain et al., 1959, Greene et al., 1962; Sugahara and Ariyoshi, 1967). Though the importance of these two conditionally nonessential amino acids is evident, the requirement for these under varying conditions is still not fully known.

2.4 Animal By-Products

One potential source of the critical nonessential amino acids is animal byproducts, specifically connective tissue and its byproducts. Connective tissue is made mostly of collagen and elastin, another fibrous protein. Each tissue's properties vary depending on the ratio of collagen to elastin, the arrangement of collagen fibers, and the presence of other tissue constituents (Reutersward, 1984). Glycine, alanine, proline, and hydroxyproline account for two thirds of the polypeptide chain, which only leaves one third for the remaining fourteen amino acids (Reutersward, 1984).

About one third of the total body protein in mammals is made up of collagen, which is the main protein in skin, bone, tendon and cartilage (Reutersward, 1984). It is predominately insoluble and a major component of connective tissue. It is comprised of three peptide chains forming a triple helix configuration. Every third residue on the peptide chain is a glycine, which makes the composition of collagen different from that

of any other protein. A small part of collagen in animal tissues is soluble, but the majority of the collagen is insoluble fibers that can be solubilized by heat treatment or enzymatic action, which does not affect protein quality. This is a slow two-step process that begins with the breakdown of the helical structure followed by the dissociation of the polypeptide chains. This end product is known as gelatin (Reutersward, 1984). Enzymatic degradation of collagen has been performed for a variety of uses including: gelatin manufacture (Balian & Bowes, 1977), leather (Gustavson, 1956), biochemistry and medicine (Weiss, 1976, Bailey & Etherington, 1980), meat tenderness (Sørensen, 1976; Sørensen 1981), and nutrition (Ashgar & Henrickson, 1982).

GELATIN

Gelatin is a purified animal protein derived from skin, bones, and connective tissue by selective hydrolysis (Boomgaardt and Baker, 1972). It is devoid of tryptophan (Reutersward, 1984) and low in most essential amino acids. In fact, the amount of essential amino acids in gelatin is less than half of that in muscle proteins (Reutersward, 1984). Despite being low in essential amino acids, it is high in arginine, glycine-serine, and proline (Boomgaardt and Baker, 1972). The order of limiting amino acids in gelatin is: tryptophan (1^{st}); sulfur amino acids (2^{nd}); isoleucine (3^{rd}); threonine, valine, and aromatic amino acids (tied for 4^{th}); leucine (7^{th}); histidine (8^{th}); and lysine (9^{th}) (Boomgaardt and Baker, 1972). Gelatin contains 90-95% crude protein (Boomgaardt and Baker, 1972), and has been found to have over 90% digestibility (Reutersward, 1984).

USE OF GELATIN IN FEEDS

The use of gelatin in animal feeds has been studied in the past. Previous attempts to feed gelatin-containing diets supplemented with amino acids known to be deficient to rats in order to promote growth have failed (Jackson et al., 1928; Kruse et al., 1934). One reason for the poor performance may be from the high digestibility of gelatin for rats and the amino acid balance and/or difference in calculated composition from analyzed values (Block and Weiss, 1956). Although some poor performance has been observed with the use of gelatin, it has also been found to enhance performance in other situations. The biological value of collagen/gelatin is low (due to no tryptophan), but it can be mixed with other proteins (keratin, blood, casein) to achieve a complementation effect (Chvapil, 1979). Reasonable performance was obtained by Ashley and Fisher (1966) when feeding chicks 10% protein from gelatin supplemented with amino acids.

It was once thought that gelatin may have an inhibitory effect caused by unusually high content of glycine and proline; however, later studies done by Summers and Fisher (1962) concluded that there was no inhibitory effect when a soy-protein diet was supplemented with glycine, proline, and hydroxyproline at levels similar to those found in gelatin (Ashley and Fisher, 1966).

2.5 Research Objectives

The following experiments were designed to evaluate the effects of coccidiosis vaccination on live performance and carcass quality of broilers and to determine if dietary nonessential amino acids in the form of gelatin and crude protein relieve any negative response to vaccination.

The first experiment was an 8 week grow-out trial with diets formulated for low and high crude protein with or without gelatin. The feeds were administered to broilers either vaccinated for coccidiosis or protected by a coccidiostat. The effect of vaccination versus a coccidiostat was examined along with any recuperative advantage provided from dietary gelatin inclusion at two crude protein levels.

The second experiment consisted of vaccinated broilers receiving one of four diets with graded levels of crude protein with or without gelatin. The objective was to determine the crude protein level that provides an optimal level of nonessential amino acids, particularly glycine and proline, when supplemented with gelatin.

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III. MANUSCRIPT 1

RESPONSE OF COCCIDIOSTAT VERSES VACCINATION-PROTECTED BROILERS TO GELATIN INCLUSION IN HIGH AND LOW CRUDE PROTEIN DIETS

3.1 ABSTRACT

Experimentation determined if gelatin supplementation to low and high crude protein (CP) feeds would relieve the impaired performance of broilers vaccinated for coccidiosis. Male and female Ross X Ross 708 broilers were fed either a 20-18-16% low CP or 23-20-18% high CP program from 0 to 8 weeks of age such that EAA levels were similar across diets and met or exceeded NRC (1994) recommendations for 0-3, 3-6, and 6-8 weeks of age. Gelatin, when employed, was included at 2% to simulate the levels of proline and glycine likely to occur from 5% meat meal. Half of the birds were spray vaccinated (live oocysts) prior to placement while the other half received a coccidiostat (salinomycin) in the feed until 6 weeks of age. Vaccination adversely affected live performance in the first 3 weeks; however, inclusion of gelatin reduced the negative response to vaccination while providing no benefit to birds receiving the coccidiostat. Gelatin inclusion during the subsequent 3-6 weeks had no

influence, but high CP improved body weight gain over low CP that was restricted to coccidiostat-protected broilers. In the last 2 weeks, vaccinated birds experienced greater gain than birds that received the coccidiostat, but complete recovery from early loss was not attained by 8 weeks of age. However, addition of gelatin to low CP feeds improved overall feed conversion to a level similar to the high CP diets. Gelatin had no affect on carcass composition, but use of low CP feeds increased abdominal fat and reduced carcass and fillet weights compared to high CP feeds. Vaccination reduced carcass weight corresponding to loss in live weight without affecting abdominal fat or skinless boneless meat yield. Generally, males were more sensitive to treatments than females. Improvements in performance from gelatin or high CP support that additional glycine and proline facilitate mucin formation and intestinal recovery after vaccination.

3.2 INTRODUCTION

Coccidiosis is the most costly disease of commercial poultry by virtue of medication and losses in performance (Williams, 1998;1999; Dalloul and Lillehoj, 2005). Management largely involves dietary anticoccidials; however, recent concern about antimicrobials and generation of resistant bacteria has led to a cautious approach toward their use. Unlike the EU and other countries, the United States allows sub-therapeutic use of antimicrobials in poultry feed, but consumer

reservations are driving the industry to seek alternative approaches to coccidiosis control.

Vaccination for coccidiosis is generally accepted as an alternative to anticoccidials; however, disadvantages exist for producers. Compared to birds protected by an anticoccidial, those given a non-attenuated vaccine exhibit reduced early weight gain and poorer feed conversion (Mathis, 1999), which may not be recovered prior to slaughter (Williams, 1998). Similar to vaccination, coccidiosis infection has been shown to shorten intestinal villi (Idris et al., 1997) resulting in loss of effective surface area for nutrient recovery which consequently impairs performance. Recovery of the intestinal mucosa involves proliferation of goblet cells along with an increase in mucin production (Miller et al., 1983; Miller and Narva, 1979).

Synthesis of intestinal mucin requires a high percentage of threonine, proline, glycine and serine, and need for these non-essential amino acids (NEAA) becomes exaggerated during the stress of vaccination (Miller et al., 1983; Neutra and Forstner, 1987). Threonine, an essential amino acid (EAA), must be present in the diet while proline, glycine, and serine can be synthesized like other NEAA but does so at a slower rate

Present experimentation focused on providing these conditionally nonessential amino acids in a greater proportion to the bird in the form of gelatin as a means to

enhance recovery from coccidial vaccination. The substantial proline and glycine-serine content (Boomgaardt and Baker, 1972) and high digestibility of gelatin enabled it to serve as a substitution for commercial animal protein meals while avoiding the variable digestibility. Previous attempts to use gelatin as a sole protein source have supported reasonable performance when diets were supplemented with deficient EAA (Ashley and Fisher, 1966).

Inclusion of commercially accessible EAA in the formulation of feeds generally leads to a reduced crude protein (CP), most of which is NEAA. Although low CP feeds assist in reducing potential N pollution, they have also been found to impair performance of coccidiosis-vaccinated broilers (Sharma et al., 1973; Richter and Wiesner, 1988; Parker et al., 2007). Implementation of low CP diets may be inappropriate given the bird's need for extensive amounts of NEAA, specifically proline, glycine, and serine, for recovery from vaccination. This experiment employed low and high CP diets to examine if gelatin incorporation would mitigate the adverse effects of low CP diets when broilers were vaccinated for coccidiosis.

3.3 MATERIALS AND METHODS

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee. Ross X Ross 708 day-old chicks (1280) were obtained from a commercial hatchery, feather-sexed, and reared sex separate. Sixty four total floor

pens each had 20 chicks in a 4.18 m^2 area with used pine shaving litter. All pens were in an open-sided house with cross ventilation and temperature control. Prior to placement in pens, one half of the birds were vaccinated against coccidiosis following manufacturer recommendations (Coccivac-D, Schering-Plough Animal Health Corporation 556 Morris Avenue, Summit, NJ 07901-1330). Birds that were not vaccinated received coccidiostat (Bio-Cox 60 Granular, Alpharma Inc., One Executive Drive, Fort Lee, NJ 07024) in the feed for the first 6 weeks. All chicks had *ad libitum* access to feed and water and received continuous lighting.

Chicks were given either a low or high CP diet from 0 to 8 weeks of age such that EAA from 0-3, 3-6, and 6-8 weeks either met or exceeded NRC (1994) recommendations. Gelatin (60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176) when employed was supplemented at 2% to simulate NEAA provided by 5% meat meal with 50% CP. Compositions of diets fed from 0-3, 3-6, and 6-8 weeks of age are detailed in Tables 1, 4, and 7, respectively. The first feed was fed as a crumble, whereas subsequent feeds were whole pellets. Feed samples were a composite of subsamples from each pen representing the respective treatments, and were analyzed for amino acid composition (Dirk Höehler, Degussa Ag Feed Additives, 1701 Barrett Lakes Blvd., Suite 340 Kennesaw, GA 30144).

Live performance was measured for each phase of production. On day 55, all birds were individually weighed and one half of the total was selected from each pen based on odd wing band number and placed in coops. The next morning, birds were "on-line" processed at the Auburn University Poultry Farm. Carcasses were chilled in static slush-ice water for three hours then drained of excess water for 3 to 5 minutes before carcass and abdominal fat pad measurements were taken. Chilled carcasses were further reduced in number by selecting alternate odd wing band numbers represented from each pen and these were held for further processing the following day. Experienced commercial plant employees used stationary cones to separate fillets, tenders, wings, drums, skinless, boneless thigh meat, and frame. Fillets were refrigerated (4°C) and held an additional 24 hours when light reflectance (L* a* b*) on the skin side surface was measured (Minolta Calorimeter CR300/DP 301, Minolta Co., Ltd. Osaka, Japan 541-8556).

Statistical Analysis

Individual floor pens served as the experimental unit. Treatments were a factorial arrangement of main factors (CP, coccidiosis protection, gelatin, and sex) in a randomized complete block design that was analyzed by an analysis of variance for dietary treatment using the General Liner Models procedure in SAS® 9.1.3 (SAS Institute, 2001). Blocks represented different locations within the house during live production, and each treatment was replicated 4 times.

3.4 RESULTS AND DISCUSSION

The current objective was to determine if proline and glycine as provided by gelatin and/or additional crude protein could relieve the stress of vaccination for coccidiosis. Starter feeds were formulated to contain either a low or high CP level each of which were supplemented with gelatin (Table 1). Analysis revealed that absolute CP levels were about 1% greater than intended; however, relative differences between the low and high levels persisted. Potentially limiting EAA were similar among all feeds and met or exceeded NRC (1994) recommendations (Table 2); thus, differences in CP were largely related to NEAA and EAA in excess of need. Greater amounts of glycine and proline at each CP level resulted from inclusion of gelatin.

During the first 3 weeks, temporary infection from vaccination created more stress than the anticoccidial medication, as evidenced by reduced body weight gain, higher feed conversion, and greater mortality (Table 3). This finding coincides with Mathis' (1999) observation and is most likely the result of damage to intestinal villi by live oocysts. Gelatin inclusion improved live performance of vaccinated birds, regardless of CP level, whereas any improvement from gelatin when using a coccidiostat for protection was not evident. Due to the similarity in limiting EAA across all diets, results suggest that increased access to glycine and proline provided by gelatin facilitated repair of mucosa damaged by vaccination. Additional proline

and glycine provides relief from *de novo* synthesis of amino acids, and is assumed to facilitate goblet cell proliferation and increased mucin production that occurs during infection (Miller et al., 1983; Miller and Narva, 1979).

Analysis of diets fed from 3-6 weeks of age verified formulation of low and high CP levels that had similar limiting EAA while glycine and proline increased with gelatin incorporation (Tables 4 and 5). Although CP level had no effect on live performance of vaccinated birds or those receiving coccidiostat during the first three weeks as gelatin did, the converse occurred during the subsequent two weeks with high CP being advantageous while gelatin had no effect (Table 6). Because intestinal maturation to support digestion is central to the post-emergent chick (Moran, 2007; Geyra et al., 2001), early lack of response to CP compared to gelatin is assumed to be the result of preferential intestinal repair and maturation rather than body protein accretion which appears to occur subsequently, particularly for males. High CP provided a significant advantage in body weight gain that was restricted to the medicated broilers, while gelatin was without effect for either level. Initial damage to mucosa from vaccination may explain the lack of response to high CP from 3-6 weeks relative to coccidiostat-protected birds.

Intended formulation of the final diets was to have low and high CP levels of 16 and 18%, respectively (Table 7); however actual values were approximately 2% higher for each diet (Table 8). As in previous diets, limiting EAA were similar while

gelatin inclusion increased glycine and proline. As a likely compensatory response to suppressed early growth, vaccinated birds expressed greater body weight gain than those that had received coccidiostat (Table 9). Regardless of method of coccidial protection, addition of gelatin to low CP diets led to decreased feed intake and improved feed conversion while no benefit occurred when gelatin was added to high CP diets. Since levels of glycine and proline in the low CP diet with gelatin and the basal high CP diet were similar, lack of improvement when gelatin was added to high CP diets may signify adequate glycine and proline.

Similar to Williams' (1998) observation, early depression in body weight gain as a result of vaccination could not be fully recovered by eight weeks (Table 10). High CP diets led to significantly increased body weight gain by 8 weeks of age compared to low CP diets, while no changes in other live performance measures were observed. Regardless of method of coccidial protection, inclusion of gelatin to the low CP diets decreased feed intake and improved feed conversion to the level of the high CP diets, both with and without gelatin. A greater proportion of NEAA, particularly glycine and proline, provided by gelatin is likely a basis for the improved the response of birds fed the low CP diet (Parr and Summers, 1991; Aftab et al., 2006). Lack of improvement from addition of gelatin to high CP diets suggests that NEAA need had been met. While feed consumption normally differs between the sexes, addition of gelatin led to reduced male feed intake compared to females, regardless of other

treatments, while the corresponding advantage that occurred in feed conversion was not statistically significant.

In addition to live performance, processing the broilers into commercial parts provided further assessment of treatment effects. Reduction in number of birds by one half based on selection of alternate wing band numbers is assumed to be representative of the original population. Chilled carcass weights after removal of abdominal fat generally resembled respective differences observed in live weight of all birds (Table 11). Low CP diets have previously been shown to adversely affect yield (Moran et al., 1992), and in present experimentation, low CP feeds increased abdominal fat together and reduced breast fillet weights(Tables 11 and 12). Vaccination reduced carcass weight to a similar extent as the low CP diet, but adverse effects on abdominal fat and fillets from vaccination did not occur. Addition of gelatin to low CP feeds had no effect on abdominal fat but decreased yield of the whole carcass. Males seemed to exhibit a more negative response to addition of gelatin than females.

Light reflectance is a means of measuring quality of breast fillets. Birds receiving high CP diets throughout production had fillets that were lighter in appearance and less yellow than fillets from birds given feed with low CP (Table 13). Addition of gelatin to high CP diets increased yellowness while no change occurred with addition of gelatin to low CP diets. In addition, fillets from males were redder

and less yellow than females. While differences were detected in light reflectance, the significance in consumer satisfaction is questionable.

Overall, performance of both sexes corresponded to expectations for this strain (Aviagen, 2007). Vaccination for coccidiosis was detrimental to early live performance; however, recovery from 0-3 weeks was facilitated by addition of gelatin so that by 8 weeks of age, recuperation from vaccination was nearly complete. While high CP diets were more advantageous than low CP diets, addition of gelatin to low CP feeds improved bird response. Gelatin was chosen to simulate the practical use of meat meal; however, employing meat meal to raise glycine, proline, and total NEAA levels may not be as viable as increasing CP from vegetable sources.

Table 1.1 Composition of experimental diets having low and high crude protein with and without gelatin (% "as is" basis) fed from 0-3 weeks

	Low CP		High CP	
	control	w/ gelatin	control	w/ gelatin
Corn	55.15	58.15	48.45	51.50
SBM	34.75	30.20	40.55	36.05
Poultry Fat	5.50	4.80	6.70	6.00
L-Lysine HCl	0.25	0.30	0.05	0.10
DL-Methionine	0.30	0.35	0.25	0.30
L-Tryptophan	0	0.05	0	0
L-Threonine	0.10	0.10	0	0
Dicalcium Phosphate	1.70	1.75	1.70	1.75
Limestone	1.40	1.40	1.40	1.40
Gelatin[1]	0	2.00	0	2.00
Constant[2]	---------- to 100% ----------			
	Calculated Composition			
ME (kcal/kg)	3200		3200	
CP (%)	21		23	

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.40% salt and 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 1.2 Amino acid composition of experimental diets fed 0-3 weeks of age (% "as is" basis)[1]

	Low CP				High CP			
	control		w/ gelatin		control		w/ gelatin	
	C[2]	V[3]	C	V	C	V	C	V
Crude Protein	22.00	21.35	21.71	21.95	24.08	23.93	23.53	23.23
				Essential Amino Acids				
Methionine	0.66	0.63	0.67	0.70	0.62	0.64	0.68	0.70
Cystine	0.35	0.33	0.32	0.31	0.37	0.37	0.35	0.34
Meth + cystine	1.01	0.96	0.99	1.02	0.98	1.01	1.03	1.04
Lysine	1.38	1.32	1.33	1.34	1.37	1.38	1.36	1.36
Threonine	0.93	0.90	0.88	0.89	0.92	0.95	0.89	0.88
Arginine	1.45	1.40	1.45	1.43	1.63	1.64	1.60	1.61
Tryptophan	0.26	0.26	0.27	0.27	0.30	0.30	0.27	0.26
Isoleucine	0.91	0.90	0.86	0.85	1.03	1.04	0.97	0.97
Leucine	1.80	1.77	1.72	1.72	1.95	1.96	1.90	1.88
Valine	1.01	1.00	0.97	0.96	1.11	1.13	1.07	1.07
Phenylalanine	1.05	1.02	0.99	0.99	1.16	1.17	1.11	1.09
Histidine	0.62	0.60	0.59	0.57	0.68	0.68	0.63	0.63
				Nonessential Amino Acids				
Proline	1.27	1.26	1.39	1.40	1.25	1.27	1.39	1.39
Glycine	0.93	0.89	1.28	1.27	1.02	1.02	1.35	1.33
Serine	1.05	1.00	1.01	1.01	1.14	1.16	1.12	1.10
Alanine	1.08	1.05	1.17	1.17	1.15	1.16	1.27	1.25
Aspartic Acid	2.22	2.16	2.12	2.09	2.47	2.54	2.39	2.38
Glutamic Acid	3.88	3.82	3.75	3.71	4.28	4.35	4.18	4.14

[1]Values for each feed represent one analysis.

[2]Control feed with 0.05% coccidiostat (Bio-Cox 60 Granular, Alpharma Inc.).

[3]Control feed without coccidiostat - birds vaccinated.

Table 1.3 Live performance of broilers receiving feeds having low and high crude protein with and without gelatin from 0-3 weeks of age[1]

CP	Protection[2]	Gelatin	Sex	Gain (g)	Feed Intake (g)	F/G	% Mort[3]
			Main Contrasts				
High	--	--	--	833	1016	1.22	1.9
Low	--	--	--	833	1002	1.21	2.3
P≤				NS	NS	NS	NS
--	Vaccination	--	--	799	1002	1.26	3.0
--	Coccidiostat	--	--	866	1017	1.17	1.3
	P≤			***	NS	***	**
--	--	No	--	824	1045	1.27	1.9
--	--	Yes	--	842	974	1.16	2.4
		P≤		*	***	***	NS
--	--	--	M	871	1056	1.22	3.3
--	--	--	F	795	962	1.21	0.9
			P≤	***	***	NS	***
	SEM			5.30	11.70	0.012	0.40
			Interactions[4]				
--	Vaccination	No	--	783	1048	1.34	2.5
--		Yes	--	816	956	1.17	3.4
--	Coccidiostat	No	--	865	1042	1.20	1.2
--		Yes	--	868	992	1.14	1.3
	P≤			*	NS	**	NS
--	--	No	M	864	1112	1.29	3.2
--	--		F	783	978	1.25	0.6
--	--	Yes	M	877	1000	1.14	3.5
--	--		F	807	947	1.17	1.2
		P≤		NS	*	*	NS
	SEM			7.5	16.6	0.017	0.56

[1] Values represent contrasts involving a total of 64 pens, each with 25 chicks averaging 41g at start of experimentation.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

[4] Only main factor interactions where $P<0.05$ are shown.

NS = $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Table 1.4 Composition of experimental diets having low and high crude protein with and without gelatin (% "as is" basis) fed from 3-6 weeks

	Low CP		High CP	
	control	w/ gelatin	control	w/ gelatin
Corn	65.20	68.55	58.80	61.85
SBM	26.60	21.90	32.40	27.85
Poultry Fat	3.70	2.90	4.80	4.10
L-Lysine HCl	0.35	0.40	0.15	0.20
DL-Methionine	0.30	0.35	0.25	0.25
L-Tryptophan	0.05	0.05	0	0.05
L-Threonine	0.15	0.20	0.05	0.10
Dicalcium Phosphate	1.25	1.30	1.20	1.25
Limestone	1.50	1.45	1.45	1.45
Gelatin[1]	0	2.00	0	2.00
Constant[2]	---------- to 100% ----------			
	Calculated Composition			
ME (kcal/kg)	3200		3200	
CP (%)	18		20	

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.40% salt and 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 1.5 Amino acid composition of experimental diets fed 3-6 weeks of age (% "as is" basis)[1]

	Low CP				High CP			
	control		w/ gelatin		control		w/ gelatin	
	C[2]	V[3]	C	V	C	V	C	V
Crude Protein	N/A[4]	19.40	19.19	18.92	21.14	21.60	20.54	20.42
				Essential Amino Acids				
Methionine	-	0.65	0.65	0.63	0.59	0.60	0.58	0.57
Cystine	-	0.33	0.28	0.28	0.35	0.33	0.29	0.30
Meth + cystine	-	0.98	0.93	0.91	0.93	0.93	0.87	0.87
Lysine	-	1.34	1.22	1.15	1.25	1.29	1.18	1.19
Threonine	-	0.89	0.85	0.83	0.88	0.89	0.84	0.83
Arginine	-	1.30	1.23	1.18	1.42	1.46	1.33	1.33
Tryptophan	-	0.27	0.24	0.23	0.27	0.27	0.26	0.25
Isoleucine	-	0.82	0.73	0.69	0.87	0.87	0.77	0.79
Leucine	-	1.68	1.57	1.55	1.81	1.83	1.63	1.65
Valine	-	0.91	0.84	0.80	0.96	0.96	0.88	0.89
Phenylalanine	-	0.95	0.86	0.83	1.04	1.05	0.92	0.93
Histidine	-	0.54	0.49	0.48	0.60	0.62	0.52	0.53
				Nonessential Amino Acids				
Proline	-	1.08	1.21	1.23	1.17	1.20	1.29	1.29
Glycine	-	0.81	1.12	1.13	0.89	0.90	1.23	1.19
Serine	-	0.92	0.85	0.85	1.05	1.08	0.94	0.94
Alanine	-	1.01	1.08	1.08	1.08	1.10	1.13	1.14
Aspartic Acid	-	1.96	1.76	1.68	2.16	2.23	1.91	1.92
Glutamic Acid	-	3.54	3.27	3.17	3.87	3.97	3.47	3.51

[1]Values for each feed represent one analysis.

[2]Control feed with 0.05% coccidiostat (Bio-Cox 60 Granular, Alpharma Inc.).

[3]Control feed without coccidiostat - birds vaccinated.

[4]Analysis not available – sample lost.

Table 1.6 Live performance of broilers receiving feeds having low and high crude protein with and without gelatin from 3-6 weeks of age[1]

CP	Protection[2]	Gelatin	Sex	Gain (g)	Feed Intake (g)	F/G	% Mort[3]
			Main Contrasts				
High	--	--	--	1869	3163	1.70	1.4
Low	--	--	--	1829	3154	1.73	1.1
P≤				*	NS	NS	NS
--	Vaccination	--	--	1838	3136	1.71	0.6
--	Coccidiostat	--	--	1859	3181	1.72	1.9
	P≤			NS	NS	NS	NS
--	--	No	--	1850	3148	1.71	1.4
--	--	Yes	--	1848	3169	1.72	1.1
		P≤		NS	NS	NS	NS
--	--	--	M	2035	3381	1.66	1.7
--	--	--	F	1662	2936	1.77	0.8
			P≤	***	***	***	NS
	SEM			13.4	30.6	0.017	0.47
			Interactions[4]				
High	Vaccination	--	--	1836	3120	1.71	0.9
	Coccidiostat	--	--	1902	3205	1.69	1.9
Low	Vaccination	--	--	1841	3152	1.72	0.3
	Coccidiostat	--	--	1816	3156	1.75	1.9
P≤				*	NS	NS	NS
High	--	--	M	2048	3415	1.67	2.2
	--	--	F	1690	2911	1.73	0.6
Low	--	--	M	2023	3347	1.65	1.2
	--	--	F	1635	2961	1.81	1.0
	P≤			NS	NS	*	NS
--	--	No	M	2059	3405	1.65	2.2
--	--		F	1640	2891	1.76	0.7
--	--	Yes	M	2011	3357	1.67	1.2
--	--		F	1685	2981	1.77	0.9
		P≤		*	NS	NS	NS
	SEM			19.0	43.2	0.024	0.66

[1] Values represent contrasts involving a total of 64 pens, each with 25 chicks at start of experimentation.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

[4] Only main factor interactions where P<0.05 are shown.

NS = $P>0.05$; * = $P<0.05$; *** = $P<0.001$

Table 1.7 Composition of experimental diets having low and high crude protein with and without gelatin (% "as is" basis) fed from 6-8 weeks

	Low CP		High CP	
	control	w/ gelatin	control	w/ gelatin
Corn	72.15	75.25	65.40	68.65
SBM	21.40	16.90	27.40	22.85
Poultry Fat	2.50	1.75	3.65	2.90
L-Lysine HCl	0.25	0.30	0.05	0.10
DL-Methionine	0.20	0.20	0.15	0.15
L-Tryptophan	0.05	0.05	0	0
L-Threonine	0.20	0.20	0.10	0.10
Dicalcium Phosphate	0	0.05	0	0
Limestone	1.00	1.00	0.95	1.00
Gelatin[1]	1.35	1.40	1.40	1.35
Constant[2]	---------- to 100% ----------			
	Calculated Composition			
ME (kcal/kg)	3200		3200	
CP (%)	16		18	

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.40% salt and 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 1.8 Amino acid composition of experimental diets fed 6-8 weeks of age (% "as is" basis)[1]

	Low CP		High CP	
	control	w/ gelatin	control	w/ gelatin
Crude Protein	18.10	17.69	19.71	18.75
		Essential Amino Acids		
Methionine	0.49	0.48	0.47	0.46
Cystine	0.29	0.28	0.33	0.29
Meth + cystine	0.77	0.76	0.80	0.75
Lysine	1.08	1.05	1.07	1.00
Threonine	0.84	0.80	0.85	0.79
Arginine	1.11	1.09	1.29	1.22
Tryptophan	0.24	0.21	0.23	0.18
Isoleucine	0.71	0.66	0.81	0.70
Leucine	1.56	1.48	1.76	1.59
Valine	0.80	0.71	0.89	0.81
Phenylalanine	0.85	0.77	0.97	0.86
Histidine	0.49	0.45	0.55	0.50
		Nonessential Amino Acids		
Proline	1.02	1.17	1.15	1.26
Glycine	0.71	1.03	0.82	1.11
Serine	0.84	0.81	0.97	0.91
Alanine	0.94	1.03	1.05	1.10
Aspartic Acid	1.68	1.53	1.95	1.74
Glutamic Acid	3.16	2.97	3.62	3.29

[1]Values for each feed represent one analysis

Table 1.9 Live performance of broilers receiving feeds having low and high crude protein with and without gelatin from 6-8 weeks of age[1]

CP	Protection[2]	Gelatin	Sex	Gain (g)	Feed Intake (g)	F/G	% Mort[3]
				Main Contrasts			
High	--	--	--	1082	2704	2.59	4.2
Low	--	--	--	1076	2730	2.59	3.4
P≤				NS	NS	NS	NS
--	Vaccination	--	--	1103	2734	2.53	3.0
--	Coccidiostat	--	--	1055	2700	2.65	4.5
	P≤			*	NS	NS	NS
--	--	No	--	1087	2726	2.55	3.3
--	--	Yes	--	1071	2708	2.62	4.3
		P≤		NS	NS	NS	NS
--	--	--	M	1270	2904	2.30	5.9
--	--	--	F	888	2530	2.88	1.7
			P≤	***	***	***	**
	SEM			16.1	24.1	0.054	1.04
				Interactions[4]			
High	--	No	--	1098	2650	2.46	3.8
	--	Yes	--	1067	2759	2.72	4.5
Low	--	No	--	1077	2802	2.64	2.8
	--	Yes	--	1075	2657	2.53	4.0
	P≤			NS	***	*	NS
	SEM			22.7	34.1	0.077	1.48

[1] Values represent contrasts involving a total of 64 pens, each with 25 chicks at start of experimentation.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

[4] Only interactions where P<0.05 are shown.

NS = $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Table 1.10 Overall live performance of broilers receiving feeds having low and high crude protein with and without gelatin from 0-8 weeks of age

CP	Protection[1]	Gelatin	Sex	Gain (g)	Feed Intake (g)	F/G	% Mort[2]
			Main Contrasts				
High	--	--	--	3784	6883	1.83	7.4
Low	--	--	--	3738	6886	1.85	6.9
P≤				*	NS	NS	NS
--	Vaccination	--	--	3741	6872	1.84	6.6
--	Coccidiostat	--	--	3781	6897	1.83	7.7
	P≤			*	NS	NS	NS
--	--	No	--	3761	6919	1.85	6.6
--	--	Yes	--	3761	6850	1.83	7.7
		P≤		NS	NS	NS	NS
--	--	--	M	4039	7341	1.82	10.9
--	--	--	F	3285	6428	1.96	3.4
			P≤	***	***	***	***
	SEM			13.9	54.6	0.012	1.16
			Interactions[3]				
High	--	No	--	3782	6835	1.82	7.5
	--	Yes	--	3787	6931	1.84	7.4
Low	--	No	--	3740	7002	1.88	5.7
	--	Yes	--	3735	6770	1.82	8.0
	P≤			NS	*	*	NS
--	--	No	M	4194	7456	1.78	10.6
--	--		F	3328	6381	1.92	2.6
--	--	Yes	M	4158	7225	1.74	11.3
--	--		F	3364	6476	1.92	4.2
		P≤		NS	*	NS	NS
	SEM			19.7	77.2	0.017	1.64

[1] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[2] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

[3] Only main factor interactions where P<0.05 are shown.

NS = $P>0.05$; * = $P<0.05$; *** = $P<0.001$.

Table 1.11 Carcass analysis of broilers receiving feeds having low and high crude protein with and without gelatin[1]

CP	Protection[2]	Gelatin	Sex	Carcass w/o fat		Abdominal Fat	
				Weight (g)	% Live Wt.	Weight (g)	% Carcass
				Main Contrasts			
High	--	--	--	2730	71.3	61.3	2.23
Low	--	--	--	2687	71.2	66.5	2.44
P≤				*	NS	**	***
--	Vaccination	--	--	2683	71.2	63.3	2.33
--	Coccidiostat	--	--	2735	71.3	64.5	2.34
	P≤			**	NS	NS	NS
--	--	No	--	2725	71.3	64.0	2.33
--	--	Yes	--	2692	71.1	63.9	2.35
		P≤		NS	NS	NS	NS
--	--	--	M	3011	71.5	61.6	1.99
--	--	--	F	2407	70.9	66.3	2.68
			P≤	***	***	*	***
Average SEM				14.6	0.13	1.35	0.043
				Interactions[3]			
High	--	No	--	2749	71.2	63.1	2.28
	--	Yes	--	2710	71.4	59.5	2.18
Low	--	No	--	2702	71.5	64.8	2.37
	--	Yes	--	2673	70.9	68.2	2.51
	P≤			NS	**	NS	NS
--	--	No	M	3066	71.8	63.1	2.00
--	--		F	2385	70.9	64.9	2.65
--	--	Yes	M	2955	71.3	60.1	1.98
--	--		F	2429	71.0	67.6	2.71
		P≤		***	NS	NS	NS
Average SEM				20.6	0.18	1.91	0.060

[1] Values are associated with approximately 10 carcasses from each of 64 pens.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3] Only main factor interactions where P<0.05 are shown.

NS = $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Table 1.12 Skinless, boneless breast meat yield of broilers receiving feeds having low and high crude protein with and without gelatin[1]

CP	Protection[2]	Gelatin	Sex	Fillet		Tender	
				Weight (g)	% Carcass	Weight (g)	% Carcass
				Main Contrasts			
High	--	--	--	741	27.2	157	5.8
Low	--	--	--	716	26.6	154	5.8
P≤				**	***	NS	NS
--	Vaccination	--	--	723	27.0	154	5.8
--	Coccidiostat	--	--	733	26.8	157	5.8
	P≤			NS	NS	NS	NS
--	--	No	--	732	26.9	157	5.8
--	--	Yes	--	725	26.9	154	5.7
		P≤		NS	NS	NS	NS
--	--	--	M	805	26.8	166	5.5
--	--	--	F	651	27.0	145	6.0
			P≤	***	NS	***	***
	Average SEM			5.2	0.98	1.4	0.04
				Interactions[3]			
--	--	No	M	820	26.8	168	5.5
--	--		F	644	27.0	146	6.1
--	--	Yes	M	791	26.8	163	5.5
--	--		F	658	27.0	145	6.0
		P≤		**	NS	NS	NS
High	--	--	M	819	27.1	169	5.6
	--	--	F	663	27.3	145	6.0
Low	--	--	M	792	26.5	163	5.4
	--	--	F	639	26.8	145	6.1
	P≤			NS	NS	NS	*
	Average SEM			7.3	0.14	1.9	0.05

[1] Values are associated with approximately 10 carcasses from each of 64 pens.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3]Onlymain factor interactions where P<0.05 are shown.

NS = *P*>0.05; * = *P*<0.05; ** = *P*<0.01; *** = *P*<0.001

Table 1.13 Light reflectance of breast fillets from broilers receiving feeds having low and high crude protein with and without gelatin[1]

CP	Protection[2]	Gelatin	Sex	L*	a*	b*
Main Contrasts						
High	--	--	--	58.08	2.71	7.05
Low	--	--	--	57.41	2.45	7.83
P≤				*	NS	**
--	Vaccination	--	--	57.76	2.53	7.47
--	Coccidiostat	--	--	57.73	2.63	7.41
	P≤			NS	NS	NS
--	--	No	--	57.74	2.47	7.26
--	--	Yes	--	57.76	2.69	7.61
		P≤		NS	NS	NS
--	--	--	M	57.61	3.05	5.56
--	--	--	F	57.89	2.11	8.32
			P≤	NS	***	***
	Average SEM			0.197	0.099	0.194
Interaction[3]						
High	--	No	--	58.18	2.47	6.58
	--	Yes	--	57.98	2.94	7.52
Low	--	No	--	57.29	2.46	7.95
	--	Yes	--	57.53	2.45	7.71
	P≤			NS	NS	*
	Average SEM			0.278	0.141	0.275

[1] Values represent light reflectance of skin side of breast fillet from approximately 10 birds from each of 64 pens.

[2] Vaccination = Coccivac-D, Schering-Plough Animal Health Corporation; Coccidiostat = Bio-Cox 60 Granular, Alpharma Inc.

[3] Only main factor interaction where P<0.05 is shown.

NS = $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

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IV. MANUSCRIPT 2

RESPONSE OF COCCIDIOSIS-VACCINATED BROILERS TO ADDITIONAL PROLINE AND GLYCINE FROM GELATIN AND INCREASING CRUDE PROTEIN

4.1 ABSTRACT

Coccidiosis-vaccinated broilers given low CP diets with gelatin have previously been shown to perform similarly to broilers receiving high CP diets without gelatin. A subsequent experiment was conducted to estimate the optimal dietary levels of CP and NEAA, particularly glycine and proline, for vaccinated birds. Male chicks were spray-vaccinated with live coccidial oocysts prior to pen placement and fed one of four diets with increasing CP: 20, 21, 22, or 23% from 0-3 weeks; 19, 20, 21, or 22% from 3-6 weeks; and 18, 19, 20, or 21% from 6-8 weeks, following respective order such that limiting EAA levels of all diets approximated NRC (1994) recommendations for age. Gelatin was included in half of the diets at 2% to increase proline and glycine without affecting CP. Increasing the level of CP improved body weight gain at each phase from 0-8 weeks of age. Feed efficiency declined as CP

increased through the first 3 weeks, but improved from 3-6 weeks while no change occurred 6-8 weeks. Gelatin provided an advantage in body weight gain only from 6-8 weeks; however, feed efficiency was improved 0-3 and 3-6 weeks, while remaining unaltered from 6-8 weeks. During the last 2 weeks, birds receiving the diets with the two lower levels of CP (20-17-15% and 21-18-16%) had reduced gain when gelatin was added while the opposite occurred when gelatin was added to the two higher CP diets (22-19-17% and 23-20-18%). Overall, both gelatin inclusion and increasing CP improved live performance. Chilled carcass, breast fillet, and tender yields improved with increasing CP levels while abdominal fat decreased. Use of gelatin independently provided additional abdominal fat while other aspects of the carcass remained unaltered. Improvements from gelatin and CP support that additional NEAA, especially glycine and proline, enhanced performance of birds vaccinated for coccidiosis. Specific estimates for optimal performance, however, could not be determined and likely vary with extent of mucosal damage.

4.2 INTRODUCTION

Vaccinating broilers for coccidiosis alleviates consumer concerns about antimicrobial use by eliminating dietary anticoccidials; however, this method of coccidiosis control hinders live performance of birds. Non-attenuated vaccines have been shown to reduce early weight gain and exacerbate feed conversion losses

compared to birds protected by an anticoccidial (Mathis, 1999), which the bird may not fully recover prior to slaughter (Williams, 1998).

Vaccination with live oocysts parallels an actual infection where intestinal mucosal damage and loss of surface area impair live performance (Apajalahti, 2004; Idris et al., 1997; Preston-Mafham and Sykes, 1970; Ruff and Wilkins, 1980). Recovery from infection involves hyperplasia of goblet cells and enhanced mucin formation (Miller et al., 1983; Miller and Narva, 1979). This additional mucin formation requires proline, glycine, serine, and threonine of which the majority of mucin is composed (Neutra and Forstner, 1987). Provision of high CP diets has improved performance of vaccinated birds (Sharma et al., 1973; Richter and Wiesner, 1988; Parker et al., 2007) by providing proline and glycine-serine for mucosal repair rather than relying on *de novo* synthesis, which may not be rapid enough to satisfy requirements of fast tissue growth.

Dietary levels of proline or glycine-serine may be raised by increasing total dietary CP or inclusion of protein sources. Though animal by-product meals may be favorable in this respect, their variable digestibility confounds experimental objectives. Gelatin, on the other hand, is highly digestible and provides much of the glycine and proline found in animal by-product meals (Boomgaardt and Baker, 1972). Previous experimentation (Lehman, unpublished results) supported that inclusion of 2%gelatin to corn-soy feed significantly alleviates early loss in gain associated with

vaccination, while gelatin included in the low CP regimen (20-18-16%) improved the response of birds to that of the high CP regimen (23-20-18%) by 8 weeks. Present experimentation involved addition of gelatin to diets of vaccinated birds with low to high graded levels of CP. Given similarity of EAA levels across diets, the CP level at which gelatin inclusion provides no benefit is assumed to represent an optimization of NEAA, particularly glycine and proline, for intestinal repair from vaccination.

4.3 MATERIALS AND METHODS

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee. Ross X Ross 708 day-old chicks (1600) were obtained from a commercial hatchery, feather-sexed, and vaccinated against coccidiosis using live oocysts following manufacturer instructions (Coccivac-D, Schering-Plough Animal Health Corporation 556 Morris Avenue, Summit, NJ 07901-1330). Vaccinated male chicks were placed in 64 total floor pens (25 birds / 4.18 m^2) with used pine shaving litter. All pens were in an open-sided house with cross ventilation and temperature control. Birds had ad libitum access to feed and water and received continuous lighting.

Chicks were given one of four diets with increasing levels of protein: 20, 21, 22, or 23% CP from 0-3 weeks; 19, 20, 21, or 22% CP from 3-6 weeks; and 18, 19, 20, or 21% CP from 6-8 weeks, following respective order. Formulation was such

that EAA approximated or exceeded NRC (1994) recommendations and were similar across all diets for each phase of production. Gelatin (60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176) was included in half of the diets at 2% to increase glycine and proline levels and simulate the use of 5% meat meal having 50% CP. Compositions of diets used from 0-3, 3-6, and 6-8 weeks are detailed in Tables 1, 4, and 7, respectively. The first feed was fed as a crumble, whereas subsequent feeds were whole pellets. Feed samples were a composite of subsamples from each pen representing the respective treatments, and were analyzed for amino acid composition (Dirk Höehler, Degussa Ag Feed Additives, 1701 Barrett Lakes Blvd., Suite 340 Kennesaw, GA 30144).

Live performance was measured after each phase of production. On day 55, all birds were individually weighed, and one half of the total was selected from each pen based on odd wing band number and placed in coops. The following morning, the selected birds were "on-line" processed at the Auburn University Poultry Farm. Carcasses were chilled in static slush-ice water for three hours then drained of excess water for 3 to 5 minutes before carcass and abdominal fat pad measurements were taken. Chilled carcasses were further reduced in number by selecting alternate odd wing band numbers represented from each pen, and then sub-sampled carcasses were held on flake ice until further processing the following morning. Experienced

commercial plant employees separated fillets, tenders, wings, drums, skinless, boneless thigh meat, and frame from the whole carcass on stationary cones.

Statistical Analysis

Individual floor pens served as the experimental unit. Treatments were a factorial arrangement of main factors (CP level and gelatin) in a randomized complete block design that was analyzed by an analysis of variance for dietary treatment using the General Liner Models procedure in SAS® 9.1.3 (SAS Institute, 2001). Regression analysis was performed on CP measurements when $P<.05$. Blocks represented different locations within the house during live production, and each treatment was replicated 8 times.

4.4 RESULTS AND DISCUSSION

Diets fed from 0-3 weeks were formulated to have four graded levels of CP (20, 21, 22, and 23%) and to include gelatin to increase glycine and proline while minimally altering other NEAA (Table 1). Although formulated to have similar EAA across all diets, analysis indicated that both 20% CP feeds were sub-marginal in meeting recommended NRC (1994) levels for threonine (0.74 and 0.75 *vs.* 0.80), isoleucine (0.75 *vs.* 0.80), and valine (0.83 and 0.84 *vs.* 0.90), whereas the higher CP feeds had satisfactory levels (Table 2). As intended, glycine and proline increased with total CP as well as with the inclusion of gelatin at each CP level. During the first

3 weeks, raising the level of CP linearly increased body weight gain, feed intake, and feed conversion (Table 3). Gelatin inclusion had no effect on gain, but it did reduce feed intake of birds to generate a noticeable improvement in feed conversion, regardless of CP. Absence of interaction between CP level and gelatin suggests that additional glycine and proline from gelatin provided an advantage to mucosal repair and nutrient recovery with increasing CP.

Similar to the first three weeks, diets fed from 3-6 weeks of age were formulated with four graded levels of CP (17, 18, 19, and 20%) and included gelatin to primarily increase glycine and proline (Table 4). Analysis indicated levels of glycine and proline increased with total CP and gelatin inclusion, but feeds with 17% CP, 18% CP with gelatin, and 19% CP with gelatin were sub-marginal in meeting NRC (1994) EAA levels for threonine (0.73 *vs.* 0.74), isoleucine (0.68, 0.64, 0.66, and 0.72 *vs.* 0.73), arginine (1.07 and 1.09 *vs.* 1.10), and valine (0.77, 0.75, and 0.76 *vs.* 0.82) or a combination of the four (Table 5). Increasing CP level linearly improved gain similar to the first three weeks and also improved feed conversion (Table 6). Inclusion of gelatin at all levels of CP adversely impacted gain and feed intake with no effect on feed conversion, contrary to the first three weeks. Lack of advantage from gelatin suggests that the need for additional glycine and proline had declined after early recovery from vaccination.

Diets fed from 42-56 days were again formulated to include gelatin to increase glycine and proline at each of four levels of CP (15, 16, 17, and 18%) that were progressively reduced from feeds fed 0-3 and 3-6 weeks (Table 7). Analysis verified increased levels of glycine and proline; however, feeds with 15% CP, 16% CP, and 17% CP with gelatin were potentially sub-marginal in threonine (0.65 and 0.67 *vs.* 0.68), isoleucine (0.58, 0.51, 0.61, 0.58, and 0.60 *vs.* 0.62), valine (0.67, 0.61, and 0.67 *vs.* 0.70), and arginine (0.95, 0.93, and 0.96 *vs.* 1.00) or a combination of those amino acids (Table 8). At each CP level, the amount of sub-marginal amino acids was generally similar between diets with and without gelatin. As with 3-6 weeks, gain increased linearly with CP from 6-8 weeks; however, improvements in feed conversion were not apparent (Table 9). Addition of gelatin aided in decreasing feed intake to improve feed efficiency. The response of gain with addition of gelatin was reduced in birds receiving the diets with the lower levels of CP (20-17-15% and 21-18-16%) but enhanced when added to diets with higher CP (22-19-17% and 23-20-18%). Sub-marginal limiting EAA in the lower CP diets may have had an influence on gain.

Increasing CP in the feeds provided from 0-8 weeks of age led to linear improvement in body weight gain of birds (Table 10). A similar increase also occurred in feed intake and resulted in improved feed conversion to gain. The advantage in feed conversion from increasing CP appeared to maximize once the 21-

18-16% CP regimen was attained, possibly from adequacy in EAA levels compared to the lower 20-17-15% CP diet. Similar to previous observations regarding supplemental NEAA to broiler feed (Fancher and Jensen, 1989a,b; Han et al., 1992; Aletor et al., 2000), addition of gelatin itself had no effect on gain, but feed conversion improved. Results suggest that increasing NEAA, particularly glycine and proline, can improve performance of vaccinated birds in diets with CP below NRC (1994) recommendations while maintaining EAA requirements.

Processing the broilers into commercial parts provided another look at treatment effects. Reduction in number of birds by one half based on selection of alternate wing band numbers is assumed to be representative of the original population. Providing CP in a linear manor increased overall carcass, fillet, and tender weights while reducing abdominal fat (Tables 11 and 12). Gelatin inclusion at each level of CP solely resulted in additional abdominal fat likely from improved nutrient recovery. Percentage of carcass from live weight linearly increased with CP; however, addition of gelatin compared to no gelatin reduced the need for CP when below 22.16% (% LW = 66.61 + .201 CP) *vs.* (% LW = 64.46 + .298 CP).

Increasing CP improved live performance and carcass meat yield of vaccinated broilers as well as reduced body fat. Inclusion of gelatin provided similar improvements in live performance as CP increased; however, it increased abdominal fat as skinless boneless breast meats remained essentially unaltered. Gelatin

predominately provided additional proline and glycine and increasing CP elevated proportions of total NEAA. When given diets adequate in EAA, broilers vaccinated for coccidiosis appeared to benefit from additional NEAA, such as those incapable of rapid *de novo* synthesis.

Table 2.1 Composition of experimental starter diets having increasing CP with and without gelatin (% "as is" basis) fed from 0-3 weeks of age

	20%		21%		22%		23%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Corn	58.3	60.0	55.1	56.8	52.0	53.7	48.9	50.4
SBM	31.5	27.1	34.3	29.9	37.0	32.7	39.8	35.6
Poultry Fat	5.40	5.90	5.90	6.40	6.41	6.90	6.90	7.46
L-Lysine HCl	0.33	0.38	0.24	0.28	0.14	0.19	0.04	0.08
DL-Methionine	0.37	0.38	0.36	0.37	0.34	0.35	0.33	0.34
L-Tryptophan	0	0.03	0	0.03	0	0.05	0	0
L-Threonine	0	0.05	0	0.08	0	0.04	0	0
Dical. Phosphate	1.76	1.81	1.74	1.79	1.72	1.77	1.7	1.75
Limestone	1.41	1.41	1.41	1.41	1.40	1.40	1.40	1.40
Salt	0.45	0.43	0.45	0.43	0.45	0.45	0.45	0.43
Gelatin[1]	0	2.00	0	2.00	0	2.00	0	2.00
Constant[2]	---------- to 100% ----------							
Calc. Kcal ME/kg	----------3200----------							

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 2.2 Amino acid composition of experimental diets fed from 0-3 weeks of age (88% DM basis)[1]

	20%		21%		22%		23%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Crude Protein	20.25	20.36	22.47	21.02	22.09	21.61	22.85	22.65
				Essential Amino Acids				
Methionine	0.63	0.63	0.65	0.63	0.62	0.62	0.63	0.63
Cystine	0.30	0.29	0.32	0.29	0.33	0.30	0.34	0.32
Meth + cystine	0.93	0.91	0.97	0.92	0.95	0.92	0.97	0.95
Lysine	1.30	1.26	1.36	1.30	1.29	1.24	1.30	1.27
Threonine	0.74	0.75	0.81	0.81	0.83	0.80	0.88	0.82
Arginine	1.28	1.30	1.41	1.36	1.46	1.39	1.54	1.52
Isoleucine	0.75	0.75	0.84	0.78	0.87	0.81	0.93	0.90
Leucine	1.61	1.58	1.72	1.62	1.77	1.65	1.85	1.76
Valine	0.83	0.84	0.92	0.87	0.95	0.90	1.01	0.99
Phenylalanine	0.91	0.89	1.00	0.92	1.02	0.95	1.09	1.02
Histidine	0.51	0.49	0.56	0.51	0.57	0.52	0.61	0.56
				Nonessential Amino Acids				
Proline	1.13	1.24	1.15	1.33	1.16	1.26	1.20	1.34
Glycine	0.80	1.04	0.86	1.15	0.89	1.14	0.94	1.24
Serine	0.97	0.95	1.05	0.97	1.07	0.99	1.13	1.06
Alanine	0.96	1.02	1.01	1.07	1.04	1.07	1.08	1.14
Aspartic Acid	1.95	1.90	2.14	1.96	2.22	2.03	2.35	2.21
Glutamic Acid	3.41	3.36	3.71	3.47	3.83	3.55	4.03	3.83

[1]Values for each feed represent one analysis.

Table 2.3 Live performance of broilers receiving feeds having increasing CP with and without supplemental gelatin from 0-3 weeks of age[1]

% CP	Gelatin	Gain (g)	Feed Intake (g)	F/G	% Mort[2]
20	--	854	1304	1.54	3.4
21	--	842	1354	1.61	3.6
22	--	902	1396	1.55	3.1
23	--	888	1517	1.70	3.3
P≤	--	*	***	**	NS
L	--	0.0165	0.0001	0.0068	-
Q	--	NS	NS	NS	-
C	--	0.0354	NS	0.0360	-
SEM	--	14.7	34.3	0.035	0.99
--	No	859	1425	1.66	3.2
--	Yes	884	1360	1.54	3.5
--	P≤	NS	NS	**	NS
--	SEM	10.4	24.2	0.025	0.70

[1]Values represent contrasts involving a total of 64 pens, each with 25 chicks averaging 42g at start of experimentation. Only main factor contrasts are given as $P > 0.05$ for CP X G. Regression analysis is provided for CP measurements when $P < 0.05$ (L= linear; Q = quadratic; C = cubic).

[2] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 2.4 Composition of experimental grower diets having increasing CP with and without gelatin (% "as is" basis) fed from 3-6 weeks of age

	17%		18%		19%		20%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Corn	68.9	70.5	65.7	67.3	62.5	64.1	59.2	60.9
SBM	23.1	18.8	26.0	21.7	28.9	24.6	31.8	27.5
Poultry Fat	3.42	3.94	3.94	4.47	4.47	4.97	4.99	5.50
L-Lysine HCl	0.43	0.48	0.33	0.38	0.23	0.27	0.13	0.17
DL-Methionine	0.36	0.37	0.34	0.35	0.33	0.34	0.32	0.32
L-Tryptophan	0.05	0.07	0.04	0.06	0.02	0.04	0.00	0.02
L-Threonine	0.14	0.18	0.10	0.13	0.06	0.09	0.01	0.04
Dical. Phosphate	1.27	1.32	1.25	1.30	1.23	1.28	1.21	1.26
Limestone	1.47	1.47	1.47	1.46	1.46	1.46	1.46	1.46
Salt	0.38	0.36	0.38	0.36	0.38	0.36	0.38	0.36
Gelatin[1]	0	2.00	0	2.00	0	2.00	0	2.00
Constant[2]	---------- to 100% ----------							
Calc. Kcal ME/kg	----------3200----------							

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 2.5 Amino acid composition of experimental diets fed from 3-6 weeks of age (88% DM basis)[1]

	17%		18%		19%		20%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Crude Protein	17.29	17.88	18.35	18.61	19.39	19.56	20.28	20.54
Essential Amino Acids								
Methionine	0.57	0.61	0.58	0.60	0.58	0.58	0.59	0.59
Cystine	0.27	0.24	0.27	0.26	0.30	0.27	0.30	0.29
Meth + cystine	0.83	0.85	0.85	0.86	0.88	0.85	0.89	0.88
Lysine	1.13	1.19	1.17	1.18	1.18	1.16	1.16	1.18
Threonine	0.74	0.73	0.74	0.75	0.77	0.75	0.76	0.76
Arginine	1.07	1.09	1.14	1.17	1.25	1.25	1.32	1.36
Isoleucine	0.68	0.64	0.73	0.66	0.76	0.72	0.85	0.79
Leucine	1.46	1.37	1.51	1.45	1.59	1.54	1.69	1.60
Valine	0.77	0.75	0.82	0.76	0.85	0.82	0.94	0.89
Phenylalanine	0.79	0.74	0.83	0.79	0.89	0.84	0.95	0.90
Histidine	0.44	0.41	0.46	0.44	0.50	0.47	0.53	0.50
Nonessential Amino Acids								
Proline	0.99	1.13	1.04	1.26	1.13	1.31	1.15	1.35
Glycine	0.68	1.05	0.72	1.11	0.77	1.17	0.83	1.22
Serine	0.80	0.74	0.82	0.84	0.92	0.89	0.94	0.94
Alanine	0.87	0.95	0.89	1.00	0.93	1.05	0.99	1.09
Aspartic Acid	1.59	1.51	1.71	1.64	1.87	1.77	2.00	1.91
Glutamic Acid	2.94	2.79	3.09	3.02	3.31	3.21	3.53	3.42

[1]Values for each feed represent one analysis.

Table 2.6 Live performance of broilers receiving feeds of increasing CP with and without gelatin fed from 3-6 weeks of age[1]

% CP	Gelatin	Gain (g)	Feed Intake (g)	F/G	% Mort[1]
20-17	--	1509	2646	1.76	0.8
21-18	--	1617	2629	1.63	2.1
22-19	--	1582	2639	1.67	2.6
23-20	--	1633	2611	1.60	3.2
P≤	--	**	NS	***	NS
L	--	0.0004	-	0.0001	-
Q	--	NS	-	NS	-
C	--	0.0130	-	0.0031	-
SEM	--	19.9	28.3	0.021	0.82
--	No	1617	2704	1.68	2.0
--	Yes	1554	2558	1.65	2.4
--	P≤	**	***	NS	NS
--	SEM	14.1	20.0	0.015	0.58

[1]Values represent contrasts involving a total of 64 pens, each with 25 chicks averaging 42g at start of experimentation. Only main factor contrasts are given as $P > 0.05$ for CP X G. Regression analysis is provided for CP measurements when $P < 0.05$ (L= linear; Q = quadratic; C = cubic).

[2] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 2.7 Composition of experimental finisher diets having increasing CP with and without gelatin (% "as is" basis) fed from 6-8 weeks of age

	15%		16%		17%		18%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Corn	75.6	77.2	72.4	74.0	69.2	70.8	66.0	67.5
SBM	18.1	13.7	20.9	16.7	23.8	19.6	26.7	22.5
Poultry Fat	2.20	2.70	2.70	3.23	3.21	3.74	3.74	4.28
L-Lysine HCl	0.35	0.40	0.25	0.30	0.15	0.19	0.05	0.09
DL-Methionine	0.36	0.37	0.32	0.33	0.30	0.29	0.29	0.28
L-Tryptophan	0.15	0.18	0.11	0.14	0.06	0.09	0.02	0.04
L-Threonine	0.04	0.08	0.05	0.07	0.03	0.05	0.01	0.02
Dical. Phosphate	1.03	1.07	1.01	1.06	0.99	1.04	0.97	1.02
Limestone	1.37	1.37	1.37	1.36	1.36	1.36	1.36	1.35
Gelatin[1]	0	2.00	0	2.00	0	2.00	0	2.00
Constant[2]	---------- to 100% ----------							
Calc. Kcal ME/kg	----------3200----------							

[1]60 bloom pure beef gelatin, Vyse Gelatin Company, Inc. 5010 N. Rose St. Schiller Park, IL 60176.

[2]Constant included 0.40% salt and 0.25% each vitamin & mineral premixes (provided the following per kg of diet: 7355 IU Vitamin A, 2200 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 36 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.05 mg biotin, 500 mg choline, 65 mg Mn, 55 mg Zn, 55 mg Fe, 6 mg Cu, 0.3 mg Se, 1 mg I).

Table 2.8 Amino acid composition of experimental diets fed from 6-8 weeks of age (88% DM basis)[1]

	15%		16%		17%		18%	
	control	w/ gel	control	w/ gel	control	w/ gel	control	w/ gel
Crude Protein	15.33	15.18	15.94	16.18	16.98	17.11	18.04	18.20
Essential Amino Acids								
Methionine	0.62	0.54	0.55	0.55	0.53	0.52	0.55	0.52
Cystine	0.25	0.23	0.25	0.23	0.28	0.24	0.28	0.26
Meth + cystine	0.87	0.76	0.80	0.78	0.81	0.76	0.83	0.77
Lysine	1.01	0.97	0.97	0.97	1.00	0.96	0.98	0.96
Threonine	0.69	0.65	0.68	0.67	0.71	0.68	0.70	0.68
Arginine	0.95	0.93	0.98	1.01	1.12	1.09	1.17	1.18
Isoleucine	0.58	0.51	0.61	0.57	0.70	0.60	0.72	0.68
Leucine	1.32	1.26	1.37	1.32	1.50	1.38	1.53	1.46
Valine	0.67	0.61	0.70	0.67	0.79	0.70	0.81	0.78
Phenylalanine	0.70	0.64	0.73	0.68	0.82	0.73	0.84	0.79
Histidine	0.41	0.37	0.41	0.39	0.46	0.42	0.47	0.44
Nonessential Amino Acids								
Proline	0.94	1.12	0.96	1.18	1.06	1.22	1.06	1.24
Glycine	0.62	0.96	0.63	1.03	0.71	1.07	0.74	1.10
Serine	0.74	0.70	0.75	0.72	0.84	0.80	0.88	0.83
Alanine	0.80	0.89	0.82	0.93	0.89	0.97	0.91	1.00
Aspartic Acid	1.40	1.27	1.47	1.37	1.68	1.51	1.76	1.65
Glutamic Acid	2.63	2.47	2.72	2.61	3.06	2.81	3.16	3.02

[1]Values for each feed represent one analysis.

Table 2.9 Live performance of broilers receiving feeds of increasing CP with and without gelatin from 6-8 weeks of age[1]

% CP	Gelatin	Gain (g)	Feed Intake (g)	F/G	% Mort[2]
Main Contrasts					
20-17-15	--	1552	3671	2.37	2.5
21-18-16	--	1641	3761	2.29	4.6
22-19-17	--	1627	3764	2.34	7.2
23-20-18	--	1669	3787	2.27	4.4
P≤	--	**	NS	NS	*
L	--	0.0023	-	-	NS
Q	--	NS	-	-	0.0364
C	--	NS	-	-	NS
SEM	--	23.6	46.4	0.043	1.12
--	No	1626	3836	2.37	4.7
--	Yes	1618	3655	2.27	4.7
--	P≤	NS	***	*	NS
--	SEM	16.7	32.8	0.030	0.79
Interactions (P<0.05)					
20-17-15	No	1613	3782	2.35	2.9
21-18-16	"	1656	3860	2.34	5.0
22-19-17	"	1589	3880	2.47	7.1
23-20-18	"	1648	3823	2.32	3.9
L		NS	-	-	-
Q		NS	-	-	-
C		NS	-	-	-
20-17-15	Yes	1492	3560	2.40	2.2
21-18-16	"	1626	3661	2.25	4.1
22-19-17	"	1665	3649	2.20	7.4
23-20-18	"	1691	3750	2.22	4.9
L		0.0002	-	-	-
Q		NS	-	-	-
C		NS	-	-	-
P≤		*	NS	NS	NS
SEM		33.4	65.6	0.060	1.58

[1]Values represent contrasts involving a total of 64 pens, each with 25 chicks averaging 42g at start of experimentation. Only main factor contrasts are given as P > 0.05 for CP X G. Regression analysis is provided for CP measurements when P < 0.05 (L= linear; Q = quadratic; C = cubic).

[2] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

NS = P > 0.05; * = P <0.05; ** = P < 0.01; *** = P < 0.001

Table 2.10 Overall live performance of broilers receiving feeds of increasing CP with and without gelatin from 0-8 weeks of age[1]

% CP	Gelatin	Gain (g)	Feed Intake (g)	F/G	% Mort[2]
20-17-15	--	3915	7622	1.95	6.8
21-18-16	--	4101	7743	1.89	9.7
22-19-17	--	4111	7799	1.90	12.4
23-20-18	--	4191	7914	1.89	10.6
P≤	--	***	*	*	NS
L	--	0.0001	0.0055	0.0177	-
Q	--	0.0431	NS	NS	-
C	--	0.0381	NS	NS	-
SEM	--	25.7	71.8	0.015	1.58
--	No	4102	7966	1.94	9.6
--	Yes	4056	7573	1.87	10.2
--	P≤	NS	***	***	NS
--	SEM	18.1	50.8	0.011	1.12

[1]Only main factor contrasts are given as P > 0.05 for CP X G. Regression analysis is provided for CP measurements when P < 0.05 (L= linear; Q = quadratic; C = cubic).

[2] Mortality percentages were statistically analyzed as the transformed value (arcsine √%), whereas SEM values are estimates based on actual data.

NS = P > 0.05; * = P <0.05; *** = P < 0.001

Table 2.11 Carcass composition and yield of broilers receiving feeds of increasing CP with and without gelatin[1]

% CP	Gelatin	Carcass w/o fat		Abdominal Fat	
		Weight (g)	% Live Wt.	Weight (g)	% Carcass
Main Contrasts					
20-17-15	--	2811	70.45	76.9	2.65
21-18-16	--	2958	70.87	73.6	2.43
22-19-17	--	2967	71.14	73.9	2.43
23-20-18	--	3020	71.27	66.1	2.14
P≤	--	***	*	***	***
L		0.0001	0.0036	0.0001	0.0001
Q		0.0423	NS	NS	NS
C		NS	NS	NS	0.0256
Average SEM		23.2	0.205	1.66	0.050
--	No	2951	71.02	71.2	2.36
--	Yes	2927	70.85	74.0	2.47
--	P≤	NS	NS	NS	*
Average SEM		16.4	0.145	1.47	0.036
Interactions (P<0.05)					
20-17-15	No	2829	70.23	72.6	2.49
21-18-16	"	2982	70.94	74.1	2.44
22-19-17	"	2992	71.75	73.4	2.40
23-20-18	"	3002	71.17	64.6	2.11
L		-	0.0032	-	-
Q		-	NS	-	-
C		-	NS	-	-
20-17-15	Yes	2792	70.68	81.2	2.82
21-18-16	"	2935	70.81	73.1	2.44
22-19-17	"	2941	70.54	74.4	2.45
23-20-18	"	3038	71.36	67.5	2.18
L		-	0.0277	-	-
Q		-	NS	-	-
C		-	NS	-	-
P≤		NS	*	NS	NS
Average SEM		32.8	0.290	2.34	2.412

[1]Values are associated with approximately 12 carcasses from each of 64 pens. Regression analysis is provided for *CP* and *CP* X *G* when ANOVA P <0.05 (L= linear; Q= quadratic; C= cubic).

NS = P>0.05; * = P<0.05; *** = P<0.001

Table 2.12 Skinless, boneless breast meat yield of broilers receiving feeds of increasing CP with and without gelatin[1]

CP	Gelatin	Fillet		Tender	
		Weight (g)	% Carcass	Weight (g)	% Carcass
20-17-15	--	717	25.5	150	5.3
21-18-16	--	777	26.0	161	5.4
22-19-17		778	26.1	164	5.5
23-20-18	--	798	26.4	165	5.4
P≤	--	***	*	***	NS
L	--	0.0001	0.0021	0.0001	-
Q	--	NS	NS	0.0223	-
C	--	NS	NS	NS	-
Average SEM		10.8	0.20	2.3	0.06
--	No	766	25.9	161	5.4
--	Yes	769	26.1	159	5.4
--	P≤	NS	NS	NS	NS
Average SEM		7.6	0.14	1.6	0.04

[1] Values are associated with approximately 8 carcasses from each of 64 pens. Regression analysis is provided for *CP* and *CP* X *G* when ANOVA P <0.05 (L= linear; Q= quadratic; C= cubic).

NS = P>0.05; * = P<0.05; *** = P<0.001

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V. CONCLUSIONS

Vaccinating broilers for coccidiosis is known to negatively affect performance compared to those receiving a coccidiostat; however, performance of vaccinated birds can be improved when a high crude protein (CP) diet is fed. Availability of nonessential amino acids in higher CP diets is believed to improve performance by facilitating production of mucin that follows infection. The previous experiments were performed to determine if the nonessential amino acids proline and glycine provided by gelatin and increased crude protein improve performance of vaccinated broilers.

The first experiment was designed to determine if vaccination impairs live performance and carcass yield more than a coccidiostat. In addition, low and high CP levels were tested to examine if gelatin could improve performance of vaccinated broilers on low protein diets to the level of birds given the high protein diets. It was concluded that vaccination impaired live performance, especially during the first three weeks, which is assumed to occur from intestinal mucosal damage and reduced nutrient recovery, much like an actual infection. Inclusion of gelatin provided the bird with additional proline and glycine, which facilitated early recovery presumably by providing access to the amino acids that are unable to be rapidly synthesized. By 8

weeks, however, vaccinated birds still had reduced live body weight and carcass weight. Addition of gelatin was successful at improving live performance of broilers receiving a low CP diet, but carcass yield was not improved with addition of gelatin.

After gelatin was found to improve the performance of broilers fed a low CP diet, a second experiment was conducted on vaccinated broilers to determine the level of CP where addition of gelatin is no longer beneficial. This level of CP was assumed to represent the optimal level of NEAA provided by gelatin and CP. Increasing CP improved live body weight gain of birds at each phase from 0-8 weeks seemingly by increasing the availability of nonessential amino acids. Gelatin provided little advantage in gain, but improved efficiency of feed to gain substantially. Carcass, breast fillet, and tender weights were increased while abdominal fat was reduced by increasing dietary CP; however, gelatin only increased abdominal fat without affecting meat yield.

Specific estimates of NEAA requirements could not be established, as gelatin seemed to provide an advantage at each CP level. Sub-marginal EAA in the diets with low CP in the second experiment as well as extent of infection from vaccination may have had an effect on performance. However, it can be concluded that, when given diets adequate in EAA, coccidiosis-vaccinated broilers benefit from additional NEAA, especially proline and glycine, provide by gelatin and crude protein.