Determination of the Optimal Digestible Isoleucine to Lysine Ratios for Yield Plus × Ross 708 Male Broilers between 1.0 and 4.0 kg Body Weight

by

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ABSTRACT

The objective of this research was to determine the optimum digestible Ile to Lys ratios for Yield Plus × Ross 708 male broilers based on growth performance and carcass characteristics from approximately 1.0 to 4.0 kg. In order to accomplish the objective, 3 experiments were conducted providing dose-response treatments with increasing digestible Ile to Lys ratios to broilers at different stages of production. Experiments (**Exp**) 1 to 3 evaluated broilers from 21 to 35, 28 to 42, and 35 to 49 d of age, respectively. Dose-response diets for all Exp were formulated with spray-dried blood cells and crystalline L-Ile to create a gradient of digestible Ile to Lys ratios (0.46 to 0.83). Digestible Lys was formulated at 1.05, 0.95, and 0.87% in all diets for Exp 1 to 3, respectively. Each Exp also contained a positive control (**PC**) diet that did not contain blood cells or feed-grade L-Ile and was formulated to contain the same dig Ile to Lys ratio as treatment 5. At the beginning and end of each experimental period, birds and feed were weighed by pen to determine growth performance parameters. Broilers were processed (Exp 1 = 9 birds/pen, 36 d of age; Exp 2 = 14 birds/pen, 43 d of age; Exp 3 = 14 birds/pen, 50 d of age) and deboned to determine carcass characteristics. For all Exp, quadratic effects ($P \leq 0.001$) on body weight gain (**BWG**), feed conversion ratio (**FCR**), breast meat weight (**BMW**), and breast meat yield (**BMY**) were observed as digestible Ile to Lys ratios increased. A pre-planned orthogonal contrast was performed between the PC and treatment 5 for each Exp and showed no effect of blood cell inclusion with the exception of FCR in Exp 1 ($P = 0.001$) and BMY in Exp 3 ($P = 0.017$). Optimum digestible Ile to Lys ratios for Exp 1 were determined to be 0.640, 0.725, 0.661, and 0.709 for BWG, FCR, BMW, and BMY, respectively from 1.0 to 2.5 kg BW ($P \leq 0.001$).

In Exp 2, optimum ratios were analyzed as 0.665, 0.671, 0.664, and 0.682 for BWG, FCR, BMW, and BMY, respectively, from 1.6 to 3.0 kg BW ($P \leq 0.001$). In Exp 3, optimum ratios were 0.625, 0.692, 0.694, and 0.730, for BWG, FCR, BMW, and BMY, respectively, from 2.7 to 3.9 kg BW ($P \leq 0.001$). Based on these findings, optimum digestible Ile to Lys ratios were determined to range from 0.63 to 0.73 for growing broilers between 1.0 and 4.0 kg BW.

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I. INTRODUCTION

Over the past 50 years, the rate and efficiency of growth for broilers have been greatly improved. Male broilers reach 2.5 kg by 33 to 35 days of age and consume less feed per kg of BW. Much of this improvement (85~90%) can be credited to genetic selection by primary breeding companies, and the remainder (10~15%) can be attributed to improvements in nutritional management (Havenstein, 2003). The principle of providing a nutrient at or near its requirement is of particular importance when it comes to amino acid (**AA**) nutrition as AA are a primary limiting factor to growth (Leeson and Summers, 2001). Dietary AA are provided by oilseed meals, cereal grains, animal protein meals, and crystalline AA. Three feed-grade AA are (L-Lys, DL-Met, and L-Thr) commonly used in poultry production; however, the use of crystalline L-Val has increased over the past decade in parallel with the practice of feeding plant-based diets. In order to properly utilize the former feed-grade AA, minimum requirements for lower limiting indispensable AA must be well understood, and Ile will be the threshold AA in formulation in corn and soybean meal-based diets (Kidd et al., 2004). As Ile is the next limiting AA after Val, it will be the threshold AA in diet formulation, especially if there is an inclusion of an animal protein meal.

Isoleucine is critical for the growth of broilers, breast tissue accretion, and has been reported to play a critical role in immune function (Hale et al., 2004; Corzo et al., 2009). Stilborn et al. (1997) reported that Ile concentrations of feathers could reach as high as 4.9% of crude protein, and whole carcass (feather-free) concentrations of Ile can be as high as 5.0% of crude protein (Stilborn et al., 2011). Because of the important role Ile plays in the growth and immunity of broilers, it is critical to understand the Ile needs of broilers to ensure optimum health and performance. There is published research concerning the Ile needs of broilers up to 1.0 kg in BW (Farran and Thomas, 1990; Barbour and Latshaw, 1992; NRC, 1994; Baker et al., 2002), however, the body of literature for birds from 1.0 to 4.0 kg BW is considerably smaller. In addition, the majority of data referencing Ile requirements for broilers of all sizes is more than 15 years old and were not performed with modern broiler strains. There are also limited data evaluating the digestible ratios of Ile to Lys for broilers in all stages of production. Therefore, 3 experiments were conducted with the objective to evaluate the optimum digestible Ile to Lys ratios for the growth performance and carcass characteristic of broilers from approximately 1.0 to 4.0 kg BW.

II. LITERATURE REVIEW

AMINO ACID DIGESTIBILITY

In order to accurately formulate poultry diets on a digestible (**dig**) amino acid (**AA**) basis, 3 basic elements are needed: nutrient requirements, nutrient composition of the feed ingredients, and the digestibility coefficients (**DC**) of given feedstuffs. When considering proteinaceous feedstuffs, a qualitative evaluation of feed ingredients is of great importance, in addition to a quantitative analysis (Ravindran and Bryden, 1999). The most basic function of dietary protein is to supply adequate AA for the synthesis of body proteins. Therefore, protein quality depends not only on its nitrogen concentration, but also on its AA composition and the availability of those AA to digestion and absorption (Friedman, 1996). Thus, the digestibility of an AA is defined by its ability to be readily digested and absorbed (Batterham, 1992a). This concept is of particular importance for AA in diet formulation. It has been established that the dig AA concentrations of feed ingredients, such as grains and animal by-product meals, are lower than total AA values (Parsons, 1996).

Due to its importance for adequate AA nutrition, AA digestibility must be evaluated for all feed ingredients in diet formulation. There are 4 primary methods for the determination of AA digestibility in poultry, and some of the first work was conducted to determine fecal digestibility by Kuiken et al. (1948). This assay evaluated the fraction of an ingested AA that is absorbed by the bird and not present in the excreta (Lemme et al., 2004). Fecal digestibility is useful for an apparent analysis of digestibility; however, it does not correct for the effects of urinary AA and hindgut microbial fermentation. Parsons et al. (1982) reported that microbial protein may contribute as much as 25% of the AA in excreta, as well as having a considerable effect on the composition of ingested proteins that are not absorbed in the small intestine. The issues of hindgut fermentation and excreted microbes can be circumvented by the use of precision-fed rooster assays (Parsons, 1986). These assays utilize cockerels that have been cecectomized in order to eliminate microbial fermentation in the hindgut to determine apparent AA digestibility (Parsons, 1986). Roosters are fed a known quantity (30-35 g) of feed, or a feed ingredient, following a fasting period, and the excreta is collected and analyzed. These assays allow for an evaluation of digestibility without the interference of microbial fermentation, therefore providing a more accurate DC (Parsons, 1986). Cececotomized roosters can also be utilized to determine True AA digestibility by accounting for the endogenous losses of birds (Parsons, 1996). In correcting for endogenous losses, roosters are either fasted or fed a nitrogen free diet, and the resulting excreta is weighed and analyzed. The AA values obtained are utilized to correct apparent AA digestibility values by accounting for endogenous loss (Parsons, 1996). However, these assays do not address the issue of AA that are absorbed in the small intestine but are not metabolized and are therefore excreted. This can be circumvented with swine, as urine is excreted separately from feces (Baker, 1996). However, because avian species produce excreta, rather than voiding urine and feces separately, it is very difficult to account for unmetabolized AA.

The majority of AA uptake occurs in the small intestine prior to the terminal ileum (Webb, 1990). Therefore, the collection of ileal digesta from the terminal ileum for use in bioassays can ameliorate the influence of urinary AA as well as microbial modification of AA in the hindgut (Ravindran et al., 1999). Ileal digesta can be collected by sacrificing the bird and extracting the digestive tract or by the surgical insertion of an ileal cannula. Sacrificing the birds is the preferred method of collection, as cannulation is a complex procedure, and it can be difficult to collect the needed volume of digesta (Ravindran and Bryden, 1999). Thus, the ileal amino acid digestibility assay was developed. This assay has many advantages including its flexibility be used to evaluate all 23 proteogenic AA simultaneously, its adaptability to many feed ingredients with the use of semi-purified diets, and its versatility to be used on growing broilers of any age (Ravindran and Bryden, 1999). Using this assay, apparent ileal AA digestibility can be determined as well as standardized ileal AA digestibility. To determine standardized ileal AA digestibility, there must be a dietary treatment devoid of protein so that endogenous losses can be measured under the same conditions (Ravindran and Bryden, 1999). This can be accomplished by fasting birds, feeding a N-free diet, or utilizing a diet with highly digestible proteins. None of the above assays accurately estimate AA availability, however, the determination of AA availability is an expensive process and can only be conducted with a single AA in each test (Batterham, 1999). While digestibility does not directly indicate AA utilization of the bird, it is considered the greatest limiting factor of AA availability, and it is the preferred indicator of AA quality in both feed ingredients and feed formulations (Batterham, 1999; Ravindran and Bryden, 1999).

ISOLEUCINE CONTENT OF COMMON FEEDSTUFFS

Total AA content of common feedstuffs, and the DC of those AA, are critical in the formulation of poultry diets. Carbonaceous feedstuffs are not included in diets for AA inclusion, however, because cereal grains have relatively high inclusion rates, they account for a substantial amount of dietary AA (Fontaine, 2003). Corn and wheat can be quite variable in CP, ranging from 7.2 to 8.6% and from 10.0 to 12.0%, respectively (Lyman et al., 1956; NRC, 1994; Rostagno et al., 2011; NRC, 2012). Total Lys and Ile contents of corn typically range from 0.21 to 0.24% and 0.24 to 0.29%, respectively. Wheat has been reported to have a total Ile content of approximately 0.45% and contain 0.35% total Lys (Rostagno et al., 2011; NRC, 2012). The DC of Ile and Lys for corn are reported as 90.8 and 85.3%, respectively, and the DC for wheat are reported as 88.6 and 82.1% for Ile and Lys, respectively (Rostagno et al., 2011). These cereal grains have relatively low concentrations of these AA with marginal to good digestibility, but corn and wheat are important contributors of dietary protein.

Even more important than the AA content of grains is the concentration of indispensable AA in protein concentrates. Ingredients such as soybean meal (**SBM**), peanut meal (**PM**), meat and bone meal (**MBM**), and poultry byproduct meal (**PBM**) are utilized primarily to supply AA to diets (Fontaine, 2003). Therefore, understanding the AA content and digestibility of these products are paramount. Crude protein and AA contents of SBM are variable based upon production practices encountered during growth (NRC, 1994; Fontaine, 2003). Crude protein concentrations of SBM can range from below 43 to 48%, while the total Lys and Ile concentrations can span from 2.74 to 2.93% and 2.10 to 2.26%, respectively (NRC, 1994; Rostagno et al., 2011; NRC, 2012). The DC of Lys and Ile are reported as 92.2 and 90.6%, respectively (Rostagno et al., 2011). Total Lys and Ile contents for PM are reported as 1.57% (DC = 78.0%) and 1.64% (DC = 87.0%), respectively. The CP of PM is also variable based on growing conditions as well as the processing techniques used, and it is typically reported between 42 and 48% (NRC, 1994; Fontaine, 2003; Rostagno et al., 2011; NRC, 2012). Crude protein and AA contents of MBM are extremely variable based on the sources and processing of the product (Fontaine, 2003). Crude protein of MBM can range from 35% to over 60% in very concentrated products. This variation is also observed in the AA content with total Ile concentrations ranging from 0.80 to 1.84% and Lys concentrations from 1.69 to 3.30% (NRC, 1994; Rostagno et al., 2011). The DC of MBM also is reported in a wide range from 73 to 83% (Rostagno et al., 2011). There is considerably less variation in the reported AA content of PBM, both for feed-grade and pet food-grade products (Fontaine, 2003). Total Ile ranges from 2.07 to 2.31% (DC = 83.5%) and Lys from 3.05 to 3.35% (DC = 80.0%). The reported CP content of PBM is reported to range from 55 to 63%, with the higher quality products typically entering the pet food market (NRC, 1994; Rostagno et al., 2011). The variations in AA content and quality observed for these protein concentrates illustrate the importance of analyzing all dietary ingredients that are used in formulation.

ISOLEUCINE FUNCTIONS AND METABOLISM

Basic Functions of Isoleucine

Isoleucine is a member of a subgroup of AA referred to as branched-chain amino acids (**BCAA**), which also includes Leu and Val. The R groups of BCAA consist of branching sections of methyl groups and are therefore extremely hydrophobic (Bender, 2012). This makes BCAA critical for the folding of globular proteins and interactions of membrane proteins with lipid bilayers. Isoleucine specifically plays a role in the production of the β-pleated sheets in the secondary protein structure (Bender, 2012). The primary function of Ile is reported to be its role in lean tissue development. Total body protein concentration of broilers ranges from 44 to 49%, and the protein concentration in edible meat ranges from 54 to 59% (Summers and Leeson, 1985; Corzo et al., 2009). Approximately 5.0% of total retained protein is comprised of Ile, and it has been reported that 4.42% of edible tissue is Ile (Bae et al., 1999). Stilborn et al. (1997) reported that the Ile concentration of feathers was 4.32% and increased to 4.82% as the birds aged from 14 to 84 d. Whole carcass Ile concentrations (feather-free) decreased from 4.30 to 4.01% as birds aged from 1 to 112 d, and displayed a significant effect of sex with females having a larger concentration (Stilborn et al., 2011). These authors also reported that the total CP percentage of the featherless carcass decreases with age. The response of breast meat yield (**BMY**) observed with increasing concentrations of dietary Ile is greater than many other AA (Kidd et al, 2004). This response indicates that Ile contributes to the accretion of lean tissue (Velu et al., 1972). These authors also reported that when the Ile concentration is increased in the diet from 0.40 to 0.70% a decrease in total fat concentration of the body occurs.

Deficiencies of Ile and Val suppressed immunity in growing mice (Petro and Bhattacharjee, 1981), and a relationship has been reported between Ile deficiencies and leukocyte and lymphocyte production in mice (Jose and Good, 1973). Similar relationships between Ile and immune function are believed to exist in poultry as well. The mechanism of action of Ile on immunity is not well understood for poultry; however, when dietary Ile concentrations become marginal, a decrease in the Ile dependent immune responses is observed (Hale et al., 2004). Unlike other indispensable AA, BCAA do not produce any unique metabolites, rather, these AA are metabolized to many common metabolic intermediates (Bender, 2012). The author also reports that Ile is both ketogenic and glucogenic, giving rise to acetyl CoA, propionyl CoA, and succinyl CoA, which are transported to the liver and used in gluconeogenesis and the synthesis of Ala.

Isoleucine is also important as a dietary nutrient. In practical broiler diets, Ile is typically the 5th limiting AA following Val; however, this is dependent on the inclusions of certain dietary ingredients (Corzo et al., 2010). When animal protein meals are included in small concentrations (0 to 3%), Ile and Val are co-fourth limiting (Kidd et al., 2000; Corzo et al., 2008). However, when inclusion of animal protein meals is in excess of approximately 3 to 4%, Ile will be 4th limiting with Val 5th limiting (Corzo et al., 2009).

Isoleucine Absorption and Catabolism

As the primary source of BCAA is intact dietary proteins, AA must be liberated by pancreatic proteases prior to absorption. Ingested proteins will be catabolized into smaller peptides by various endopeptidases. Then, the individual BCAA are cleaved from the peptide by carboxypeptidase A, which has an affinity for large neutral residues (Lipscomb, 1989). Branched-chain AA are primarily absorbed in the jejunum but cannot penetrate the mucosal membrane passively. Therefore, BCAA must bind to the Large Neutral AA Transporter Protein (**LAT1**) in order to enter the enterocytes (Bender, 2012). Branched-chain AA are preferentially bound by LAT1; however, this protein is also responsible for the transport of other neutral and aromatic AA (Brosnan and Brosnan, 2006). The affinity of AA to LAT1 allows for large concentrations of this transport protein to be present in the brush border for optimum absorption to occur (Napolitano et al., 2015). These authors also reported that LAT1 synthesis is susceptible to AA deficiencies including Thr and sulfur AA, thus the digestibility of BCAA can also be impaired by these deficiencies. Once BCAA have been absorbed, they are transmitted through the blood stream mainly to skeletal muscles where they will undergo metabolism (Bender, 2012).

Unlike most AA, which are transaminated and deaminated mainly in the liver, catabolism occurs almost exclusively in skeletal muscle for BCAA (Bender, 2012). In the fasting state, BCAA provide amino groups for the transamination of pyruvate arising from glycolysis of muscle glycogen to Ala, which is transported to the liver for gluconeogenesis (Bender, 2012). Branched-chain AA can also be metabolized to branched-chain oxo-acids (**BCOA**) to provide an energy source to muscle tissue or transported to the liver where they can be transaminated back to AA through reaction with Ala (Cynober and Harris, 2006). The oxo-acid pathway works as a mechanism to conserve Ile and Val, and its metabolites are primarily reconverted to these AA in the liver. However, oxo-methylvaleric acid, which also arises from Ile metabolism, is primarily transported to the liver as a precursor to acetyl CoA and succinyl CoA. These metabolites enter the TCA cycle but can also be further metabolized in skeletal and cardiac muscle for the production of ATP (Mansoorabadi et al., 2005). Isoleucine can also be more directly metabolized to succinyl CoA and acetyl CoA through the methylmalonyl CoA mutase pathway, which is vitamin B_{12} dependent (Banerjee, 2003). To enter this pathway, Ile must first be transaminated and dehydrogenated to create tiglyl CoA. Tiglyl CoA is dehydrogenated and then cleaved by β-ketothiolase and CoASH to create acetyl CoA and propionyl CoA. Propionyl CoA is then carboxylated to create methylmalonyl CoA, which is in turn converted to succinyl CoA by methylmalonyl CoA mutase (Banerjee, 2003). This pathway, or the production of oxo-acids, are the primary metabolic pathways Ile follows in a non-fasting state (Bender, 2012).

Isoleucine Synthesis

The BCAA synthesis pathway is the primary method in which Ile can be synthesized, but there are many metabolites that can enter this pathway (Bender, 2012). The BCOA (direct metabolites of BCAA) can enter Ile synthesis as 2-oxobutyrate (**OB2**). Threonine can also enter this pathway when it is converted to OB2 by reaction with Thr deaminase, and this is the primary source of OB2 to Ile synthesis (Jander and Joshi, 2010; Bender, 2012). In specific situations, such as Na deficiencies or dehydration, Met can also be a substrate Ile synthesis. Methionine can be converted to OB2 by reaction with methionine γ-lyase; however, this process is not common under normal conditions and is regulated by the concentration of Thr deaminase present in the cytosol (Joshi and Jander, 2009).

The final substrate that must enter the pathway is hydroxyethyl thiamin diphosphate, which is the product of pyruvate binding to thiamin diphosphate on the enzyme complex acetohydroxy acid synthase (Chipman et al., 2005). These authors reported that the acetohydroxy acid synthase complex binds the hydroxyethyl residue from pyruvate with OB2 to produce aceto-hydroxybutyrate and the thiamin diphosphate is released. Then, the synthesis of Ile follows the same pathway, no matter the initial substrate. Aceto-hydroxybutyrate is then converted to dihydroxy-methylvalerate by the enzyme acetohydroxy acid reducto-isomerase (**AARI**), which is common in the synthesis of all BCAA (Tyagi et al., 2005). Dihydroxy-methylvalerate is then dehydrated by another common enzyme, dihydroxyacid dehydrase, to create oxomethylvalerate, which is then transaminated to produce Ile (Duan et al., 2009). The last step of this pathway is the only reversible reaction. The reverse of this reaction frequently occurs in muscle tissue as a first step to Ile catabolism (Hyduke et al., 2007).

The regulation of the Ile synthesis pathway is common with parallel pathways for the synthesis of Val and Leu, hence, competition exists between pyruvate and OB2 for enzymatic binding sites (Bender, 2012). These enzymes have a lower affinity for pyruvate relative to OB2, and with equal concentrations of the 2 substrates, the pathway will proceed forward primarily with Ile synthesis (Barak et al., 1987). However, these authors note that *in vivo* the intracellular concentration of pyruvate is considerably higher than that of OB2 causing the metabolic flux of the parallel pathways to be close to equal. Acetohydroxy acid reducto-isomerase has a similar affinity for both aceto-hydroxybutyrate and acetolactate (common substrate for Val and Leu synthesis). However, its activity with aceto-hydroxybutyrate is 5 to 8 times higher than for acetolactate, thus, Ile synthesis is preferred by AARI (Tyagi et al., 2005).

Isoleucine acts to control the provision of aceto-hydroxybutyrate by a negative feedback inhibition of the deamination of Thr to OB2 by Thr deaminase to ensure adequate synthesis of the other BCAA (Dumas et al., 2001). Isoleucine synthesis is also inhibited by feedback inhibition of acetohydroxyacid synthase by BCAA. This enzyme has 2 binding sites for inhibition; 1 can only bind Leu and the other can bind either Ile of Val (Slutzker et al., 2011). Both sites must be bound for inhibition of acetohydroxyacid synthase and stop the production of reaction intermediates for all BCAA. Leucine synthesis is the slowest to initiate, as it has the lowest affinity to the common enzymes in these pathways. Therefore, it must be present in an adequate concentration for the total inhibition of these pathways to occur (Chipman et al., 2005; He et al., 2010).

IDEAL PROTEIN CONCEPT

Ideal protein was first conceptualized as the concept that AA needs could be represented as proportions to each other (Almquist and Grau, 1944). Cole (1980) proposed that ideal AA ratios could be used in the formulation of swine diets, and this concept was further popularized by the Agricultural Research Council (1981). Currently, ideal AA ratios, with Lys as the reference AA, are used throughout the world for the diet formulation of both swine and poultry (Baker, 2003). There are many reasons why ideal protein is appealing for diet formulation. When diets are formulated based on dig AA, there is a need to define the requirement of each indispensable AA at various stages of growth, maintenance, and egg production (Baker and Han, 1994). However, this is not necessary using the ideal protein concept, as Lys is the reference AA.

Lysine is almost exclusively utilized for body protein accretion, and it is only affected in very limited capacity by other metabolic activities. Also, Lys has no metabolic interactions with other AA, and it is easy to identify through analysis (Baker et al., 2002). Thus, empirical evidence can be used to set Lys concentrations and all other AA can be based on the ideal AA ratios observed to optimize performance (Baker, 2003). Therefore, using ideal AA ratios, unlike requirements, would not change based on the energy or CP concentration of the diet, and would remain constant regardless of the genetic potential of the bird (Emmert and Baker, 1997). These authors also reported that extreme temperatures, stocking density, and disease, which will typically suppress feed intake, are unlikely to have an impact on the ideal ratios, though they may change the Lys requirement. However, previous research reported that Lys requirements expressed as a percentage of the diet change negligibly due to heat stress and disease challenges (Han and Baker, 1993; Webel et al. 1998). Male broilers have higher Lys requirements (10% in excess of females) as a dietary percentage, especially at older ages (Han and Baker, 1994; Dozier et al., 2008). Due to these factors, Lys is the reference AA from which ideal AA ratios are based.

ISOLEUCINE REQUIREMENTS

Several statistical analyses can be used to determine the dietary requirement of a given nutrient for broilers (Agostini et al., 2019). Multiple regression techniques (linear, non-linear regression, and broken-line analysis) are typically used with dose response studies (Edwards et al., 1997; Baker et al., 2002). Amino acid requirements are typically determined from growth and/or meat yield responses of birds being fed graded concentrations of a limiting AA (Baker, 1996). These studies can be designed based on a total or dig AA basis; however, formulating based on dig values is more effective at providing accurate results (Oviedo-Rondon and Waldrup, 2002). Requirements can be expressed as a percentage of the diet or as an optimum ratio to Lys though this must be considered in the design of the experiment (Liebert et al., 2009). Many studies focus on a targeted period because requirements expressed as a percentage of the diet change over time. Linear regression is typically utilized to represent a relationship between increasing concentrations of an individual dietary AA with the response obtained by the birds (Mercer, 1982). Quadratic regression can be used in a dose response study to estimate requirements by calculating 95% of the AA concentration that exhibits the greatest response (Baker, 1996). For both linear and quadratic broken line regression, 6 to 10 test diets should be employed ranging from approximately 75% of the assumed requirement to 30% in excess of that value (Portz et al., 2000). Experiments utilizing quadratic regression use a similar number of treatments; however, these types of analysis are able to be used with a smaller range of AA concentrations. Both quadratic and broken-line regression techniques can be used to estimate requirements or ideal AA ratios.

Requirement for Maintenance

Precise feed formulation requires an adequate understanding of the dietary requirements of AA, and knowledge of how consumed AA are partitioned between maintenance and growth. A maintenance requirement (**MR**) is typically described as the concentration of an AA that produces a nitrogen balance of zero when fed as the first limiting AA (Heuser, 1941). This method has been criticized as it is difficult to equate to practical diets, however, there has been little improvement in the methodologies to determine MR since the work of Leveille and Fisher (1959). Leveille and Fisher (1960) reported the MR for Ile for adult cockerels was 78 mg/kg body weight/day. Moreover, Ishibashi and Kametaka (1973) observed the Ile MR of cockerels to be 40 mg/kg/d. Later, MR were reported as 203 and 58 mg/kg body weight/day for adult cockerels and growing broilers respectively (Owens et al., 1985; Kebreab et al., 2008). Viewing these requirements as a function of body weight/day provided adequate results for one point in time, however, these findings were not able to be applied to birds of other ages and varied environmental conditions (Burnham and Gous, 1990). These authors reported the MR both as a function of daily mass (60 mg/kg/d), and as a function of maintenance protein/day (300 mg/ $P_m^{0.73}u$/d) for adult cockerels. This unit allows MR estimates to be scaled based on mature protein weight (P_m) and the degree of protein maturity (u), which offers adaptability to birds of various ages and varying dietary conditions. A similar experiment was conducted to determine the MR of broilers from 1 to 21 d of age (Lima et al., 2016). These authors found the MR to be 134 mg/unit $P_m^{0.73}u$/d, as well as reporting a requirement of 20 mg/kg/d as a comparison with other published MR. These reports of the MR of Ile for poultry vary by more than 40% for broilers and cockerels. Marcato et al. (2008) attributes these differences to the wide range of analytical techniques used and types of birds tested.

Starter Period

Development of the broiler is critical, as birds will increase body mass by 10 to 15-fold during the first 2 weeks of life. Chicks must not only develop the bone and muscle tissue to support this growth but must also enlarge their digestive tracts (Picard et al., 1999). Development of the gastrointestinal tract is greatest relative to other growth in the starter period, and it is critical for development as it is responsible for the uptake of nutrients (Dibner et al., 1996). Thus, inadequate AA nutrition in the starter period can have a detrimental effect on the growth in later production periods. Hence, there are many studies investigating the Ile requirements of broilers less than 1 kg BW (Barbour and Latshaw, 1992; Baker, Baker and Han, 1994; Baker, 1996; Baker et al., 2002).

Total Ile requirements expressed as a percentage of the diet have been reported to range from 0.72 to 0.84% from 1 to 21 d of age (Farran and Thomas, 1990; Barbour and Latshaw, 1992; NRC, 1994). Total Ile requirements from 1 to 21 d of age were reported as 0.66% (Bae et al., 1999) and 0.68% (NRC, 1994) for high producing broiler strains. From 1 to 16 d of age, Park and Austic (2000) observed the total Ile requirement to range from 0.63 to 0.65%. Sedghi et al. (2015) determined the total Ile requirements range from

0.85 to 0.95% of the diet from 1 to 7 d of age. The dig Ile requirement was estimated as 0.68% from 7 to 21 d of age (Baker and Han, 1994; Baker et al., 2002). Studies utilizing ideal AA ratios report the optimum dig Ile to Lys ratio from 1 to 21 as 0.67 (Baker and Chung, 1992; Baker, 1996; Baker, 1997). The NRC (1994) also reports an optimum ratio of 0.67 for broilers in the first 3 weeks of production. An optimum ratio from 7 to 21 d of age has been reported as 0.67 (Baker and Han, 1994). These data indicate that the dig Ile requirement for broilers under 1 kg BW is within 0.65 to 0.67% of the diet and the optimum dig Ile to Lys ratio is 0.67.

Grower and Finisher Periods

Due to the increasing breast tissue growth in the later periods, understanding the requirement of dietary Ile is critical, especially for breast meat yield (Kidd et al., 2004). However, limited data exist in the literature investigating Ile requirements in the grower and finisher periods, and even fewer studies on the optimal concentration of dietary Ile for breast muscle development. Kidd et al. (2004) reported that the total Ile requirement from 18 to 30 d of age ranged from 0.67 to 0.71% of the diet. Likewise, Berres et al. (2010) reported the optimum dig Ile to Lys ratio from 14 to 35 d of age as 0.65. Digestible Ile requirements have been reported as 0.66 (Kidd et al., 2004) and 0.71% (NRC, 1994) from 30 to 42 and 21 to 42 d of age, respectively. Other research has suggested the optimal dig Ile to Lys ratios from 20 to 42 d of age are 0.69 (Baker, 1996), 0.66 (Mack et al., 1999), and 0.67 (Miranda et al., 2015) for growth performance. Mejia et al. (2011) determined optimum Ile ratios at 0.69 for growth performance parameters and 0.72 for breast meat yield from 28 to 42 d of age. Dozier et al. (2012) also evaluated the effects of dig Ile and Val on growth performance and breast meat yield from 28 to 42 d of age. These authors reported that dig Ile to Lys ratios may be in higher than 0.67 for total breast meat yield. The total Ile requirement of birds larger than 3 kg is not well studied but has been reported to be between 0.55 and 0.66% of the diet from 42 to 56 d of age (Kidd et al., 2004). With limited data for the grower period, the Ile requirement appears to range from 0.66 to 0.70% and the optimum dig Ile to Lys ratio in the grower period is 0.65 (Baker, 1996). From 28 to 48 d of age, the total Ile requirement ranges from 0.66 to 0.71% for birds in this period and closer to 0.60% for birds older than 48 d of age (Kidd et al., 2004; Berres et al., 2010). The optimum dig Ile to Lys ratio for growth performance appears to be between 0.66 and 0.69 and slightly higher for breast meat yield (Baker, 1996; Miranda et al., 2015).

BRANCHED-CHAIN AMINO ACID INTERACTIONS

It has long been understood that excess Leu has an antagonistic effect on the other BCAA (Harper et al., 1955). Fisher et al. (1960) reported that excess Leu depresses feed intake and BWG of chicks being fed diets with marginal CP concentrations. Further investigation revealed that excess Leu reduced chick weights more dramatically when Val was 4th limiting in chick diets (Mathieu and Scott, 1968) Moreover, Bray (1970) observed that excess dietary Leu increased the need for Ile and Val in laying pullets. These findings suggested that Leu had an antagonistic effect on Val and Ile when it was in excess of its dietary requirement. This concept was further validated with the quantification of Leu effect on the efficacy (capacity of an AA to promote weight gain) of Ile and Val in broiler diets (Allen and Baker, 1972). These authors found that the efficacy of dig Ile was reduced to 80% with only 3% excess total dietary Leu, and likewise total Val efficacy was reduced to 74% at 5.57% excess Leu. D’Mello and Lewis

(1970) demonstrated that excess dietary Leu had the capacity to reduce the blood plasma concentrations of Val. These authors reported that a 5% excess of Leu reduced the plasma concentration of Val by over 30%. Excess Leu had no effect on plasma Ile concentrations; however, excess Ile had a similar effect to Leu and lowered Val plasma concentrations by nearly 26% (D'Mello and Lewis, 1970). This response is likely driven by the affinity of LAT1 in the small intestine being greater for Leu and Ile relative to Val (Bender, 2012). This also indicates that an antagonistic effect of Leu on Ile occurs after the AA have been absorbed into the blood. It has been hypothesized that the antagonism stems from a suppression of feed intake caused by excess Leu reducing the relative concentration of Ile being consumed (Fisher et al., 1960).

The effects of Leu on the muscle protein degradation pathways are more likely the source of reduced performance (Zeitz et al., 2019). These authors report a relationship between excess dietary Leu and transcription of protein kinase B/Akt, which positively regulates forkhead box protein 01. This protein is primarily responsible for the degradation of proteins in muscle tissue (Zeitz et al., 2019). There is also evidence that a direct metabolite of Leu, 3-hydroxy-3-methylbutyrate increases the rate of proteolysis in the muscle tissues of chicks resulting in reduced weight gain (Ostaszewski et al., 2000). These authors also reported that Leu increased protein turnover rate of muscles, particularly glycolytic muscles. These findings preceded additional work into further quantifying the impacts of excess Leu, and to identify strategies to ameliorate the negative effects of Leu.

The relative concentrations of Ile and Leu in corn gluten meal and other corn byproducts infer that an imbalance between them is likely when these products are a primary constituent of diets (Burnham et al., 1990). D'Mello and Lewis (1970) suggested that increased dietary concentrations of Ile and Val could be beneficial in the alleviation of the effects of excess Leu inclusion. These authors observed that the inclusion of supplemental Val restored the growth performance of broilers; however, supplemental Ile did not restore performance. Only in combination with Val was Ile able to restore growth, inferring the relationship between Leu and Val is more pronounce (D'Mello and Lewis, 1970). In another experiment, these authors observed that the relative requirements of Ile and Val were increased 13 and 31%, respectively, when Leu concentrations increased from 1.40 to 3.40% (D'Mello and Lewis, 1970). Tuttle and Balloun (1976) reported similar results in turkey poults with the supplementation of Val at 11% above its requirement alleviating excess Leu, but Ile supplementation was insufficient to alleviate the negative effects of Leu.

It has also been suggested that BCAA antagonisms can occur without excess Leu, when Ile and Val concentrations are very high (Smith and Austic, 1978). However, Barbour and Latshaw (1992) demonstrated that when Ile is adequate in diets excess Val has no significant effect on growth performance. These authors reported that the negative feed intake response observed with excess Val was more related to an inadequacy of Ile. In agreement, Burnham et al. (1970) reported that diets marginal in Ile that contained excess Val showed depressed growth and feed intake. Again, this response was likely more related to the suboptimal Ile concentration rather than the excess in Val. Burnham et al. (1970) also reported that the optimum concentration of Ile will increase proportionally with increases in excess Leu up to 10% beyond the requirement, but excess beyond 10% will have little effect.

KNOWLEDGE GAPS IN THE LITERATURE

Knowledge gaps exist in the literature concerning the optimum concentrations of dig Ile for growing broilers. Published literature has evaluated dig Ile requirements and optimum dig Ile to Lys ratios for broilers up to 1 kg BW (Farran and Thomas, 1990; Baker and Chung, 1992; Barbour and Latshaw, 1992; NRC, 1994; Baker, 1996, 1997; Park and Austic, 2000; Baker et al., 2002). Total Ile requirements and optimum dig Ile to Lys ratios have also been assessed for broilers from 1.0 to 3.5 kg BW (NRC, 1994; Baker, 1996; Kidd et al., 2004; Berres et al., 2010; Miranda et al., 2015). However, a disproportionate amount of the published data are for broilers under 1.0 kg. Additionally, published Ile requirements are more than 15 years old and were not conducted with modern broiler strains. Moreover, there is a small body of literature for growing broilers from 1.5 to 2.5 kg BW and broilers approaching market weights at 3.0 and 4.0 kg based on growth performance and carcass characteristics. With the utilization of supplemental L-Val increasing in broiler diets, understanding the needs of broilers for Ile is critical as it will be the threshold AA for formulation.

In order to address these knowledge gaps, the proposed research will determine the optimal dig Ile to Lys ratios for broilers from 1.0 to 2.5, 1.5 to 3.1, and 2.5 to 4.0 kg BW. Optimum ratios will be determined based on responses of growth performance and carcass characteristics to increasing dig Ile to Lys ratios. These data will provide novel information to estimate dig Ile needs of modern broilers.

REFERENCES

Agricultural Research Council. 1981. The Nutrient Requirements of Pigs. Commonwealth Agricultural Bureaux, Slough.

Agostini, P. S., R. R. Santos, D. R. Khan, D. Siebert, and P. van der Aar. 2019. The optimum valine: lysine ratios on performance and carcass traits of male broilers based on different regression approaches. Poult. Sci. 98: 1310-1320.

Allen, N. K., and D. H. Baker. 1972. Quantitative efficacy of dietary isoleucine and valine for chick growth as influenced by variable quantities of excess dietary leucine. Poult. Sci. 51: 1292-1298.

Almquist, H. J., and C. R. Grau. 1944. The amino acid requirements of the chick. J. Nutr. 28: 325-331.

Bae, S. H., J. H. Kim, I. S. Shin, and K. Han. 1999. Parition of amino acid requirements of broilers between maintenance and growth. V. isoleucine and valine. Asian Australasian J. Anim. Sci. 12: 388-394.

Baker, D. H. 1996. Advances in Amino Acid Nutrition and Metabolism of Swine and Poultry. Nutrient Management of food Animals to Enhance and Protect the Environment. CRC Press Inc. 41-54.

Baker, D. H. 2003. Ideal amino acid patterns for broiler chicks. Amino Acids in Animal Nutrition, 2nd Edition. CAB International. 223-236.

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. BioKyowa Technical Review-9. BioKyowa Inc. 154-178.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81: 485-494.

Baker, D. H., and T. K. Chung. 1992. Ideal protein for swine and poultry. Biokyowa Technical Review, 4: 16.

Baker, D. H., S. R. Fernandez, C. M. Parsons, H. M. Edwards, III, J. L. Emmert, and D. M. Webel. 1996. Maintenance requirement for valine and efficiency of its use above maintenance for accretion of whole-body valine and protein in young chicks. J. Nutr. 126: 1844–1851.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult Sci. 74: 1441-1447.

Banerjee, R., A. Dybala-Defratyka, and P. Paneth. 2006. Quantum catalysis in B12 dependent methylmalonyl-CoA mutase: experimental and computational insights. Phil. Trans. R. Soc. B. 361: 1333-1339.

Barak, Z., D. M. Chipman, and N. Gollop. 1987. Physiological implications of the specificity of acetohydroxy acid synthase isozymes of enteric bacteria. Am. Soc. Microbio. 169: 3750-3756.

Barbour, G., and J. D. Latshaw. 1992. Isoleucine requirement of broiler chicks as affected by the concentrations of leucine and valine in practical diets. Br. Poult. Sci. 33: 561-568.

Batterham, E. 1992. Availability and utilization of amino acids for growing pigs. Nutr. Res. Rev. 5: 1-18.

Bender, D. A. 2012. The Branched-Chain Amino Acids: Leucine, Isoleucine and Valine. Amino Acid Metabolism, Third Edition. John Wiley & Sons, Ltd. 279-302.

Berres, J., S. L. Vieira, W. A. Dozier III, R. M. E. M. Cortês, De Barros, E. T. Nogueira, and M. Kutschenko. 2010. Broiler responses to reduced-protein diets supplemented with valine, isoleucine, glycine, and glutamic acid. J. Appl. Poult. Res. 19: 68-79.

Bray, D. J. 1970. The isoleucine and valine nutrition of young laying pullets as influenced by excessive dietary leucine. Poult. Sci. 49: 1334-1341.

Brosnan, J. T., and M. E. Brosnan. 2006. Branched-chain amino acids: enzyme and substrate regulation. J. Nutr. 136: 207S–211S.

Burnham, D., G. C. Emmans, and R. M. Gous. 1970. Isoleucine requirements of the chicken: the effect of excess leucine and valine on the response to isoleucine. Br. Poult. Sci. 33: 71-87.

Burnham, D., and R. M. Gous. 1990. Isoleucine requirements of the chicken: requirement for maintenance. Br. Poult. Sci. 33: 59-69.

Chipman, D. M., R. G. Duggleby, and K. Tittmann. 2005. Mechanisms of acetohydroxyacid synthase. Cur. Op. Chem. Bio. 9: 475-481.

Cole, D. J. A. 1980. The amino acid requirements of pigs – the concept of an ideal protein. Pig News and Information. 1: 201-205.

Corzo, A., R. E. Loar II, and M. T. Kidd. 2009. Limitations of dietary isoleucine and valine in broiler chick diets. Poult. Sci. 88: 1934-1938.

Corzo, A., W. A. Dozier, III, M. T. Kidd, and D. Hoeler. 2008. Impact of dietary isoleucine status on heavy-broiler production. Inter. J. Poult. Sci. 7: 526-529.

Corzo, A., W. A. Dozier, III, R. E. Loar, M. T. Kidd, and P. B. Tillman. 2010. Dietary limitation of isoleucine and valine in diets based on maize, soybean meal, and meat and bone meal for broiler chickens. Br. Poult. Sci. 51: 558-563.

Cynober, L., and R. A. Harris. 2006. Symposium on Branched-Chain Amino Acids: Conference Summary. J. Nutr. 136: 333S–336

Dibner, J. J., M. L Kitchell, C. A. Atwell, and F. J. Ivey. 1996. The effect of dietary ingredients and age on the microscopic structure of the gastrointestinal tract in poultry. J. Appl. Poult. Res. 5: 70-77.

D'Mello, J. P. F., and D. Lewis. 1970. Amino acid interactions in chick nutrition: Interrelationships between leucine, isoleucine, and valine. Br. Poult. Sci. 11: 313 323.

Dozier III, W. A., A. Corzo, M. T. Kidd, and M. W. Schilling. 2008. Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. Poult. Sci. 87: 1385-1391.

Dozier III, W. A., P. B. Tillman, and J. Usry. 2012. Interactive effects of digestible valine-and isoleucine-to-lysine ratios provided to male broilers from 4 to 6 weeks of age. J. Appl. Poult. Res. 21: 838-848.

Duan, Y., Y. Duan, F. Li. 2009. Effects of supplementation with branched-chain amino acids to low-protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids. 48: 2131–2144.

Dumas, R., V. Biou, F. Halgand, R. Douce, and R. G. Duggleby. 2001. Enzymology, structure, and dynamics of acetohydroxy acid isomeroreductase. Am. Chem. Soc. 34: 399-408.

Edwards III, H. M., D. H. Baker, S. R. Fernandez, and C. M. Parsons. 1997. Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. Br. J. Nutr. 78: 111-119.

Emmert, J. L., and D. H. Baker. 1997. Use of ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6: 462-470.

Farran, M. T., and O. P. Thomas. 1990. Dietary requirements of leucine, isoleucine, and valine in male broilers during the starter period. Poult. Sci. 69: 757-762.

Fisher, H., P. Griminger, G. A. Leveille, and R. Shapiro. 1960. Quantitative aspects of lysine deficiencies and amino acid imbalance. J. Nutr. 71: 213-220.

Fontaine, J. 2003. Amino Acids Analysis of Feeds. Amino Acids in Animal Nutrition. CAB International. 15-25.

Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. . Agric. Food Chem. 44: 6-29.

Hale, L. L., G. T. Pharr, S. C. Burgess, A. Corzo, and M. T. Kidd. 2004. Isoleucine needs of thirty- to forty-day-old female chickens: immunity. Poult. Sci. 83: 1979-1985.

Han, Y., and D. H. Baker. 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. Poult. Sci. 73: 1739-1745.

Han, Y., and D. H. Baker. 1993. Effects of sex, heat stress, body weight, and genetic strain on the dietary lysine requirement of broiler chicks. Poult. Sci. 72:701-708.

Harper, A. E., D. A. Benton, and C. A. Elvehjem. 1955. L-leucine, an isoleucine antagonist in the rat. Arch. Biochem. Biophsys. 57: 1-12.

He, Y., B. Chen, Q. Pang, J. M. Strul, and S. Chen. 2010. Functional specification of Arabidopsis isopropylmalate isomerases in glucosinolate and leucine biosynthesis, Pl. Cell Phys. 51: 1480–1487.

Heuser, G. F. 1941. Protein in Poultry Nutrition – a review. Poult. Sci. 20: 362-368.

Hyduke, D. R., L. R Jarboe, L. M. Tran, K. J. Chou, and J. C. Liao. 2007. Integrated network analysis identifies nitric oxide response networks and dihydroxyacid dehydratase as a crucial target in Escherichia coli. Nat. Acad. Sci. USA. 104: 8484-8489.

Ishibashi, T., and M. Kametaka. 1973. Effect of dietary conditions on metabolism of phenylalanine, tyrosine and isoleucine in the growing chick. Nip. Nog. Kai. 47: 103-109.

Jander, G., and V. Joshi. 2010. Recent progress in deciphering the biosynthesis of aspartate derived amino acids. Molec. Plant 3: 54-65.

Jose, D. G., and R. A. Good. 1973. Quantitative effect of nutritional essential amino acid deficiency upon immune responses to tumors in mice. J. Exp. Med 137: 115 121.

Joshi, V., and G. Jander. 2009. Arabidopsis methionine g-lyase is regulated according to isoleucine biosynthesis needs but plays a subordinate role to threonine deaminase. Plant Physiol. 151: 367-378.

Kebreab, E., J. France, H. Kuhi, and S. Lopez. 2008. A comparative evaluation of functions for partitioning nitrogen and amino acid intake between maintenance and growth in broilers. J. Agri. Sci. 146: 163-170.

Kidd, M. T., D. J. Burnham, and B. J. Kerr. 2004. Dietary isoleucine responses in male broiler chickens. Br. Poult. Sci. 45: 67-75.

Kidd, M. T., B. J. Kerr, J. P. Allard, S. K. Rao, and J. T. Halley. 2000. Limiting amino acid responses in commercial broilers. J. Appl. Poult. Res. 9: 223-233.

Kuiken, K. A., C. M. Lyman, S. Dieterich, M. Bradford, and M. Trant. 1948. Availability of amino acids in some foods. J. Nutr. 36: 359-368.

Lemme, A., V. Ravindran, and W. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. Worlds Poult. Sci. J. 60: 423-438.

Leveille, G A., and H. Fisher. 1959. Amino Acid Requirements for Maintenance in the Adult Rooster: II. The Requirements for Glutamic Acid, Histidine, Lysine and Arginine. J. Nutr. 69: 289–294.

Leveille, G. A., and H. Fisher. 1960. Amino acid requirement for maintenance in the adult rooster: III. The requirements for leucine, isoleucine, valine and threonine, with reference also to the utilization of the D-isomers of valine, threonine and isoleucine. J. Nutr. 70: 135–140.

Liebert, F. 2009. Amino acid requirement studies in Oreochromis niloticus by application of principles of the diet dilution technique. J. Anim. Phys. Anim. Nutr. 93: 787-793.

Lima, de M. B., N. K. Sakomura, J. C. P. Dorigam, E. P. da Silva, N. T. Ferreira, and J. B. K. Fernandes. 2016. Maintenance valine, isoleucine, and tryptophan requirements for poultry. Poult. Sci. 95: 842-850.

Lipscomb, D. W. 1989. Carboxypeptidase A. Am. Chem. Soc. 22: 62-69.

Lyman, C. M., K. A. Kuiken, F, Hale.1956. Essential amino acid content of farm feeds. Agric. Food Chem. 4: 1008-1013

Mack, S., D. Bercovici, G. De Groote, B. Leclercq, M. Lippens, M. Pack, J. B. Schutte, and S. Van Cauwenberghe. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. Br. Poult. Sci. 40: 257 265.

Mansoorabadi, S. O., R. Padmakumar, N. Fazliddinova, M. Vlasie, R. Banerjee, and G. H. Reed. 2005. Characterization of a Succinyl-CoA Radical−Cob(II)alamin Spin Triplet Intermediate in the Reaction Catalyzed by Adenosylcobalamin-Dependent Methylmalonyl-CoA Mutase. Biochem. 44: 3153-3158.

Marcato, S. M., N. K. Sakomura, D. P. Munari, J. B. K. Fernandes, I. M. Kawauchi, and M. A. Bonato. 2008. Growth and body deposition of two broiler commercial lines. Braz. J. Poult. Sci. 10: 117-123.

Mathieu, D., and H. M. Scott. 1968. Growth depressing effect of excess leucine in relation to amino acids composition of diet. Poult. Sci. 47: 1694.

Mejia, L., C. D. Zumwalt, E. J. Kim, P. B. Tillman, and A. Corzo. 2011. Digestible isoleucine-to-lysine ratio effects in diets for broilers from 4 to 6 weeks posthatch. J. Appl. Poult. Res. 20: 485-490.

Mercer, L.P. 1982. The quantitative nutrient-response relationship. J. Nutr. 112: 560-566.

Miranda, D. J. A., S. L. Vieira, A. Favero, C. R. Angel, C. Stefanello, and E. T. Nogueira. 2015. Performance and meat production of broiler chickens fed diets formulated at different crude protein levels supplemented or not with L-valine and L-isoleucine. Anim. Feed Sci. Tech. 206: 39-47.

Napolitano, L., M. Scalise, M. Galluccio, L. Pochini, L. M. Albanese, and C. Indiveri. 2015. LAT1 is the transport competent unit of the LAT1/CD98 heterodimeric amino acid transporter. Int. J. Biochem. Cell Bio. 67: 25-33.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

National Research Council. 2012. Proteins and amino acids. In: Nutrient requirements of swine, 11 rev. edth edn. The national Academies Press, Washington DC, 15-44.

Ostaszewski, P., S. Kostuik, B. Balasinska, M. Jank, I. Paret, and F. Glomot. 2000. The leucine metabolite 3-hydroxy-3-methylbutyrate (HMB) modifies protein turnover in muscles of laboratory rate and domestic chickens *in vitro*. J. Anim. Physiol. and Anim. Nutr. 84: 1-8.

Oviedo-Rondon, E. O., and P. W. Waldroup. 2002. Models to estimate amino acid requirements for broiler chickens: a review. Int. J. Poult. Sci. 1: 106-113.

Owens, F. N., J. E. Pettigrew, S. G. Cornelius, and R. L. Moser. 1985. Amino acid requirements for growth and maintenance of rats and chicks. J. Anim. Sci. 61 (suppl. 1): 312.

Park, B. C., and R. E. Austic. 2000. Isoleucine imbalance using selected mixtures of imbalancing amino acids in diets of the broiler chick. Poult. Sci. 79: 1782- 1789.

Parsons, C. M. 1986. Determination of digestible and available amino acids in meat meal using conventional and caecectomized cockerels or chick growth assays. Br. J. Nutr. 56: 227-240.

Parsons, C. M. 1996. Digestible Amino Acids for Poultry and Swine. Anim. Feed Sci. Tech. 59: 147-153.

Parsons, C. M., L. M. Potter, and R. D. Brown, Jr. 1982. Effect of dietary and intestinal microflora on excretion of amino acids in poultry. Poult. Sci. 61: 939-946.

Parsons, C. M., L. M. Potter, R. D. Brown, Jr., T. D. Wilkins, and B. A. Bliss, 1982. Microbial contribution to dry matter and amino acid content of poultry excreta. Poult. Sci. 61: 925-932.

Petro, T. M., and J. K. Bhattacharjee. 1981. Effect of dietary essential amino acid limitations upon the susceptibility to salmonella typhimurium and the effect upon humoral and cellular immune responses in mice. Infect. Immun. 32: 251-259.

Picard, M., P. B Siegal, C. Leterrier, and P. Geraet. 1999. Diluted starter diet, growth performance, and digesive tract development in fast and slow-growing broilers. J. Appl. Poult. Res. 8: 122-131.

Portz, L., C. T. D. S. Dias, and J. E. P. Cyrino. 2000. Regressao segmentada como modelo na determinacao de exigencies nutricionais peixes. Scientia Agricola. 57: 601-607.

Ravindran, V., and W. L. Bryden. 1999. Amino acid availability in poultry—in vitro and in vivo measurements. Aust. J. Agric. Res. 50: 889-908.

Ravindran, V., L.I. Hew, G. Ravindran, and W.L. Bryden. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry, Br. Poult. Sci. 40: 266-274.

Robbins, K. R.. A. M. Saxton, and L. L. Southern. 2006. Estimation of nutrient requirements using broken-line regression analysis. J. Anim. Sci. 84 (suppl_13): E155–E165

Rostagno, H. S. 2011. Brazilian Tables for Poultry and Swine, composition of feedstuffs and nutrient requirements, 3rd edition. Referencias bibliogrficas. 233-255

Sedghi, M., A. Golian, F. Kolahan, and A. Afsar. 2015. Optimisation of broiler chicken responses from 0 to 7 d of age to dietary leucine, isoleucine and valine using Taguchi and mathematical methods. Br. Poult. Sci. 56: 696-707.

Slutzker, A., M. Vyazmensky, D. M. Chipman, and Z. Barak. 2011. Role of the C terminal domain of the regulatory subunit of AHAS isozyme III: Use of random mutagenesis with in vivo reconstitution (REM-ivrs). BBA-Protiens and Proteomics. 1814: 449-455.

Smith, T. K., and R. E. Austic. 1978. The branched-chain amino acid antagonism in chicks. J. Nutr. 108: 1180-1191.

Stilborn, H. L., E. T. Moran, R. M. Gous, and M. D. Harris. 1997. Effest of age on feather amino acid content in two broiler strain crosses and sexes. J. Appl. Poult. Sci. 6: 205-209.

Stilborn, H. L., E. T. Moran, R. M. Gous, and M. D. Harris. 2010. Influence of age on carcass (feather-free) amino acid content for two broiler strain-crosses and sexes. J. Appl. Poult. Sci. 19: 13-23.

Summers, J. D., and S. Leeson. 1985. Broiler carcass composition as affected by amino acid supplementation. Can. J. Anim. Sci. 65: 717-723.

Tuttle, W. L., and S. L. Balloun. 1976. Leucine, isoleucine and valine interactions in turkey poults. Poult. Sci. 55: 1737-1743.

Tyagi, R., T. U. Lee, L. W. Guddat, and R. G. Duggleby. 2004. Probing the mechanism of the bifunctional enzyme ketol-acid reductoisomerase by site-directed mutagenesis of the active site. FEBS J. 272: 593-602.

Velu, J. G., H. M. Scott, and D. H. Baker. 1972. Body composition and nutrient utilization of chicks fed amino acid diets containing graded amounts of either isoleucine or lysine. J. Nutr. 102: 741–747.

Webb, Jr. K. E. 1990. Intestinal absorption of protein hydrolysis products: a review. J. Anim. Sci. 68: 3011–3022.

Webel, D. M., R. W. Johnson, and D. H. Baker. 1998. Lipopolysaccharide-Induced reductions in food intake do not decrease the efficiency of lysine and threonine utilization for protein accretion in chickens. J. Nutr. 128: 1760–1766.

Zeitz, J. O., S. Käding, I. R. Niewalda, V. Machander, J. C. de Paula Dorigam, and K. Eder. 2019. Effects of leucine supplementation on muscle protein synthesis and degradation pathways in broilers at constant dietary concentrations of isoleucine and valine. Arch. Anim. Nutr. 73: 75-87.

III. DETERMINATION OF THE OPTIMAL DIGESTIBLE ISOLEUCINE TO LYSINE RATIOS FOR YIELD PLUS × ROSS 708 MALE BROILERS FROM APROXIMATELY 1 TO 4 KG BODY WEIGHT

ABSTRACT

Three experiments (**Exp**) were conducted to determine optimal digestible Ile to Lys ratios for male Yield Plus × Ross 708 broilers from approximately 1.0 to 4.0 kg BW. Broilers were fed dose-response diets with inclusions of blood cells that were formulated to contain a gradient of digestible Ile to Lys ratios (0.46 to 0.83). Treatments for Exp 1 to 3 were fed from 21 to 35, 28 to 42, and 35 to 49 d of age, respectively, to target market weights from 2.5 to 4.0 kg. Experiments utilized positive control (**PC**) diets that did not contain blood cells and were formulated to the same Ile ratios as treatment 5. Birds and feed were weighed by pen on the first and last days of the experimental period to determine growth performance. Broilers were processed and deboned to determine carcass characteristics. For all Exp, quadratic effects ($P \leq 0.001$) were observed with BW gain, feed conversion ratio (**FCR**), breast meat weight, and breast meat yield (**BMY**) as digestible Ile to Lys ratios increased. Pre-planned orthogonal contrasts between PC and Treatment 5 for each Exp showed no effect of blood cell inclusion with the exception of FCR in Exp 1 ($P = 0.001$) and BMY in Exp 3 ($P = 0.017$). Optimum digestible Ile to Lys ratios for Exp 1 were determined to range from 0.640 to 0.725 for growth from 1.0 to 2.5 kg BW ($P \leq 0.001$) and breast meat characteristics. In Exp 2, optimum ratios ranged from

0.664 to 0.682 for growth and breast meat characteristics from 1.6 to 3.1 kg BW ($P \leq 0.001$). For growth and breast meat characteristics of broilers in Exp 3, optimum ratios ranged from 0.625 to 0.730, from 2.6 to 3.9 kg BW ($P \leq 0.001$). Based on these findings, optimum digestible Ile to Lys ratios were determined to range from 0.63 to 0.73 for broilers from 1.0 to 4.0 kg BW.

INTRODUCTION

Dietary Ile is the 4th or 5th limiting amino acid (**AA**) in corn and soybean-meal based diets with inclusions of animal protein meals fed to broilers (Kidd et al., 2000; Corzo et al., 2009). Isoleucine is a member of a sub-group of AA known as branched-chain AA, that also includes Val and Leu. The primary function of Ile is contributing to lean tissue development and is a substantial portion of edible tissue (Bae et al., 1999). Isoleucine is reported to play an important role in active immunity and the production of immune cells (Hale et al., 2004; Bender, 2012).

Digestible (**dig**) Ile requirements and optimum dig Ile to Lys ratios have been reported for broilers up to 1.0 kg BW (Farran and Thomas, 1990; Baker and Chung, 1992; Barbour and Latshaw, 1992; NRC, 1994; Baker, 1996, 1997; Baker et al., 2002). In addition, published research has reported total and dig Ile requirements and optimum dig Ile to Lys ratios for broilers from 1.0 to 3.5 kg BW (NRC, 1994; Baker, 1996; Kidd et al., 2004; Berres et al., 2010; Miranda et al., 2015). However, a disproportionate amount of the existing literature are for broilers under 1.0 kg BW, and many of these published articles are over 15 years old. It is now common practice to formulate poultry diets using ideal ratios of limiting AA with Lys as a reference AA (Baker, 2003); however, there is limited published research reporting optimum ratios for dig Ile in broilers greater than 1.0 kg.

Previous research has demonstrated that Ile requirements for breast muscle accretion are greater than for growth (Kidd et al., 2004; Mejia et al., 2011; Dozier et al., 2012). However, published data are sparse reporting optimum dig Ile ratios for growth performance and breast meat yields of broilers grown greater than 2.5 kg. Therefore, determining optimum dig Ile to Lys ratios for broilers from 1.0 to 4.0 kg BW is paramount for accurately formulating broiler diets and optimizing meat yields. Objectives of these studies were to determine the optimum dig Ile to Lys ratios of broilers approaching market weights from 1.0 to 2.5, 1.5 to 3.0, and 2.5 to 4.0 kg utilizing responses of growth and carcass parameters.

MATERIALS AND METHODS

All procedures involving live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2019-3555, PRN 2020-3655)

Common Procedures

Three experiments (**Exp**) were conducted utilizing Yield Plus × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) obtained from a commercial hatchery at 1 d of age. At the hatchery, all chicks received vaccinations for Marek's disease, Newcastle disease, and infectious bronchitis. Broiler chicks were placed into floor pens (Exp 1 = 72 pens, 30 birds/pen, 0.11 m^2/bird; Exp 2 = 64 pens, 25 birds/pen, 0.09 m^2/bird; Exp 3 = 72 pens, 30 birds/pen, 0.11 m^2/bird) of a solid sided house. Each pen was equipped with a tube feeder, a nipple drinker line, and litter from 1 previous flock. Experimental facilities consisted of a negative-pressure ventilation system equipped with vent boards, exhaust fans, evaporative cooling pads, and an electronic controller to maintain the temperature and ventilation needs of the birds. House temperature at chick placement was maintained at 33 ºC and was gradually reduced to 20 ºC based on bird comfort. The photoperiod was set at 23L:1D for the first 7 d post-hatch and 20L:4D was maintained for the duration of the Exp. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 d of age through the duration of the Exp, respectively. Feed and water were provided ad libitum throughout the experimental periods. Broiler chicks were fed common starter and grower diets until the beginning of the experimental periods (Exp 1 = 1 to 20 d of age; Exp 2 = 1 to 14 and 15 to 27 d of age; Exp 3 = 1 to 18 and 19 to 34 d of age), formulated to meet or exceed the nutrient recommendations of the NRC (1994). The incidence of mortality was recorded daily throughout each Exp.

Dietary Treatments

Experimental diets were corn and soybean-meal based with inclusions of spray-dried blood cells (Table 3.1). Corn and soybean meal were analyzed using near infrared reflective spectroscopy (Evonik Nutrition AMINO*NIR*®) to determine total AA concentrations. Blood cells (American Protein Corporation, Arion, IA) were analyzed via HPLC (method 982.30 E (a,b,c); AOAC International, 2006) to determine the total AA content. Digestible AA contents of the blood cells, corn, and soybean meal were determined by multiplying the total AA concentrations by digestibility coefficients adapted from the Brazilian Tables for Poultry and Swine (2017). Blood cells were used due to their low concentration of dig Ile (0.40 to 0.75%). This enabled a small inclusion of blood cells to create a dig Ile deficient test diet (Negative Control).

In Exp 1, 2, and 3, dig Ile to Lys ratios of titrated diets were formulated to range from 0.48 to 0.83, 0.46 to 0.82, and 0.48 to 0.83, respectively (Table 3.2). Experimental diets were created by the mixing of negative control (**NC**) (deficient in dig Ile) and summit (excess dig Ile) diets in varying proportions. Each experimental diet was formulated to contain 95% of the recommended dig Lys concentration to prevent birds from overconsuming Lys (Baker and Han, 1994). For Exp 1, the NC diet was formulated to contain 0.50% dig Ile and 1.05% dig Lys, and the summit was formulated to contain 0.87 and 1.05% dig Ile and Lys, respectively. Treatments 1 to 8 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively, and the remaining proportion of each treatment (**Trt**) was comprised of the summit diet. In Exp 2, the NC diet was formulated to contain 0.44% dig Ile and 0.95% dig Lys, and the summit diet was formulated to contain 0.78% dig Ile and 0.95% dig Lys. Diets were consisted of 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet for Trt 1 to 7, respectively, and the summit diet comprised the remainder. In Exp 3, the NC diet was formulated to contain 0.42 and 0.87% dig Ile and Lys, respectively, and the summit was formulated to contain 0.72 and 0.87% dig Ile and Lys, respectively. Treatments 1 to 8 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively, and the remainder was made up of summit diet. All Exp had a positive control (**PC**) (Exp 1 = Trt 9; Exp 2 = Trt 8; Exp 3 = Trt 9) that was formulated without blood cells to have the same dig Ile to Lys ratio as Trt 5. For Exp 1, 2, and 3, PC diets were formulated to contain dig Ile to Lys ratios of 0.68, 0.70, and 0.68, respectively.

Measurements

Birds and feed were weighed by pen at the beginning and end of each experimental period (Exp 1 = 21 and 35 d of age; Exp 2 = 28 and 42 d of age; Exp 3 = 35 and 49 d of age) in order to determine BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**). At the end of each experimental period (Exp 1 = d 36; Exp 2 = d 43; Exp 3 = d 50), birds were randomly selected to be processed to asses carcass characteristics (Exp 1 = 9 birds/pen; Exp 2 = 14 birds/pen; Exp 3 = 14 birds/pen). Birds were processed in a pilot processing facility at the Auburn University Poultry Research Unit following a 12-hour feed withdrawal period. Broilers were electronically stunned, exsanguinated, scalded, picked mechanically, eviscerated mechanically, and placed on ice. Carcasses were chilled in ice water for a period of 3 hours and then drained of excess water for approximately 3 minutes. The abdominal fat pad was removed and weighed separately from the chilled carcass to determine the fat percentage. Carcasses were deboned the following day to obtain breast fillets (*pectoralis major*), tenders (*pectoralis minor*), wings, drums, and boneless-skinless thigh meat by experienced personnel utilizing stationary cones (Exp 1). For Exp 2 and 3, breast fillets and tenders were weighed. Tender and breast fillet weights were combined for the analysis of total breast meat weight. Meat yield percentages were based on live weight at 35, 42, and 49 d of age for Exp 1, 2, and 3, respectively.

Apparent Ileal Amino Acid Digestibility Assays

For all Exp, apparent ileal AA digestibility was determined for the NC and summit diets. At 38, 45, and 52 d of age for Exp 1, 2 and 3, respectively, ileal digesta was collected from 6 birds per pen from the NC and summit Trt (Exp 1 = 1 and 8; Exp 2 = 1 and 7; Exp 3 = 1 and 8). Birds were euthanized via CO_2 asphyxiation and digesta was collected by gently flushing a section of the terminal ileum (terminal 1/3 between the Meckel's diverticulum and 2 cm proximal to the ileo-cecal junction) with deionized water. Diets and digesta were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA), and then ground in an electric coffee grinder (Hamilton Beach, Glen Allen, VA). The dried digesta was analyzed in duplicate and dried diets were analyzed in quadruplicate for TiO_2 concentration using the method described by Short et al. (1996). Absorbance was measured on a SPECTRAmax Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, CA), using 1.0 mL of solution in a cuvette reader. A standard curve was used to create a regression equation ($R^2 = 0.985$) as a reference to calculate TiO_2 concentrations in diets and digesta. Digesta and diet samples were also analyzed in duplicate for AA profile using HPLC (method 982.30 E (a,b,c); AOAC International, 2006). These values were used to calculate apparent ileal AA digestibility using the following formula:

$$AIAAD\ \% = \left[1 - \left(\frac{Ti_i}{Ti_o}\right) \times \left(\frac{AA_o}{AA_i}\right)\right] \times 100$$

where Ti_i represents the TiO_2 concentration in the input (diet), Ti_o represents the TiO_2 concentration in the output (digesta), AA_o represents the concentration of the AA in the output, and AA_i represents the concentration of the AA in the input (Dilger et al., 2004).

Statistical Analysis

All 3 Exp were conducted as a randomized complete block design with pen location as the blocking factor. Pen was considered the experimental unit. Regression analysis and contrasts were performed with the PROC REG and PROC MIXED procedures of SAS 9.4 (2017). For all Exp, a pre-planned orthogonal contrast was performed between the PC and Trt 5 in order to determine the effects of blood cell inclusion. The dose response dietary Trt (Exp 1 = 1 to 8; Exp 2 = 1 to 7; Exp 3 = 1 to 8) were delineated for optimum dig Ile to Lys ratios using linear and quadratic regression. For these analyses, mortality was arcsine of the square root transformed. The determination of the optimum ratios for all Exp was performed via linear and quadratic broken-line regression using Programa Prático de Modelagem (2015) on the dose response diets. For all statistical processes, significance was considered at P-value ≤ 0.05.

RESULTS AND DISCUSSION

For Exp 1, analyzed dig Lys and Ile values of test diets were lower than the formulated concentrations (Table 3.3). For Exp 2, analyzed dig Lys values for the test diets were in agreement with the calculated values as was the dig Ile concentration of the summit; however, analyzed values for Ile in the NC were lower than formulated. Analyzed dig Lys concentrations for Exp 3 were in agreement with calculated values as was the dig Ile concentration in the NC; however, the analyzed Ile concentration of the summit was lower than formulated. These discrepancies in analyzed dig Ile values may be attributed to the lower digestibility coefficients of the NC diets as Table 3.3 illustrates that the digestibility coefficients of Ile are considerably lower for the NC relative to the summit for all 3 Exp. These diets were formulated with a single assumed digestibility coefficient of Ile in blood cells (Rostagno et al., 2017); however, the progressive additions of L-Ile increased the digestibility of Ile in the diets as a smaller percentage of Ile was originating from intact proteins.

Experiment 1

Positive linear and quadratic effects (*P* < 0.001) were observed for BW and BWG as dig Ile to Lys ratios increased in the diet from 1.0 to 2.5 kg BW (Table 3.4). Similarly, FI increased linearly and quadratically (*P* < 0.001) as dietary Ile ratios increased. Broilers fed increasing dig Ile to Lys ratios displayed linear and quadratic decreases in FCR (*P* < 0.001) as BW increased from approximately 1.0 to 2.5 kg. Positive linear and quadratic effects were also observed (*P* < 0.001) for dig Ile intake. There was no effect (*P* = 0.74) of dietary dig Ile to Lys ratios on mortality. The pre-planned orthogonal contrast elucidated differences between the PC and Trt 5 for FI (*P* < 0.001), FCR (*P* < 0.001), and daily dig Ile intake (*P* = 0.022) where the responses of the PC were smaller for each parameter. No differences (*P* > 0.05) were observed between the PC and Trt 5 for BW, BWG, or mortality.

Positive linear and quadratic effects (*P* < 0.001) were observed for carcass weight, carcass yield, breast meat weight (**BMW**), and breast meat yield (**BMY**) as dig Ile to Lys ratios increased in the diet from 1.0 to 2.5 kg BW (Table 3.5). Similarly, drum weight, thigh meat weight, drum yield, and thigh meat yield had linear and quadratic responses (*P* < 0.001) to increasing dietary dig Ile to Lys ratios. For broilers being fed progressive additions of dig Ile, increasing linear and quadratic responses of wing weight were observed (*P* < 0.001); however, dig Ile ratios had no effect on wing yield (*P* = 0.77). Abdominal fat yield displayed negative linear and quadratic effects (*P* < 0.001) as dig Ile to Lys ratios increased in the diet; however, there was no effect of dietary Ile ratio on abdominal fat weight (*P* = 0.64). The pre-planned orthogonal contrast explicated differences between the PC and Trt 5 for wing yield (PC > Trt 5) and abdominal fat weight and yield (PC < Trt 5) (*P* = 0.020). There were no differences observed for any of the other carcass parameters (*P* > 0.05).

The optimum dig Ile to Lys ratio for BWG was determined to be 0.64 for broilers from 1.0 to 2.5 kg BW (*P* < 0.001) utilizing linear broken-line regression (Table 3.6). For FCR, the optimum dig Ile to Lys ratio was calculated as 0.62 (*P* < 0.001) utilizing linear broken-line regression analysis. The optimum dig Ile to Lys ratio for BMW of broilers was estimated to be 0.66 (*P* < 0.001) with linear broken-line analysis. A greater optimum dig Ile to Lys ratio for BMY was determined as 0.71 (*P* < 0.001) for broilers from 21 to 35 d of age. The optimum dig Ile to Lys ratios obtained were based on the calculated dig Ile to Lys ratios of the diets.

In agreement, Kidd et al. (2004) reported the total Ile requirement to range from 0.71 to 0.76% for growth performance and carcass characteristics from 18 to 30 d of age with a total Lys concentration of 1.09%. However, the reported daily Ile intakes to reach optimum performance, 750 to 850 mg/day, were much lower than responses obtained in the current study (950 to 1,000 mg/day for growth performance and BMW; 1,050 mg/day for BMY). This deviation indicates that more dietary Ile is required to obtain similar growth responses in modern broiler strains. Elevated concentrations of dig Ile increased meat yields for all carcass parts except for wings. The inclusion of blood cells to Trt 5 may have caused an elevated FI response relative to the PC. There was no effect on the growth rate of the broilers, thus the lower FI caused broilers provided the PC diet to have a lower FCR than those fed Trt 5. Birds fed Trt 5 had lower daily dig Ile intakes when compared with the broilers consuming the PC even with the greater FI of birds caused by Trt 5 containing a lower concentration of dig Ile than it was formulated to contain. There were also differences observed for abdominal fat weight and yield, with broilers provided Trt 5 having the greater responses. This may be due to broilers fed Trt 5 having a greater FI and a larger nutrient intake than birds provided the PC.

Optimum dig Ile to Lys ratios were 0.64 and 0.66 for BWG and BMW, respectively, in agreement with a previously reported optimum of 0.65 for broilers from 14 to 35 d of age (Berres et al., 2010). The optimum dig Ile to Lys ratio obtained for BMY of 0.71 is greater than previously reported optimum dig Ile ratios for broilers in this age range (Baker and Chung, 1992; Baker, 1997). In other research, broilers of larger weight classes displayed estimates of optimum ratios that were greater for breast meat yield than for BWG (Mejia et al., 2011; Miranda et al., 2015). Mejia et al. (2011) reported an optimum dig Ile to Lys ratio of 0.72 for BMY, however, these data evaluated broilers from 1.5 to 3.1 kg BW. In the current research, the response of FCR produced an estimated optimum ratio of 0.62, which is lower than both previous reports and the optimum for BWG. Previous studies have found the optimum responses for FCR are in agreement with BWG for broilers under 3.0 kg BW (Baker and Chung, 1992; Baker, 1996, 1997; Corzo et al., 2002; Kidd et al., 2004). This deviation in the estimated optimum ratio for FCR may be due to FI being maximized at a ratio of 0.63 while BWG continued to increase until a dig ratio of 0.67. The result was FCR responses that plateaued earlier than hypothesized.

For all parameters tested, optimum ratios were determined utilizing linear broken-line regression analysis. The quadratic analysis fit a significant model, however, it yielded higher standard errors (0.006 vs. 0.018) and therefore had larger 95% confidence intervals. This may have occurred because there were not adequate additional data points above the break point of the fit line causing an over estimation of the optimum ratios. An optimal dig Ile to Lys ratio of 0.66 to 0.68 is appropriate for broilers from 1.0 to 2.5 kg BW.

Experiment 2

Positive linear and quadratic responses were observed (*P* < 0.001) for BW and BWG as dig Ile to Lys ratios increased in the diet from 1.6 to 3.1 kg BW (Table 3.7). Similarly, FI and daily dig Ile intake displayed increasing linear and quadratic effects (*P* < 0.001) as dig Ile to Lys ratios were elevated from Trt 1 to 7. The FCR of broilers that consumed increasing dig Ile to Lys ratios were observed to decrease linearly and quadratically (*P* < 0.001). No effect (*P* = 0.60) of dietary dig Ile to Lys ratios was noted for the incidence of mortality of broilers. The preplanned orthogonal contrast displayed a difference between broilers consuming the PC and Trt 5 for daily dig Ile intake (*P* < 0.001), with the PC fed birds consuming 136 mg more dig Ile. In addition, there was a numerical decrease (*P* = 0.051) of 6 points in FCR for the PC birds relative to those on Trt 5.

Broilers fed increasing dig Ile to Lys ratios displayed positive linear and quadratic effects for carcass weight and yield (*P* < 0.001) (Table 3.8). Likewise, BMW and BMY responses increased linearly and quadratically (*P* < 0.001) as dig Ile to Lys ratio increased in the diet from 1.6 to 3.1 kg BW. There was no effect of increasing dig Ile to Lys ratios on abdominal fat yield (*P* = 0.52); however, positive linear and quadratic effects were observed (*P* = 0.003) for abdominal fat weight as dietary dig Ile to Lys ratios were elevated. The pre-planned orthogonal contrast did not display any differences between birds the PC and Trt 5 for the carcass characteristic of broilers in this Exp (*P* >

0.05). Broilers fed progressive additions of dig Ile led to an optimum dig Ile to Lys ratio of 0.67 for BWG ($P < 0.001$) from 1.5 to 3.1 kg BW. Similarly, optimum dig Ile to Lys ratios were determined to be 0.67, 0.66, and 0.68 FCR, BMW, and BMY, respectively, based on linear broken-line regression.

In agreement, Kidd et al. (2004) reported the total Ile requirement to range from 0.64 to 0.69% for growth performance and carcass characteristics from 30 to 42 d of age with a total Lys concentration of 1.05%. These authors also determined that optimum performance for broilers from 30 to 42 d of age could be obtained with a total daily Ile intake of 1,100 mg. This is considerably lower than the values of 1,600 to 1,650 mg/day obtained from broilers in the present research. This indicates that modern broiler strains have a greater dietary need for Ile to optimize growth relative to genetic strains of the past. There was an increase of abdominal fat weight as dig Ile to Lys ratios were elevated, however, there was no effect on the fat yield observed. This result is in agreement with what was reported for broilers from 22 to 42 d of age, where dietary Ile concentration affected abdominal fat weight, but did not change yield (Mejia et al., 2011; Miranda et al., 2015). Birds fed Trt 5 had a reduced daily dig Ile intake compared with the broilers provided the PC diet; however, this was not caused by a reduction in FI. This may be due to Trt 5 containing a lower dig Ile concentration compared with the calculated values and was not similar in dig Ile with the PC diet. A numerical decrease was observed for the birds provided the PC diet compared with broilers consuming Trt 5 in FCR, and though it was not a significant effect there was an improvement of 6 point of FCR in the absence of blood cells.

Estimated optimum dig Ile to Lys ratios ranged from a 0.66 to 0.68 for BWG, FCR, BMW, and BMY. These estimates are congruent with previously reported optimum ratios that range from 0.66 to 0.69 for broilers from 1.0 to 3.0 kg BW (Baker, 1996; Mack et al., 1999; Miranda et al., 2015), though dietary dig Lys concentrations varied. Mejia et al. (2011) reported higher optimum ratios of 0.69 for growth performance and 0.72 for BMY while utilizing a similar high producing broiler strain as the present study. Estimated optimum ratios were obtained utilizing linear broken-line regression analysis, as quadratic analysis did not produce a good fit with the response criteria (BWG SEM = 0.029 vs. 0.092). The treatment design may not have allowed an adequate number of data points to be above the breaking point to provide a good fit for a quadratic model. This was addressed in the experimental designs of Exp 1 and 3 with the addition of an 8th titrated diet above the hypothesized optimum ratio. However, this did not allow the quadratic model to have a better fit for any of the present Exp. These data indicate an optimal dig Ile to Lys ratio of 0.66 to 0.68 is appropriate for broilers from 1.6 to 3.1 kg BW.

Experiment 3

Positive linear and quadratic effects were observed for BW and BWG ($P < 0.001$) of broilers as dig Ile to Lys ratios increased in the diet from 2.6 to 3.9 kg BW (Table 3.9). Similarly, dig Ile intake displayed linear and quadratic responses ($P < 0.001$), where values increased linearly and quadratically from Trt 1 to 8. For FI of broilers, quadratic responses were observed ($P < 0.001$), however, there was not a linear relationship between FI and dig Ile to Lys ratio ($P = 0.15$). Broilers that were fed progressive additions of dig Ile displayed decreasing linear and quadratic responses ($P < 0.001$) for

FCR. There was no effect of increasing dig Ile to Lys ratios on the mortality of broilers. A difference was elucidated between birds provided the PC and Trt 5 for the daily dig Ile intake of broilers ($P < 0.001$), with the PC producing a larger dig Ile intake response. Blood cell inclusion had no effect on any other growth performance parameters of broilers from 2.6 to 3.9 kg BW ($P > 0.05$).

Broilers being fed increasing dig Ile to Lys ratios displayed linear and quadratic responses ($P < 0.001$) for carcass weight from 2.6 to 3.9 kg BW (Table 3.10). However, there was no effect of dig Ile to Lys ratios on carcass yield ($P = 0.30$) as dig Ile increased in the diet. Positive linear and quadratic effects were observed ($P < 0.001$) for BMW and BMY of broilers when dietary Ile concentrations were increased. There was no effect of dig Ile to Lys ratios observed for abdominal fat weights ($P = 0.85$) or yields ($P = 0.12$). The pre-planned orthogonal contrast may have revealed an effect of blood cells inclusion for both carcass yield ($P = 0.016$) and BMY ($P = 0.017$) between broilers fed Trt 5 and the PC. No other differences were observed between these diets for carcass characteristics ($P > 0.05$). The optimum dig Ile to Lys ratio for BWG was determined to be 0.63 ($P < 0.001$) utilizing linear broken-line regression from 2.6 to 3.9 kg BW (Table 3.6). Similarly, the optimum did Ile to Lys ratio for FCR was calculated as 0.69 ($P < 0.001$).The optimum dig Ile to Lys ratios for BMW and BMY were observed at 0.69 and 0.73 ($P < 0.001$), respectively, with linear analysis.

Kidd et al. (2004) reported the total Ile requirement of broilers from 42 to 56 d of age to range from 0.57 to 0.67% with a dietary Lys concentration of 0.90%. These authors reported daily Ile intake necessary to obtain optimum performance of 1,154 mg, which agrees with the range of 1,100 to 1,200 mg/day that was obtained in the current study. However, broilers from the previous study were grown approximately 400 g larger than birds from Exp 3. Therefore, modern broiler strains required more dietary Ile per unit of growth compared with genetic strains of the past. The daily dig Ile intakes of birds fed the PC were higher compared with Trt 5 broilers; however, there was no effect observed for FI. This is likely caused by Trt 5 containing an analyzed dig Ile to Lys ratio of 0.66, rather than the ratio of 0.68 to which it was formulated. Increases in carcass and breast meat yields were also observed for birds consuming the PC compared with Trt 5. This response may be due to Trt 5 having a lower dig Ile concentration than it was formulated to contain.

Optimum dig Ile to Lys ratios ranged from 0.69 to 0.73 for FCR, BMW, and BMY. Body weight gain produced an optimum ratio of 0.63, which is lower than previously reported ratios for this time period of 0.68 and 0.69 (Baker, 1996, 1997). The estimated optimum ratios for FCR and BMW were at 0.69, which is in agreement with previous reports for broilers grown to approximately 3.5 kg BW (Baker, 1997). The difference observed between responses of BWG and FCR have been observed previously for broilers of > 3.0 kg (Kidd et al., 2000, 2004). These authors reported that the difference can be rationalized by increased FI responses to compensate for the limiting nutrient. Breast meat yield produced an optimum dig Ile to Lys ratio of 0.73, which is higher than previously published data (Corzo et al., 2002, 2008), however, it is in agreement with the dig Ile ratio obtained by Mejia et al. (2011) of 0.72 for broilers from 28 to 42 d of age. Based on these data, an optimal dig Ile to Lys ratio of 0.69 to 0.70 is appropriate for broilers from 2.5 to 3.9 kg BW.

Statistical Models

Consideration must be given to the statistical model chosen to evaluate optimum dig AA ratios, as it can affect the results obtained. Broken-line regression analysis is commonly used as it provides a function that describes the responses to nutrient doses across all concentrations and provides a break point estimate of the optimum with an associated standard error (Robbins et al., 2006). However, the type of broken-line model fit to a given data set can change the output of the analysis based on the shape of the data. Linear broken-line regression presumes that the responses to a nutrient dose are linear, when in most cases of dose response designs responses are curvilinear in nature (Robbins et al., 2006). In these cases, linear broken-line analysis can still provide a satisfactory fit to the data, however, this model can underestimate a requirement compared to a quadratic model that achieves a significant fit. The issue that is observed with quadratic broken-line models is that a minimum of 3, and preferably 4, data points are required above the break point of the model for it to accurately predict an optimum requirement (Robbins et al., 2006). These authors report that problems can also be observed if there are large variations in the responses of broilers above a hypothesized optimum that effect the shape of the plateau. Many of these issues occurred in the present research, and likely effected the ability of the quadratic broken-line model to accurately predict the optimum dig Ile to Lys ratios of broilers. Additionally, Ile concentrations being analyzed lower than formulated values may have contributed to a linear broken-line model better fitting the data. The lower values shifted the data points down so that there were insufficient data above the break point for a quadratic model to estimate the optimums. Based on the responses observed linear broken-line analysis provided accurate estimates of dig Ile ratios from 1.0 to 4.0 kg BW (Table 3.6).

Response Criteria

In the current research, optimum dig Ile to Lys ratios vary based on the response criteria. Optimum dig Ile to Lys ratios are more pronounced with breast meat yield compared with growth performance (Kidd et al., 2004; Mejia et al., 2011; Miranda et al., 2015). This response was observed for all the present studies, with optimum ratios for BMY being 3 to 5 points greater than growth performance characteristics. Corzo et al. (2002) reported that Ile needs of broilers heavier than 3.0 kg are greater for breast tissue development relative to other growth. However, similar responses have been reported for broilers from 1.5 to 2.5 kg as well (Kidd et al., 2004). This is critical, especially if final market weights larger than 3.0 kg are being targeted. Feeding higher dig Ile to Lys ratios through the grower and finisher periods may help to optimize breast meat yields.

In conclusion, data from these 3 Exp indicated that the optimum dig Ile to Lys ratios for growth performance are largely in agreement with previous research (NRC, 1994; Baker, 1996,1997; Miranda et al., 2015). However, the Ile intake required to optimized growth and feed efficiency is greater in modern broiler strains (Kidd et al., 2004). Estimated optimums for BMY in the current studies are greater than previously reported for broilers (Baker, 1996; Mejia et al., 2011). Corn and soybean meal-based diets formulated with a dig Ile to Lys ratio of 0.67 to 0.69 will be adequate for broilers to reach optimum growth performance from 1.0 to 4.0 kg BW.

REFERENCES

Association of Official Analytical Chemists International. 2006. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA. 982.30 E (a, b, c).

Bae, S. H., J. H. Kim, I. S. Shin, and K. Han. 1999. Partition of amino acid requirements of broilers between maintenance and growth. V. isoleucine and valine. Asian Australasian J. Anim. Sci. 12: 388-394.

Baker, D. H. 1996. Advances in Amino Acid Nutrition and Metabolism of Swine and Poultry. Pages 41-54 in Nutrient Management of food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. CRC Press Inc. Boca Raton, Florida

Baker, D. H. 2003. Ideal amino acid patterns for broiler chicks. Amino Acids in Animal Nutrition, 2nd Edition. CAB International. 223-236.

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. Pages 154-178 in BioKyowa Technical Review *9*. BioKyowa Inc. Cape Girardeau, Missouri.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81: 485-494.

Baker, D. H., and T. K. Chung. 1992. Ideal protein for swine and poultry. Biokyowa Technical Review, 4: 16.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult Sci. 74: 1441-1447.

Barbour, G., and J. D. Latshaw. 1992. Isoleucine requirement of broiler chicks as affected by the concentrations of leucine and valine in practical diets. Br. Poult. Sci. 33: 561-568.

Bender, D. A. 2012. The Branched-Chain Amino Acids: Leucine, Isoleucine and Valine. Amino Acid Metabolism, Third Edition. John Wiley & Sons, Ltd. 279-302.

Berres, J., S. L. Viera, M. T. Kidd, D. Taschetto, D. M. Freitas, R. Barros, and E. T. Nogueira. 2010. Supplementing L-valine and L-isoleucine in low-protein corn and soybean meal all-vegetable diets for broilers. J. Appl. Poult. Res. 19: 373 379.

Corzo, A., R. E. Loar, II, and M. T. Kidd. 2009. Limitations of dietary isoleucine and valine in broiler chick diets. Poult. Sci. 88: 1934-1938.

Corzo, A., W. A. Dozier, III, M. T. Kidd, and D. Hoeler. 2008. Impact of dietary isoleucine status on heavy-broiler production. Int. J. Poult. Sci. 7: 526-529.

Corzo, A., E. T. Moran, and D. Hoehler, Jr. 2002. Isoleucine needs of broiler males from 42 to 56 days of age. Poult. Sci. 80: 25. Abstr.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715-724.

Dozier III, W. A., P. B. Tillman, and J. Usry. 2012. Interactive effects of digestible valine-and isoleucine-to-lysine ratios provided to male broilers from 4 to 6 weeks of age. J. Appl. Poult. Res. 21: 838-848.

Farran, M. T., and O. P. Thomas. 1990. Dietary requirements of leucine, isoleucine, and valine in male broilers during the starter period. Poult. Sci. 69: 757-762.

Garcia-neto, M., Perri, S. H. V. 2015. PPM: Programa prático de modelagem. Disponivel em https://sites.google.com/site/programapraticodemodelagem/. Acesso em 1 Jun-20.

Hale, L. L., G. T. Pharr, S. C. Burgess, A. Corzo, and M. T. Kidd. 2004. Isoleucine needs of thirty- to forty-day-old female chickens: immunity. Poult. Sci. 83: 1979-1985.

Kidd, M. T., D. J. Burnham, and B. J. Kerr. 2004. Dietary isoleucine responses in male broiler chickens. Br. Poult. Sci. 45: 67-75.

Kidd, M. T., B. J. Kerr, J. P. Allard, S. K. Rao, and J. T. Halley. 2000. Limiting amino acid responses in commercial broilers. J. Appl. Poult. Res. 9: 223-233.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Mack, S., D. Bercovici, G. De Groote, B. Leclercq, M. Lippens, M. Pack, J. B. Schutte, and S. Van Cauwenberghe. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. Br. Poult. Sci. 40: 257 265.

Mejia, L., C. D. Zumwalt, E. J. Kim, P. B. Tillman, and A. Corzo. 2011. Digestible isoleucine-to-lysine ratio effects in diets for broilers from 4 to 6 weeks posthatch. J. Appl. Poult. Res. 20: 485-490.

Miranda, D. J. A., S. L. Vieira, A. Favero, C. R. Angel, C. Stefanello, and E. T. Nogueira. 2015. Performance and meat production of broiler chickens fed diets formulated at different crude protein levels supplemented or not with L-valine and L-isoleucine. Anim. Feed Sci. Tech. 206: 39-47.

Petro, T. M., and J. K. Bhattacharjee. 1981. Effect of dietary essential amino acid limitations upon the susceptibility to salmonella typhimurium and the effect upon humoral and cellular immune responses in mice. Infect. Immun. 32: 251 259.

Robbins, K. R., A. M. Saxton, and L. L. Southern. 2006. Estimation of nutrient requirements using broken-line regression analysis. J. Anim. Sci. 84(E. Suppl.): E155-E165.

Rostagno, H. S. 2011. Brazilian Tables for Poultry and Swine, composition of feedstuffs and nutrient requirements, 3rd edition. Referencias bibliograficas.

Rostagno, H. S. 2017. Brazilian Tables for Poultry and Swine, composition of feedstuffs and nutrient requirements, 4th edition. Referencias bibliograficas.

SAS Institute. 2017. SAS® User's Guide. Version 9.4 ed. SAS Institute Inc., Cary, NC.

Short, F. J., P. Gorton, J. Wiseman, J., and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215-221

Stilborn, H. L., E. T. Moran, R. M. Gous, and M. D. Harris. 2010. Influence of age on carcass (feather-free) amino acid content for two broiler strain-crosses and sexes. J. Appl. Poult. Sci. 19: 13-23.

Table 3.1. Analysis of amino acid and crude protein concentrations of primary ingredients used in the formulation of diets fed to Yield Plus × Ross 708 male broilers from 1.0 to 4.0 kg body mass.

	Corn		SBM[1]		Blood Cells[2]	
Experiment 1[3]						
Nutrient, % "as fed"	Total[4]	Dig[4]	Total[4]	Dig[4]	Total[5]	Dig[6]
Crude Protein	7.95	---	43.86	---	96.75	---
SAA	0.370	0.329	1.220	1.015	1.820	1.638
Lys	0.260	0.219	2.710	2.350	9.240	8.316
Thr	0.290	0.244	1.750	1.440	4.400	3.960
Val	0.400	0.359	2.160	1.827	9.100	8.190
Ile	0.290	0.270	2.090	1.814	0.410	0.369
Trp	0.080	0.067	0.600	0.521	1.680	1.512
Arg	0.380	0.337	3.090	2.818	3.600	3.240
His	0.230	0.214	1.120	0.993	6.410	5.769
Phe	0.400	0.367	2.210	1.960	7.540	6.786
Leu	0.970	0.895	3.370	2.956	13.000	11.700
Experiment 2						
Crude Protein	8.50	---	47.75	---	93.67	---
SAA	0.335	0.281	1.250	1.062	1.736	1.562
Lys	0.261	0.201	2.862	2.518	8.518	7.667
Thr	0.273	0.216	1.798	1.528	4.196	3.776
Val	0.364	0.309	2.118	1.864	7.783	7.005
Ile	0.261	0.225	2.077	1.807	0.833	0.750
Trp	0.047	0.039	0.672	0.584	1.587	1.428
Arg	0.375	0.326	3.358	2.955	3.364	3.028
His	0.205	0.174	1.178	1.013	5.776	5.198
Phe	0.341	0.303	2.438	2.170	6.343	5.709
Leu	0.898	0.826	3.533	3.145	11.820	10.638
Experiment 3						
Crude Protein	7.11	---	46.56	---	90.00	---
SAA	0.320	0.278	1.260	1.064	1.900	1.710
Lys	0.240	0.197	2.820	2.482	8.520	7.668
Thr	0.255	0.209	1.780	1.495	4.200	3.780
Val	0.340	0.299	2.200	1.936	5.000	4.500
Ile	0.245	0.216	2.110	1.836	0.690	0.621
Trp	0.055	0.042	0.630	0.554	1.450	1.305
Arg	0.360	0.317	3.350	3.015	3.400	3.060
His	0.210	0.189	1.200	1.056	5.580	5.265
Phe	0.330	0.297	2.380	2.118	6.860	6.174
Leu	0.830	0.772	3.500	3.080	12.110	10.899

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[1] Soybean Meal

[2] American Protein Corporation, Arion, IA

[3] Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively.

[4] Digestible values determined using Evonik Nutrition AMINO*NIR*® near infrared reflective spectroscopy.

[5] Values obtained from HPLC analysis of ingredients (method 982.30 E (a,b,c); AOAC International, 2006)

[6] Values obtained by multiplying total amino acid concentrations by digestibility coefficients adapted from the Brazilian Tables (2017) for blood cells.

Table 3.2. Ingredient and nutrient composition of dietary treatments fed to Yield Plus × Ross 708 male broilers from 1.0 to 4.0 kg body mass[1].

	Experiment 1[2]			Experiment 2			Experiment 3		
	NC[3]	Summit	PC[4]	NC	Summit	PC[4]	NC	Summit	PC[4]
Ingredient, % "as-fed"									
Corn	76.25	76.07	62.46	79.10	78.95	64.28	81.10	80.93	69.80
Soybean Meal	15.52	15.52	29.99	12.92	12.52	28.81	11.15	11.15	24.03
Vegetable Oil	0.45	0.25	3.12	0.45	0.25	3.57	0.91	0.77	3.32
Limestone	0.67	0.67	0.68	0.66	0.66	0.60	0.81	0.81	0.79
Defluorinated Phosphate	1.00	1.00	0.98	1.49	1.49	1.29	0.84	0.85	0.76
Sodium Bicarbonate	0.27	0.27	0.29	0.34	0.34	0.32	0.37	0.37	0.37
Salt, NaCl	0.18	0.18	0.21	0.17	0.17	0.23	0.18	0.18	0.25
DL-Methionine	0.43	0.43	0.35	0.39	0.39	0.30	0.33	0.33	0.23
L-Lysine, HCl	0.40	0.40	0.27	0.40	0.40	0.12	0.33	0.33	0.17
AU Trace Mineral Premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Threonine	0.23	0.23	0.15	0.23	0.23	0.09	0.18	0.18	0.08
AU Vitamin Premix[6]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Phytase[7]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Val	0.11	0.11	0.09	0.16	0.16	0.05	0.16	0.16	0.005
L-Ile	0.01	0.38	---	0.01	0.36	---	0.02	0.33	---
L-Trp	0.05	0.05	0.01	0.08	0.08	0.01	0.04	0.04	---
L-Arg	0.60	0.60	0.31	0.42	0.42	0.06	0.30	0.30	---
Gly	0.68	0.69	0.41	0.50	0.50	0.09	0.32	0.32	---
Blood Cells[8]	2.50	2.50	---	2.00	2.00	---	2.25	2.25	---
Titanium Dioxide	0.50	0.50	---	0.50	0.50	---	0.50	0.50	---

Table 3.2. Continued

Calculated Analysis, % (unless otherwise noted)									
AME, kcal/kg	3,120	3,120	3,120	3,155	3,152	3,152	3,196	3,196	3,196
Crude protein	18.09	18.32	19.88	17.17	17.39	19.84	15.53	15.71	17.19
Digestible Lys[9]	1.05	1.05	1.05	0.95	0.95	0.95	0.87	0.87	0.87
Digestible SAA	0.87	0.87	0.86	0.78	0.78	0.78	0.71	0.71	0.68
Digestible Thr	0.74	0.74	0.74	0.67	0.67	0.67	0.60	0.60	0.58
Digestible Val	0.87	0.87	0.86	0.78	0.78	0.78	0.71	0.71	0.68
Digestible Ile[10]	0.50	0.87	0.71	0.44	0.78	0.67	0.42	0.72	0.59
Digestible Leu	1.44	1.44	1.45	1.28	1.28	1.44	1.22	1.22	1.28
Ca	0.76	0.76	0.76	0.90	0.90	0.86	0.76	0.76	0.76
Non-phytate P	0.40	0.40	0.40	0.43	0.43	0.43	0.38	0.38	0.38
Na	0.21	0.21	0.21	0.23	0.23	0.23	0.23	0.23	0.23

[1] Experimental diets 1 to 8 for experiments 1 and 3 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively and diets 1 to 7 in experiment 2 contained 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet, respectively, and remaining space in all diets was comprised of summit diet to create diets of intermediate digestible Ile to Lys ratios.

[2] Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively, in 8 replications/treatment.

[3] Negative control

[4] Positive control diets formulated to have a digestible Ile to Lys ratio of 0.68, 0.70, and 0.68 for Experiments 1, 2, and 3, respectively.

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

[6] Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D3(cholecalciferol), 6,614 IU; Vitamin E (DL-alphatocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12(cyanocobalamin), 0.03 mg; folacin (folic acid), 2.6mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate),5.5 mg; biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[7] Quantum Blue Phytase, AB Vista, Marlborough, UK

[8] American Protein Corporation, Arion, IA

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[9] Analyzed digestible Lys concentrations were 0.94 and 0.92, 0.94 and 0.92, and 0.92 and 0.84% for basal and summit diets of Experiments 1 to 3, respectively.

[10] Analyzed digestible Ile concentrations were 0.48 and 0.68, 0.39 and 0.77, and 0.42 and 0.62% for basal and summit diets of Experiments 1 to 3, respectively.

Table 3.3. Amino acid digestibility analysis of negative control and summit diets fed to Yield Plus × Ross 708 male broilers from 1.0 to 4.0 kg body mass[1].

	Experiment 1[2]				Experiment 2				Experiment 3			
	NC[3]		Summit[4]		NC		Summit		NC		Summit	
Amino Acid	DC[5]	dig AA[6]	DC	dig AA	DC	dig AA	DC	dig AA	DC	dig AA	DC	dig AA
	%	%	%	%	%	%	%	%	%	%	%	%
Lys	89.85	1.01	89.73	1.00	90.31	0.94	90.07	0.92	91.29	0.92	90.73	0.88
SAA	88.69	0.70	88.51	0.70	93.81	0.70	93.57	0.72	91.27	0.71	90.48	0.68
Thr	82.40	0.66	82.55	0.66	83.62	0.65	82.07	0.60	84.73	0.60	83.55	0.58
Val	85.82	0.77	85.36	0.78	86.47	0.73	86.81	0.79	87.67	0.78	86.38	0.78
Ile	80.01	0.45	86.99	0.72	80.43	0.39	88.37	0.77	82.87	0.41	88.26	0.65
Trp	83.21	0.18	82.21	0.18	86.39	0.21	85.67	0.20	86.23	0.15	87.77	0.15
Arg	93.31	1.30	92.78	1.29	91.57	1.05	91.70	1.09	93.45	0.99	92.54	0.96
Leu	86.79	1.32	87.31	1.33	87.50	1.22	87.43	1.32	87.83	1.22	86.02	1.21
Phe	88.13	0.78	87.31	0.75	87.11	0.63	86.83	0.65	88.64	0.70	86.98	0.67
His	88.19	0.43	87.27	0.42	89.52	0.38	89.34	0.39	88.95	0.40	87.32	0.39

[1] Experimental diets 1 to 8 for experiments 1 and 3 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively and diets 1 to 7 in experiment 2 contained 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet, respectively, and remaining space in all diets was comprised of summit diet to create diets of intermediate digestible Ile to Lys ratios.

[2] Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively in 8 replication/treatment.

[3] Negative control diets were formulated to contain 1.05% Lys and 0.50% Ile; 0.95% Lys and 0.44% Ile; and 0.87% Lys and 0.42% Ile on a digestible basis for Experiments 1 to 3, respectively.

[4] Summit diets were formulated to contain 1.05% Lys and 0.87% Ile; 0.95% Lys and 0.78% Ile; and 0.87% Lys and 0.72% Ile on a digestible basis for Experiments 1 to 3, respectively.

[5] Digestibility coefficient, obtained by titanium dioxide assay according to method of Short et al. (1996).

[6] Concentration of digestible amino acid included in the diet. Obtained by multiplying total amino acid concentration from HPLC analysis by the digestibility coefficient.

Table 3.4. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 1.0 to 2.5 kg BW[1].

Dietary Treatments[2]	BW	BWG[3]	FI[4]	Dig Ile Intake[5]	FCR[6]	Mortality
	(kg)	(kg)	(kg)	(mg/day)	(kg/kg)	(%)
1) dIle:Lys ratio 0.44	2.063	1.082	2.051	613	1.897	0.42
2) dIle:Lys ratio 0.48	2.199	1.198	2.123	679	1.772	0.86
3) dIle:Lys ratio 0.51	2.389	1.355	2.254	766	1.663	0.86
4) dIle:Lys ratio 0.55	2.451	1.437	2.302	852	1.602	0.00
5) dIle:Lys ratio 0.60	2.498	1.484	2.334	935	1.573	0.86
6) dIle:Lys ratio 0.63	2.467	1.465	2.283	964	1.558	0.45
7) dIle:Lys ratio 0.67	2.471	1.459	2.262	1,012	1.551	0.83
8) dIle:Lys ratio 0.72	2.490	1.493	2.292	1,108	1.536	0.00
9) Positive Control	2.473	1.446	2.190	963	1.515	1.82
SEM[7]	0.027	0.015	0.022	8	0.009	0.026
			Probabilities			
Regression Analysis						
Linear	0.001	0.001	0.001	0.001	0.001	0.59
Quadratic	0.001	0.001	0.001	0.001	0.001	0.74
Pre- planned orthogonal contrast						
PC vs. Trt 5	0.33	0.08	0.001	0.022	0.001	0.18
			Coefficient of Determination			
R^2 Linear	0.673	0.711	0.395	0.968	0.782	0.005
R^2 Quadratic	0.848	0.905	0.648	0.972	0.944	0.010

[1] Values represent least-square means for 8 replicate pens with 30 chicks per pen at 1 d of age.

[2] Treatments 1 to 8 are represented by calculated digestible Ile to Lys ratios.

[3] Body weight gain

[4] Feed intake

[5] Determined using digestible Ile concentration of each treatment

[6] Feed conversion ratio corrected for mortality

[7] Pooled standard error

Table 3.5. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 1.0 to 2.5 kg BW[1].

		Carcass		Breast Meat		Wing		Drum		Thigh Meat		Abdominal Fat	
Response Parameter	BW	Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield
	kg	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%
Dietary Treatments[2]													
1) dIle:Lys ratio 0.44	2.063	1.500	72.69	0.466	22.57	0.161	7.79	0.198	9.60	0.206	9.98	0.021	1.36
2) dIle:Lys ratio 0.48	2.199	1.598	72.67	0.514	23.36	0.168	7.66	0.206	9.40	0.216	9.81	0.021	1.33
3) dIle:Lys ratio 0.51	2.389	1.754	73.45	0.581	24.32	0.180	7.54	0.215	9.01	0.232	9.71	0.025	1.44
4) dIle:Lys ratio 0.55	2.451	1.799	73.38	0.610	24.86	0.189	7.71	0.223	9.08	0.241	9.81	0.022	1.21
5) dIle:Lys ratio 0.60	2.498	1.855	74.25	0.646	25.85	0.191	7.66	0.223	8.92	0.241	9.64	0.021	1.13
6) dIle:Lys ratio 0.63	2.467	1.820	73.77	0.644	26.11	0.189	7.66	0.221	8.94	0.237	9.61	0.020	1.08
7) dIle:Lys ratio 0.67	2.471	1.844	74.62	0.650	26.30	0.200	7.58	0.220	8.90	0.238	9.64	0.021	1.12
8) dIle:Lys ratio 0.72	2.490	1.859	74.67	0.656	26.39	0.193	7.74	0.224	8.97	0.240	9.65	0.022	1.18
9) Positive Control	2.473	1.836	74.21	0.638	25.77	0.194	7.83	0.223	9.00	0.232	9.36	0.015	0.84
SEM[3]	0.027	0.023	0.34	0.010	0.26	0.004	0.05	0.003	0.07	0.004	0.13	0.001	0.05
	Probabilities												
Regression Analysis													
Linear	0.001	0.001	0.001	0.001	0.001	0.001	0.77	0.001	0.001	0.001	0.001	0.47	0.001
Quadratic	0.001	0.001	0.001	0.001	0.001	0.001	0.58	0.001	0.001	0.001	0.001	0.64	0.001
Pre-planned orthogonal contrast													
PC vs. Trt 5	0.49	0.55	0.95	0.83	0.81	0.70	0.020	0.89	0.43	0.13	0.12	0.001	0.001
	Coefficient of Determination												
R^2 Linear	0.586	0.623	0.349	0.743	0.684	0.423	0.002	0.418	0.538	0.367	0.236	0.007	0.279
R^2 Quadratic	0.796	0.785	0.350	0.870	0.723	0.495	0.018	0.570	0.644	0.518	0.252	0.014	0.305

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[1] Values are least-square means of 8 replicate pens, with 9 birds being selected and processed at d 35.

[2] Treatments 1 to 8 are represented by calculated digestible Ile to Lys ratios.

[3] Pooled standard error

Table 3.6. Optimal digestible Ile to Lys ratios for male Yield Plus × Ross 708 broilers based on growth performance and carcass characteristics from 1.0 to 4.0 kg body mass.

Response	Estimated Ratio[1]	95% CI[2]	SEM[3]	R^2 (%)	*P*-value
		Experiment 1[4]			
Linear					
BWG[5], kg	0.640	0.628 to 0.652	0.006	92.27	< 0.001
FCR, kg:kg	0.621	0.607 to 0.635	0.007	93.54	< 0.001
TBMW[6], kg	0.662	0.641 to 0.684	0.011	87.17	< 0.001
TBMY[7], %	0.708	0.677 to 0.739	0.016	81.35	< 0.001
Quadratic					
BWG, kg	0.708	0.679 to 0.737	0.018	92.02	< 0.001
FCR, kg:kg	0.725	0.702 to 0.748	0.012	95.03	< 0.001
TBMW, kg	0.756	0.710 to 0.801	0.023	87.26	< 0.001
TBMY, %	0.848	0.752 to 0.934	0.049	81.10	< 0.001
		Experiment 2			
Linear					
BWG, kg	0.665	0.609 to 0.722	0.029	96.03	< 0.001
FCR, kg:kg	0.671	0.617 to 0.725	0.028	96.66	< 0.001
TBMW, kg	0.664	0.627 to 0.701	0.019	91.90	< 0.001
TBMY, %	0.682	0.590 to 0.775	0.047	92.28	< 0.001
Quadratic					
BWG, kg	0.802	0.621 to 0.984	0.092	91.46	< 0.001
FCR, kg:kg	0.808	0.644 to 0.979	0.083	92.58	< 0.001
TBMW, kg	0.783	0.649 to 0.917	0.068	95.86	< 0.001
TBMY, %	0.755	0.550 to 0.959	0.104	88.35	0.002
		Experiment 3			
Linear[8]					
BWG, kg	0.625	0.568 to 0.683	0.029	98.63	< 0.001
FCR, kg:kg	0.692	0.623 to 0.762	0.035	91.92	< 0.001
TBMW, kg	0.694	0.623 to 0.765	0.036	91.90	< 0.001
TBMY, %	0.730	0.652 to 0.807	0.039	92.81	< 0.001

[1] Values obtained using linear and quadratic broken-line modelling.

[2] 95% confidence intervals for the optimal digestible Ile to Lys ratios.

[3] Standard error of the estimate

[4] Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively.

[6] Total breast meat weight

[7] Total breast meat yield

[8] Quadratic broken-line regression did not fit the data for Experiment 3 and yielded insignificant results.

Table 3.7. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 1.6 to 3.1 kg BW[1].

Dietary Treatments[2]	BW (kg)	BWG[3] (kg)	FI[4] (kg)	Dig Ile Intake[5] (mg/day)	FCR[6] (kg/kg)	Mortality (%)
1) dIle:Lys ratio 0.42	2.732	1.178	2.279	903	1.940	4.0
2) dIle:Lys ratio 0.49	2.735	1.195	2.286	1,232	1.920	4.0
3) dIle:Lys ratio 0.56	2.939	1.377	2.436	1,437	1.771	3.5
4) dIle:Lys ratio 0.63	3.063	1.486	2.536	1,516	1.707	1.0
5) dIle:Lys ratio 0.69	3.117	1.521	2.533	1,718	1.665	1.0
6) dIle:Lys ratio 0.76	3.144	1.557	2.557	1,841	1.643	3.0
7) dIle:Lys ratio 0.83	3.097	1.514	2.518	1,948	1.665	3.5
8) Positive Control	3.119	1.541	2.472	1,862	1.605	1.5
SEM[7]	0.036	0.028	0.034	20.56	0.022	0.017
			Probabilities			
Regression Analysis						
Linear	0.001	0.001	0.001	0.001	0.001	0.60
Quadratic	0.001	0.001	0.001	0.001	0.001	0.39
Pre-planned orthogonal contrast						
PC vs. Trt 5	0.98	0.57	0.18	0.001	0.051	0.84
			Coefficient of Determination			
R^2 Linear	0.598	0.650	0.427	0.938	0.657	0.007
R^2 Quadratic	0.668	0.733	0.510	0.962	0.732	0.002

[1] Values represent least-square means for 8 replicate pens with 25 chicks per pen at 1 d of age.

[2] Treatments 1 to 7 are represented by analyzed digestible Ile to Lys ratios.

[3] Body weight gain

[4] Feed intake

[5] Determined using digestible Ile concentration of each treatment

[6] Feed conversion ratio corrected for mortality

[7] Pooled standard error

Table 3.8. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 1.6 to 3.1 kg BW[1].

Response Parameter	Live Weight, kg	Carcass		Breast Meat		Abdominal Fat	
		Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Percentage, %
Dietary Treatments[2]							
1) dIle:Lys ratio 0.42	2.720	2.028	74.57	0.685	25.19	0.026	0.94
2) dIle:Lys ratio 0.49	2.790	2.081	74.60	0.708	25.41	0.026	0.92
3) dIle:Lys ratio 0.56	2.982	2.240	75.22	0.786	26.38	0.027	0.92
4) dIle:Lys ratio 0.63	3.112	2.348	75.50	0.831	26.70	0.032	1.04
5) dIle:Lys ratio 0.69	3.137	2.385	75.91	0.861	27.56	0.030	0.95
6) dIle:Lys ratio 0.76	3.161	2.450	75.84	0.852	26.98	0.033	1.03
7) dIle:Lys ratio 0.83	3.162	2.391	75.56	0.852	26.93	0.030	0.94
8) Positive Control	3.160	2.392	75.70	0.873	27.57	0.026	0.81
SEM[3]	0.037	0.026	0.29	0.017	0.46	0.002	0.05
	Probabilities						
Regression Analysis							
Linear	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.35
Quadratic	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.005	0.52
Pre-planned Orthogonal Contrast							
PC vs. Trt 5	0.64	0.83	0.61	0.60	0.98	0.07	0.07
	Coefficient of Determination						
R^2 Linear	0.635	0.684	0.206	0.685	0.404	0.136	0.002
R^2 Quadratic	0.716	0.771	0.237	0.789	0.494	0.153	0.012

[1] Values are least-square means of 8 replicate pens, with 14 birds being selected and processed at d 42.

[2] Treatments 1 to 7 are represented by analyzed digestible Ile to Lys ratios.

[3] Pooled standard error

Table 3.9. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 2.6 to 3.9 kg BW[1].

Dietary Treatments[2]	BW	BWG[3]	FI[4]	Dig Ile Intake[5]	FCR[6]	Mortality
	(kg)	(kg)	(kg)	(mg/day)	(kg/kg)	(%)
1) dIle:Lys ratio 0.45	3.621	0.988	2.177	693	2.214	2.55
2) dIle:Lys ratio 0.53	3.744	1.068	2.324	777	2.192	1.15
3) dIle:Lys ratio 0.56	3.850	1.219	2.422	923	1.988	6.25
4) dIle:Lys ratio 0.60	3.882	1.221	2.408	971	1.979	2.20
5) dIle:Lys ratio 0.66	3.921	1.268	2.406	1,051	1.902	4.80
6) dIle:Lys ratio 0.70	3.931	1.311	2.393	1,136	1.832	3.85
7) dIle:Lys ratio 0.72	3.980	1.308	2.426	1,215	1.857	1.40
8) dIle:Lys ratio 0.74	3.900	1.222	2.232	1,119	1.831	1.50
9) Positive Control	3.892	1.261	2.370	1,150	1.882	4.25
SEM[7]	0.034	0.028	0.030	12.15	0.044	0.045
				Probabilities		
Regression Analysis						
Linear	0.001	0.001	0.15	0.001	0.001	0.60
Quadratic	0.001	0.001	0.001	0.001	0.001	0.21
Pre-planned orthogonal contrast						
PC vs. Trt 5	0.54	0.85	0.40	0.001	0.75	0.82
				Coefficient of Determination		
R^2 Linear	0.429	0.426	0.033	0.864	0.503	0.004
R^2 Quadratic	0.574	0.629	0.459	0.928	0.523	0.051

[1] Values represent least-square means for 8 replicate pens with 30 chicks per pen at 1 d of age.

[2] Treatments 1 to 8 are represented by analyzed digestible Ile to Lys ratios.

[3] Body weight gain

[4] Feed intake

[5] Determined using digestible Ile concentration of each treatment

[6] Feed conversion ratio corrected for mortality

[7] Pooled standard error

Table 3.10. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 2.6 to 3.9 kg BW[1].

Response Parameter	Live Weight, kg	Carcass Weight, kg	Carcass Yield, %	Breast Meat Weight, kg	Breast Meat Yield, %	Abdominal Fat Weight, kg	Abdominal Fat Percentage, %
Dietary Treatments[2]							
1) dIle:Lys ratio 0.45	3.678	2.801	76.28	0.987	26.82	0.034	0.92
2) dIle:Lys ratio 0.53	3.777	2.879	76.25	1.017	26.95	0.037	0.97
3) dIle:Lys ratio 0.56	3.937	3.014	76.53	1.075	27.28	0.035	0.88
4) dIle:Lys ratio 0.60	3.950	3.008	76.15	1.076	27.24	0.035	0.89
5) dIle:Lys ratio 0.66	3.967	3.031	76.37	1.086	27.39	0.033	0.82
6) dIle:Lys ratio 0.70	4.026	3.085	76.71	1.122	27.81	0.032	0.81
7) dIle:Lys ratio 0.72	4.045	3.101	76.84	1.120	27.72	0.034	0.85
8) dIle:Lys ratio 0.74	3.991	3.050	76.46	1.108	27.77	0.036	0.90
9) Positive Control	3.970	3.065	77.29	1.113	28.10	0.033	0.82
SEM[3]	0.038	0.030	0.27	0.014	0.22	0.002	0.05
				Probabilities			
Regression Analysis							
Linear	< 0.001	< 0.001	0.12	< 0.001	< 0.001	0.85	0.12
Quadratic	< 0.001	< 0.001	0.30	< 0.001	< 0.001	0.82	0.12
Pre-planned orthogonal contrast							
PC vs. Trt 5	0.95	0.42	0.016	0.13	0.017	0.98	0.99
				Coefficient of Determination			
R^2 Linear	0.447	0.447	0.037	0.507	0.283	0.001	0.039
R^2 Quadratic	0.567	0.560	0.039	0.584	0.288	0.007	0.066

[1] Values are least-square means of 8 replicate pens, with 14 birds being selected and processed at d 50.

[2] Treatments 1 to 8 are represented by analyzed digestible Ile to Lys ratios.

[3] Pooled standard error

IV. CONCLUSIONS

For Exp 1, 2, and 3, optimum dig Ile to Lys ratios for growth performance were determined to range from 0.64 to 0.66, 0.66 to 0.67, and 0.63 to 0.69, respectively. These estimated optimum ratios are in agreement with previously reported optimum dig Ile ratios of 0.65, 0.67 and 0.69 for each of the experimental periods tested. However, consideration must be given to dig Lys concentrations utilized in experimental diets as this will have an effect on the dig Ile need. Additionally, daily intakes of dig Ile needed to obtain optimum responses are higher in the current research than for previously reported studies. This supports the concept that modern genetic strains of broilers have greater amino acid needs to support accelerated growth and lean tissue accretion. Broilers fed progressive additions of dig Ile displayed optimum dig Ile to Lys ratios ranging from 0.68 to 0.73 for BMY from 1.0 to 3.9 kg BW, which are higher than estimates from previous research of 0.67 to 0.70. However, the literature base is relatively sparse concerning optimum dig Ile ratios for BMY in these weight ranges of broilers. This increase may be due to an increased genetic potential of breast tissue growth for late-developing broiler strains. These data provide further evidence that optimum dig Ile to Lys ratios are higher for breast tissue accretion than for BWG and FCR. In each Exp, the optimum ratios determined for BMY were 3 to 5 ratio points higher than for growth parameters. This response was observed regardless of broiler age.

These results were obtained utilizing linear broken-line regression analysis. In Exp 1 and 2, quadratic broken-line models also significantly fit these data sets; however, the linear broken-line model produced less variation in the estimates. For Exp 3, the quadratic broken-line model was unable to achieve an acceptable fit to these data. This may have occurred because there were inadequate data points beyond the break point of the model for the rate constant of the curve to be minimized. This is likely due to the dig Ile ratios being analyzed below the formulated values, resulting in fewer points beyond the plateau. It is also possible that these responses did not have an adequate plateau for the quadratic curve to best fit these data. This should be given consideration in the design of future Exp, as dose response treatment designs with Ile and other similarly limiting amino acids have produced curvilinear data in the past, which were fit with reliable quadratic broken-line models.

For future research, there is a need to utilize the results herein to develop dietary treatments for a phase feeding schedule for broilers grown to 50 d of age. The ranges evaluated in the present experiments represent grower phases 1 and 2 as well as the finisher phase in a 7-week production period. Results observed from the current research can be used to improve dietary feeding programs used by the broiler industry. Additionally, these data provide valuable insight into the dig Ile ratios necessary to optimize breast tissue accretion. The information gained from this research can help the poultry industry provide an efficient and affordable protein source to consumers.