

EFFECTS OF LOW CRUDE PROTEIN DIETS WITH AMINO ACID
SUPPLEMENTATION ON BROILER PERFORMANCE IN THE STARTER PERIOD

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by
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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

**EFFECTS OF LOW CRUDE PROTEIN DIETS WITH AMINO ACID
SUPPLEMENTATION ON BROILER PERFORMANCE IN THE STARTER
PERIOD**

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LIST OF TABLES

Table	Page
1. Effect of Low Crude Protein Diets on Live Performance of Broilers.....	26
2. Amino Acid Levels of 15% Crude Protein Diets Experiments 1 and 2.....	34
3. Composition of Experimental Diets; Experiments 1 and 2.....	35
4. Performance of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1.....	36
5. Performance of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2.....	40
6. Amino Acid Levels of Experimental Diets for Broilers fed 13% Crude Protein; Experiment 1.....	55
7. Composition of Experimental Diets for Broilers fed 13% Crude Protein; Experiment 1.....	56
8. Composition of Experimental Diets for Broilers fed 13% Crude Protein; Experiment 2.....	57
9. Performance of Broiler Chicks Fed a 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1.....	58
10. Performance of Broiler Chicks Fed a 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2.....	62
11. Composition of Diets for Broilers Consuming 22% or 15% Crude Protein with the Addition or Exclusion of Meat and Bone Meal.....	72
12. Performance of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age.....	73
13. Composition of Diets for Broilers Consuming 15% Crude Protein Diets with or without Additional Dietary Fat	83
14. Performance of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age.....	84

LIST OF FIGURES

Figure	Page
1. Body Weight Gain (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1.....	37
2. Feed Intake (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1.....	38
3. Feed:Gain of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1.....	39
4. Body Weight Gain (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2.....	41
5. Feed Intake (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2.....	42
6. Feed:Gain of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2.....	43
7. Body Weight Gain (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1.....	59
8. Feed Intake (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1.....	60
9. Feed:Gain of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1.....	61
10. Body Weight Gain (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2.....	63
11. Feed Intake (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2.....	64
12. Feed:Gain of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2.....	65
13. Body Weight Gain (g) of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age.....	74
14. Feed Intake (g) of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age.....	75

15. Feed:Gain of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age.....	76
16. Body Weight Gain (g) of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age.....	85
17. Feed Intake (g) of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age.....	86
18. Feed:Gain of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age.....	87

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	iv
LIST OF FIGURES.....	v
CHAPTER	
1. LITERATURE REVIEW.....	1
Introduction.....	1
Protein and Amino Acids in Poultry Nutrition.....	3
<i>Essential versus Nonessential Amino Acids</i>	4
Protein and Amino Acid Requirements.....	6
<i>Factors Affecting Protein and Amino Acid Requirements</i>	6
<i>Amino Acid Interactions, Imbalances, and Antagonisms</i>	8
<i>Limiting Amino Acids for Poultry</i>	11
<i>Amino Acid Requirements and the Protein and Energy</i> <i>Relationship</i>	13
<i>Amino Acid Requirements as Related to Protein Level</i>	14
Amino Acid Digestibility.....	15
The Ideal Protein Concept.....	16
Low Protein Diets.....	19
Summary.....	24
2. EFFECT OF 15% CRUDE PROTEIN CORN AND SOYBEAN MEAL DIETS WITH AMINO ACID SUPPLEMENTATION ON BROILERS IN THE STARTER PERIOD.....	27
Abstract.....	27

Introduction.....	28
Materials and Methods.....	29
Results and Discussion.....	31
3. EFFECTS OF 13% CRUDE PROTEIN CORN AND SOYBEAN MEAL DIETS WITH AMINO ACID SUPPLEMENTATION ON BROILERS IN THE STARTER PERIOD.....	44
Abstract.....	44
Introduction.....	46
Materials and Methods.....	47
Results and Discussion.....	50
4. EFFECTS OF LOW CRUDE PROTEIN DIETS WITH AND WITHOUT THE ADDITION OF MEAT AND BONE MEAL ON BROILERS IN THE STARTER PERIOD.....	66
Abstract.....	66
Introduction.....	67
Materials and Methods.....	68
Results and Discussion.....	70
5. EFFECTS OF LOW CRUDE PROTEIN RATIONS WITH VARIOUS LEVELS OF FAT ADDITION ON BROILERS IN THE STARTER PERIOD.....	77
Abstract.....	77
Introduction.....	78
Materials and Methods.....	79
Results and Discussion.....	81
6. SUMMARY.....	88
REFERENCES.....	90

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Worldwide poultry production has increased significantly over the past fifty years to accommodate rising demand. Broilers make up a large part of the industry with chicken meat accounting for 86% of the world poultry meat output (Economic Research Service/USDA, 2001). In the United States, the broiler industry has also experienced impressive growth. Chicken consumption on a per capita basis has more than tripled with an average of 86 pounds consumed in 2006 versus 28 pounds in 1960, as chicken products became more convenient and diverse through further processing techniques while remaining a healthy, affordable alternative to pork and beef products. However, although annual broiler production in terms of pounds of meat continues to rise, annual production growth has decreased since the mid-1990s. New challenges to the industry like the slowing of production and productivity growth and rising feed and energy costs have led to increases in retail prices for poultry products that more closely follow increases for other food prices than they have in the past (MacDonald, 2008). In order to maintain growth and profitability, it is becoming increasingly important to find new ways to stay competitive within the industry and decrease the costs of production as much as possible while producing a high quality product for consumers.

Meeting the nutritional requirements for growing birds constitutes the majority of costs associated with poultry production (May *et al.*, 1998), accounting for around 75 percent of the expense (Nakaue and Arscott, 1991), and certainly is becoming an issue of even greater significance as the prices of feed ingredients continue to rise. A large portion of that cost involves meeting the amino acid requirements of the birds (Corzo *et al.*, 2004; Firman and Boling, 1998; Eits *et al.*, 2005; Firman, 1994). By reducing the level of crude protein in the diet it is possible to achieve significant cost savings. Firman (1994) reported that it is possible to save five dollars per ton of feed by reducing the protein level in the diet of turkeys by one percent. In addition to reducing feed costs, the ability to lower crude protein in the diet can result in decreased nitrogen excretion (Kidd *et al.*, 1996; Ferguson *et al.*, 1998; Nahm, 2002; Namroud, *et al.*, 2008), improved ability to cope with heat stress, and allow for the use of a greater variety of feedstuffs (Kidd *et al.*, 1996), which can be valuable in itself as a method to increase flexibility in the choice of locally available feedstuffs, potentially decreasing transportation costs.

As advances in feeding and formulation techniques have been made, it has become a relatively easy task in areas such as the United States that have access to a variety of quality feed ingredients to meet the nutritional requirements of poultry. Compared to many other industries, the poultry industry is considerably advanced in terms of understanding how to feed birds to meet maximum growth. Indeed, one needs only to feed all nutrients in excess to meet such growth, although this is a far from ideal method for a variety of reasons, including efficiency and expense. As a result, the current goal has shifted away from just feeding to reach certain growth standards to meeting maximum growth in the most cost efficient manner, or finding the least cost per unit of

gain. Developing feeding programs that utilize concepts such as ideal protein, formulation programs that calculate the ingredient combinations that will closely meet the birds' nutritional requirements at the least possible cost (Pesti and Miller, 1992), digestible amino acid values, and crystalline amino acid supplementation has allowed the poultry industry to reduce dietary crude protein to decrease excess amounts of amino acids and the cost of rations (Kidd *et al.*, 1996). However, the lowest level to which crude protein can be reduced with amino acid supplementation in broiler diets without reducing bird performance is still unknown, and additional research on the subject could yield significantly greater cost savings in the future.

PROTEIN AND AMINO ACIDS IN POULTRY NUTRITION

Protein is a critical component of poultry rations, and along with the other major nutrient classes of carbohydrates, fats, vitamins, minerals, and water, is essential for life (Cheeke, 2005; North and Bell, 1990, Pond *et al.*, 1995). Proteins are polymers that are made up of α -amino acids in covalent linkage by peptide bonds, and the structure of individual α -amino acids includes an amino group and a carboxyl group linked to an α -carbon, as well as a side chain that differs for each amino acid (Perry *et al.*, 2003). Intact proteins are broken down by hydrolysis during digestion to yield these amino acids, which are then utilized in the body to fulfill a variety of functions in poultry, including as structural components of skin, feathers, and muscle, as well as filling important metabolic

roles as blood plasma proteins, enzymes, hormones, and immune antibodies which are all individually involved in specific functions in the body (Pond *et al.*, 1995).

Although it was recognized early on that at least a few individual amino acids played an important role in animal performance, a majority of the preliminary research in poultry nutrition was conducted with the purpose of establishing minimum levels of protein that would support performance. At the University of Missouri, Funk (1932) provided chicks with a free selection of diets that varied in protein content, and found that an 18-19% protein diet was chosen with significantly greater frequency. Other research investigated levels of protein that allowed maximal growth. Swift and coworkers (1931) reported maximal weight gain in chicks fed a 22.9% crude protein ration, while Carver and coworkers (1932) found the greatest weight gain occurred when chicks consumed a diet containing 18% crude protein. Similar results were seen in a trial in which chicks fed a 20% CP diet exhibited superior performance compared to other treatments (Norris and Heuser, 1930). All of these groups saw a significant reduction in the performance of birds fed low protein rations, a decreased requirement as the birds aged, and a decreased efficiency of protein and overall feed utilization as the birds aged. The variation in reported protein levels for optimal growth in this early literature may be partially due to differences in the protein levels tested, as well as factors such as protein source or other dietary nutrient and ingredient levels that may have differed among experiments.

Essential versus Nonessential Amino Acids

The National Research Council (1994) states that poultry do not actually have a protein requirement as much as an amino acid requirement, and it is these amino acids

that are used in the body for a variety of functions. While all of the twenty amino acids commonly present in proteins are required in the body, only some, termed the essential or indispensable amino acids, must be provided in the diet. The importance of amino acids as opposed to crude protein in nutrition and feeding of animals has been recognized and investigated since the early twentieth century (Osborne and Mendel, 1914), as well as the effects of reduced levels of specific essential amino acids on performance. The work of Buckner and coworkers (1915) demonstrated the essentiality of lysine for broiler chicks, reporting significantly decreased growth and activity in chicks fed grain diets low in lysine. Similar results were reported in rats fed rations deficient in lysine and tryptophan (Osborne and Mendel, 1916). Essential amino acids are those that cannot be synthesized by the body either at all or in amounts great enough to meet physiological need, and include valine, tryptophan, threonine, isoleucine, leucine, lysine, arginine, histidine, phenylalanine, methionine and, in some cases for poultry, glycine and serine (Cheeke, 2005). The nonessential amino acids, which include proline, alanine, cystine, tyrosine, glutamic acid and aspartic acid, can be synthesized from other amino acids or nitrogen in the bird, and so are not necessarily required in the diet. However, it is important to keep in mind that if the diet does not provide nonessential amino acids or an additional source of nitrogen, the nonessentials must be synthesized from other amino acids including those that are essential. Therefore, in order to prevent deficiencies in either essential or nonessential amino acids, it is common to provide an overall protein requirement as well as requirements for the essential amino acids when formulating rations (NRC, 1994).

PROTEIN AND AMINO ACID REQUIREMENTS

Nutrient requirements are often defined as the minimum dietary concentration required for maximum performance, and nutritionists frequently provide a margin of safety above the requirement when formulating to avoid deficiencies and the resultant decreases in performance (Sterling *et al.*, 2005). A protein deficiency, caused by either one or more limiting amino acids or an overall inadequate consumption of protein, will result in decreases in parameters such as rate of growth, nitrogen retention, feed consumption and feed utilization (Church, 1991), while an over-consumption of protein results in the catabolism of amino acids through deamination and excretion as uric acid which is both energetically and economically inefficient (Sklan and Plavnik, 2002), or, in severe cases, ammonia toxicity (Perry *et al.*, 2003). It is essential to try to meet the requirement of the bird as closely as possible in order to maximize production and profitability.

Factors Affecting Protein and Amino Acid Requirements

A number of factors influence the protein and amino acid requirements of poultry. Changes in requirements occur with bird variation in age, gender, production status, size, species, and strain (Samadi and Liebert, 2006; Kidd *et al.*, 2005; NRC, 1994), as well as variation in protein quality and digestibility. Temperature may also affect the amino acid requirements, as intake is often decreased in excessive heat and increased during periods of cold (Hurwitz *et al.*, 1980; Furlan *et al.*, 2004). As a result, adjustments to amino acid levels in the ration may be required in periods of heat and cold stress, although

contradictions concerning this approach exist in the literature. Research conducted by Cheng and others (1997a), in which the effects of feeding increased levels of protein in response to decreased feed intake in heat-stressed male broiler chicks was investigated, reported that elevated temperatures significantly decreased body weight gain, feed intake, and feed conversion, and that an increase in dietary protein and amino acids further depressed performance. When the effects of increasing essential amino acid levels to 110% of the expected requirement while maintaining constant crude protein were tested, researchers found no differences in live performance; however, abdominal fat was increased in the treatment receiving increased levels of amino acids suggesting an improvement in recovery of productive energy from the dietary metabolizable energy, which was deposited as fat rather than muscle (Zarate *et al.*, 2003). Additional research by Cheng and coworkers (1997b) found that feeding lower crude protein diets with methionine, lysine, threonine, tryptophan, and arginine supplementation did not improve weight gain of heat-stressed broilers and produced negative effects on feed conversion and body fat deposition, suggesting that other amino acids might be limiting. However, other research indicates that reducing the heat increment of the diet by reducing crude protein and providing a well-balanced amino acid supply that closely matches the requirement of the bird can alleviate the poor performance associated with heat stress (Gous and Morris, 2005), and that the weight gain and feed efficiency of heat-stressed birds can be improved with low crude protein diets that are properly balanced for amino acids, with no excess (Waldroup *et al.*, 1976). The lack of agreement on this subject may be at least partially due to differences in the degree of severity and the timing of the heat-stress applied to the birds in many experiments (Gonzalez-Esquerria and Leeson, 2006).

Other factors that affect feed consumption, and consequently amino acid consumption, include health status of the bird, the form of the feed, (such as mash versus pellets) (Maiorka, *et al.*, 2005), and a variety of environmental stressors. Fortunately, poultry in the United States are typically grown under fairly standard conditions using relatively similar basic diets regardless of the area, which allows for more refined dietary requirements than might be seen in other types of livestock production (Church and Varela-Alvarez, 1991).

Amino Acid Interactions, Imbalances, and Antagonisms

When attempting to meet the amino acid requirements of poultry, the interactions between amino acids are an important consideration, and may result in imbalances or antagonisms (Harper, 1956). A deficiency of one amino acid is enough to cause problems with the entire diet, and birds may attempt to make up for the deficiency by consuming more feed, thereby reducing the efficiency of the diet (Almquist, 1952). Conversely, an amino acid imbalance arises with changes in the proportion of amino acids in the ration, usually because one amino acid will be deficient and others provided in excess (Boorman and Burgess, 1986). Imbalances cause deleterious effects in performance resulting from reduced feed intake likely due to changes in the pattern of amino acids in the plasma which may affect satiety, and may be overcome by supplementation of the most limiting amino acid(s) (Harper, 1958; Pond *et al.*, 1995).

Two main theories have been proposed to more precisely explain the mechanisms behind the reduced feed intake seen in birds consuming imbalanced rations. The anabolic theory, proposed by Harper and Rogers (1965), suggests that an excess of amino acids

will stimulate synthesis or suppress breakdown of protein in the liver, leaving more of the limiting amino acid there. This then leads to a reduced concentration of the first-limiting amino acid in the blood. The resulting altered amino acid pattern may be detected by the brain, consequently depressing feed intake (Austic, 1986). An alternative explanation is presented in the catabolic theory, proposed by Lewis and D'Mello (1967). This theory states that an excess of one amino acid will enhance the catabolism and excretion of the other amino acids and consequently encourage loss of the target amino acid, which disrupts the pattern of free amino acids in the plasma and tissue causing reduced intake and performance.

The effect of amino acid imbalance on feed intake and consequent performance has been demonstrated experimentally by Sugahara and coworkers (1969), who found that consumption of poorly balanced diets containing individual amino acid deficiencies was decreased, while consumption of diets with a proper balance of amino acids, even included at deficient levels, was no different from that of the control treatment. Imbalances have been observed in some cases with studies utilizing low protein diets in which the protein became unbalanced due to the addition of amino acids or an unbalanced protein (Harper, 1958); however, it may be possible to improve the overall amino acid balance and reduce crude protein level in poultry diets with careful addition of synthetic amino acids (Waldroup *et al.*, 2005a).

Amino acid antagonisms involve interactions in which an increase in the requirement of one indispensable amino acid results from the addition of another amino acid that is structurally related (Harper, 1956). Common examples of amino acid antagonisms in poultry include the lysine and arginine antagonism (O'Dell and Savage,

1966; Austic and Scott, 1975), and the branched-chain amino acid antagonisms between leucine, isoleucine, and valine (Smith and Austic, 1978). The interaction between lysine and arginine arises from excessive lysine in relation to arginine, increasing the requirement for arginine through intensified competition for reabsorption in the renal tubules and enhanced activity of renal arginase, which degrades arginine to ornithine and urea (Austic and Scott, 1975). This type of interaction is very difficult to create using practical ingredients. Casein is one of a very small number of ingredients that contain a significantly greater amount of lysine compared to arginine (Waldroup, 2002).

Branched-chain amino acid antagonisms typically result from excessive levels of leucine inhibiting utilization of isoleucine and valine (D'Mello and Lewis, 1970). In chicks, high levels of leucine, as opposed to isoleucine and valine, have been shown to increase activity of muscle branched-chain amino acid aminotransferase and catabolism of isoleucine and valine through oxidation, although the biochemical explanation behind this phenomenon is still somewhat unclear (Smith and Austic, 1978). This type of antagonism, especially that between leucine and isoleucine, is much more likely to occur in a practical diet than a lysine-arginine antagonism as corn is significantly higher in leucine than isoleucine (1.00% and 0.29%, respectively) (NRC, 1994).

Other relationships between amino acids that may affect their requirements include those between methionine and cystine, phenylalanine and tyrosine, and glycine and serine. Methionine can be used to synthesize cystine in the body; therefore the requirement for cystine can be met by either cystine or methionine (Boorman and Burgess, 1986), and it is common to see requirements stated for methionine plus cystine or total sulfur amino acids. However, the requirement for methionine must be met by

methionine, and it has been reported that the digestible cystine can supply no more than 52% of the total digestible sulfur amino acid requirement of growing chicks (Baker *et al.*, 1996). It is also common to see the requirements for phenylalanine and tyrosine as an additive measure or as total aromatic amino acids, as it is possible to meet the total needs for phenylalanine and tyrosine with phenylalanine (Sasse and Baker, 1972). Tyrosine is the first degradation product of phenylalanine (Nelson and Cox, 2008), however alone it is not capable of meeting a significant amount of the aromatic amino acid requirement (Perry *et al.*, 2003). Glycine can be converted to serine in the body in a reversible reaction (Nelson and Cox, 2008).

Limiting Amino Acids for Poultry

The limiting amino acid of a protein or whole feed can be defined as the essential amino acid found in the smallest quantity relative to its requirement (Bender, 2005). Other essential amino acids can only be used towards meeting their requirements to the point that the first limiting amino acid is present in the ration. The order of limitation can vary among individual ingredients or, in a complete feed, the level and combination of ingredients as well as overall protein level. Corn and soybean meal (SBM) are the two most commonly utilized ingredients for broiler diets in the U.S., and a great deal of research has been conducted in an effort to determine the limiting amino acids in such rations. Typically, the order of limitation in corn-SBM broiler diets containing 20-23% crude protein and approximately 75-85% of the total dietary amino acids provided by SBM is methionine, lysine, threonine, and valine (Baker *et al.*, 1993; Fernandez *et al.*, 1994).

This commonly utilized level of crude protein in corn-SBM diets usually will not result in a deficiency of the less limiting amino acids (Kidd *et al.*, 2000). However, interest in reducing the protein level in poultry diets has grown as nutritionists and producers attempt to decrease diet costs, environmental impact, and excess dietary amino acids which are utilized inefficiently (Perry *et al.*, 2004) and can cause reduced feed intake and body weight gain (Waldroup *et al.*, 1976). The advent of more commercially available synthetic amino acids has made it possible to decrease crude protein to a degree, but has also furthered the need for the assessment of the limiting amino acids in low protein corn-SBM diets. The two most common methods of determining limiting amino acids are addition assays, in which amino acids are added individually and in combination to a low protein diet or one that is deficient in a particular amino acid, and deletion assays, which employ a ration that initially meets all amino acid requirements and then from which individual amino acids are systematically removed (Edmonds, *et al.*, 1985; Baker, 1989) and the effects on performance are measured.

Inconsistent results have been reported for the order of amino acid limitation in low protein corn and soybean meal diets. Han and others (1992) fed broiler chicks a 19% crude protein diet, and determined that methionine and lysine were first and second limiting, respectively. Arginine, valine, and threonine were limiting as well, although it was indeterminable in this study the exact order of limitation of the three. Fernandez and coworkers (1994) reported an order of limitation for corn-SBM diets of methionine, threonine, lysine, valine, arginine, and tryptophan, with 10, 11.5, or 12.5% crude protein diets formulated based on ideal protein using digestible amino acid values. Addition and deletion studies conducted at the University of Illinois in which broiler chicks were fed

16% crude protein diets yielded overall results that indicated an order of limitation of methionine, lysine, arginine, valine, and threonine (Edmonds *et al.*, 1985). However, their results varied from trial to trial depending on whether or not glutamic acid was added and whether it was an addition or deletion trial. Addition trials clearly showed methionine as first limiting and arginine as second limiting, while deletion trials presented arginine as first limiting and methionine and lysine equally second limiting when glutamic acid was withheld, and methionine and lysine equally first limiting when it was added. The researchers observed that feed intake was higher in addition trials as well. This may indicate an imbalance of amino acids with some being provided in excess on top of a possible deficiency, causing a misinterpretation of the order of amino acid limitation. It is obvious from the variation in these results that the order of amino acid limitation depends on a variety of factors including method of formulation, type of assay used, ingredients utilized, and ingredient inclusion level, which will affect the amount of crude protein in the ration and the amino acid profile.

Amino Acid Requirements and the Protein and Energy Relationship

Early use of fat in poultry rations as a means to boost the energy content and corresponding decreases in feed intake that were observed encouraged researchers to investigate the relationship between energy and protein, and several groups came to the conclusion that if fat was added to a ration, the percentage of protein should also be adjusted (Sunde, 1954; Hill and Dansky, 1954; March and Biely, 1954). March and Biely (1954) reported that when fat was added to a diet low in protein, birds failed to grow at the same rate. However, fat additions to a high protein diet resulted in improved

growth, indicating the existence of a relationship between protein and energy. Changes in dietary metabolizable energy will affect feed intake, and therefore the intake of amino acids. The effects of metabolizable energy content of diets on growth response of chicks to graded doses of SAA was investigated by Boomgardt and Baker (1973), who reported that different concentrations of metabolizable energy resulted in different rates of growth. As energy concentration of the diets increased, weight gain decreased as a result of decreased feed intake.

Amino Acid Requirements as Related to Protein Level

Another factor that may affect the requirements for amino acids is the level of total protein. Amino acid requirements have been shown to fluctuate with the level of protein in the diet; specifically, the amino acid requirement as a percentage of the diet will increase with the concentration of dietary crude protein (Grau, 1948; Almquist, 1952; Hurwitz *et al.*, 1998; Morris *et al.*, 1999; Sklan and Noy, 2003). Almquist (1952) also stated that when the amino acid requirements are expressed as a percentage of the protein in the diet, the requirements are not as affected. It appears important that amino acids remain balanced not only relative to each other, but to the level of dietary protein as well.

It is also apparent that amino acid supplementation can affect the requirement for protein. Early research with turkeys concluded that the 28% crude protein requirement for turkeys could be reduced to 20% with proper amino acid supplementation with similar performance (Baldini *et al.*, 1954). Similar work in broilers has shown that crude protein in diets can be reduced to a point without harming performance with the addition

of lysine and methionine (Lipstein and Bornstein, 1975; Uzu, 1982) or a combination of essential amino acids and a source of non-essential amino acids/nitrogen (Corzo *et al.*, 2005). It has been suggested that when the level of total dietary protein (amino acids) is reduced, the requirement for each amino acid also decreases due to the depression in growth resulting from a single or many amino acid deficiencies, and that by supplementing each amino acid individually to a low protein diet it is possible to improve the overall balance of the ration (Hurwitz *et al.*, 1998). Again, additional research is necessary to determine to what extent crude protein may be reduced without a corresponding reduction in performance.

AMINO ACID DIGESTIBILITY

Computer diet formulation programs utilize the nutrient profiles of different feed ingredients to calculate ingredient combinations that will closely meet the birds' nutritional requirements, including the amino acid profiles. However, this approach does not account for the actual availability or digestibility of the feed ingredients *in vivo* which, in addition to knowledge of the requirements of the birds, is important for maximizing the efficiency of formulation and production (Firman, 1994). Amino acid requirements such as those found in the NRC (1994) are provided on a total basis, and so do not account for endogenous loss in the bird or those that are passed through to the excreta. In 1986, Sibbald published the methodology for an assay which may be used to determine the amino acid digestibility in feed ingredients or mixed feeds using adult

cecectomized roosters, and since then a number of studies have been conducted that present the importance of formulating rations based on digestible amino acids rather than total amino acids (Fernandez *et al.*, 1995; Rostagno *et al.*, 1995; Dari *et al.*, 2005; Maiorka *et al.*, 2005). The term “digestibility” as it is used in animal nutrition refers to the percentage of a nutrient or a feed that is available for absorption and use by the body (Schneider and Flatt, 1975), and is therefore one of the determining factors of the nutritive value of a feedstuff (Schneider and Flatt, 1975; Chung and Baker, 1992). Digestibility can be affected by a number of factors, including processing of the feedstuff (Maiorka *et al.*, 2005), age of the animal (Batal and Parsons, 2002), species (Kluth and Rodehutsord, 2006), strain, sex and physiological state (Firman, 1992). By utilizing digestible values for formulation, these factors are accounted for (Sibbald, 1986), and amino acid overfeeding is prevented. Digestible amino acid values are also very useful and are superior to total amino acid values when formulating diets containing animal byproducts such as meat and bone meal, which can vary widely in digestibility (Parsons *et al.*, 1997), or when feedstuffs with differing amino acid digestibility values are used to replace higher quality ingredients such as corn and soybean meal (Rostagno *et al.*, 1995; Emmert and Baker, 1997).

THE IDEAL PROTEIN CONCEPT

An additional application of digestible amino acid values is towards formulation of diets on an ideal protein basis, which is one of the more recent steps in the direction of

truly precise amino acid requirements. The development of an ideal protein, or an ideal combination of proteins, is an admirable, though difficult to achieve, goal. Mitchell and Scott developed a concept for ideal protein in the late 1950s and 1960s in which the main goal was to provide a combination of indispensable amino acids that precisely meets an animal's requirement for protein accretion and maintenance while avoiding deficiencies or excesses (Emmert and Baker, 1997). This type of pattern would allow all of the amino acids to be equally limiting (Fuller *et al.*, 1989; Wang and Fuller, 1989), which would help reduce the level of excess amino acids that must be catabolized (Fuller *et al.*, 1989). This would also prove valuable as a standard profile or reference protein when evaluating the quality of other dietary proteins (Wang and Fuller, 1989).

The search for the perfect balance of amino acids has been an ongoing process. Mitchell and Block first developed a chemical method for evaluating the quality of a protein by comparison to a reference protein, in this case whole egg protein, in 1946, and then Mitchell (1964) later recognized that what was needed was an amino acid mixture that was equivalent to the amino acid requirements of the animal for growth and maintenance. For many years, researchers attempted to find this balance of amino acids using carcass composition data (Price *et al.*, 1953; Summers and Fisher, 1961; Robel and Menge, 1973). Robel and Menge (1973) used the amino acid profile from chick carcasses as a model for a diet that would closely meet the birds' requirements. However, while carcass composition data is a good starting point for an ideal ratio, it does not take into account factors affecting a live animal such as maintenance costs (Firman and Boling, 1998). Subsequent research has focused on establishing ideal amino acid ratios for chicks and pigs (Fuller *et al.*, 1989; Wang and Fuller, 1989; Chung and Baker, 1992;

Baker and Han, 1994), and then using those ratios for precision diet formulation and measuring the effects on performance (Lopez *et al.*, 1994; Emmert and Baker, 1997; Kerr and Kidd, 1999b; Lemme *et al.*, 2003; Wijtten *et al.*, 2004; Dari *et al.*, 2005).

To use the ideal protein concept in diet formulation, all of the indispensable amino acids are expressed as ideal ratios, or percentages, of lysine. Baker and coworkers (1993) report several advantages to this method over other requirements. First, more is known about the amount of lysine in feed ingredients, as well as the lysine requirement of poultry of various ages, than is for any other amino acid. Lysine is the second-limiting amino acid in commercial corn-soybean meal poultry diets, and dietary lysine functions solely in protein synthesis. Secondly, factors such as sex, genetics, environmental conditions, caloric density and dietary protein level can effect amino acid requirements, and the ideal protein method can take these factors into account allowing more accurate formulation. Finally, use of the ideal protein concept helps prevent over-formulation which will minimize nitrogen excretion in waste.

One should be mindful of certain considerations when employing the ideal protein concept for feed formulation. Obviously the lysine requirement must be very accurate for the birds being fed as it is the basis for the requirements for all of the other indispensable amino acids, so any error in the lysine requirement will translate into errors for all other amino acids (Baker *et al.*, 1993; Emmert and Baker, 1997). Additionally, the ideal pattern of amino acids is based on digestible levels of dietary amino acids (Baker *et al.*, 1993; Emmert and Baker, 1997), which eliminates differences in absorption and utilization from various protein sources or if synthetic amino acids are utilized (Emmert and Baker, 1997). Nutritionists must formulate on a digestible basis, using the digestible amino acid

contents of the feedstuffs in the diet, or the ratios lose their usefulness. Finally, the values should be considered minimum requirements for each amino acid, not exact requirements, which can only be obtained by significantly reducing crude protein and adding back large amounts of crystalline amino acids, which is not currently economically efficient (Firman, 1997).

Use of the ideal protein concept can allow for determination of digestible amino acid requirements for birds at any age period (Baker, 2003) and the formulation of diets on a digestible basis, which has been discussed in this review. As the requirements of all indispensable amino acids are related to lysine, they can be easily and quickly modified as the requirement for lysine changes. The ideal protein concept can be used to formulate low protein diets with crystalline amino acids added back. Furthermore, it has been shown to be useful in tracking the order of limitation of amino acids as they might change in diets where the level of protein or the ingredient profile, and therefore the amino acid profile, changes (Han *et al.*, 1992; Baker *et al.*, 1993; Wang *et al.*, 1998).

LOW PROTEIN DIETS

Low protein diets have been used by researchers for a variety of functions, some of which have been discussed in this review, including the determination of the order of amino acid limitation in different types of rations, effects of various imbalances or antagonisms, effects on the performance of birds under different environmental conditions such as heat stress, effects of protein level on the efficiency of nutrient

utilization, and how amino acids interact with other nutrients such as metabolizable energy, and all of which have improved and increased the information available on amino acid requirements for poultry. Much of the current desire to continue studying the possibilities of reducing the level of crude protein further in poultry rations revolves around the desire to maximize the use of amino acids for protein synthesis as opposed to their less efficient use as an energy source, decreased environmental pollution, decreased requirements for the limiting amino acid, and reduced feed costs (Dari *et al.*, 2005). Certainly the combined use of digestible amino acid values for feed formulation, the ideal protein concept, and the increased availability and affordability of several supplemental amino acids can allow a decrease in crude protein to a point, but the extent to which protein can be decreased while still realizing maximal growth is still unknown.

It is evident from the literature that lowering crude protein without supplementation of amino acids is detrimental to broiler performance (Kerr and Kidd, 1999a), but that crude protein can be successfully reduced to a point with synthetic amino acid supplementation and result in similar performance to standard diets with higher levels of crude protein (Lipstein and Bornstein, 1975; Waldroup *et al.*, 1976; Han *et al.*, 1992; Kerr and Kidd, 1999b; Aleator *et al.*, 2000; Dean *et al.*, 2006; Namroud *et al.*, 2008), with the reduction in crude protein ranging from just a few percentage points up to a 25% reduction in the case of Dean and others (2006). However, a number of researchers have reported decreased performance in birds fed low protein, amino acid supplemented diets with reductions of crude protein in some cases of only 3 or 4 percent (Fancher and Jensen, 1989; Pinchasov *et al.*, 1990; Ferguson *et al.*, 1998; Bregendahl *et al.*, 2002; Si *et al.*, 2004). Some of the more recent research reports that investigated the

effects of low crude protein rations for broilers, modeled after a similar table by Aftab and coworkers (2006), are summarized in Table 1.

A number of explanations for the discrepancies in performance between experiments have been proposed. Corzo and coworkers (2005) suggest that differences in the level of crude protein, amino acid fortification, dietary ingredients, chosen amino acid requirements, as well as bird age and strain may have contributed to some of the variation in reported performance. Aletor and others (2000) suggest that in addition to differences in the degree of crude protein reduction and the age and class of the birds, some of the discrepancy may be due to the inclusion or exclusion of the crude protein and metabolizable energy contributions from the amino acid supplements and whether or not the ratios of the intact protein sources are kept constant to minimize amino acid imbalances.

While many of these factors are under the control of the group conducting the experiment, there are additional possibilities to explain the reduction in live performance parameters and carcass yield in birds fed low crude protein diets. In a recent review of the effects of low crude protein diets for broilers, Aftab and collaborators (2006) discuss eight main possibilities that may explain the negative effects of LCP on performance. The first of these involves changes in the dietary electrolyte balance, or dEB, ($K+Na-Cl$) or dietary potassium levels. Diets low in crude protein often replace soybean meal with synthetic amino acids, resulting in a reduction in dietary K and an increase in the levels of Cl^- supplied by supplemented amino acids, lowering the dEB in these type of diets, which may cause the depression in performance (Patience, 1990; Aftab *et al.*, 2006). However, it has been reported that maintaining dEB in low protein diets did not restore

performance to the level of control diets (Han *et al.*, 1992; Si *et al.*, 2004), and that increasing dEB in low protein diets may actually depress feed intake and body weight gain (Adekunmisi and Robbins, 1987).

The second possible explanation for the decrease in performance seen in birds fed decreased levels of crude protein reported by Aftab and coworkers (2006) is an insufficiency of non-specific nitrogen for the synthesis of nonessential amino acids (NEAA). In many cases, the addition of glutamic acid helped improve performance of birds consuming low protein diets to a degree, but failed to fully restore levels up to those seen with control diets (Kerr and Kidd, 1999a; Namroud *et al.*, 2008). Kerr and Kidd (1999a) found that a reduction in crude protein of a diet, regardless of glutamic acid supplementation, resulted in decreased carcass yields, increased abdominal fat, and decreased breast meat yield. Namroud and others (2008) found that while performance was unaffected by a decrease in crude protein to 19% with amino acid supplementation, decreases beyond this drastically reduced growth and feed intake even with the addition of glutamic acid.

A third explanation for the decreased performance is that broilers tend to decrease voluntary feed intake when consuming low crude protein diets (Aftab *et al.*, 2006). Some disagreement in the literature exists over this explanation, although it is likely that reduced feed intake is at least partially responsible for the decreased growth that is observed. Several theories for why intake is affected have been proposed, including amino acid imbalances (previously discussed), increases in blood ammonia as a result of high levels of crystalline amino acids (Namroud, *et al.*, 2008), changes in dEB that

promote water intake over feed, and changes in the ratio of net energy to metabolizable energy (Aftab *et al.*, 2006).

A fourth explanation for decreased performance in low crude protein diets is a deficiency of glycine. Increased performance has been observed in birds consuming low protein diets when the level of glycine was increased, suggesting that glycine may have a more specific role than previously believed and that the NRC-suggested requirements may be too low (Corzo *et al.*, 2004; Waldroup *et al.*, 2005b; Aftab *et al.*, 2006; Dean *et al.*, 2006). The formulation of low crude protein diets involves a decrease in intact protein sources such as soybean meal that contain relatively high levels of glycine compared to other ingredients, so it may be important to consider glycine levels specifically, rather than just total NEAA levels, when formulating low crude protein diets.

The fifth possible reason for the depressed performance of broilers consuming low protein, amino acid-supplemented diets is an improper ratio of nonessential amino acids (NEAA) to essential amino acids (EAA) (Aftab *et al.*, 2006). It has long been asserted that low crude protein diets needed supplementation of NEAA (Stucki and Harper, 1961), and various ratios have since been suggested. A ratio near 50:50 is often used, although it is not uncommon to see up to 5% variation in either direction.

The final three suggestions proposed by Aftab and coworkers are a deficiency of an essential amino acid, decreased efficiency of utilization of free amino acids as compared to that of amino acids from intact protein sources, and a disruption of the ratio of net energy to metabolizable energy, which also may help explain the increase in body fat yield in broilers fed low crude protein diets.

The issue of carcass composition is another important consideration when discussing low crude protein diets. It has been proposed that when amino acid levels are below the requirement, feed intake will increase in an attempt to obtain the deficient amino acids, and the extra energy consumed will be deposited as fat (Bartov, 1979). Similar results in fat deposition have been observed by other researchers (Moran *et al.*, 1992; Hurwitz *et al.*, 1998; Kidd and Kerr, 1999a; Aleator *et al.*, 2000). Conversely, it has been demonstrated that increasing amino acid density above adequate levels can increase body weight and breast meat yield (Kidd *et al.*, 2004; Corzo *et al.*, 2004), although with rising feed costs it may not be economically beneficial to increase the cost of the diet by increasing protein and amino acid density for the level of return on the increased meat yield.

SUMMARY

In order to meet increasing worldwide demand for poultry and maintain profitability, it is important to find new ways to stay competitive within the industry and decrease the costs of production as much as possible while achieving a high quality product for consumers. The development of low protein broiler diets is essential for combating rising feed and production costs, as well as environmental concerns. It is not entirely clear at this point why low crude protein diets supplemented with amino acids have been unable to provide the same level of production in broiler chickens as standard high crude protein diets. Research with turkeys has shown that crude protein can be

reduced from 28% to 10% with essential amino acid supplementation with similar performance (Moore *et al.*, 2001). If a diet low enough in crude protein can be developed that still achieves adequate performance, it would be possible to more closely determine the digestible requirements for the essential amino acids by using that diet to individually study each amino acid through titration experiments. This would eventually allow formulation of economically efficient diets that precisely meet the birds' requirements with little or no excess.

The lowest level to which crude protein can be reduced with amino acid supplementation in broiler diets without reducing bird performance is still unknown, and additional research on the subject could yield significantly greater cost savings in the future.

Table 1. Effect of Low Crude Protein Diets on Live Performance of Broilers

CPI ^A (CPA) ^B %	Control CP %	CPA/ Control	ME/CP	Age, Days	Gain ----- CPA/Control group	FE	Reference
19.0	23.12	0.82	167	10-28	0.99	1.00	Namroud <i>et al.</i> (2008)
16.2(16.6)	22.2	0.75	193	0-18	0.99	1.05*	Dean <i>et al.</i> (2006)
22.69	24.28	0.93	141	1-21	0.99	1.02	Waldroup <i>et al.</i> (2005b)
17.6	21.2	0.83	178	5-21	0.96	1.03	Corzo <i>et al.</i> (2005)
18.2	19.0	0.96	170	21-42	1.00	1.01	Dari <i>et al.</i> (2005)
17.3	19.3	0.90	155	0-21	1.06	1.01	Aftab <i>et al.</i> (2004a) ^C
15.3	17.2	0.89	180	21-42	1.04	0.99	Aftab <i>et al.</i> (2004b) ^C
17.6(18.3)	23.4	0.78	175	7-21	0.97	0.94*	Bregendahl <i>et al.</i> (2002)
15.3(16.0)	22.7	0.70	194	21-42	1.01	1.07*	Aletor <i>et al.</i> (2000)

^ACrude protein from intact protein.

^BCrude protein from diet analysis (including crystalline amino acids).

^CLow-ME (2700-2750 kcal ME per kg) diets.

* Statistically different

CHAPTER 2

EFFECT OF 15% CRUDE PROTEIN CORN AND SOYBEAN MEAL DIETS WITH AMINO ACID SUPPLEMENTATION ON BROILERS IN THE STARTER PERIOD

ABSTRACT

Previous research conducted by this laboratory (Brooks, 2003) indicated that dietary crude protein can be as low as 15% and achieve similar broiler performance as a 23% CP diet. Two experiments were conducted with the objective of testing the effects of feeding a 15% CP diet with crystalline amino acid supplementation on the performance of broilers from 0-3 weeks of age. In both experiments, commercial broilers were fed a diet formulated to meet NRC requirements for the first seven days. The diet contained 23% CP and 3200 kcal/kg ME, and also served as the positive control diet (PC). On day 7, birds were sorted by weight into battery pens with 5 birds per pen. Both experiments utilized the same six dietary treatments with eight replicates per treatment for a total of 48 pens. The remaining treatments consisted of: a 15% CP negative control diet with crystalline amino acids added back to meet required levels (NC), a NC diet + .1% cystine (NC + C), a NC diet + .1% threonine (NC + T), a NC diet + .1% glycine (NC + G), and a NC diet + .1% cystine, threonine, and glycine (NC + C,T,G). Glutamic acid was added to all diets to maintain a 20% protein equivalent. All diets were formulated on a digestible basis, and were designed to be isocaloric. At the conclusion of the experiments, body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were

measured. In Experiment 1, significant differences ($P < 0.05$) were found in BWG between the PC treatment and PC + C,T,G, although no significant differences in FI or F:G were observed. There were no significant differences ($P > 0.05$) in BWG, FI, or F:G among any of the other treatments. In Experiment 2, treatments had no effect ($P > 0.05$) on performance. Overall, these results suggest that feeding a 15% CP diet + crystalline amino acids to broilers in the starter period can yield similar performance to a 23% CP diet.

INTRODUCTION

Meeting the nutritional requirements for broiler chickens constitutes a large percentage of the cost of production. Reducing the level of crude protein (CP) in the diet may allow a reduction in feed costs, use of alternate feedstuffs, and an improved ability to cope with heat stress (Kidd *et al.*, 1996). It is known that there is no requirement for protein in the diet, *per se*, but actually a requirement for the amino acids found in protein (NRC, 1994). Because of this, it may be possible to supplement low CP diets with crystalline amino acids and achieve similar performance as with diets higher in CP. Research from the University of Missouri found that the amount of CP in turkey diets can be reduced from 28% to 10% with the addition of essential amino acids and achieve adequate performance (Moore *et al.*, 2001). Previous research with broilers indicated that it may be possible to feed broilers a 15% crude protein diets and obtain similar performance to birds consuming a standard diet (Brooks, 2003). However, the requirements of amino acids must be well defined for these diets to be successful.

Feeding a broiler diet low enough in CP that individual amino acids can be titrated may lead to a better understanding of these requirements and to diets that more closely meet the birds' requirements. Additional research is needed in order to discover the minimum levels of amino acids necessary to achieve maximum growth and efficiency. The objective of Experiment 1 was to examine the effects of feeding a 15% CP diet with crystalline amino acid supplementation on the performance of broilers from 0-3 weeks of age. Experiment 2 was conducted in an attempt to validate the results obtained from Experiment 1.

MATERIALS AND METHODS

Day-old straight run broiler chicks were obtained from a commercial hatchery and fed a NRC-type corn and soybean meal diet until seven days of age. On day seven, birds were wing-banded and weighed. A computer sorting program was used to sort birds by weight and assign them to pens (5 birds per pen) to obtain similar starting pen weights. Each trial utilized 240 birds to provide eight replications of six treatments. Chicks were provided access to experimental diets and water *ad libitum* for fourteen days, and trials were terminated on day 21. Feed intake, body weight gain, and feed:gain were measured. Feed:gain was adjusted for mortality by adding each mortality weight to the appropriate pen gain then dividing feed consumed by gain.

Diets were formulated on a digestible basis utilizing least-cost formulation software. The amino acid digestibility values used in this experiment for the corn and SBM were obtained previously by precision feeding a known sample of each ingredient

to cecectomized roosters that had been removed from feed for 24 hours to clear the gut. Excreta were collected for 48 hours after precision feeding, and were then dried in a forced air oven and ground. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratory for a complete amino acid analysis, and digestibility values were then determined.

In each experiment, a 23% protein, 3200 kcal/kg ME NRC-type diet was utilized as the positive control (PC). For all other diets, a 15% crude protein ration was formulated, and the levels of amino acids that were supplied by the corn and SBM were determined. The essential amino acid levels were then brought up to the total digestible levels found in a 15% CP diet from previous research conducted at the University of Missouri (Table 2). After careful examination of this diet, cystine, threonine, and glycine were determined to be at levels slightly below NRC requirements, and so four treatments were designed to account for these deficiencies. The remaining treatments consisted of: a 15% CP negative control diet with crystalline amino acids added back to meet the levels found in the previous trial (NC), a NC diet + .1% cystine (NC + C), a NC diet + .1% threonine (NC + T), a NC diet + .1% glycine (NC + G), and a NC diet + .1% cystine, threonine, and glycine (NC + C,T,G). Glutamic acid was added to the diets in order to maintain a 20% protein equivalent and to prevent confounding of results due to a generalized nitrogen deficiency, and all amino acids were added at the expense of sucrose as its energy content is comparable to that of crystalline amino acids. The compositions of dietary treatments are shown in Table 3.

Chicks were housed in stainless steel batteries with 24 hours of fluorescent lighting. The room was thermostatically controlled with temperatures maintained near 90

degrees F for the first week post-hatch and a two degree reduction in temperature every four days thereafter. Birds were cared for using husbandry guidelines derived from University of Missouri standard operating procedures.

Data were analyzed with pen gain as the experimental unit using the JMP statistical analysis software package. Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed, and the level of significance was established at $P < 0.05$. Mean comparisons for all pairs were conducted using the Least Significant Difference test.

RESULTS AND DISCUSSION

In these experiments, body weight gain, feed intake, and feed:gain were measured in order to determine whether or not a 15% crude protein corn and soybean meal diet with crystalline amino acid supplementation can support similar performance to that achieved with a NRC-type diet.

In Experiment 1, significant differences ($P < 0.05$) in BWG were observed between the PC treatment and the NC + .1% C,T,G treatment (Table 4, Figure 1). All other treatments were statistically the same ($P > 0.05$). There were no differences ($P > 0.05$) among treatments with respect to feed intake (Table 4, Figure 2) or feed:gain (Table 4, Figure 3).

Experiment 2 was conducted in order to test the results obtained in Experiment 1. In Experiment 2, no significant differences ($P > 0.05$) were seen in gain (Table 5, Figure

4), feed intake (Table 5, Figure 5), or feed:gain (Table 5, Figure 6) among any of the dietary treatments.

One of the most important questions raised by these experiments is why the 15% CP rations resulted in body weight gain values that, although not statistically different from the control, were consistently lower than control values. One possible answer for this question is that the amino acid levels in the 15% CP ration were so near the true requirement that any slight difference in ingredient amino acid levels could cause a deficiency and therefore growth depression. This might also help explain the differences in results from the first and second trials in which different batches of corn and SBM were utilized. The margin of safety that nutritionists put into place with a 23% CP diet is essentially removed with a 15% CP diet, leaving any variation in the amino acid content or digestibility of the ingredients capable of depressing performance.

It is unclear why the NC + .1% C,T,G treatment resulted in decreased growth when compared to the other treatments in Experiment 1. It is possible that an amino acid imbalance may have caused the depression in growth, but it is difficult to draw any conclusions on this issue, especially considering that these results were not duplicated in Experiment 2.

Overall, these results indicate that a 15% crude protein is capable of supporting performance of broilers in the starter period that is similar to that of a 23% protein diet. A number of researchers have obtained similar results (Lipstein and Bornstein, 1975; Waldroup *et al.*, 1976; Han *et al.*, 1992; Kerr and Kidd, 1999b; Dean *et al.*, 2006; Namroud *et al.*, 2008). In the case of Dean and others, (2006), a 25% reduction in crude protein with amino acid supplementation and higher than normal levels of glycine +

serine was successful in supporting performance similar to that observed in birds consuming high protein control rations. Parr and Summers (1991) utilized a 23% CP control diet and low CP diets ranging from 16.5% to 21% CP in which the essential amino acids were kept balanced. They observed no significant differences between the control treatment and the low CP treatments.

Other researchers have reported decreased performance in birds fed low protein, amino acid supplemented diets with reductions of crude protein in some cases of only 3 or 4 percent (Fancher and Jensen, 1989; Pinchasov *et al.*, 1990; Ferguson *et al.*, 1998; Bregendahl *et al.*, 2002; Si *et al.*, 2004). A number of explanations for the discrepancies in performance between low CP experiments have been proposed, including differences in the level of crude protein and amino acid fortification, dietary ingredients utilized, chosen amino acid requirements, as well as bird age and strain (Corzo *et al.*, 2005). Aleator and others (2000) suggest that in addition to differences in the degree of crude protein reduction and the age and class of the birds, some of the discrepancy may be due to the inclusion or exclusion of the crude protein and metabolizable energy contributions from the amino acid supplements and whether or not the ratios of the intact protein sources were kept constant to minimize amino acid imbalances.

The results from these experiments indicate that a 15% CP diet with crystalline amino acid fortification can be utilized for broilers in the starter period and will support similar growth to an NRC-type ration. Additional research is necessary to determine if additional reductions in intact protein are possible.

Table 2. Amino Acid Levels of 15% Crude Protein Diets (Experiments 1 and 2)

Treatment	NC
Crude protein	15
Protein Equivalent	20
Amino Acid	
Lysine	1.434
Methionine	0.622
Cystine	0.248
Met + Cys	0.870
Threonine	0.782
Valine	1.052
Arginine	1.673
Leucine	1.984
Histidine	0.652
Isoleucine	1.005
Phenylalanine	1.594
Tyrosine	0.444
Phe + Tyr	2.042
Tryptophan	0.324
Glycine	0.748
Serine	0.358
Gly + Ser	1.106

* All values are expressed on a digestible basis

Table 3. Composition of Experimental Diets (Experiments 1 and 2)

Treatment:	PC	NC	NC + C	NC + T	NC + G	NC + C,T,G
Corn	51.624	64.704	64.704	64.704	64.704	64.704
Soybean Meal	39.822	21.104	21.104	21.104	21.104	21.104
Lard	4.648	2.431	2.431	2.431	2.431	2.431
Dicalcium Phosphate	1.699	1.861	1.861	1.861	1.861	1.861
Limestone	1.174	1.198	1.198	1.198	1.198	1.198
Sodium Bicarbonate	0.3	1.0	1.0	1.0	1.0	1.0
Salt	0.256	0.26	0.26	0.26	0.26	0.26
Sucrose ³		1.752	1.752	1.752	1.752	1.752
Coban	0.075	0.075	0.075	0.075	0.075	0.075
Vitamin Premix ¹	0.075	0.075	0.075	0.075	0.075	0.075
Choline Chloride	0.05	0.05	0.05	0.05	0.05	0.05
Calcium Trace Mineral ²	0.10	0.10	0.10	0.10	0.10	0.10
Selenium Premix ²	0.03	0.03	0.03	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013	0.013	0.013	0.013
DL Methionine	0.116	0.329	0.329	0.329	0.329	0.329
Arginine		0.674	0.674	0.674	0.674	0.674
Glycine		0.304	0.304	0.304	0.404	0.404
Histidine		0.221	0.221	0.221	0.221	0.221
Isoleucine		0.312	0.312	0.312	0.312	0.312
Leucine		0.491	0.491	0.491	0.491	0.491
Lysine		0.567	0.567	0.567	0.567	0.567
Phenylalanine		0.797	0.797	0.797	0.797	0.797
Threonine		0.198	0.198	0.298	0.198	0.298
Tryptophan		0.177	0.177	0.177	0.177	0.177
Valine		0.272	0.272	0.272	0.272	0.272
Cystine			0.1			0.1
Glutamic Acid		0.906	0.806	0.806	0.806	0.608
Calculated to contain						
Crude Protein, % ⁴	23	15	15	15	15	15
Protein Equivalent, % ⁵	23	20	20	20	20	20
ME, kcal/kg	3200	3200	3200	3200	3200	3200
Calcium, %	1.0	1.0	1.0	1.0	1.0	1.0
Available Phosphorus, %	0.45	0.45	0.45	0.45	0.45	0.45

¹ Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

² Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

³ Synthetic amino acids and glutamic acid added at expense of sucrose.

⁴ Crude protein values calculated from protein provided from corn and soybean meal.

⁵ Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids.

Table 4. Performance of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
PC	614 ^a	807 ^a	1.31 ^a
NC	601 ^{ab}	803 ^a	1.34 ^a
NC + C	594 ^{ab}	801 ^a	1.35 ^a
NC + T	599 ^{ab}	791 ^a	1.32 ^a
NC + G	577 ^{ab}	812 ^a	1.33 ^a
NC + C,T,G	572 ^b	767 ^a	1.34 ^a
Pooled SEM	.0094	.0113	.0185

Values with differing letters are significantly ($P < 0.05$) different

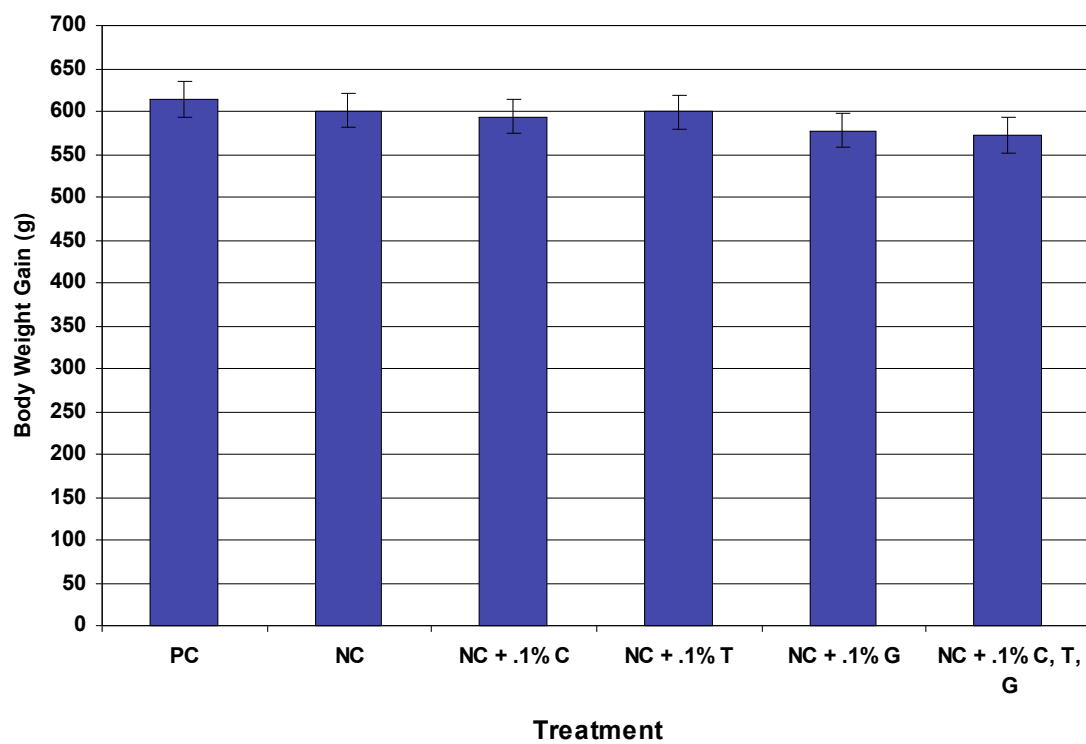


Figure 1. Body Weight Gain (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1

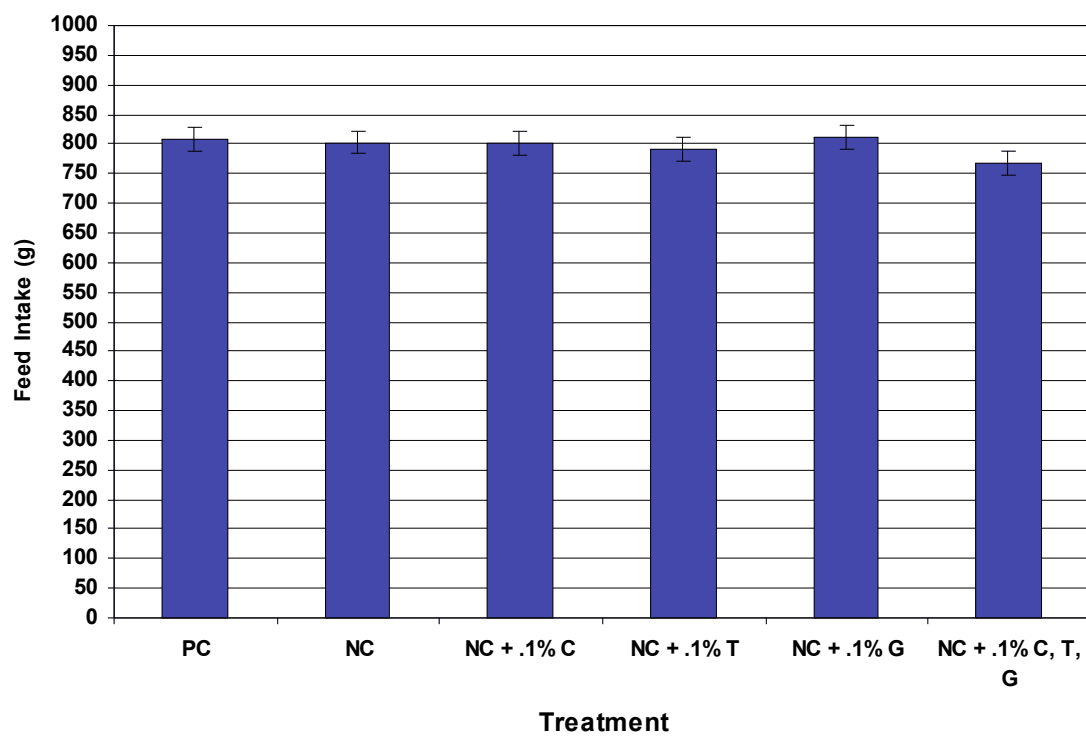


Figure 2. Feed Intake (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1

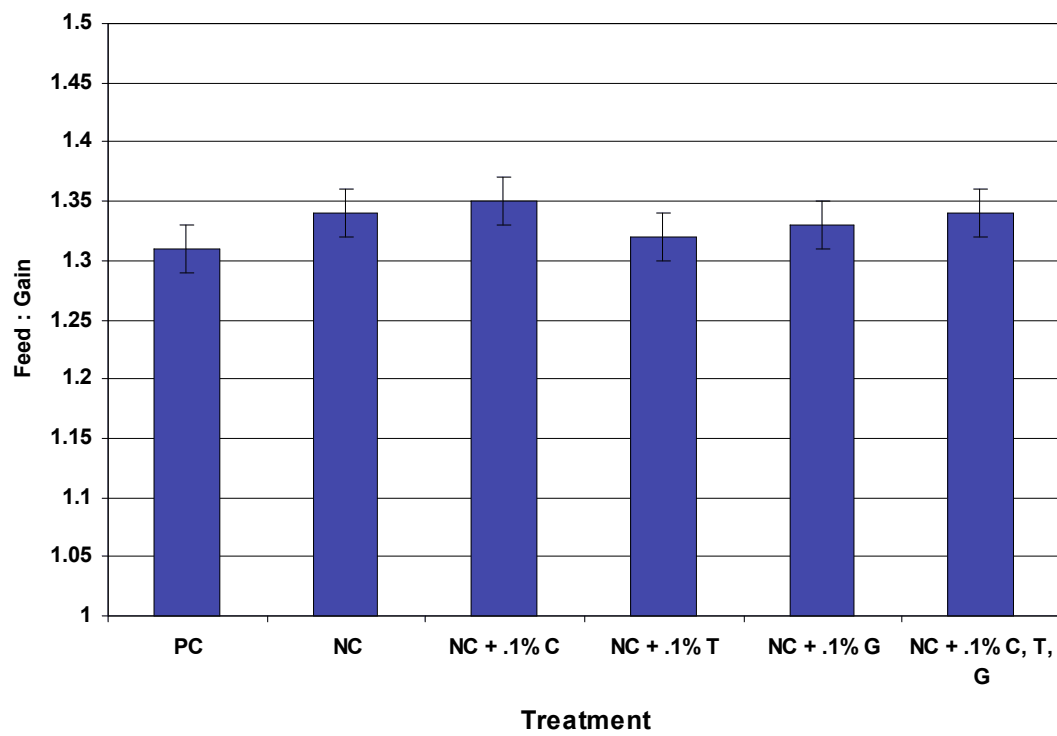


Figure 3. Feed:Gain of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 1

Table 5. Performance of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age;
Experiment 2

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
PC	657 ^a	856 ^a	1.30 ^a
NC	642 ^a	847 ^a	1.34 ^a
NC + C	630 ^a	850 ^a	1.34 ^a
NC + T	636 ^a	826 ^a	1.34 ^a
NC + G	645 ^a	858 ^a	1.30 ^a
NC + C,T,G	645 ^a	861 ^a	1.32 ^a
Pooled SEM	.0102	.0117	.0164

Values with differing letters are significantly ($P < 0.05$) different

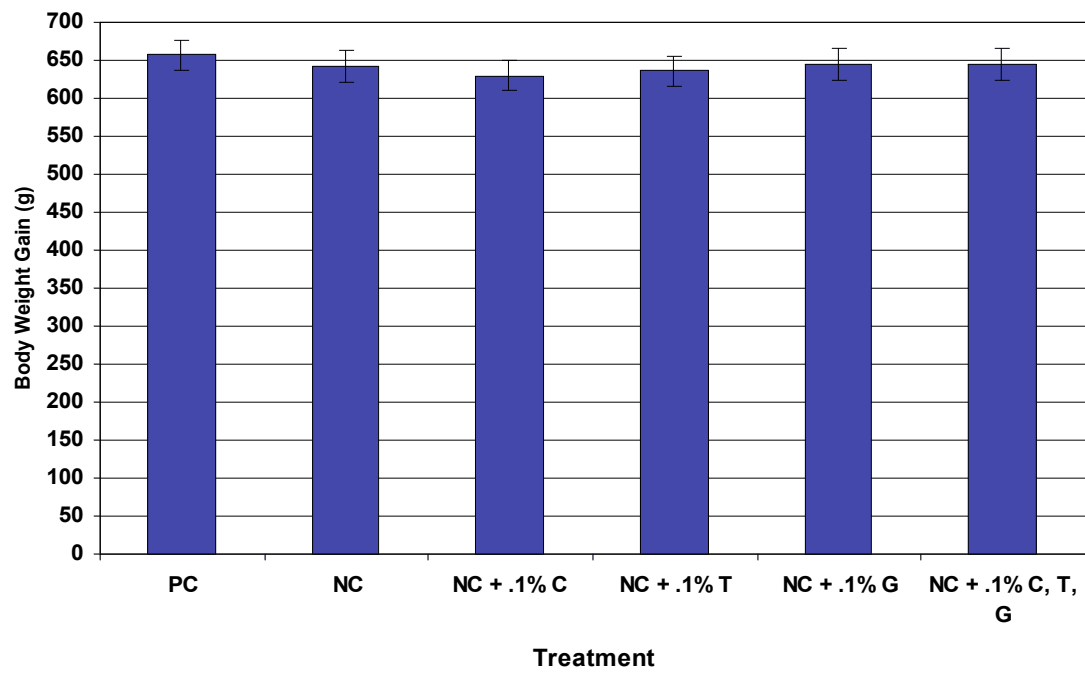


Figure 4. Body Weight Gain (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2

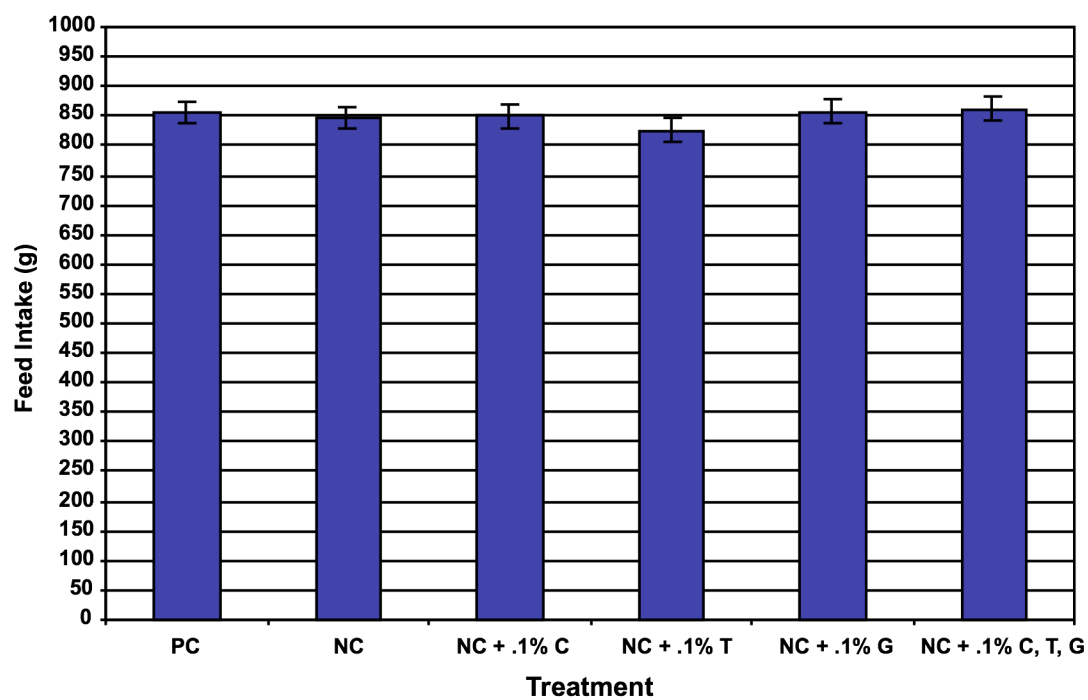


Figure 5. Feed Intake (g) of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2

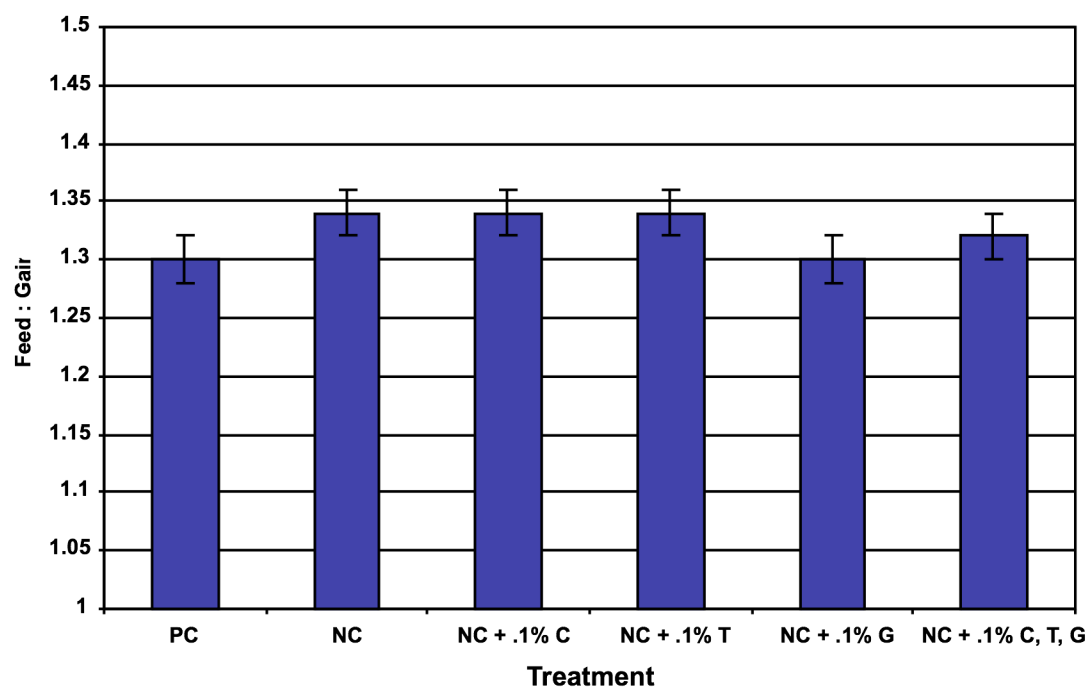


Figure 6. Feed:Gain of Broiler Chicks Fed Low Protein Diets from 7-21 Days of Age; Experiment 2

CHAPTER 3

EFFECTS OF 13% CRUDE PROTEIN CORN AND SOYBEAN DIETS WITH AMINO ACID SUPPLEMENTATION ON BROILERS IN THE STARTER PERIOD

ABSTRACT

Two experiments were conducted with the objective of testing the effects of feeding 13% CP diets with crystalline amino acid supplementation and various protein equivalents on the performance of broilers in the starter growth period. In each experiment, commercial broilers were fed a diet formulated to meet NRC requirements for the first seven days. The diet contained 23% CP and 3200 kcal/kg ME, and also served as the positive control diet (PC). On day 7, birds were sorted by weight into battery pens with 5 birds per pen. In the first experiment, six dietary treatments were utilized with eight replicates per treatment for a total of 48 pens. For the remaining dietary treatments, 13% CP diets were formulated and various levels of crystalline amino acids were added back to meet either digestible amino acid levels from a 22% CP diet from previous experiments from our lab at the University of Missouri (Guaiume, 2007) or digestible amino acid requirements set by Baker and coworkers (1993) using the ideal protein concept. One treatment using the University of Missouri values contained no glutamic acid and a low protein equivalent of 15.5% (MLPE), while others contained varying levels of glutamic acid to achieve a high protein equivalent of 20% (MHPE) or a mid-level protein equivalent of 18% (MMPE). Similarly, two treatments were developed

using Baker and coworkers (1993) amino acid values and glutamic acid to achieve a 20% high protein equivalent (BHPE) or an 18% mid-level equivalent (BMPE). In Experiment 2, four dietary treatments with 12 replicates were utilized for a total of 48 pens. The same 23% CP diet used as the PC in Experiment 1 was utilized in Experiment 2. The remaining treatments in Experiment 2 consisted of 13% crude protein diets with crystalline amino acids added back to meet control levels and either no glutamic acid to yield a protein equivalent of 17.5% (PE-17.5), or glutamic acid added to meet an 18.75% (PE-18.75) or 20% (PE-20) protein equivalent. All diets were formulated on a digestible basis and were designed to be isocaloric. Birds received feed and water *ad libitum*. At the conclusion of each experiment, body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were measured. In Experiment 1, birds consuming the PC treatment achieved significantly greater ($P < 0.05$) BWG than birds in any other treatment. A significant difference ($P < 0.05$) in intake was seen between the BMPE treatment and all others. A significantly improved F:G ($P < 0.05$) was observed in the PC treatment. Additionally, the BMPE treatment resulted in impaired F:G ($P < 0.05$) when compared to the MMPE and MHPE treatments. In Experiment 2, birds receiving the PE-17.5 treatment gained significantly less weight ($P < 0.05$) than those consuming other dietary treatments. There were no significant differences ($P > 0.05$) in feed intake. Birds in the PC groups displayed significantly improved F:G over all other treatments ($P < 0.05$).

INTRODUCTION

The first two trials in this series of experiments indicated that a 15% CP diet with crystalline amino acid supplementation can yield similar performance in broilers from 0-3 weeks of age. Two additional experiments were conducted with the objective of testing the effects of feeding a 13% CP diet with crystalline amino acid supplementation on the performance of broilers in the same growth period. Use of the ideal protein concept can allow for determination of digestible amino acid requirements for birds at any age period (Baker, 2003) and the formulation of diets on a digestible basis, which can help reduce the level of excess protein in the diet. Using this method, the requirements of all indispensable amino acids are related to lysine and can be easily and quickly modified as the requirement for lysine changes. One of the practical uses of the ideal protein concept is to formulate low protein diets with crystalline amino acids added back. Furthermore, it has been shown to be useful in tracking the order of limitation of amino acids as they might change in diets in which the level of protein or the ingredient profile, and therefore the amino acid profile, changes (Han *et al.*, 1992; Baker *et al.*, 1993; Wang *et al.*, 1997). It is possible to use the ideal protein concept to feed animals more precisely and avoid the use of excessive levels of crude protein to meet amino acid requirements. However, in order to truly establish exact amino acid requirements, it is necessary to significantly reduce crude protein and add back large amounts of crystalline amino acids in order to make it possible to titrate individual amino acids. These experiments were conducted in an attempt to reduce the level of crude protein that can be fed with various levels of amino acid supplementation. Additionally, these experiments examined the importance

of the level of protein equivalence in low crude protein rations. When birds are fed standard levels of crude protein, nonessential amino acids can be formed in the body from excess essential amino acids. However, when low crude protein diets are fed, this excess is reduced, leaving less essential amino acids available for conversion to the nonessentials. To prevent a deficiency of essential amino acids and the resultant poor performance, many researchers add supplemental non-essential amino acids, a non-specific nitrogen source such as glutamic acid, or both to low crude protein diets. While a number of researchers have reported the importance of supplementing nonessential amino acids to low crude protein diets for improving live performance (Han *et al.*, 1992, Aleator *et al.*, 2000, Dean *et al.*, 2006), others have suggested that glutamic acid or other non-specific nitrogen sources result in little or no advantage when added to low protein broiler rations (Kerr and Kidd, 1999) or swine rations (Kephart and Sherritt, 1990). For this reason, a variety of protein equivalencies, achieved by the addition of glutamic acid, will be used in these trials and performance will be measured. The overall objective of this research is to eventually determine the lowest possible level of crude protein that may be fed to broilers that will achieve similar growth to those consuming standard diets, allowing the establishment of precise digestible amino acid requirements.

MATERIALS AND METHODS

Two experiments were conducted with the objective of testing the effects of feeding a 13% CP diet with crystalline amino acid supplementation on the performance of broilers in the starter growth period. In each experiment, day-old straight run broiler

chicks were obtained from a commercial hatchery and fed a NRC-type corn and soybean meal diet until seven days of age. On day seven, birds were wing-banded and weighed. Birds were sorted by weight and assigned to pens (5 birds per pen) to obtain similar starting pen weights. The first trial utilized 240 birds to provide eight replicates of six treatments, while the second trial utilized 240 birds to provide 12 replicates of 4 treatments. Chicks were provided access to experimental diets and water *ad libitum* for fourteen days, and trials were terminated on day 21. Feed intake, body weight gain, and feed:gain were measured. Feed:gain was adjusted for mortality by adding each mortality weight to the appropriate pen gain then dividing feed consumed by gain.

Diets were formulated on a digestible basis utilizing least-cost formulation software and were designed to be isocaloric. The amino acid digestibility values used in this experiment for the corn and SBM were obtained previously by precision feeding a known sample of each ingredient to cecectomized roosters that had been removed from feed for 24 hours to clear the gut. Excreta were collected for 48 hours after precision feeding, and were then dried in a forced air oven and ground. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratory for a complete amino acid analysis, and digestibility values were then determined.

In each experiment a 23% protein, 3200 kcal/kg ME NRC-type diet served as the positive control (PC). In Experiment 1, the remaining dietary treatments consisted of 13% CP diets to which varying levels of crystalline amino acids were added back to meet either digestible amino acid levels from a 22% CP diet used in previous experiments from our lab at the University of Missouri (Guaiume, 2007) or digestible amino acid requirements established by Baker and coworkers (1993) using the ideal protein concept.

The total amino acid levels of the dietary treatments for Experiment 1 are provided in Table 6. One treatment using the University of Missouri values contained no glutamic acid and a low protein equivalent of 15.5% (MLPE), while others contained varying levels of glutamic acid to achieve a high protein equivalent of 20% (MHPE) or a mid-level protein equivalent of 18% (MMPE). Similarly, two treatments were developed using Baker and coworkers (1993) amino acid values and glutamic acid to achieve a 20% high protein equivalent (BHPE) or an 18% mid-level equivalent (BMPE). The ingredient and nutrient compositions of the experimental diets in Experiment 1 are provided in Table 7. Experiment 2 used the same positive control diet (PC), as well as three additional treatments, which consisted of 13% crude protein diets with crystalline amino acids added back to meet control levels and either no glutamic acid to yield a protein equivalent of 17.5% (PE-17.5), or glutamic acid added to meet an 18.75% (PE-18.75) or 20% (PE-20) protein equivalent. The ingredient and nutrient composition of dietary treatments for Experiment 2 are provided in Table 8.

Chicks were housed in stainless steel batteries with 24 hours of fluorescent lighting. The room was thermostatically controlled with temperatures maintained near 90 degrees F for the first week post-hatch and a two degree reduction in temperature every four days thereafter. Birds were cared for using husbandry guidelines derived from University of Missouri standard operating procedures.

Data were analyzed with pen gain as the experimental unit using the JMP statistical analysis software package. Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed, and the level of significance was

established at $P < 0.05$. Mean comparisons for all pairs were conducted using the Least Significant Difference test.

RESULTS AND DISCUSSION

These experiments were conducted in order to determine whether or not 13% crude protein corn and soybean meal diets with crystalline amino acid supplementation can support similar performance to that achieved with a NRC-type diet. Body weight gain, feed intake, and feed:gain were measured to determine any differences in bird performance.

In Experiment 1, birds consuming the PC treatment had significantly greater ($P < 0.05$) BWG than birds in any other treatment (Table 9, Figure 7). A significant difference ($P < 0.05$) in intake was seen between the BHPE treatment, which displayed the lowest FI, and the BMPE treatment, which displayed the highest feed intake (Table 9, Figure 8). A significantly improved F:G ($P < 0.05$) was observed in the PC treatment. Additionally, the BMPE treatment resulted in impaired F:G ($P < 0.05$) when compared to the MMPE and MHPE treatments (Table 9, Figure 9).

In Experiment 2, birds receiving the PE-17.5 treatment gained significantly less body weight ($P < 0.05$) than those consuming the PC treatment (Table 10, Figure 10). There were no significant differences ($P > 0.05$) in feed intake among any of the treatments (Table 10, Figure 11). Birds in the PC group displayed significantly improved F:G ($P < 0.05$) over all other treatments (Table 10, Figure 12).

The results of these experiments indicate that a 13% CP diet with crystalline amino acid supplementation did not support similar performance to a 23% CP industry-type diet. In Experiment 1, the addition of glutamic acid to achieve various protein equivalencies failed to bring performance up to control levels. However, the diets with the lowest protein equivalencies in both those formulated with amino acid levels from the University of Missouri and Baker's ideal ratios resulted in numerically worse feed conversions, although statistically these treatments were similar to others. In Experiment 2, the birds receiving the PE-17.5 treatment, which contained amino acids added to meet levels found in the control but no glutamic acid, gained significantly less weight than birds in the PC group. This is in disagreement with some of the previously mentioned literature concerning the importance of adding a non-specific nitrogen source to diets with low levels of CP; however, the 13% crude protein used in these experiments is significantly lower than those reported in the literature, which ranged from 15.3% CP (Aletor *et al.*, 2000) up to just a two or three percent reduction in CP, and may have resulted in a deficiency in one or more essential amino acid that was not observed when higher levels of crude protein were fed.

It is unknown why a 13% crude protein diet with amino acid supplementation is unable to provide similar performance in broilers as a 23% crude protein diet, especially as trials conducted at the University of Missouri using turkeys indicate that protein can be reduced from 28% to 10% with the addition of essential amino acids and achieve adequate performance (Moore *et al.*, 2001). A variety of explanations have been hypothesized and discussed in more detail in the literature review. Two of these include a deficient amount of nitrogen for synthesis of nonessential amino acids, leading to a

deficiency of essential amino acids, or an unfavorable dietary electrolyte balance (dEB). In these experiments, the diets low in crude protein replaced soybean meal with a range of crystalline amino acids. This can result in a reduction in dietary K and an increase in the levels of Cl⁻ supplied by those amino acids, lowering the dEB in these types of diets, which may cause the depression in performance (Patience, 1990; Aftab *et al.*, 2006). However, it has been reported that maintaining dEB in low protein diets did not restore performance to the level of control diets (Han *et al.*, 1992; Si *et al.*, 2004), indicating that a disruption in dEB is not the cause of depressed performance in birds consuming low crude protein rations. As a precaution, 1.5% sodium bicarbonate was added to the experimental diets in Experiment 2 (as opposed to 1.0% to the diets in Experiment 1) to prevent a disruption in the metabolic acid-base balance due to high levels of added amino acids.

Another hypothesis is that the requirement for glycine is actually higher in low CP diets than typical high CP diets, and a glycine deficiency is the main cause behind the depressed performance observed with the use of diets significantly low in CP. Increased performance has been observed in birds consuming low protein diets when the level of glycine was increased, suggesting that glycine may have a more specific role than previously believed and that the NRC-suggested requirements may be too low (Corzo *et al.*, 2004; Waldroup *et al.*, 2005b; Aftab *et al.*, 2006; Dean *et al.*, 2006; Namroud *et al.*, 2008). Namroud and collaborators (2008) suggest that adding significant amounts of crystalline amino acids to low intact CP diets increases blood and excretory ammonia concentrations, which may cause a reduction in growth and appetite due to negative effects on tissue metabolism. They state that in birds, the conversion of ammonia to uric

acid requires 1 glycine molecule in chicks, resulting in a greater than expected glycine requirement. This may be one explanation why researchers have seen improved performance when birds have been fed diets with increased glycine supplementation. The formulation of low crude protein diets involves a decrease in intact protein sources such as soybean meal that contain relatively high levels of glycine compared to other ingredients, so it may be important to consider glycine levels specifically, rather than just total NEAA levels, when formulating low crude protein diets.

While an amino acid deficiency might play a role in the decreased performance observed in birds consuming the 13% CP rations, the ratio between nonessential amino acids (NEAA) and essential amino acids (EAA) may also be an important factor. A ratio near 50:50 NEAA:EAA is often suggested, although a 5% variation in either direction is not uncommon (Aftab *et al.*, 2006). In Experiment 1, the NEAA:EAA ratio ranged from approximately 17:83 for the MLPE treatment to approximately 42:58 for the MHPE diets, while the BHPE treatment had a ratio near 49:51 and the BMPE treatment ratio was near 41:59. Interestingly, the wide variation of the NEAA:EAA ratios in the dietary treatments in this experiment did not seem to cause any clear discrepancies in performance.

Some researchers propose that decreased feed intake, which might occur for a variety of reasons, may be to blame for decreased performance. However, there is little agreement in the literature as to why this might occur; indeed, some researchers have found decreased feed intake in birds consuming low CP diets while others have not. In Experiment 1 and 2, the PC treatment did not result in significantly increased ($P < 0.05$) feed intake than other treatments.

It is still unclear at this time why a 13% CP ration with amino acid supplementation cannot yield similar results to a 23% CP ration. It may be that the minimum amount of intact protein that is required in the diet to achieve performance similar to that from a 23% CP diet is above 13%. Further research is necessary in this area. It does not appear that using a non-specific nitrogen source such as glutamic acid to increase the protein equivalent of a 13% CP ration can alleviate the resulting depression in performance.

Table 6. Amino Acid Levels of Experimental Diets for Broilers fed 13% Crude Protein (Experiment 1)

Treatment	Experimental Diets, %					
	PC ¹	MLPE	MHPE	MMPE	BHPE	BMPE
Crude protein	23	13	13	13	13	13
Protein Equivalent	23	15.5	20	18	20	18
Amino Acid						
Lysine	1.36	1.09	1.09	1.09	1.12	1.12
Methionine	0.50	0.50	0.50	0.50	0.405	0.405
Threonine	0.851	0.728	0.728	0.728	0.75	0.75
Valine	1.143	0.905	0.905	0.905	0.86	0.86
Arginine	1.543	1.342	1.342	1.342	1.18	1.18
Leucine	2.02	1.758	1.758	1.758	1.24	1.24
Histidine	0.625	0.514	0.514	0.514	0.35	0.35
Isoleucine	1.054	0.817	0.817	0.817	0.75	0.75
Phenylalanine	1.168	0.956	0.956	0.956		
Tryptophan	0.241	0.22	0.22	0.22	0.18	0.18
Glycine	0.636	0.437	0.437	0.437		
Glutamic Acid	0	0	4.524	2.503	5.491	3.471

* All values are expressed an a digestible basis

Table 7. Composition of Experimental Diets for Broilers fed 13% Crude Protein
(Experiment 1)

Treatment:	PC	MLPE	MHPE	MMPE	BHPE	BMPE
Corn	51.624	69.285	69.285	69.285	69.285	69.285
Soybean Meal	39.822	16.151	16.151	16.151	16.151	16.151
Lard	4.648	1.755	1.755	1.755	1.755	1.755
Dicalcium Phosphate	1.699	1.9	1.9	1.9	1.9	1.9
Limestone	1.174	1.206	1.206	1.206	1.206	1.206
Sodium Bicarbonate	0.3	1.0	1.0	1.0	1.0	1.0
Salt	0.256	0.26	0.26	0.26	0.26	0.26
Sucrose ³		5.28	0.756	2.777	0.797	2.817
Coban	0.075	0.075	0.075	0.075	0.075	0.075
Vitamin Premix ¹	0.075	0.075	0.075	0.075	0.075	0.075
Choline Chloride	0.05	0.149	0.149	0.149	0.149	0.149
Calcium Trace Mineral ²	0.10	0.10	0.10	0.10	0.10	0.10
Selenium Premix ²	0.03	0.03	0.03	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013	0.013	0.013	0.013
DL Methionine	0.116	0.227	0.227	0.227	0.132	0.132
Arginine		0.477	0.477	0.477	0.317	0.317
Glycine		0.039	0.039	0.039	0.15	0.15
Histidine		0.165	0.165	0.165		
Isoleucine		0.215	0.215	0.215	0.174	0.174
Leucine		0.383	0.383	0.383		
Lysine		0.444	0.444	0.444	0.482	0.482
Phenylalanine		0.25	0.25	0.25		
Threonine		0.209	0.209	0.209	0.231	0.231
Tryptophan		0.098	0.098	0.098	0.058	0.058
Valine		0.214	0.214	0.214	0.169	0.169
Cystine						
Glutamic Acid			4.524	2.503	5.491	3.471
Calculated to contain						
Crude Protein, % ⁴	23	13	13	13	13	13
Protein Equivalent, % ⁵	23	15.5	20	18	20	18
ME, kcal/kg	3200	3200	3200	3200	3200	3200
Calcium, %	1.0	1.0	1.0	1.0	1.0	1.0
Available Phosphorus, %	0.45	0.45	0.45	0.45	0.45	0.45

¹ Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

² Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

³ Synthetic amino acids and glutamic acid added at expense of sucrose.

⁴ Crude protein values calculated from protein provided from corn and soybean meal.

⁵ Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids.

Table 8. Composition of Experimental Diets for Broilers fed 13% Crude Protein
(Experiment 2)

Treatment:	PC	PE-17.5	PE-18.75	PE-20
Corn	51.642	68.231	68.231	68.231
Soybean Meal	39.822	16.322	16.322	16.322
Lard	4.648	2.138	2.138	2.138
Dicalcium Phosphate	1.699	1.902	1.902	1.902
Limestone	1.174	1.204	1.204	1.204
Sodium Bicarbonate	0.3	1.50	1.50	1.50
Salt	0.256	0.261	0.261	0.261
Sucrose ³	0	3.085	1.86	0.598
Coban	0.075	0.075	0.075	0.075
Vitamin Premix ¹	0.075	0.075	0.075	0.075
Choline Chloride	0.05	0.149	0.149	0.149
Calcium Trace Mineral ²	0.1	0.1	0.1	0.1
Selenium Premix ²	0.03	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013	0.013
DL Methionine	0.116	0.229	0.229	0.229
Arginine		0.680	0.680	0.680
Glycine		0.239	0.239	0.239
Histidine		0.307	0.307	0.307
Isoleucine		0.533	0.533	0.533
Leucine		0.651	0.651	0.651
Lysine		0.786	0.786	0.786
Phenylalanine		0.463	0.463	0.463
Threonine		0.333	0.333	0.333
Tryptophan		0.119	0.119	0.119
Valine		0.453	0.453	0.453
Cystine		0.122	0.122	0.122
Glutamic Acid			1.225	2.487
Calculated to contain				
Crude Protein, % ⁴	23.0	13	13	13
Protein Equivalent, % ⁵	23.0	17.5	18.75	20.0
ME, kcal/kg	3200	3200	3200	3200
Calcium, %	1.0	1.0	1.0	1.0
Available Phosphorus, %	0.45	0.45	0.45	0.45

¹ Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

² Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

³ Synthetic amino acids and glutamic acid added at expense of sucrose.

⁴ Crude protein values calculated from protein provided from corn and soybean meal.

⁵ Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids.

Table 9. Performance of Broiler Chicks Fed a 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
PC	752 ^a	988 ^{ab}	1.31 ^a
MLPE	643 ^b	982 ^{ab}	1.52 ^{bc}
MHPE	667 ^b	973 ^{ab}	1.47 ^b
MMPE	660 ^b	988 ^{ab}	1.48 ^b
BHPE	639 ^b	961 ^b	1.50 ^{bc}
BMPE	651 ^b	999 ^a	1.53 ^c
Pooled SEM	0.6578	0.0085	.0143

Values with differing letters are significantly ($P < 0.05$) different

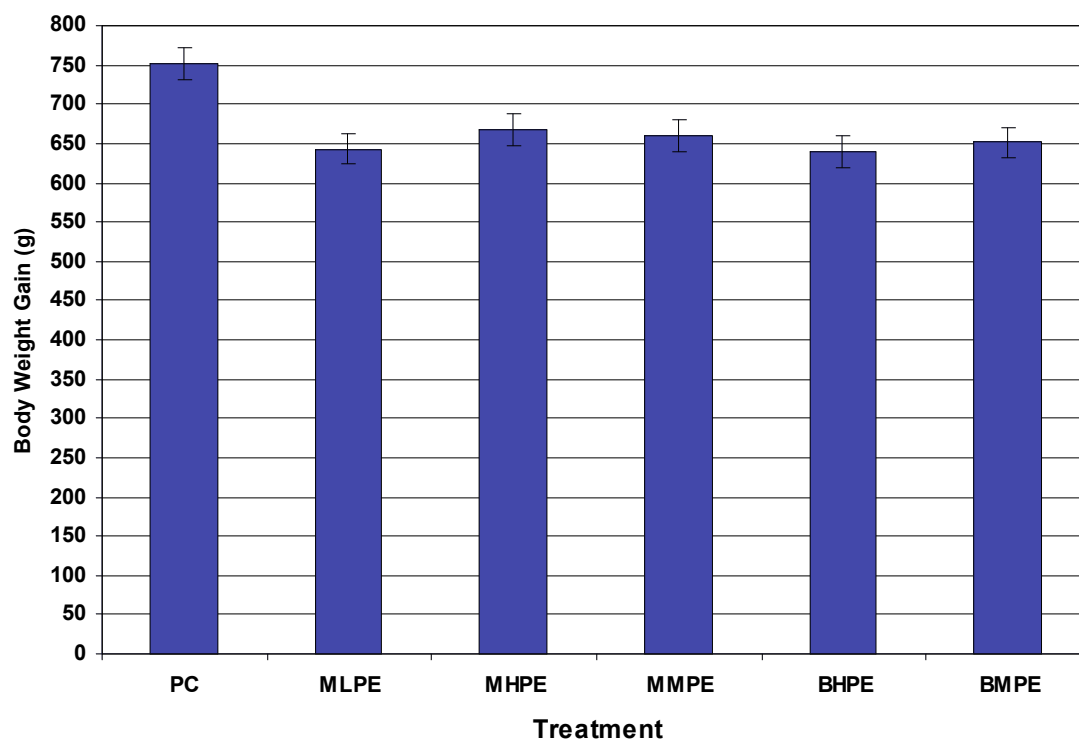


Figure 7. Body Weight Gain (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1

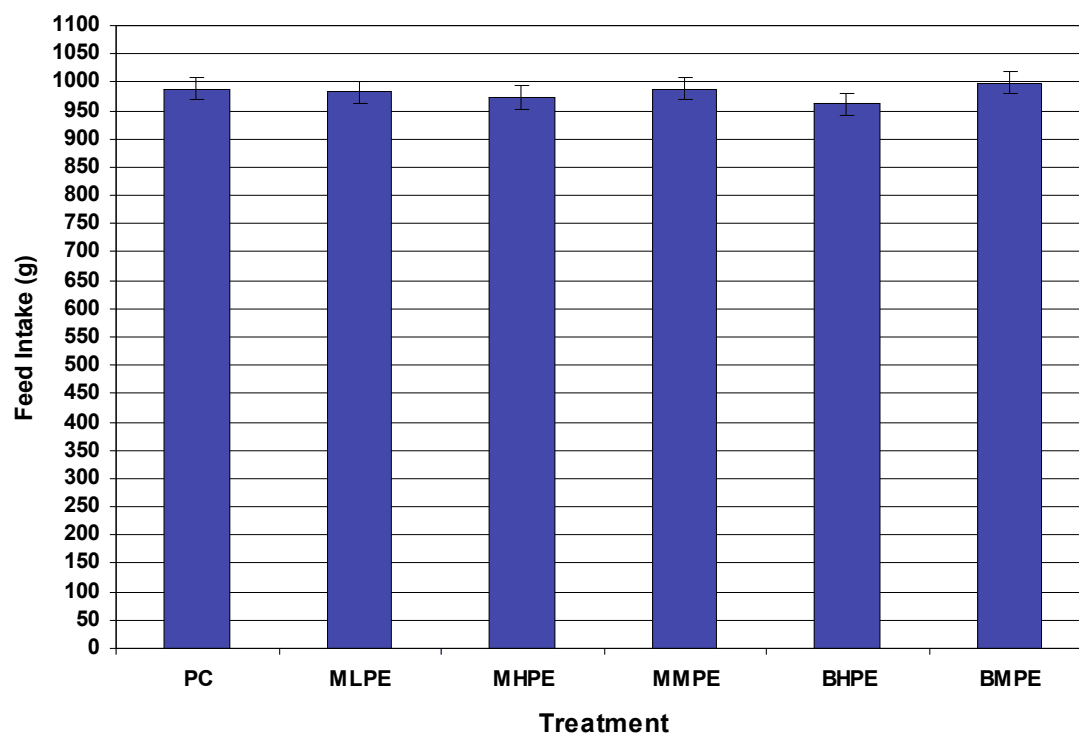


Figure 8. Feed Intake (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1

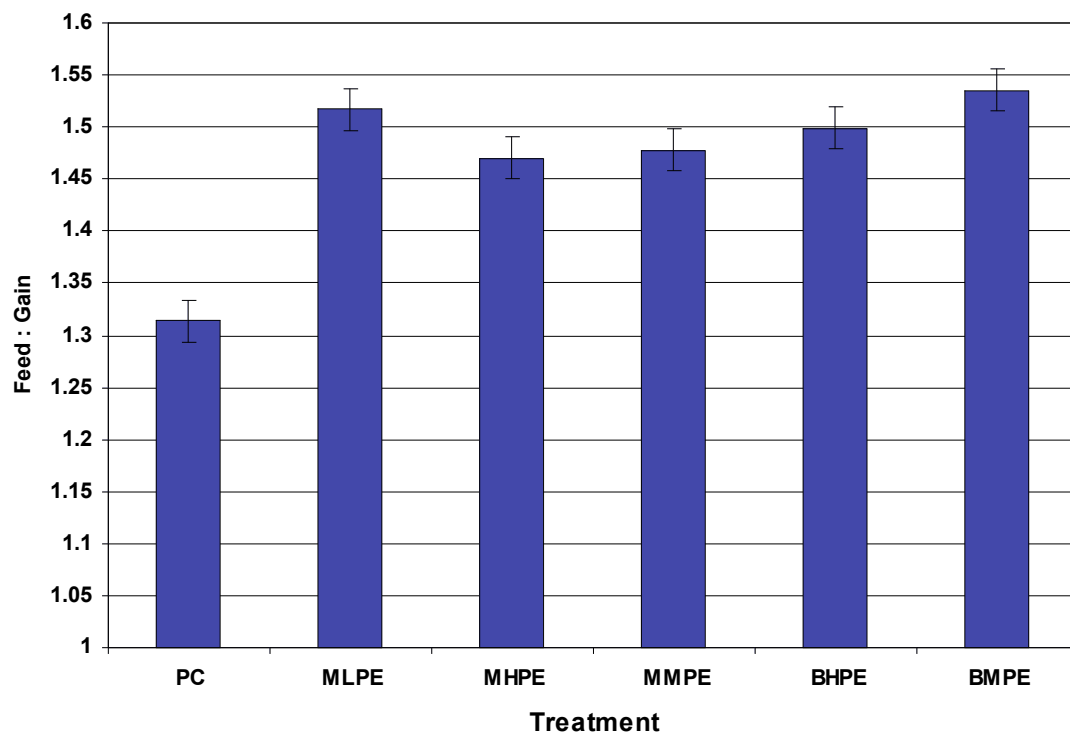


Figure 9. Feed:Gain of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 1

Table 10. Performance of Broiler Chicks Fed a 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
PC	655 ^a	815 ^a	1.25 ^a
PE-17.5	606 ^b	803 ^a	1.31 ^b
PE-18.75	634 ^{ab}	813 ^a	1.29 ^b
PE-20	630 ^{ab}	825 ^a	1.30 ^b
Pooled SEM	.0085	.0114	.0095

Values with differing letters are significantly ($P < 0.05$) different

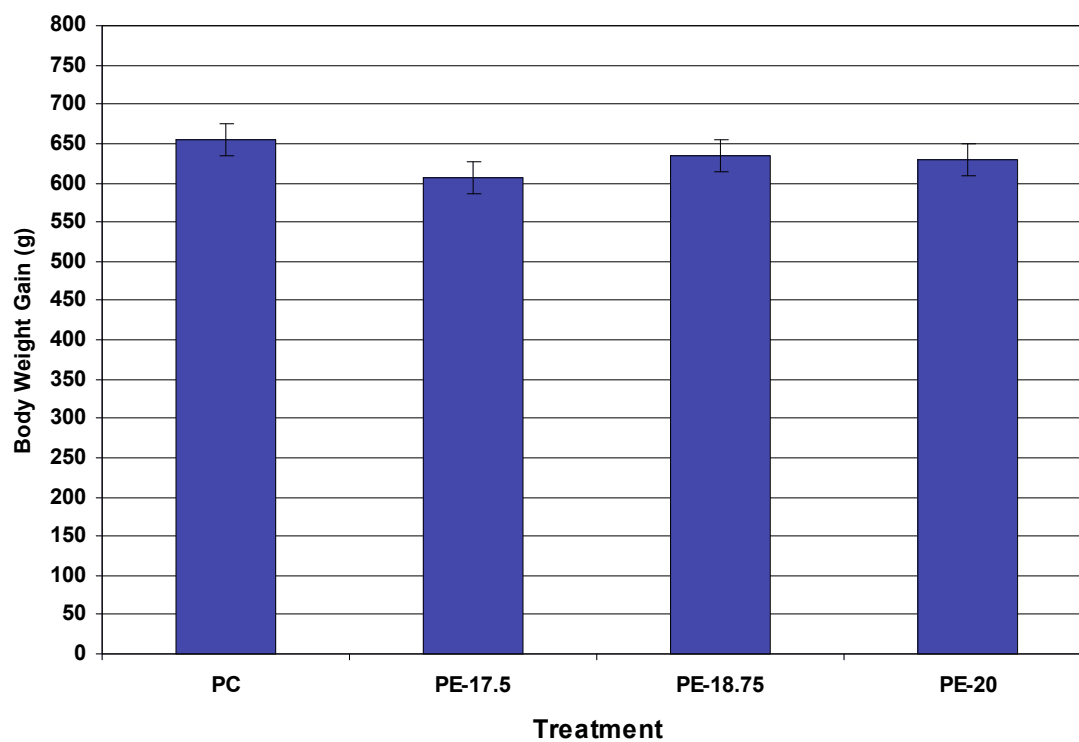


Figure 10. Body Weight Gain (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2

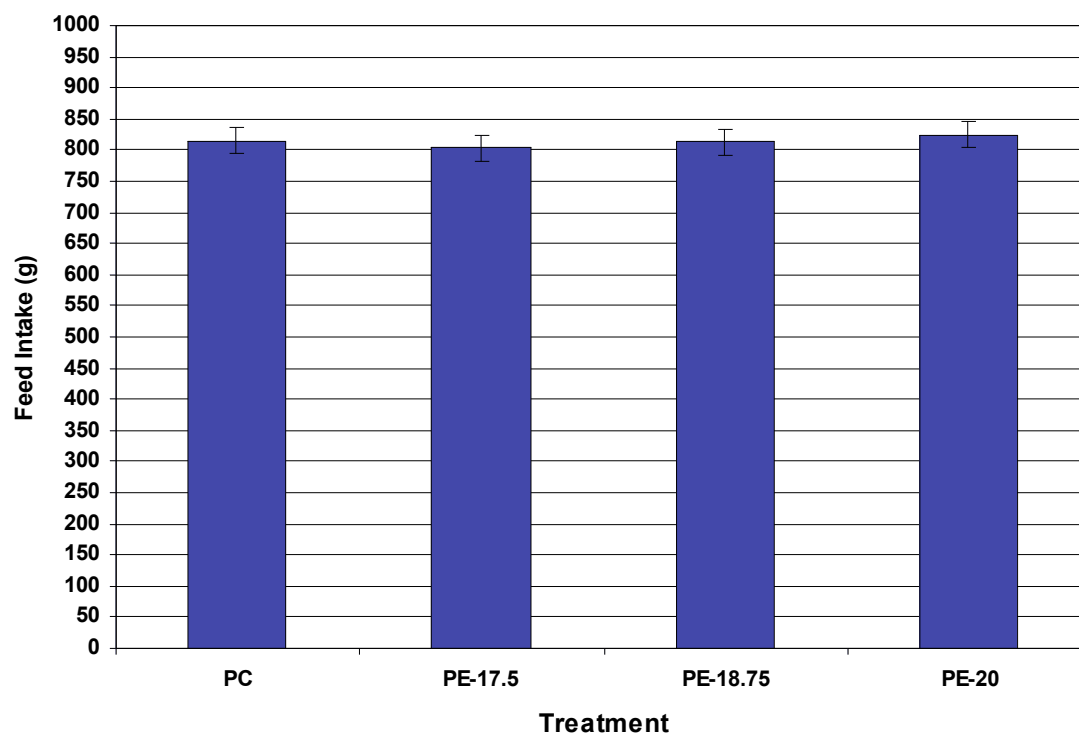


Figure 11. Feed Intake (g) of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2

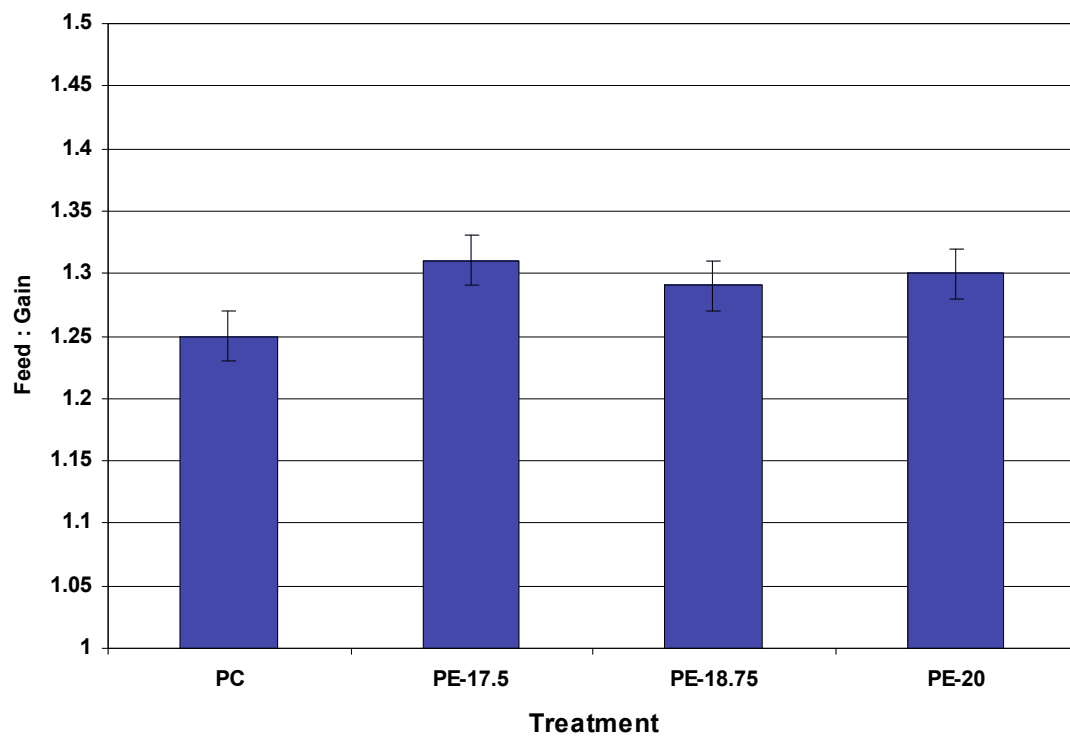


Figure 12. Feed:Gain of Broiler Chicks Fed 13% Crude Protein Diets from 7-21 Days of Age; Experiment 2

CHAPTER 4

EFFECTS OF LOW CRUDE PROTEIN DIETS WITH AND WITHOUT THE ADDITION OF MEAT AND BONE MEAL ON BROILERS IN THE STARTER PERIOD

ABSTRACT

A study was conducted in order to determine how the addition or exclusion of meat and bone meal (MBM) may affect performance in broilers consuming diets low in crude protein. Commercial broilers were fed an NRC-type diet for the first seven days of age. On day 7, birds were sorted by weight into battery pens with 5 birds per pen. Four dietary treatments were utilized with 12 replicates per treatment for a total of 48 pens. The treatments consisted of a 22% CP diet which did not contain MBM (22-MBM), a 15% CP diet with crystalline amino acids added back that did not contain MBM (15-MBM), a 22% CP diet with MBM (22+MBM), and a 15% CP diet with amino acids added back that did include MBM (15+MBM). In the 15% CP diets, the essential amino acid levels were brought up to levels found in a 15% CP diet from previous research conducted at the University of Missouri (Brooks, 2003). All diets were formulated on a digestible basis and were designed to be isocaloric. Birds received feed and water *ad libitum* during the course of the study. At the conclusion of the experiment, body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were measured. Birds consuming the 22-MBM treatment achieved significantly greater ($P < 0.05$) BWG than birds consuming either treatment with MBM added. The 15-MBM treatment resulted in similar ($P > 0.05$)

BWG as the 22-MBM and 22+MBM treatments. A significant difference ($P < 0.05$) in BWG and F:G was observed between the 15+MBM treatment and all other treatments. All treatments had similar F:G ($P > 0.05$) with the exception of the 15-MBM and 22+MBM treatments, in which case significantly improved F:G ($P < 0.05$) was observed in birds in the 15-MBM treatment groups over those in the 22+MBM groups. Overall, these results indicate that the addition of MBM to a low CP ration did not improve the performance of broiler chicks in the starter period.

INTRODUCTION

The first two trials in this series of experiments indicated that feeding a 15% crude protein diet to broilers in the starter period results in similar performance to birds consuming a standard diet, with the negative control treatments (15% CP) achieving approximately 98% of the performance of the positive control industry-type corn and SBM diet. Meat and bone meal (MBM) is a commonly utilized ingredient in practical poultry rations that is high in protein. The addition of animal protein sources such as MBM may improve performance over standard corn and soybean meal diets, and this improvement is thought to be the result of high availability of some of the limiting amino acids and/or the reduction of the amount of poorly digested carbohydrates from soybean meal (Firman, 2006). Although samples of MBM often vary somewhat in amino acid digestibility, formulation on a digestible basis together with the utilization of crystalline amino acids can allow for balanced, high quality diets that are economically beneficial (Rostagno *et al.*, 1995). Few studies have been conducted in poultry with low CP rations

that utilize protein by-product meals. The current study was designed with the purpose of investigating the effects of MBM incorporation into diets significantly low in CP on performance of broilers in the starter period when compared to NRC-type diets.

MATERIALS AND METHODS

Day-old straight run broiler chicks were obtained from a commercial hatchery and fed a NRC-type corn and soybean meal diet until seven days of age. On day seven, birds were wing-banded and weighed. Birds were sorted by weight and assigned to pens (5 birds per pen) to obtain similar starting pen weights. Each trial utilized 240 birds to provide 12 replications of four treatments. Chicks were provided access to experimental diets and water *ad libitum* for fourteen days, and the trial was terminated on day 21. Feed intake, body weight gain, and feed:gain were measured. Feed:gain was adjusted for mortality by adding each mortality weight to the appropriate pen gain then dividing feed consumed by gain.

Diets were formulated on a digestible basis utilizing least-cost formulation software. The amino acid digestibility values used in this experiment for the corn, SBM, and MBM were obtained previously by precision feeding a known sample of each ingredient to cecectomized roosters that had been removed from feed for 24 hours to clear the gut. Excreta was collected for 48 hours after precision feeding, and were then dried in a forced air oven and ground. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratory for a complete amino acid analysis, and digestibility values were then determined.

The four dietary treatments consisted of a 22% protein NRC-type diet without MBM (22-MBM), a 15% CP diet with crystalline amino acids added back that did not contain MBM (15-MBM), a 22% CP diet with MBM (22+MBM), and a 15% CP diet with amino acids added back that included MBM (15+MBM). In the 15% CP diets, the essential amino acid levels were brought up to levels found in a 15% CP diet from previous research conducted at the University of Missouri (Brooks, 2003). Glutamic acid was added to the diets in order to maintain a 20% protein equivalent and to prevent confounding of results due to a generalized nitrogen deficiency, and all amino acids were added at the expense of sucrose as its energy content is comparable to that of crystalline amino acids. The compositions of dietary treatments are shown in Table 11.

Chicks were housed in stainless steel batteries with 24 hours of fluorescent lighting. The room was thermostatically controlled with temperatures maintained near 90 degrees F for the first week post-hatch and a two degree reduction in temperature every four days thereafter. Birds were cared for using husbandry guidelines derived from University of Missouri standard operating procedures.

Data were analyzed with the pen gain as the experimental unit using the JMP statistical analysis software package. Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed, and the level of significance was established at $P < 0.05$. Mean comparisons for all pairs were conducted using the Least Significant Difference test.

RESULTS AND DISCUSSION

This study was designed with the purpose of investigating the effects of MBM incorporation into low CP diets on the performance of broilers in the starter period when compared to NRC-type diets. At the conclusion of the experiment, body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were measured to determine differences in performance.

The results for BWG are displayed in Table 12 and Figure 13. Birds consuming the 22-MBM and 15-MBM treatments achieved statistically similar ($P > 0.05$) BWG, and the greatest gain with 648g and 623g, respectively. The BWG for the 15-MBM treatment was also similar ($P > 0.05$) to the 22+MBM treatment. The birds consuming the 15+MBM treatment gained significantly less weight ($P < 0.05$) than any other treatment with only 576g. The 15+MBM treatment also resulted in significantly reduced ($P < 0.05$) feed intake (Table 12, Figure 14) compared with all other treatments. All treatments had similar F:G ($P > 0.05$) with the exception of the 15-MBM and 22+MBM treatments, in which case significantly improved F:G ($P < 0.05$) was observed in birds in the 15-MBM treatment groups over those in the 22+MBM groups (1.368 versus 1.406, respectively). Feed conversion data is shown in Table 12 and Figure 15.

The results from this trial indicate that the addition of MBM to the low protein ration was detrimental to bird performance. It also resulted in reduced body weight gain in birds fed a 22% CP ration compared to birds fed a 22% CP ration without MBM. A great deal of variation can exist in the protein and amino acid quality of MBM due to differences in processing, making feed formulation on a digestible amino acid basis of

great importance (Wang and Parsons, 1998). In a trial conducted with cecectomized birds by Parson and collaborators (1997), it was shown that the true digestibility of amino acids as a percentage varied greatly among MBM samples, with the mean (and range) for lysine, methionine, and cystine being 81 (73-88), 85 (77-91), and 58% (37-72%), respectively. While the diets in this trial were formulated on a digestible amino acid basis, the values utilized were from previous research conducted with MBM. It is possible that calculated protein and amino acid values were greater than the actual content of those in the specific MBM used in this trial. It is apparent that if MBM is to be utilized, each batch of MBM must be analyzed for digestible amino acid content for accurate formulations to be made, especially when using low protein rations in which any excess amino acids that might provide a margin of safety against deficiencies is eliminated.

Table 11. Composition of Diets for Broilers Consuming 22% or 15% Crude Protein with the Addition or Exclusion of Meat and Bone Meal

Treatment:	22-MBM	15-MBM	22+MBM	15+MBM
Corn	53.706	65.601	58.426	69.35
Soybean Meal	37.359	20.959	27.147	10.582
Meat and Bone Meal			9.0	9.218
Lard	3.512	1.68	2.575	1.125
Dicalcium Phosphate	1.719	1.859		
Limestone	1.178	1.2	0.344	0.92
Sodium Bicarbonate	0.3	1.0	0.3	1.0
Salt	0.256	0.26	0.25	0.3
Sucrose ³	1.5	1.66	1.5	1.634
Coban	0.075	0.075	0.075	0.075
Vitamin Premix ¹	0.075	0.075	0.075	0.075
Choline Chloride	0.05	0.149	0.05	0.149
Calcium Trace Mineral ²	0.1	0.1	0.1	0.1
Selenium Premix ²	0.03	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013	0.013
DL Methionine	0.127	0.326	0.114	0.315
Arginine		0.672		0.644
Glycine		0.302		
Histidine		0.278		0.319
Isoleucine		0.367		0.458
Leucine		0.479		0.498
Lysine		0.721		0.760
Phenylalanine		0.795		0.847
Threonine		0.196		0.199
Tryptophan		0.178		0.198
Valine		0.270		0.295
Cystine				
Glutamic Acid		0.755		0.836
Calculated to contain				
Crude Protein, % ⁴	22.0	15.0	22.0	15.0
Protein Equivalent, % ⁵	22.0	20.0	22.0	20.0
ME, kcal/kg	3166	3166	3166	3166
Calcium, %	1.0	1.0	1.0	1.0
Available Phosphorus, %	0.45	0.45	0.45	0.45

¹ Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

² Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

³ Synthetic amino acids and glutamic acid added at expense of sucrose.

⁴ Crude protein values calculated from protein provided from corn and soybean meal.

⁵ Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids.

Table 12. Performance of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
22-MBM	648 ^a	878 ^a	1.372 ^{ab}
15-MBM	627 ^{ab}	857 ^a	1.368 ^b
22+MBM	605 ^b	849 ^a	1.406 ^a
15+MBM	576 ^c	807 ^b	1.400 ^{ab}
Pooled SEM	.0076	.0101	.0094

Values with differing letters are significantly ($P < 0.05$) different

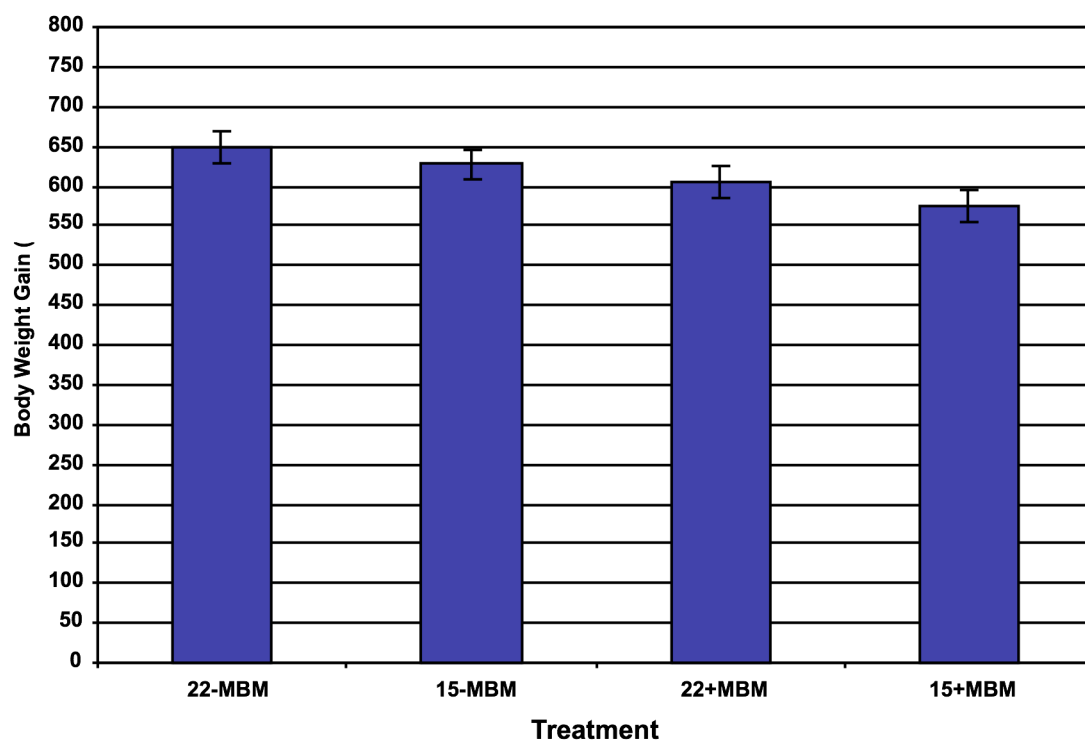


Figure 13. Body Weight Gain (g) of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age

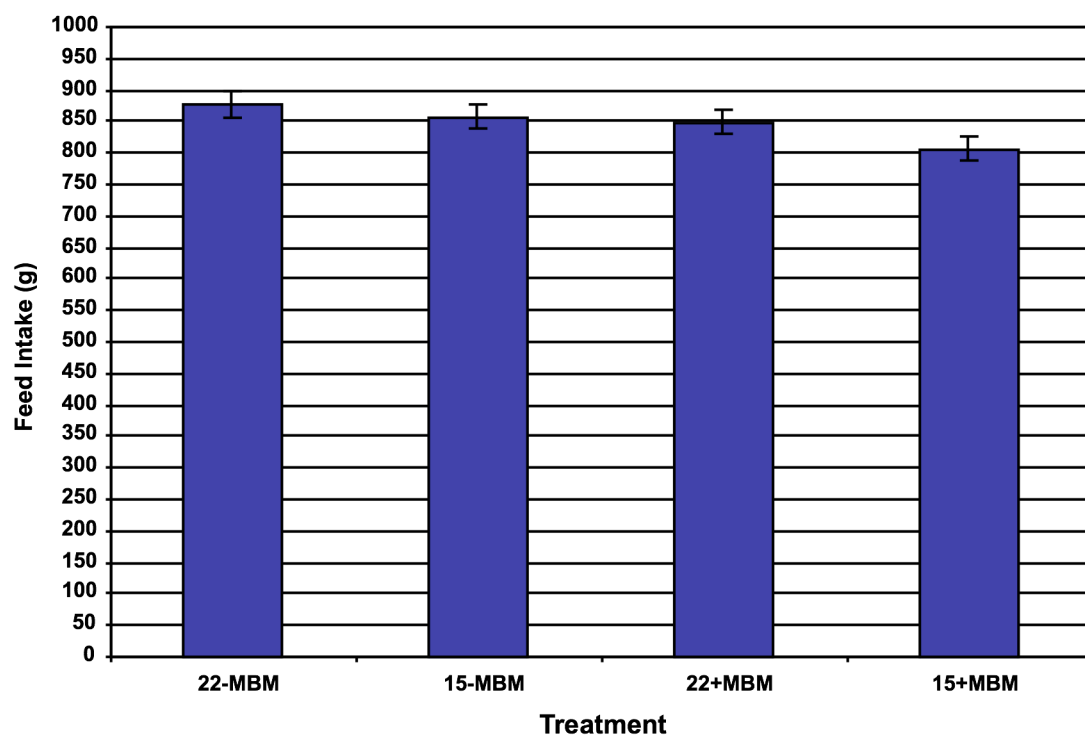


Figure 14. Feed Intake (g) of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age

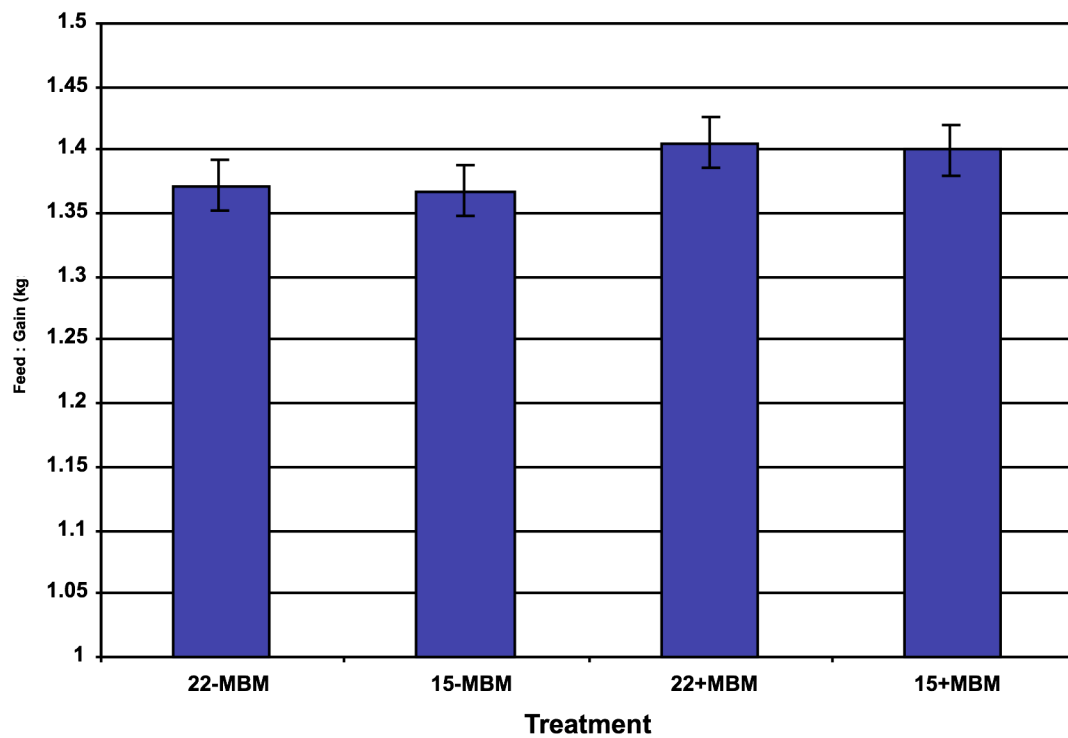


Figure 15. Feed:Gain of Broiler Chicks Fed 22% or 15% Crude Protein Diets with the Addition or Exclusion of Meat and Bone Meal from 7-21 Days of Age

CHAPTER 5

EFFECTS OF LOW CRUDE PROTEIN RATIONS WITH VARIOUS LEVELS OF FAT ADDITION ON BROILERS IN THE STARTER PERIOD

ABSTRACT

A study was conducted in order to determine if the addition of an increased level of fat similar to that found in the NRC-type control ration could improve performance of broilers consuming low crude protein diets in the starter period. Commercial broilers were fed an NRC-type diet for the first seven days of age. On day 7, birds were sorted by weight into battery pens with 5 birds per pen. Three treatments were utilized with 16 replicates per treatment for a total of 48 pens. The dietary treatments consisted of a 22% CP ration that contained approximately 3.5% added fat (22-HF). The remaining treatments consisted of a 15% CP ration that contained approximately 1.6% added fat (15-LF) and a 15% CP ration with 3.5% added fat (15-HF). Least-cost computer formulation was used to formulate all rations, and in the 22-HF and 15-LF treatments, the computer was allowed to add fat without restrictions. For the 15-HF ration, fat was forced into the ration at a level similar to that in the control. In the 15% CP diets, the essential amino acid levels were brought up to levels found in a 15% CP diet from a previous research conducted at the University of Missouri (Brooks, 2003). All diets were formulated on a digestible basis and were designed to be isocaloric. Birds received feed and water *ad libitum* during the course of the study. At the conclusion of the experiment,

body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were measured. Birds consuming the 22-HF treatment had significantly higher BWG ($P < 0.05$) than birds on the other treatments. Significant differences in FI ($P < 0.05$) were observed between the 22-HF and 15-HF treatments, with the 22-HF treatment resulting in greater intake. Additionally, the 22-HF treatment resulted in significantly improved F:G ($P < 0.05$) over either of the low CP treatments.

INTRODUCTION

The first two trials in this series of experiments indicated that feeding a 15% crude protein diet to broilers in the starter period results in similar performance to birds consuming a standard diet, with the negative control treatments (15% CP) achieving approximately 98% of the performance of the positive control industry-type corn and SBM diet. It was observed in previous trials from this series of experiments that feed from the low CP treatments visually appeared drier and dustier, and that birds consuming these treatments had significantly dirtier water troughs from beak washing than those consuming the control rations. It is known that added fat to poultry rations lends physical improvement to feed by reducing dustiness and particle separation and increasing palatability, and can reduce the amount of feed lost in waterers (Fuller, 1996). Additionally, added fat can slow gut motility, allowing increased time for nutrient absorption, and the micelles themselves may help in the transport of amino acids to the gut wall (Firman and Remus, 1994). After examining the diets from the previous trials, it was determined that the control diets contained over twice the amount of added fat as the

low CP diets. The current study was developed with the purpose of investigating the effects of the addition of fat levels similar to those found in a standard, NRC-type control diet into diets significantly low in CP on performance of broilers in the starter period.

MATERIALS AND METHODS

Day-old straight run broiler chicks were obtained from a commercial hatchery and fed a NRC-type corn and soybean meal diet until seven days of age. On day seven, birds were wing-banded and weighed. Birds were sorted by weight and assigned to pens (5 birds per pen) to obtain similar starting pen weights. Each trial utilized 240 birds to provide 16 replications of three treatments. Chicks were provided access to experimental diets and water *ad libitum* for fourteen days, and the trial was terminated on day 21. Feed intake, body weight gain, and feed:gain were measured. Feed:gain was adjusted for mortality by adding each mortality weight to the appropriate pen gain then dividing feed consumed by gain.

Diets were formulated on a digestible basis utilizing least-cost formulation software. The amino acid digestibility values used in this experiment for the corn and SBM were obtained previously by precision feeding a known sample of each ingredient to cecectomized roosters that had been removed from feed for 24 hours to clear the gut. Excreta was collected for 48 hours after precision feeding, and were then dried in a forced air oven and ground. Samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratory for a complete amino acid analysis, and digestibility values were then determined.

The three dietary treatments consisted of a 22% protein NRC-type diet that contained approximately 3.5% added fat (22-HF), a 15% CP ration that contained approximately 1.6% added fat (15-LF) and a 15% CP ration with 3.5% added fat (15-HF). Least-cost computer formulation was used to formulate all rations, and in the 22-HF and 15-LF treatments, the computer was allowed to add fat without minimum or maximum restrictions. For the 15-HF ration, fat was forced into the ration at a level similar to that in the control. In the 15% CP diets, the essential amino acid levels were brought up to levels found in a 15% CP diet from a previous research conducted at the University of Missouri. Glutamic acid was added to the diets in order to maintain a 20% protein equivalent and to prevent confounding of results due to a generalized nitrogen deficiency, and all amino acids were added at the expense of sucrose as its energy content is comparable to that of crystalline amino acids. The compositions of dietary treatments are shown in Table 13.

Chicks were housed in stainless steel batteries with 24 hours of fluorescent lighting. The room was thermostatically controlled with temperatures maintained near 90 degrees F for the first week post-hatch and a two degree reduction in temperature every four days thereafter. Birds were cared for using husbandry guidelines derived from University of Missouri standard operating procedures.

Data were analyzed with the pen gain as the experimental unit using the JMP statistical analysis software package. Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed, and the level of significance was established at $P < 0.05$. Mean comparisons for all pairs were conducted using the Least Significant Difference test.

RESULTS AND DISCUSSION

This study was designed with the purpose of investigating the effects of increased levels of added fat in low CP diets on the performance of broilers in the starter period when compared to NRC-type diets containing similar levels of fat. At the conclusion of the experiment, body weight gain (BWG), feed intake (FI), and feed:gain (F:G) were measured to determine differences in performance.

The results for BWG are displayed in Table 14 and Figure 16. Birds consuming the 22-HF treatment achieved significantly higher BWG ($P < 0.05$) than birds on the other treatments. Significant differences in FI ($P < 0.05$) were observed between the 22-HF and 15-HF treatments, with the 22-HF treatment resulting in greater intake (803g for the 22-HF treatment versus 778g for the 15-HF treatment). The data for FI can be seen in Table 14 and Figure 17. Additionally, the 22-HF treatment resulted in significantly better F:G ($P < 0.05$) than either of the low CP treatments (Table 14, Figure 18).

Unexpectedly, the increase in the level of added fat to the 15% CP diet resulted in depressed BWG and FI and significantly poorer ($P < 0.05$) feed conversion. Adding dietary fat at higher than normal levels can decrease feed intake by increasing the energy density of the diet, however at the current levels this would not be an expected outcome. It has been proposed that there is a minimum level of intact protein required in poultry diets, and that there may actually be a minimum ratio of intact protein to that supplied from the nitrogen of crystalline amino acids which, if not met, can negatively affect performance by altering the absorption of amino acids in the body (Colnago *et al.*, 1991).

However, in this experiment, the amount of crystalline amino acids added in the 15-LF diet and the 15-HF diet differed only slightly (5.339% versus 5.345%, respectively). It is unknown at this time why additional dietary fat led to decreased performance.

Table 13. Composition of Diets for Broilers Consuming 15% Crude Protein Diets with or without Additional Dietary Fat

Treatment:	22-HF	15-LF	15-HF
Corn	53.706	65.601	57.963
Soybean Meal	37.359	20.959	20.066
Wheat Midds			6.742
Lard	3.512	1.68	3.5
Dicalcium Phosphate	1.719	1.859	1.795
Limestone	1.178	1.2	1.229
Sodium Bicarbonate	0.3	1.0	1.0
Salt	0.256	0.26	0.263
Sucrose ³	1.5	1.66	1.485
Coban	0.075	0.075	0.075
Vitamin Premix ¹	0.075	0.075	0.075
Choline Chloride	0.05	0.149	0.149
Calcium Trace Mineral ²	0.1	0.1	0.1
Selenium Premix ²	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013
DL Methionine	0.127	0.326	0.336
Arginine		0.672	0.661
Glycine		0.302	0.293
Histidine		0.278	0.287
Isoleucine		0.367	0.376
Leucine		0.479	0.527
Lysine		0.721	0.734
Phenylalanine		0.795	0.809
Threonine		0.196	0.205
Tryptophan		0.178	0.173
Valine		0.270	0.278
Cystine			
Glutamic Acid		0.755	0.666
Calculated to contain			
Crude Protein, % ⁴	22.0	15.0	15.0
Protein Equivalent, % ⁵	22.0	20.0	20.0
ME, kcal/kg	3166	3166	3166
Calcium, %	1.0	1.0	1.0
Available Phosphorus, %	0.45	0.45	0.45

¹ Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

² Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm.

³ Synthetic amino acids and glutamic acid added at expense of sucrose.

⁴ Crude protein values calculated from protein provided from corn and soybean meal.

⁵ Protein equivalent calculated from protein provided from corn and soybean meal plus protein from synthetic amino acids.

Table 14. Performance of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age

Treatment	Weight Gain (g)	Feed Intake (g)	Feed:Gain
22-HF	604 ^a	803 ^a	1.33 ^a
15-LF	566 ^b	787 ^{ab}	1.39 ^b
15-HF	555 ^b	778 ^b	1.41 ^b
Pooled SEM	.0062	.0050	.0153

Values with differing letters are significantly ($P < 0.05$) different

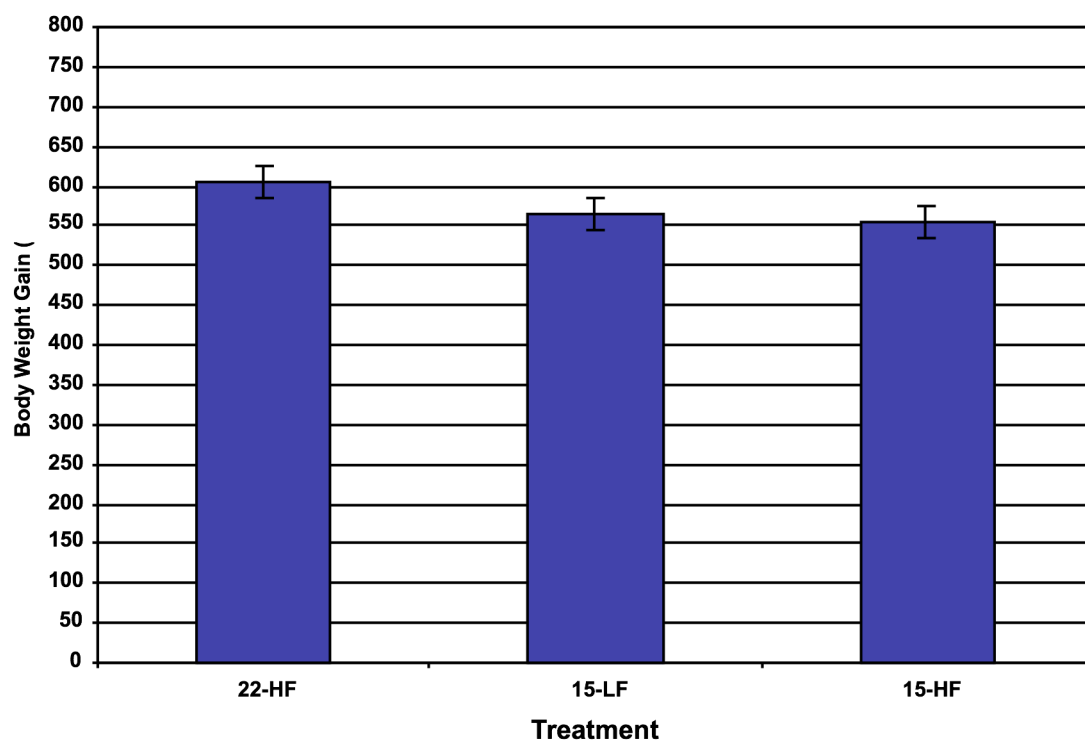


Figure 16. Body Weight Gain (g) of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age

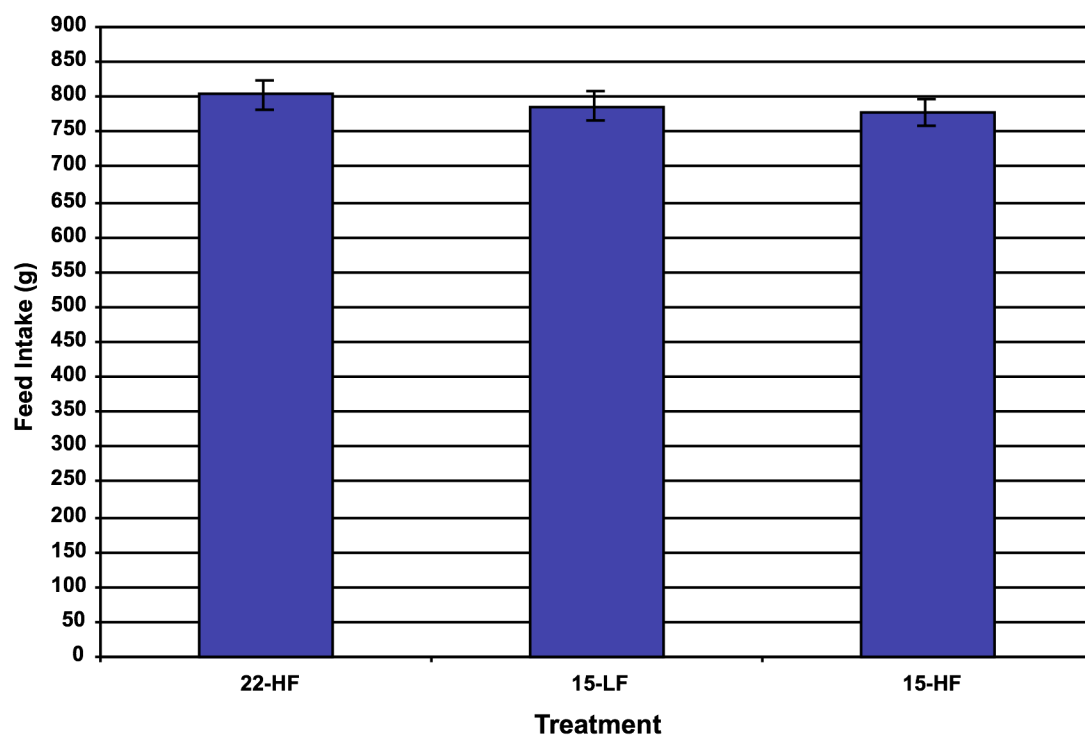


Figure 17. Feed Intake (g) of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age

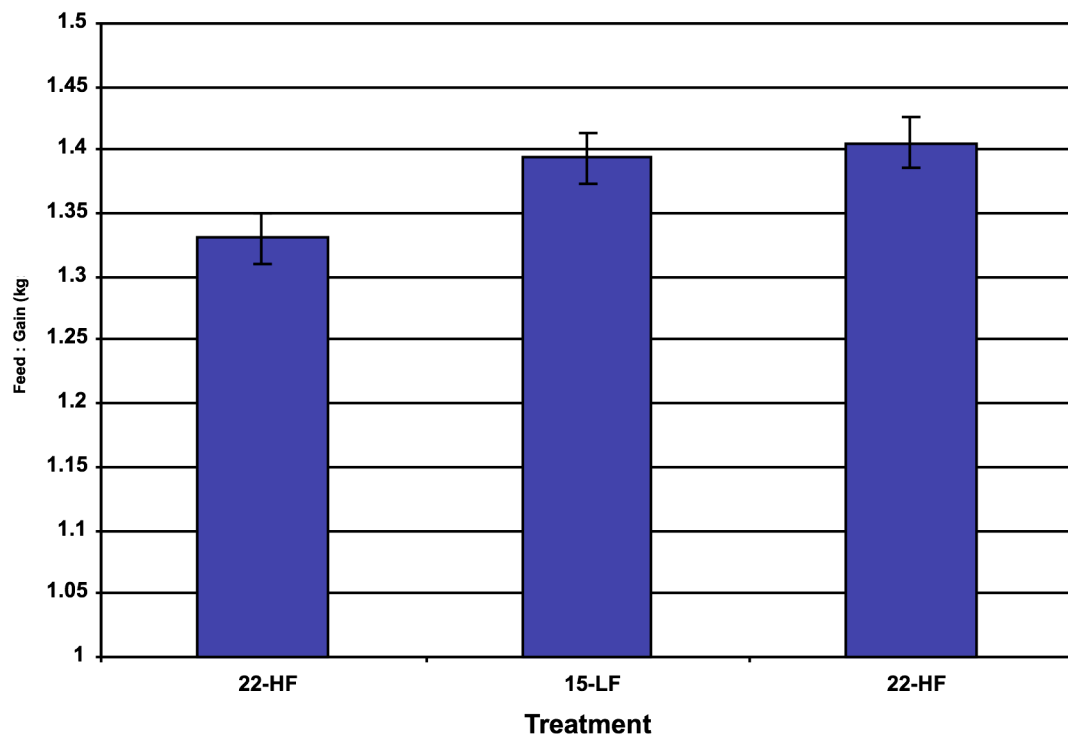


Figure 18. Feed:Gain of Broiler Chicks Fed 15% Crude Protein Diets with or without Additional Dietary Fat from 7-21 Days of Age

SUMMARY

In order to meet increasing worldwide demand for poultry and maintain profitability, it is important to find new ways to stay competitive within the industry by decreasing the costs of production while still producing a high quality product. Meeting the requirements for protein and amino acids is one of the largest costs associated with feeding poultry, making the development of low protein broiler diets essential for combating rising feed and production costs. Additionally, low crude protein diets are an important step towards combating environmental pollution concerns surrounding poultry production. It is not entirely clear at this point why low crude protein diets have been unable to provide the same level of production in broiler chickens as standard high crude protein diets, especially as research with turkeys has shown that crude protein can be reduced from 28% to 10% with essential amino acid supplementation and still achieve similar performance (Moore *et al.*, 2001). If a diet low enough in crude protein can be developed that still achieves adequate performance, it would be possible to more closely determine the digestible requirements for the essential amino acids by using that diet to individually study each amino acid through titration experiments. This would eventually allow formulation of economically efficient diets that precisely meet the birds' requirements with little or no excess.

The lowest level to which crude protein can be reduced with amino acid supplementation in broiler diets without reducing bird performance is still unknown, and additional research on the subject could yield significantly greater cost savings in the future. The results from this series of experiments indicate that a 15% crude protein diet

is capable of supporting performance that is similar to that of birds fed 23% crude protein diets formulated to meet or exceed NRC requirements, and may be close to the level at which individual amino acids can be titrated in order to determine more precise requirements.

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