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# Effects of Supplementing Crystalline L-Valine and L-Isoleucine and a Novel Threonine Biomass in Reduced Crude Protein Diets fed to Broilers

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# Effects of Supplementing Crystalline L-Valine and L-Isoleucine and a Novel Threonine Biomass in Reduced Crude Protein Diets fed to Broilers

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Poultry Science

by

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This thesis is approved for recommendation to the Graduate Council.

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# ABSTRACT

Poultry nutritionists continue to identify methods to lower feed price without compromising broiler growth performance, processing yields, or animal well-being. A common approach is to reduce dietary crude protein (**CP**), the second most expensive component in a broiler diet. Subsequently, reducing CP will reduce the inclusion levels of intact protein sources (e.g., soybean meal [**SBM]**), which can be accomplished by supplementing individual amino acids (**AA**). This methodology not only reduces diet cost, pending if feed-grade AA are more economical, but can contribute to improving broiler health and environmental sustainability. Therefore, two experiments were conducted to evaluate individual feed-grade AA in commercial reduced CP diets fed to broilers from 0 to 48 d. In experiment 1, L-Val and L-Ile were supplemented in addition to L-Met, L-Lys, and L-Thr to further reduce dietary CP in corn and SBM-based diets. Peanut meal or animal protein blend was used to replace partial amounts of SBM. Results confirmed that broilers maintained performance when fed reduced CP diets, independent of diet composition. Furthermore, broilers fed L-Val and L-Ile had increased breast meat yield and lower nitrogen excretion. The partial replacement of SBM with animal protein blend alleviated footpad dermatitis, whereas feed-grade L-Val and L-Ile did not. In experiment 2, L-Val, L-Ile, and L-Arg were supplemented after the additions of L-Met, L-Lys, and L-Thr to further reduce dietary CP. One experimental diet was devoid of supplemental Thr to demonstrate the necessity of maintaining digestible Thr levels. For two experimental diets, a novel, alternative Thr biomass was used in place of a traditional crystalline L-Thr as a Thr source in reduced CP diets. The Thr biomass not only supplied Thr but energy and other nutrients such as essential and nonessential AA. The Thr biomass was fed in two dietary treatments but formulated differently: in one diet, the biomass was formulated only on the Thr and energy

contributions while the other diet considered the full nutrient matrix. Results confirmed that broilers maintained performance when fed reduced CP diets and that broilers need a minimum dietary Thr. The Thr biomass was efficacious in replacing crystalline L-Thr.

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Most importantly, to my wife, Katie Lee; thank you for taking this journey with me. You are my rock, best friend, and biggest supporter. You believe in me when I am not able to at times. I am excited to see what the future holds.

# DEDICATION

I dedicate my thesis to my dad, Keith Lee. Thank you for always believing in, supporting, and challenging me. Love you, Dad.

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# CHAPTER I: INTRODUCTION

Approximately 60 to 70% of the cost of live poultry production is associated with feed. Protein is considered the second most expensive component in broiler diets after energy. Traditionally, dietary protein was quantified and qualified by the percentage of crude protein (**CP**) in the diet, but CP only accounts for the amount of nitrogen (**N**) and not necessarily the quality of available proteins. Researchers have recognized that broilers do not require CP per se but a balance, or an ideal profile, of digestible amino acids (**AA**). Therefore, reducing the amount of intact protein (e.g., soybean meal [**SBM]**) while meeting individual AA requirements can create potential cost savings. Reducing dietary CP and intact protein inclusions not only reduces diet cost but reduces the amount of excess N and phosphorus excreted, which has a significant impact on bird health as well as environmental sustainability.

Amino acids are known as the "building blocks" that make up the polypeptide chain of proteins. In nature, there are over 300 AA but only 20 serve as the standard "building blocks" for protein synthesis (Wu, 2009), with each having a specific aminoacyl-tRNA synthetase in the body. These 20 AA are categorized as either dietary essential (indispensable) or non-essential (dispensable) AA. Essential AA (**EAA**), which are Met, Lys, Thr, Val, Ile, Arg, Trp, Phe, His, and Leu for chickens, are defined as those that cannot be synthesized or are inadequately synthesized *de novo* to meet metabolic requirements and must be obtained via dietary intake. Non-essential AA (**NEAA**), which are Ala, Asp, Glu, Ser, Tyr, Cys, Gln, and Asn for chickens, are those that can be synthesized *de novo* in adequate amounts to meet metabolic requirements. A sub-category of AA are considered conditionally EAA, such as Gly, that may be needed by young but not older broilers to maximize performance; other potential conditionally EAA (e.g., Cys, Pro, Glu, Gln, or Tau) may be required under special conditions such as injury, infection, heat or cold stress, etc (Dean et al., 2006; Wu, 2009, 2013a).

The reduction of CP and, in turn, the reduction of intact proteins will predominantly be guided by the amount of limiting EAA supplied by the diet. In the corn and soybean meal (**CSBM**) based diets typically used in the United States, the first three limiting AA are Met, Lys, and Thr. Their feed-grade forms have been commercially adopted into formulation due to their ability to replace intact proteins while maintaining performance (Kidd et al., 2013). Currently, sources of other feed-grade AA beyond Met, Lys, and Thr such as Val and Ile are becoming economically feasible to meet these essential AA minimums, which can subsequently reduce the amount of intact proteins in commercial diets. Both Val and Ile are considered the 4th or 5th limiting AA in traditional broiler diets and have significant roles in the body. The additions of feed-grade Val and Ile can facilitate additional reductions of CP; however, the supplementation of these AA needs to be further assessed in reduced CP diets when fed to broilers from placement until market age. In addition, there is a lack of research comparing broiler performance when fed CSBM diets with or without the use of alternative proteins.

The use of alternative protein ingredients such as peanut meal and animal protein meal to partially replace SBM can be cost-effective and provide other nutritional benefits. However, the replacement of SBM with alternative proteins can alter the EAA and NEAA profile of the diet and may influence the success of CP reduction potential. In several reports, young broilers fed reduced CP diets have experienced a reduction in live performance, with Gly cited as a limiting factor (Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Ospina-Rojas et al., 2014a). Therefore, the use of alternative ingredients having higher Gly levels than SBM may be beneficial in reduced CP, CSBM-based diets.

The majority of feed-grade AA are produced via bacterial fermentation (D'Este et al., 2018). During this process, selected bacteria are placed in a controlled environment and given nutrients to promote fermentation and thus the creation of a fermented biomass. The biomass is then purified to extract the desired AA in the crystalline form and the remaining (e.g., cell mass and culture broth) is typically considered waste (Hermann, 2003). However, this biomass contains additional AA, energy, and potentially bioactive molecules that can be utilized by the bird. Therefore, feeding the complete biomass without the AA purification step could provide to broilers additional nutrients to facilitate a greater reduction of other intact proteins while improving diet and manufacturing cost.

Threonine, the 3rd limiting AA, is an essential component in the body and must be in the diet as birds are unable to synthesize Thr *de novo*. The predominant supplemental form of L-Thr is the purified crystalline; however, a Thr biomass is available. It is therefore important to confirm that Thr, the key nutrient, in the biomass is as efficacious as crystalline L-Thr in supporting broiler performance and yield. This is even more important in reduced protein diets in which a higher proportion of the bird's Thr requirement is met by supplementation rather than intact proteins. As such, the objectives of this thesis are as follows:

1. The first experiment evaluates three main areas: 1) assess the effects of feed-grade L-Val and L-Ile in reduced dietary CP, CSBM-based diets on broiler performance, 2) evaluate performance and possible improvements in the severity of pododermatitis given the partial replacement of SBM with alternative proteins, and 3) determine if feeding broilers reduced CP diets can lower N excretion.
2. The second experiment evaluates three main components: 1) assess the effects of feed-grade L-Val, L-Ile, and L-Arg in reduced dietary CP, CSBM-based diets on

broiler performance, 2) evaluate the effects of reducing dietary Thr by removing supplemental Thr in the diet, and 3) determine if a novel Thr biomass product can effectively replace crystalline L-Thr.

# CHAPTER II: LITERATURE REVIEW

## IDEAL PROTEIN CONCEPT

Over 50 years ago, Mitchell (1964) and Dean and Scott (1965) proposed the ideal protein concept (**IPC**). The goal of IPC is to ideally meet the bird’s AA requirements for maintenance and protein accretion, as well as maximize growth performance without having any limiting or excess AA that may be used instead for energy, fat deposition, or excreted as waste (Emmert and Baker, 1997). The concept has been practiced in swine (ARC, 1981; Chung and Baker, 1992), then in broiler chicks (Baker and Han, 1994; Emmert and Baker, 1997), and is now widely used in other agricultural animals. This can be accomplished by formulating AA as a ratio to a reference AA such as Lys. This allows essential AA balanced in the formulation to be co-limiting for performance so that the AA supply exactly meets AA requirements (Milgen and Dourmad, 2015). Additionally, IPC can be used to reduce the environmental impact by lowering N excretion of the animal.

As reviewed by Baker and Han (1994), the rationale for formulating an AA as a ratio to Lys is that factors such as diet (e.g., protein and energy levels), environment (e.g., disease and heat stress), and genetics (e.g., sex and genetic traits) may affect AA requirements, but the ideal ratios of AA to Lys should remain generally similar. However, the period of growth and intended criterion (e.g., body weight gain [**BWG**], FCR, or meat yield) of the animal may alter the ratio (Baker, 2009). Lysine was adopted as the reference AA because it is relatively simple to analyze, deficient in many feed ingredients (thus a multitude of available data exists for Lys needs), primarily used for body protein accretion, defined maintenance requirement, and has a lack of precursor roles or interactions with other AA to be utilized (Baker and Han, 1994; Emmert and Baker, 1997; Mack et al., 1999). Additionally, Lys is the first limiting AA in swine, whereas it is second for poultry (Han et al., 1992) when fed a CSBM diet. Methionine, the first limiting AA in poultry, is not used as a reference AA due to being a precursor to Cys, its participation as a methyl donor, and being more complicated to analyze than Lys. Lastly, from an industry perspective, IPC simplifies a nutritionist's job by not requiring knowledge of specific quantities of each AA but the change in the digestible Lys requirement over time and knowing that all other AA ratios will change proportionally (Milgen and Dourmad, 2015).

## FORMULATING TO DIGESTIBLE AMINO ACIDS

Traditional formulations using total analyzed AA for CSBM diets appear to be successful for broiler performance (Parsons, 2020). However, corn and SBM, unlike other ingredients, have high AA digestibility (the ability to chemically and enzymatically breakdown proteins to smaller peptides and free AA that are suitable for absorption). Furthermore, these conventional diets have higher levels of dietary CP to provide sufficient amounts of essential AA for performance (Parsons, 2020). Using ingredients other than SBM may appear to have a balanced total AA composition but can be misleading since proteins in alternative feed ingredients are often less digestible, leading to excretion of undigested AA into the environment (Wu, 2009).

Formulation based on the bioavailability (the ingested AA that is absorbed and utilized in the body) of an AA is the best option, but is difficult to measure. A few methods to best estimate the AA digestibility (the movement of AA from the intestinal lumen into the mucosa) include total fecal collection, cecectomized rooster, and ileal collection assay (Lemme et al., 2004). Total fecal collection is not recommended, as the majority of AA are absorbed rapidly in the duodenum and jejunum but slower in the distal end of the ileum (Wu, 2013b). The remaining AA that continue into the colon and cecum are not utilized by the bird, and the hindgut (colon and cecum) microflora are able to metabolize undigested AA (Kan, 1975). Parsons et al. (1982)

estimated that 25% of the AA in the feces are from microbial origin. To mitigate the microbial influence, cecectomized roosters or collection of prececal digesta from the ileum can be used (Parsons et al., 1982). However, an additional concern is the correction for endogenous losses (e.g., sloughed epithelial cells, enzymes, mucin proteins, bile, and albumin) that are components of the digesta. Ignoring these endogenous loses will potentially underestimate the AA digestibility. These loses can be accounted for and methods to do so have been described elsewhere (Lemme et al., 2004; Adedokun et al., 2011; Adeola et al., 2016).

It is clear that formulating AA to their digestible vs. total content more closely meets the bird's AA needs (Rostagno et al., 1995). Currently, there is still debate on the exact method to estimate the AA digestibility of feed ingredients, but advancements in research and technology are improving these estimates. Better estimations are paramount as the industry implements more reduced protein diets with inclusions of various protein sources or use of novel supplemental AA. Indeed, not only can this improve diet cost but contribute to improving bird welfare and environmental sustainability.

## ADVANTAGES OF REDUCED CRUDE PROTEIN DIETS

In addition to reducing diet cost, other benefits of feeding reduced CP diets to broilers include improving broiler welfare and environmental sustainability. It has been documented that higher intake of dietary CP or intact proteins will increase N excretions as well as water consumption, and therefore, increase litter moisture (Alleman and Leclercq, 1997; Ferguson et al., 1998a; Shepherd and Fairchild, 2010). Broiler paws, hocks, and breasts are regularly in contact with the litter, and the combination of high litter moisture and the irritation effect of ammonia from the urea in litter can induce burns or skin dermatitis. Consequently, these conditions can depress live performance as well as negatively impact animal welfare and processing characteristics (Haslam et al., 2007; Shepherd and Fairchild, 2010; de Jong et al., 2014). This commonly occurs on the skin of broiler feet and is termed pododermatitis or footpad dermatitis (**FPD**). Shepherd and Fairchild (2010) extensively reviewed the literature and found high correlations between the occurrence of FPD and high litter moisture conditions.

The consumption of SBM has been credited for high water excretion due to its fiber, fermentable sugar, and potassium content (Francesch and Brufau, 2004). Nagaraj et al. (2007) found that partially replacing SBM with poultry by-product meal and reducing CP improved FPD, with no significant association found between wet litter conditions and FPD development. This indicates that SBM has a considerable impact on FPD. Furthermore, high amounts of dietary SBM have been suggested to cause “sticky” excreta due to the soluble nonstarch polysaccharide and oligosaccharide content of SBM, which increases digesta viscosity that more readily adheres fecal and litter microbes to poultry paws (Jensen et al., 1970; Choct et al., 2010). This increase in digesta viscosity not only slows the digesta passage but creates low oxygen tension in the small intestine, thus providing a stable environment for undesirable microflora to establish; inversely, nutrient (fat, starch, and protein) digestion and absorption are reduced (Choct et al., 2010).

In poultry excreta (urine and feces), uric acid and undigested proteins comprise 70 and 30% of the total fecal N, respectively (Koerkamp, 1994). Broilers appear to average 60.2% N retention, which is more than turkeys (56.8%) and laying hens (45.6%) (Powers and Angel, 2008). Once digesta is excreted, microbial enzymes in the litter can degrade uric acid into urea to produce ammonia ($NH_3$) and carbon dioxide ($CO_2$) as a byproduct (Koerkamp, 1994; Naseem and King, 2018). Formation of these compounds depends on the litter pH (around 8.5), water and oxygen content, microbial enzyme activity (urease), temperature, humidity, and urea from uric acid (Nahm, 2003; Naseem and King, 2018). It is estimated that 50 to 80% of N in excreta is converted to ammonia (Ritz et al., 2004). Broilers experiencing ammonia levels at 25 ppm or greater have been shown to experience snickering, tracheal irritation, air sac inflammation, conjunctivitis, dyspnea, eye damage, and possibly reduced broiler performance (Ritz et al., 2004).

Ammonia, once emitted, can rapidly react with acidic compounds found in the atmosphere, such as nitric acid and sulfuric acid, and can be deposited back in the soil in a matter of days (Ritz et al., 2004). Excess environmental N can negatively affect plant diversity, soil leaching, plant growth, and can increase plant disease susceptibility (Bobbink et al., 2010). Nitrate contamination in groundwater has been linked to methemoglobinemia, toxicities in livestock and aquatic species, and excess growth of algal blooms (Chalova et al., 2016). Additionally, ammonia can cause acid rain that can lead to forest diebacks (Nahm, 2003).

In recent years, the German Federal Ministry of Food and Agriculture has set an ordinance to limit the amount of total N excretion per broiler to 385 to 413 g/farm/year (Lemme et al., 2019). In coming years, pressure will increase from government agencies as well as the public to regulate, control, and reduce N excretion. As previously mentioned, formulating diets with digestible AA, applying the IPC, and supplementing individual AA to replace intact protein sources and reduce dietary CP are acceptable approaches to managing N excretion. Ferguson et al. (1998b) fed diets supplemented with Met, Lys, Thr, and Trp to broilers from 0 to 42 d and found that a percentage point reduction in dietary CP led to a 7% reduction in litter N. Others have similarly suggested that reducing CP by 1 percentage unit will reduce N excretion ranging from 4.8 to 7.9% (Bregendahl et al., 2002; Si et al., 2004; Hernandez et al., 2012; Kriseldi et al., 2018).

# THREONINE

Methionine and Lys sources are routinely supplemented in broiler diets and were commercially adopted in the 1950s and 1970s, respectively (Kidd et al., 2013). Currently, L-Thr is recognized as the last widespread, commercially adopted (1990s) AA in broiler diets (Kidd et al., 2013), though adoption of L-Val is increasing rapidly. Threonine ($C_4H_9NO_3$) is generally considered to be the 3rd limiting AA in reduced CP, CSBM-based diets (Han et al., 1992; Fernandez et al., 1994), due to ingredients such as corn, wheat, sorghum, barley, and peanut meal being low in Thr (NRC, 1994). Hegsted (1944) showed that chicks needed dietary Thr to maintain performance, while Kidd et al. (2002) confirmed that supplemental L-Thr was able to replace Thr from intact protein sources. Work by various authors have found broilers fed diets with low to deficient amounts of Thr had reduced live performance, carcass yield, and breast yield (Çiftci and Ceylan, 2004; Mejia et al., 2012; Ospina-Rojas et al., 2014b).

W. C. Rose identified the last discovered EAA, Thr (McCoy et al., 1935). Threonine is a glucogenic AA that can be catabolized to pyruvate, acetyl-CoA, propionyl-CoA, or succinyl-CoA to yield energy or be used in the synthesis of Ser, glucose, Gly, or feather and muscle protein (Fisher et al., 1981; Kidd and Kerr, 1996; Stilborn et al., 2010). Threonine serves as a precursor for Gly, which is an essential component for the formation of uric acid (Baker et al., 1972). Therefore, a dietary excess of N can increase Thr requirements if the quantity of Gly is low (Corzo et al., 2009), but not when Gly is present in adequate amounts (Hilliar et al., 2019). The majority (60 to 80%) of dietary Thr is utilized by the portal-drained viscera (stomach, intestines, pancreases, and spleen), but primarily by the small intestine for mucus production (Schaart et al., 2005; Law et al., 2007). Mucus is made up of mucin glycoproteins that are synthesized by goblet cells, and 26% of the mucus protein is made up of Thr (Mantle and Allen, 1981). Glycoproteins serve primarily as a protective barrier but also assist with nutrient transport and host enzymes and receptors (Strous and Dekker, 1992). It has been shown that increasing dietary Thr can increase the quantity of goblet cells as well as improve digestion by increasing the villus height to crypt depth ratio (Abbasi et al., 2014). However, mucus Thr is not digested and lost in the excreta or fermented by hindgut microbes, deeming it indigestible (Siriwan et al., 1993; Bortoluzzi et al., 2018). Additionally, Thr is an integral component of immunoglobulins (Bortoluzzi et al., 2018). As such, dietary Thr needs may change when broilers experience gut health challenges associated with environmental challenges (Kidd et al., 2003). Corzo et al. (2007b) found that the digestible Thr requirement of broilers (21 to 42 d) was 0.63 to 0.66% when reared on new litter versus 0.65 to 0.70% when reared on used litter.

Currently, Aviagen (2019) recommends a digestible Thr to Lys ratio of 67 for each growth phase, whereas Cobb-Vantress (2018) recommends a ratio of 68 for the starter phase (0 to 8 d) and a ratio of 65 for the remaining phases. When estimating requirements, it has been suggested that there is an interaction between Thr and CP levels though controversial results have been reported (Kidd et al., 2001; Abbasi et al., 2014; Sigolo et al., 2017). In review of recent digestible Thr to Lys ratio estimates, Dozier et al. (2015) found male Hubbard × Cobb 500 broilers had optimal digestible Thr to Lys ratios for body weight gain (**BWG**) and feed conversion ratio (**FCR**) from 0 to 14 d of 66 and 69 or 74 and 73 for linear broken-line or quadratic-line model, respectively. Ospina-Rojas et al. (2014b) evaluated male Cobb 500 broilers from 22 to 42 d fed a digestible Thr to Lys ratio ranging from 57 to 67.5 and found that the ratio of 57 was sufficient to maximize performance. Mejia et al. (2012) evaluated male Ross 708 broilers fed a digestible Thr to Lys ratios ranging from 35 to 74 and the ratio of 50 in broiler diets was able to maintain BWG, feed intake (**FI**), and FCR. However, when the ratio was reduced to 43, BWG and FCR, but not processing measurements, were significantly impacted. Mejia et al. (2012) further reduced the ratio from 43 to 35 and was able to elicit a reduction in yield for breast meat.

It was previously suggested that the Thr requirement for Ross broilers was lowest for weight gain, moderate for breast meat, and highest for FCR (Kidd, 2000; Mack et al., 1999). Çiftci and Ceylan (2004) reduced dietary Thr in diets of Ross PM3 broilers (male and female) from 0 to 42 d, and a 15% reduction of Thr only impacted FCR, but a 23% reduction of Thr elicited responses in FI, BWG, and breast weight. In the study by Ospina-Rojas et al. (2014b), a linear response was detected for FCR but not for BWG or breast yield when Cobb 500 male broilers were fed digestible Thr to Lys ratios reduced from 67.5 to 57. In another study, Ross 308 broilers were provided Thr levels at 100, 110, 120, and 130% of Ross recommendations from 0 to 42 d and average daily FI, average daily gain, and gain to feed suffered linearly by increasing Thr levels above recommendation (Sigolo et al., 2017). In the same trial, increasing Thr levels did not improve breast or other parts yield. Under heat stress, the Thr requirement for FCR is greater than breast development in male Ross broilers (Dozier et al., 2000). Additionally, Ross males have higher Thr requirements than females for breast yield (Dozier et al., 2001).

## VALINE AND ISOLEUCINE

The supplementation of Met, Lys, and Thr will make the next limiting EAA a pressure point for the amount of intact protein pulled into the diet with least-cost formulation. Depending on ingredient composition, Val is considered 4th limiting while Ile is 5th limiting in broiler diets consisting of CSBM (Kidd and Hackenhaar, 2006). However, inclusion of an animal protein meal can alter this ratio to cause Ile to become 4th limiting or co-limiting with Val (Kidd and Hackenhaar, 2006; Dozier et al., 2011). Valine, Ile, and Leu are referred to as the branched chain

AA (**BCAA**) as these three AA are catabolized by the same enzymes (excluding the α-ketoisocaproate dioxygenase enzyme for Leu). The first catabolic step is carried out by BCAA aminotransferase (**BCAT**), while the second is carried out by the branched chain α-keto acid dehydrogenase (**BCKD**) (Harper et al., 1984; Duan et al., 2016). Unlike Thr, which has been extensively reviewed in poultry (Kidd and Kerr, 1996; Kidd, 2000; Qaisrani et al., 2018), a comparable in-depth review of the BCAA in poultry has not currently been published.

Valine ($C_5H_{11}NO_2$) was initially discovered by von Gorup-Besanez in 1856 and Ile ($C_6H_{13}NO_2$) by Felix Ehrich in 1903 (Vickery and Schmidt, 1931). Valine is glycogenic (succinyl-CoA), Leu is ketogenic (acetyl-CoA), and Ile is both glycogenic and ketogenic (D’Mello, 2003). All BCAA are EAA for poultry and therefore must be supplied by the diet. Leucine is the least likely to be limited in practical CSBM diets due to having a high content in both corn and SBM; therefore, Leu does not need to be supplemented in practical diets (NRC, 1994; Kidd et al., 2013). Valine has been commercially available since 2008 (Kidd et al., 2013), and Ile is expected to become economically feasible in broiler diets in the near future. A diet deficient in Val causes feather and leg abnormalities, reduced broiler performance, and higher mortality when compared to decreasing the three BCAA simultaneously (Farran and Thomas, 1992a; b). Creating an Ile deficient diet caused a negative response in broiler BWG and FCR, but a consistent effect on carcass characteristics was not achieved (Kidd et al., 2004; Corzo et al., 2008a).

It is worth mentioning that the BCAA have interactions among themselves that can cause antagonisms. Most notably, the antagonistic relationship is most prominent for Val-Leu and Ile-Leu (D’Mello and Lewis, 1970; Burnham et al., 1992; D’Mello, 2003) and to a lesser extent for Val-Ile (Berres et al., 2010; Dozier et al., 2011, 2012). Excess or limitations of one or more of these BCAA have been shown to cause negative effects, the most prominent when Leu is fed in excess of Val and Ile (D'Mello and Lewis, 1970). Metabolically, a plausible reason for the antagonistic relationship is the common catabolism pathways. An excess of any one of the BCAA would cause transamination (**BCAT**) and possibly oxidation (**BCKD**) of all three BCAA. (Smith and Austic, 1978; Calvert et al., 1982; Harper et al., 1984; D'Mello, 2003). For example, excess dietary Leu leads to greater Val and Ile catabolism, thus decreasing the plasma concentration of these AA (Smith and Austic, 1978). Harper et al. (1984) states that only the Leu-induced antagonism can be demonstrated without careful manipulation of dietary AA composition.

The metabolism and use of BCAA are unique among AA. Unlike other AA, BCAA are poorly metabolized during the first pass through the liver as the liver expresses low levels of BCAT (Harper et al., 1984; Brosnan and Brosnan, 2006). Therefore, BCAA are transported to and oxidized in skeletal muscle and other peripheral tissues (Harper et al., 1984; Herman et al., 2010; Zhang et al., 2017). The BCAA comprise about 35% of essential AA in muscle tissue (Harper et al., 1984). In addition to muscle protein regulation and providing energy when necessary, BCAA are involved in brain function, immunity, insulin secretion, glucose transport, gut function, and lipid metabolism regulation (Fernstrom, 2005; Li et al., 2007; Bai et al., 2015; Zhang et al., 2017).

The BCAA are widely known to participate in the mammalian target of the rapamycin (**mTOR**) signaling pathway, which is the major nutrient-sensitive regulator of cell growth and proliferation (Sabatini, 2017). The BCAA and particularly Leu can stimulate protein synthesis (Atherton et al., 2010) and prevent proteolysis (Nakashima et al., 2005). However, it was recently demonstrated in poultry that providing excess dietary Leu did not improve growth performance or processing characteristics even with (Zeitz et al., 2019b) or without (Zeitz et al., 2019a) adjusting Val and Ile ratios. Ospina-Rojas et al. (2019) found that increasing standardized ileal digestible Leu levels from 1.00 to 1.91% stimulated the expression of genes involved in the mTOR signaling pathway of the breast muscle tissue, but this response did not translate to positive results in broiler performance or meat yield. This is possibly due to the fact that all AA must be available for protein synthesis and adequate energy must be readily available (Ospina-Rojas et al., 2019).

Interestingly, the supplementation of Ile has increased breast meat yield in some studies (Kidd et al., 2000, 2004; Corzo et al., 2010; Dozier et al., 2012), however, this exact mechanism has not been defined in poultry. In swine, the supplementation of Val, Ile, and Leu up-regulated the appetite regulatory genes (Agrp and NPY) and the genes inhibiting FI (MC4R and CART) were down-regulated (Zheng et al., 2016). Furthermore, these authors suggest the increase in muscle growth may be due to the activation of translation initiation factors from the mTOR pathway. In a similar study in swine, Zheng et al. (2017) stated that the skeletal muscles catabolize BCAA for the synthesis of Ala, Glu, Gln, Asp, and Asn, and the supplementation of BCAA increased the muscle net-AA fluxes. This led to the elevation of muscle protein deposition. For poultry, further research is needed in this area.

Currently, Aviagen (2019) recommends a digestible Val to Lys ratio of 75 for the starter phase (0 to 10 d) and 76 for the remaining phases, whereas Cobb-Vantress (2018) recommends a ratio of 73 for the starter phase (0 to 8 d) and 75 for the remaining phases. In review of recent literature, the suggested optimal ratio of digistible Val to Lys for young broilers is 80 (0 to 12 d; male Cobb 500; Agostini et al., 2019), 77 (0 to 21 d; male Cobb 500; Ospina-Rojas et al., 2019), and 77 (8 to 21 d; Cobb 500; Tavernari et al., 2013). For moderately older broilers, ratios of 65

(10 to 20 d; male Ross 308; Pastor et al., 2013), 78 (8 to 21 d; male New Hampshire x Columbian Plymouth Rock; Baker et al., 2002), and 75 (0 to 28 d; male Cobb 500; Agostini et al., 2019) have been reported. For older broilers, ratios of 72 (25 to 35 d; male Ross 308; Pastor et al., 2013), 68 (21 to 42 d; male Ross 508; Thornton et al., 2006), 78 (21 to 42 d; Ross 708; Corzo et al., 2007a), 74 (26 to 42 d; male Ross 708; Dozier et al., 2012), 76 (30 to 43 d; Cobb 500; Tavernari et al., 2013), and 78 (0 to 35 and 0 to 42 d; male Cobb 500; Agostini et al., 2019) have been reported.

For the digestible Ile to Lys ratio, Aviagen (2019) recommends an increasing ratio of 67, 68, and 69 for the starter (0 to 10 d), grower (11 to 24 d), and finisher (25 d to market age) phases, respectively, whereas Cobb-Vantress (2018) recommends an increasing ratio of 63, 64, 65, and 66 for the starter (0 to 8 d), grower (9 to 18 d), finisher 1 (19 to 28 d), and finisher 2 (29 d to market age) phases, respectively. Compared with Val, Ile dietary requirements have not been as extensively evaluated. Recent literature suggests that the optimal ratio of digistible Ile to Lys for young broilers is 66 (7 to 21 d; male Cobb 500; Tavernari et al., 2012) and 61 (8 to 21 d; male New Hampshire x Columbian Plymouth Rock; Baker et al., 2002). The suggested optimal ratio for older broilers is 72 (22 to 42 d; male Cobb 500; Duarte et al., 2015), 69 (28 to 40 d; male Cobb 500;Campos et al., 2012), greater than 67 (28 to 42 d; male Ross 708; Dozier et al., 2012), 68 (30 to 42 d; female Ross 708, Hale et al., 2004), and 68 (30 to 43 d; male Cobb 500; Tavernari et al., 2012). It is important to note that the digistible AA to Lys ratios will change depending on, but not limited to, response criteria, growth period, and analytical methods.

Corzo et al. (2010) suggests that broiler live performance is more sensitive to Val, while breast meat yield is maximized with Ile supplementation. Other studies have also shown that Val requirements are higher for growth (Corzo et al., 2008b; Dozier et al., 2012; Tavernari et al., 2013; Agostini et al., 2019) , while Ile requirements are higher for meat yield (Kidd et al., 2000, 2004; Corzo et al., 2010; Dozier et al., 2012). However, there are a few exceptions (Hale et al., 2004; Dozier et al., 2011; Tavernari et al., 2012; Duarte et al., 2015). The apparent ability of Ile to increase breast meat yield is interesting but still not completely understood (Kidd et al., 2000, 2004; Corzo et al., 2010; Dozier et al., 2012). Nonetheless, meeting optimal digestibility levels of both Val and Ile is needed to maximize growth and processing performance (Corzo et al., 2010; Dozier et al., 2011, 2012).

## AMINO ACID FERMENTATION AND PRODUCTION

It is clear that feeding feed-grade individual AA is an effective strategy for replacing intact proteins. The predominant production of these supplemental AA are by a microbial fermentation (Hashimoto, 2017; D’Este et al., 2018). Other AA production methods include extraction from protein hydrolysates, chemical synthesis, and enzymatic synthesis (Ivanov et al., 2013; D’Este et al., 2018). The advantages of attaining AA by fermentation are its simplicity, economics, high production capacity, and use of cheap materials (Ivanov et al., 2013). Most importantly, production via fermentation results in the nutritionally valuable L-isomer form of the AA, which are specifically needed for feed-grade Thr and Lys due to poultry’s lack of an aminotransferase to convert the D-isomer to L-isomer form of Thr and Lys (Huang, 1961; D’Mello, 2003). In agreement, research has found that feeding Thr in the DL-isomer form was found to be half as active as the L-isomer form of Thr (Grau, 1949).

There was strong demand for monosodium glutamate (**MSG**) beginning in 1908 as a food seasoner in Japan. Manufacturing MSG prior to the 1950s was by extraction or protein hydrolysis such as hydrolyzing wheat gluten or defatted soybean by hydrochloric acid (Ivanov et al., 2013; Hashimoto, 2017). However, these methods depended on limited raw materials and produced hydrogen chloride gas and large volume of waste (Ivanov et al., 2013; Hashimoto, 2017). In 1956, Dr. Shukuo Kinoshita with Kyowa Hakko Kogyo Co., Ltd. of Japan discovered a soil bacterium *Corynebacterium glutamicum* that produced glutamic acid (Kinoshita, 1959). Soon after the first symposium presentation on *C. glutamicum* fermentation in 1957, numerous researchers and companies devoted time and resources into fermentation research, which has been referred to as a “gold rush” (Hashimoto, 2017).

Today, the fermentation production of crystalline AA begins with using a bacterium largely of *Eschericha coli* or *C. glutamicum* (Debabov, 2003) and a culture medium containing carbon, N, sulfur, phosphorus, and trace elements (Leuchtenberger et al., 2005). After fermentation of the nutrients, a fermented biomass is created and then purified to extract the desired or intended AA (e.g., L-Thr) (Hermann, 2003). The biomass is often considered waste, but a large quantity of the residual biomass is rich in other EAA, NEAA, in addition to partial retention of the desired AA (Hermann, 2003; Almeida et al., 2014). Thus the biomass, prior to purifying, can be a valuable nutrient source while reducing manufacturing cost. Almeida et al. (2014) fed a Thr biomass (analyzed: 80% CP, 5.20% Lys, 5.10% Val, 4.52% Thr, 4.15% Ile, 1.06% Trp, and other AA) in place of fish meal to weanling pigs and found pigs fed the Thr biomass had greater apparent and standardized ileal AA digestibility except for Trp, Gly, and Pro. Dozier et al. (2003b) measured the relative bioavailability of a Thr by-product fermentation broth relative to crystalline L-Thr (98.5% digestible) in diets fed to broilers from 0 to 21 d and estimated it to be from 98.7 to 100.2% of L-Thr when based on either BW, FCR, or percentage of N retention. Wensley et al. (2019) evaluated supplementing a Thr biomass (75% digestible Thr) as a replacement to crystalline L-Thr (98.5% digestible) fed to broilers from 0 to 28 d, which resulted in similar performance. However, these authors formulated the Thr biomass solely on the digestible Thr and energy contribution and did not consider the full nutrient matrix of the biomass.

## ALTERNATIVE PROTEIN SOURCES

Many nutritionists, especially in the United States, predominantly use SBM as the main protein source in diets; it is considered the “gold standard” due to being well-balanced in digestible and readily available AA (Cromwell, 2012). However, SBM is one of the most expensive protein sources on the market (USDA; March 2019 and 2020; USDA Market News) making the inclusion of alternative proteins such as poultry (or by-product) meal, blood meal, feather meal, animal protein blends (e.g., meat, meat by-product, and meat and bone meal), corn gluten meal, sunflower meal, canola meal, peanut meal, and even dried distillers grains with solubles economically attractive to replace partial amounts of SBM. Despite the advantages, a number of studies have found large nutrient variability among alternative protein ingredients (Hendriks et al., 2002; Dozier et al., 2003a; Huang et al., 2005). The varying nutrient composition, particularly the AA content, resulting from the use of alternative protein ingredients will influence the AA profile and EAA limiting order of the diet (Kidd and Hackenhaar, 2006). Currently, there is lack of published comparisons of broilers fed traditional diets (CSBM) to broilers fed diets with the inclusion of an alternative protein source such as animal proteins or peanut meal to ensure optimum performance, especially as the industry advances towards greater reductions of dietary CP.

In the 1930-40s, broiler performance was vastly improved when animal proteins were added to all-vegetable diets; animal proteins were credited for supplying higher levels of calcium, phosphorus, B-complex vitamins, and Met and Lys than in common plant protein sources (Leeson and Summers, 2001). Additionally, animal proteins can provide greater quantities of Gly, a conditionally EAA in reduced CP, CSBM-based diets (NRC, 1994; Dean et al., 2006). Animal proteins can provide sufficient quantities of calcium and phosphorous to reduce or eliminate the supplementation of inorganic sources (e.g., defluorinated phosphate, tricalcium phosphate, or dicalcium phosphate) that are needed in all-vegetable diets (Sell and Jeffrey, 1996; Leeson and Summers, 2001). This is of tremendous importance given the growing fears of phosphorus reserves being potentially limited in 30 to 400 years (Rengel and Zhang, 2011; Cordell and White, 2013; Li et al., 2016). There are only a few recent studies that have compared broilers fed a CSBM diet with or without the inclusion of an animal protein where the inclusion of an animal protein negatively affected broiler performance (Nagaraj et al.,2007), whereas others have reported similar performance (Drewyor and Waldroup, 2000; Vieira and Lima, 2005; Caires et al., 2010) and processing characteristics (Caires et al., 2010) with the inclusion of animal proteins. As of today, there is still concern about disease transmission originating from animal ingredients (Beski et al., 2015), as well as the novelty to the consumer of broilers being fed all-vegetable diets.

In parts of the world, peanut (groundnut) meal is the standard protein source to which other ingredients are compared (Pesti et al., 2003; Toomer et al., 2020). Peanut meal is rich in Arg but low in Met, Lys, and Thr (Batal et al., 2005). Accordingly, researchers often use peanut meal to create Met, Lys, and Thr deficient basal diets in AA response studies (Kidd et al., 1999, 2000; Corzo et al., 2007a), but only a few studies have evaluated efficacy of peanut meal in replacing SBM for broilers. Costa et al. (2001) showed that broiler performance could be maintained when PM was fed up to 20% in CSBM diets when supplemented with Met, Lys, and Thr. Negatively, because peanuts are grown underground, they are susceptible to *Aspergillus* fungal growth that can produce aflatoxins. Other possible negative factors are the heat sensitive trypsin inhibitors and high oxidative potential if not stored in proper conditions (Ata, 2016).

In conclusion, SBM is a valuable protein ingredient for supplying well-balanced digestible AA. However, feeding SBM as the sole intact protein ingredient may not be economically feasible as some alternative protein ingredients are more cost-effective. These alternative ingredients may present challenges such as different AA profiles and digestibility, but studies have shown that alternative ingredients can replace partial amounts of SBM (and in some instances be the sole protein ingredient) and sustain broiler performance. Conversely, alternative ingredients can provide benefits that SBM cannot, such as higher amounts of Gly and phosphorous.

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# CHAPTER III:

# INFLUENCE OF BRANCHED CHAIN AMINO ACID INCLUSION IN DIETS VARYING IN INGREDIENT COMPOSITION ON BROILER PERFORMANCE, PROCESSING YIELDS, AND PODODERMATITIS AND LITTER CHARACTERISTICS

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Key words: valine, isoleucine, peanut meal, animal protein blend, footpad dermatitis, broiler

## ABSTRACT

The supplementation of L-Val and L-Ile is progressively becoming an economically feasible strategy to reduce the dietary CP content of broiler diets. In the current experiment, the influence of supplementing the feed-grade branched chain amino acids (**BCAA**) Val and Ile was evaluated in corn and soybean meal-based diets (**CSBM**), with or without the inclusion of either peanut meal (**PM**) or an animal protein blend (**APB**), on broiler growth performance (0 to 48 d), processing characteristics, and incidence of pododermatitis and litter characteristics. Supplementation of BCAA reduced analyzed CP by an average of 1.62 percentage units across four feeding phases. Overall (0 to 48 d) broiler body weight gain and FCR were unaffected when fed supplemental BCAA; however, feed intake was increased for those supplemented with BCAA. Overall growth performance was similar when including either PM or APB in CSBM diets. At processing, broilers fed BCAA diets had increased relative and absolute breast fillets and total white meat weights, as well as heavier fat pad weights. Nitrogen excretion was reduced when BCAA were supplemented, but no differences were observed for footpad dermatitis (**FPD**) lesion scores. Interestingly, APB inclusion did not impact litter moisture or nitrogen but improved FPD scores compared to birds fed CSBM, with PM-fed birds having intermediate FPD scores. In conclusion, these data indicate that BCAA inclusion can support CP reductions while maintaining growth performance, improving breast fillet yield, and reducing nitrogen excretion in broilers reared to market ages.

## INTRODUCTION

A continuing challenge for poultry nutritionists is to identify methods for lowering feed prices without compromising broiler growth performance, processing yields, or animal well-being. One strategy is to reduce dietary CP, which in addition to reducing diet cost, can lower nitrogen (**N**) excretion (Hernandez et al., 2012) and ammonia emissions (Ferguson et al., 1998a) and alleviate the incidence and severity of pododermatitis (i.e., footpad dermatitis [**FPD]**) (Lemme et al., 2019). Furthermore, broilers may have improved gut health when the amount of undigested protein available for microbial fermentation in the lower intestine is reduced (Gilbert et al., 2018).

A common approach to reduce dietary CP is to supplement individual amino acids (**AA**) to lower the amount of intact protein sources (e.g., soybean meal [**SBM**]) needed to meet essential AA requirements. Poultry nutritionists adopted supplementation of Met, Lys, and Thr, the first 3 limiting AA in corn and soybean meal-based diets (**CSBM**), in the 1950s, 1970s, and 1990s, respectively (Kidd et al., 2013). The 4th and 5th limiting AA in CSBM diets are generally considered to be Val and Ile (Kidd and Hackenhaar, 2006). Feed-grade Val has been commercially available since 2008 and is becoming more frequently used in commercial broiler feed formulations (Kidd et al., 2013), and feed-grade Ile is expected to become economically feasible for broiler diets in the near future. Miranda et al. (2014) demonstrated supplementing L-Val and L-Ile from 0 to 43 d reduced dietary CP by 1.63 percentage units and sustained broiler performance and processing measurements. Further work by these authors also supported the concept of reducing dietary CP using these AA while maintaining broiler performance (Miranda et al., 2015). However, Berres et al. (2010) found that L-Val and L-Ile supplementation from 0 to 42 d increased FCR of broilers by 3.3 points, with no effects on BWG or FI.

The aforementioned research on Val and Ile supplementation was conducted using CSBM diets, though it is often beneficial to incorporate intact protein sources other than SBM such as peanut meal (**PM**) or an animal protein meal in broiler feeds. Indeed, it has been demonstrated that high dietary SBM concentrations can increase the incidence of broiler FPD (Jensen et al., 1970), which can be ameliorated by partially replacing dietary SBM with animal protein (Eichner et al., 2007; Nagaraj et al., 2007). In addition to providing essential AA, calcium, and phosphorus, animal proteins serve as a good source of Gly + Ser, which may be conditionally limiting AA for young broilers fed reduced CP, CSBM-based diets (Dean et al., 2006). Peanut meal is a regionally important, vegetable-based ingredient that also has a moderately higher Gly + Ser concentration compared to SBM (Li et al., 2011). Thus, use of these feedstuffs will alter the essential and nonessential AA provided by intact ingredients and may influence the success of CP reduction using Val and Ile. Therefore, the objective of this experiment was to evaluate the supplementation of branched chain AA (**BCAA**) L-Val and L-Ile in CSBM-based diets, with or without PM or an animal protein blend (**APB**), as a strategy to reduce dietary CP throughout 4 feeding phases for broilers reared to 48 d post-hatch. It was hypothesized that broilers fed reduced CP diets through BCAA supplementation would have similar growth performance and processing yields, combined with reduced N excretion, when compared to birds fed higher CP diets, regardless of ingredient composition. It was also hypothesized that a reduction in dietary SBM content through either BCAA supplementation or the inclusion of PM or APB would improve litter characteristics and lessen the severity of FPD in broilers.

## MATERIALS AND METHODS

All procedures involving live birds were approved by University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

### *Bird Husbandry*

A total of 1,512 male chicks from Ross 708 breeder line (Aviagen North America, Sallisaw, OK) were obtained at hatch and transported to the University of Arkansas poultry research farm. Birds were vaccinated in ovo for Marek’s disease. Upon arrival, chicks were allocated to 72 floor pens (0.09 $m^2$/bird; 21 birds/pen) in a solid-sided research barn. Chicks were selected and weighed such that each group weight of 21 chicks fell within 3% of the expected average based on a preliminary weight of approximately 20% of the population. The barn was equipped with exhaust fans, radiant tube heaters, vent boards, and an electronic controller to maintain target environmental temperatures. Each pen contained one hanging feeder, a section (5 nipples/pen) of a continuous nipple drinker line that extended through each row of pens, and bedding of unused pine shavings. Feed and water access were provided ad libitum for the duration of the experiment. Ambient temperature at chick placement was set at 33°C and gradually decreased to maintain bird comfort as they aged until a final set point of 20°C was reached. The photoperiod was set at 23L:1D from placement to 7 d of age, 16L:8D from 8 to 28 d of age, and 18L:6D from 29 d until the end of the experiment. Light intensity was set at 27 lux from 1 to 7 d of age, 16 lux from 8 to 14 d of age, and 1 lux from 15 d to the end of the trial.

### ***Dietary Treatments***

On d of placement, all birds were provided 1 of 6 dietary treatments within a block of pens, with 12 replicate pens per treatment. Treatments were maintained throughout the entire feeding schedule, which consisted of starter (0 to 14 d), grower (14 to 28 d), finisher (28 to 39 d), and withdrawal (39 to 48 d) phases. Dietary treatments were a factorial arrangement of 3 diet types (CSBM, PM, and APB) with or without supplementation of the branched chain AA Val and Ile and were as follows: 1) CSBM, 2) CSBM + BCAA, 3) CSBM + PM, 4) CSBM + PM + BCAA, 5) CSBM + APB, 6) CSBM + APB + BCAA. To improve the accuracy of diet formulations, samples of corn, SBM, PM, and APB were analyzed for total AA profile and CP content (ATC Scientific, North Little Rock, AR) before formulation. Experimental feeds from each growth phase were sampled and sent for nutrient analysis at the same laboratory. All nutrient requirements were formulated to meet or exceed primary breeder recommendations (Aviagen, 2019) and to be isocaloric with the same minimum digestible AA to digestible Lys ratios within each phase. Starter diets were formulated to contain 1.28% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 74, 67, 75, 67, 107, and 16% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 3.1). Grower diets were formulated to contain 1.15% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 76, 67, 76, 68, 107, and 16% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 3.2). Finisher diets were formulated to contain 1.02% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 78, 67, 76, 69, 107, and 16% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 3.3). Withdrawal diets were formulated to contain 0.96%

digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 78, 67, 78, 69, 108, and 16% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 3.4).

### ***Determination of Growth Performance and Occurrence of Footpad Dermatitis***

Birds and feed were weighed at placement, 14, 28, 39, and 48 d to determine BWG, feed intake (**FI**), and mortality corrected FCR. Intake of N was determined by multiplying the FI by the analyzed N values per growth phase. Estimates of digestible AA intake for TSAA, Lys, Thr, Val, Ile, Arg, Trp, and Gly + Ser were calculated by multiplying the calculated complete diet digestibility coefficient of each AA by its analyzed total intake (FI × analyzed AA concentration) from 0 to 28 d (not shown) and 0 to 48 d. Mortality was recorded twice daily, and weights of mortality were used to correct FCR. On d 48, all birds were subjected to FPD lesion scoring using a 3-point scale system based on that of Nagaraj et al. (2007). Scores included 0 (no lesions), 1 (mild lesions; ≤ 1.5 cm,), or 2 (severe lesion; > 1.5 cm).

### ***Processing***

On d 48, after all birds were weighed by pen, 6 birds per pen (432 birds) were randomly selected for processing and wing-banded. On d 49, following 10 hr of feed removal, banded birds were transported to the University of Arkansas Pilot Processing Plant and weighed individually prior to processing. Birds were humanely electrically stunned prior to exsanguination via jugular vein incision. Thereafter, birds were scalded and defeathered and the neck, heads, and feet were removed. After hand-removal of the viscera and the lungs, hot carcass and fat pad weights were recorded. Hot carcasses were submerged in an ice water bath for 4 hr, and chilled carcasses were weighed and manually deboned to collect weights of breast fillets, tenders, wings, and leg quarters. Percentage yield of each of these parts was determined relative to individual back dock live weight.

### *Litter Characteristics*

Litter was sampled on d 48 at three locations within each pen. Samples were collected between the feeder and water lines at a depth of 5.08 cm in order to fill a 0.946 L size freezer bag. After collection, samples were placed in a cooler at 6°C until analysis of litter moisture, N, and pH. Litter moisture was assayed by drying a 50 g sample at 105°C for 12 hr in a drying oven. Litter N was determined on ground samples weighing 1 g at the University of Arkansas Central Analytical Laboratory (Fayetteville, AR, USA). Litter pH was measured using 4 g of homogenized litter mixed with 40 mL of deionized water. The solution was agitated for 15 min and allowed to settle for 30 min before a portable pH meter (Seven2Go pro, Mettler Toledo, Columbus, OH, USA) was used to determine pH.

### *Statistical Analysis*

Dietary treatments were arranged in a randomized complete block design and pen location served as the blocking factor. Data were analyzed by two-way ANOVA using MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC) as a factorial arrangement of 3 diet types (CSBM, PM, and APB) with or without BCAA supplementation. Fixed effects in the model included main effects of diet type and BCAA supplementation and their interaction. When appropriate, means were then separated using Tukey's HSD multiple comparison test. Mortality was analyzed by the percentage of deceased by treatment per pen. Mortality and FPD scores were subjected to a square root arcsine transformation prior to analysis. Statistical significance was considered at $P \leq 0.05$ in all cases.

# RESULTS

## *Diet Analysis*

In general, the analyzed total AA concentrations of Val and Ile were in good agreement among the CSBM, PM, and APB diets, though all diets contained slightly higher than calculated AA, as well as CP (Tables 3.1 – 3.4). The analyzed CP values averaged 1.39, 0.69, 0.41, and 0.41 percentage units higher than calculated for the starter, grower, finisher, and withdrawal phases, respectively. Nonetheless, BCAA supplementation reduced the overall (0 to 48 d) average analyzed CP by 1.62 percentage units, which was close to the calculated reduction of 1.71 percentage units. For each phase, analyzed CP was reduced by 1.51, 1.48, 1.92, and 1.58 percentage units for the starter, grower, finisher, and withdrawal phases, respectively. The overall analyzed CP reductions for each diet type were 1.68, 1.53, and 1.66 percentage units for CSBM, PM, and APB diets, respectively.

## *Growth Performance*

***General***. There were no interactive effects ($P > 0.05$) between diet type and BCAA supplementation on 0 to 48 d broiler live performance or mortality, processing measurements, FPD lesion scores, or litter characteristics. An interaction was observed for mortality for the 0 to 14 d ($P = 0.020$), 0 to 28 d ($P = 0.012$), and 0 to 39 d ($P = 0.036$) periods, but Tukey's HSD only separated means for 0 to 39 d mortality. During this period, on an average pen basis, birds fed APB had the highest mortality (8.0%), APB + BCAA-fed birds had the lowest (2.1%), and all other treatments were intermediate (2.5 to 5.3%). The overall (0 to 48 d) mortality was 6%.

***0 to 14 d.*** For the starter period, diet type influenced FI ($P = 0.032$) and FCR ($P < 0.001$), but not BWG ($P > 0.05$) (Table 3.5). Feed intake of broilers was highest for CSBM-fed birds, lowest for APB-fed birds, and intermediate for PM-fed birds. Broiler FCR of PM-fed and APB-fed birds were similar, and both were lower than that of CSBM-fed birds. There were no effects of BCAA supplementation on starter performance (*P* > 0.05), except a tendency (*P* = 0.057) for BCAA inclusion to increase FI.

***0 to 28 d.*** Main effects of diet type were observed for BWG (*P* = 0.014), FI (*P* = 0.005), and FCR (*P* < 0.001) (Table 3.6). Broiler BWG was highest for PM-fed birds, lowest for APB-fed birds, and intermediate for CSBM-fed birds. Broiler FI was higher in CSBM-fed and PM-fed birds than in APB-fed birds. Broiler FCR was highest for CSBM-fed birds, lowest for PM-fed birds, and intermediate for APB-fed birds. The supplementation of BCAA increased broiler FI (*P* = 0.029) and FCR (*P* = 0.015) but had no impact on BWG (*P* > 0.05).

***0 to 39 d.*** In contrast to 0 to 28 d, 0 to 39 d live performance of broilers was not impacted (*P* > 0.05) by diet type (Table 3.7). Supplementing BCAA increased the FI (*P* = 0.003) of broilers but did not influence BWG or FCR (*P* > 0.05).

***0 to 48 d.*** Similar to 0 to 39 d responses, diet type did not (*P* > 0.05) influence broiler growth performance (Table 3.8). The inclusion of BCAA increased bird FI (*P* = 0.014) but did not influence bird BWG or FCR (*P* > 0.05). Cumulative N intake was highest (*P* = 0.017) for birds fed PM, lowest for CSBM-fed, and intermediate for APB-fed, and broilers supplemented with BCAA had lower (*P* < 0.001) N intake than those not fed supplemental BCAA (Table 3.8).

The main effects and interactions of dietary treatment on digestible intakes of TSAA, Lys, Thr, Val, Ile, Arg, Trp, and Gly + Ser from 0 to 48 d are presented in Table 3.9. Diet type × BCAA supplementation interactions were observed (*P* < 0.05) for Lys, Thr, Ile, Arg, and Trp. For TSAA, only a main effect of diet type was observed (*P* < 0.001), with the CSBM-fed birds having higher dTSAA intake than PM or APB-fed birds. Main effects of both diet type and BCAA supplementation were observed (*P* < 0.05) on dGly + Ser intake. Intake of dGly + Ser was highest for APB-fed birds, lowest for CSBM-fed birds, and intermediate for PM-fed birds, while BCAA supplementation reduced dGly + Ser intake.

***Processing characteristics.*** Live ($P = 0.025$), hot carcass ($P = 0.010$), and chilled carcass ($P = 0.013$) weights were impacted by diet type (Table 3.10). Weights were heaviest for birds fed PM, lowest for APB-fed birds, and intermediate for CSBM-fed birds, although no differences in percentage yield ($P > 0.05$) were observed. Additionally, absolute and relative fat pad weights were not affected by diet type ($P > 0.05$), but both were increased ($P = 0.002$) with the supplementation of BCAA.

For deboning measurements, diet type affected tender ($P = 0.044$) and leg quarter ($P = 0.002$) weights but did not affect yields ($P > 0.05$) of these parts (Table 3.11). Birds fed APB had the lowest tenders and leg quarters weights, while CSBM-fed birds and PM-fed birds were similar in weights. Supplemental BCAA increased fillets ($P = 0.032$) and total white meat ($P = 0.042$) weights and percentage yield ($P < 0.05$), while weights and yields of tenders, wings, and leg quarters were unaffected ($P > 0.05$) by BCAA supplementation.

***Litter Characteristics.*** Litter moisture and pH on d 48 were unaffected ($P > 0.05$) by diet type or BCAA supplementation (Table 3.12). However, litter N was lowered ($P < 0.001$) by 8.5% when birds were supplemented with BCAA, with no differences among diet types ($P > 0.05$).

***Footpad Lesions.*** Broiler FPD lesions were influenced by diet type ($P = 0.041$) but not by BCAA ($P > 0.05$) (Table 3.12). A score of 2 was not recorded for any bird and accordingly was removed from Table 3.12. The incidence of FPD was highest for birds fed CSBM, lowest for the APB-fed, and intermediate for PM-fed.

## DISCUSSION

The objective of the current study was to evaluate the use of supplemental L-Val and L-Ile in order to maintain the digestible content of these AA in reduced CP diets when varying ingredient composition. The SBM inclusion and calculated CP levels of diets were generally higher than typically observed in practice due to lower than expected analyzed Val and Ile values for SBM. Peanut meal was included at 5% for each diet phase, whereas the inclusions of APB were 5, 4, 3, and 2.75% for the starter, grower, finisher, and withdrawal phases, respectively, in order to prevent dietary excesses of calcium and phosphorus that would have occurred if 5% APB was used across all phases. Diet composition also altered the 4th and 5th limiting AA of the experimental diets by changing the quantity of supplemental AA supplied to meet minimum AA requirements. For the starter diets, the 4th limiting AA for CSBM and PM diets was Val while Ile was 4th limiting in the APB diet. This varied somewhat among phases due to changing proportions of corn and SBM, and for the withdrawal diets, Ile was 4th limiting and Val was 5th limiting in all diet types.

In this experiment, there were no effects of PM or APB inclusion on broiler performance from 0 to 48 d post-hatch or on processing yields, though carcass, tenders, and leg quarters weights were reduced for birds fed APB. During the early growth phases (0 to 28 d), broilers fed PM diets had improved FCR, which could have been due to several factors including the higher intake of Arg from 0 to 28 d ($P < 0.001$; data not shown) and 0 to 48 d for these birds as Arg and other essential AA beyond Val and Ile met or exceeded requirements but were not balanced in this study. Indeed, PM is rich in Arg but is low in Met, Lys, and Thr (Batal et al., 2005). Accordingly, researchers often use PM to create Met, Lys, and Thr deficient basal diets in AA response studies (Kidd et al., 1999, 2000; Corzo et al., 2007), but only a few studies have evaluated efficacy of PM in replacing SBM for broilers. The results reported herein support those of Costa et al. (2001) who showed that broiler performance could be maintained when PM was fed up to 20% in corn-soy diets when supplemented with Met, Lys, and Thr.

The inclusion of an animal protein meal can be valuable due to its protein and phosphorus content. When comparing broilers fed a CSBM diet with or without the inclusion of an animal protein, some have found the inclusion of animal protein negatively affected broiler performance (Nagaraj et al.,2007), whereas others have reported similar performance (Drewyor and Waldroup, 2000; Vieira and Lima, 2005; Caires et al., 2010) and processing characteristics (Caires et al., 2010) with the inclusion of an animal protein. For this study, the reduction in processing weights, but not yield, of APB-fed birds could be due to the reduction in early (0 to 28 d) FI, and consequently, digestible Lys intake from 0 to 28 d ($P < 0.001$; data not shown) and 0 to 48 d, which has been previously shown to reduce carcass/parts weights of broilers (Tesseraud et al., 1992). Interestingly, broilers fed APB diets without BCAA supplementation had the highest mortality, while those fed the APB + BCAA had the lowest mortality among all groups. Though most mortalities were necropsied, the treatment design and measurements in the current study were not sufficient to confirm if these responses in mortality were associated with dietary treatment.

The reduction of CP through BCAA supplementation did not influence cumulative BWG or FCR in this study. Impaired broiler performance has been observed when lowering CP by more than 3 percentage units (Bregendahl et al., 2002; Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008) or reducing CP by more than 20% in diets of young broilers (Bregendahl et al., 2002; Si et al., 2004; Sterling et al., 2005; Waldroup et al., 2005) even when essential AA levels were maintained (Bregendahl et al., 2002; Si et al., 2004; Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008). Aftab et al. (2006) reviewed the possible factors that limit the growth of broilers fed reduced CP diets and suggested the ratio of non-essential to essential AA, lack of non-essential N, lack of Lys, insufficiency of essential AA, or ratio of ME to NE to be potential causes. Additionally, another often-mentioned factor is insufficient Gly + Ser, as these nonessential AA may be conditionally-essential for young chicks fed a reduced CP, CSBM-based diet (Dean et al., 2006). Broilers supplemented with BCAA had a lower intake of Gly + Ser, but 0 to 14 d performance was unaffected. Broiler intake of Gly + Ser when varying diet composition was lowest for CSBM, intermediate for PM, and highest for APB diets. Broiler BWG was unaffected but FCR was lower for PM and APB-fed than CSBM-fed from 0 to 14 d. Due to the design of this study, it would not be possible to solely attribute differences in performance among diet types to Gly + Ser intake, but nonetheless, this factor did not appear to markedly impact 0 to 14 d performance in the current trial.

Supplementation of BCAA to reduce dietary CP did increase the FI and FCR of young broilers (0 to 28 d). By d 48, BCAA-fed broilers had increased FI (1.37%), but the corresponding increase in BWG (0.81%), though not statistically significant, was sufficient to prevent an impact on FCR (0.45% increase). Similarly, Ospina-Rojas et al. (2014) reported that supplementing L-Val and L-Ile increased FCR of young broilers (experiment 1: 0 to 21 d) but not older broilers (experiment 2: 22 to 42 d). However, Miranda et al. (2015) noted that supplementing L-Val and L-Ile from 0 to 21 d increased overall (0 to 42 d) broiler BWG at similar FI and FCR, but supplementing L-Val and L-Ile from 22 to 42 d increased overall (0 to 42 d) FI and FCR. Berres et al. (2010) supplemented L-Val and L-Ile in broiler diets from 0 to 42 d to reduce CP by an average of 2.9 percentage units (1 to 7 d: 25.8 to 23.1% CP) and observed an increase in FCR from 0 to 21 d and 0 to 42 d, with no differences observed for BWG and FI. Miranda et al.

(2014) also reported that broiler performance was sustained from 0 to 43 d when CP was reduced by an average of 1.63 percentage units (1 to 7 d: 25.3 to 23.1% CP) using L-Val and L-Ile. It appears that this experiment agrees with existing literature that supplementing BCAA does not negatively affect BWG of broilers but may transiently affect FCR at earlier ages with similar FCR at market age.

The increase in FI with BCAA supplementation may have been due to several factors. Summers et al. (1992) supports previous data that broilers may increase FI to satisfy essential AA needs, which may negatively impact FCR. In the current study, the increase in FI with BCAA supplementation was associated with a numerical increase in BWG that led to marginal impact on FCR. In piglets, Zheng et al. (2016) reported that supplementation of Val, Ile, and Leu up-regulated the hypothalamic appetite regulatory gene and down-regulated the genes inhibiting FI, although the mechanistic effects of BCAA on FI and muscle development have not been extensively studied in poultry.

The increase in FI with BCAA supplementation may have also contributed to the increase in breast fillets and total white meat yield of these birds in the current study. Others have reported reduced leg quarter yield (Berres et al., 2010; Ospina-Rojas et al., 2014), fillet yield (Ospina-Rojas et al., 2014), or no differences in weight or yield (Miranda et al., 2014, 2015) when Val and Ile were supplemented. Breast fillet development has been shown to be more sensitive to Ile in diets deficient in Val and Ile (Corzo et al., 2010). Increasing incremental additions of Ile close to or above requirement have improved fillet yield (Dozier et al., 2012), whereas incremental additions of Val in Ile-limiting diets reduced breast fillet weight and yield (Corzo et al., 2011).

It is well documented that lowering CP will increase fat pad deposition (Moran and Stilborn, 1996; Namroud et al., 2008; Darsi et al., 2012; Kriseldi et al., 2017; Belloir et al., 2017). In this study, both breast fillet yield and abdominal fat deposition were increased when birds were fed reduced CP diets, which was not observed in related studies (Berres et al., 2010; Miranda et al., 2014; Ospina-Rojas et al., 2014), excluding one (Miranda et al., 2015). Namroud et al. (2008) stated that birds fed greater protein levels experienced an increased heat increment involved in the deamination and transamination of surplus AA to other metabolites and finally uric acid. Research by Bartov et al. (1974) and more recently Belloir et al. (2017) supports the concept that a dietary increase in calorie to protein ratio will increase fat deposition, and typically broilers will increase FI when fed reduced CP diets and will consequently consume more ME that will be subsequently stored as fat.

The reduction in CP with L-Val and L-Ile supplementation in the current experiment lowered broiler N intake by 12 g and litter N by 8.5%. Ferguson et al. (1998b) fed diets supplemented with Met, Lys, Thr, and Trp to broilers from 0 to 42 d and found that one percentage unit reduction in dietary CP led to a 7% reduction in litter N. Bregendahl et al. (2002) evaluated apparent N excretion by whole-body composition and showed that a unit reduction in CP achieved a 6.3% lowering in N excretion. Others have similarly suggested that reducing CP by 1 percentage unit will reduce N excretion ranging from 4.8 to 7.9% (Si et al., 2004; Hernandez et al., 2012; Kriseldi et al., 2018). In the current study, 1 percentage unit reduction in dietary CP lowered litter N by 5.25%. However, given that dietary CP reductions may lead to an increase in FI of broilers as previously discussed, N intake and excretion may not be a direct function of dietary CP content. However, there were only slight deviations between regression equations describing litter N responses to either dietary CP or N intake in the current experiment

(Figure 3.1). It is important to note that larger CP reductions with additional AA supplementation may lead to stronger relationships between N intake and litter N than observed in the current study, as well as greater differences between regression slopes when dietary CP or N intake is used as the independent variable.

The supplementation of BCAA reduced intake of N and litter N and numerically reduced litter moisture with no effect on the severity of FPD in the current experiment. Reducing CP, and hence litter N, has been linked to improving FPD (Shao et al., 2018), but broilers fed higher CP diets will generally increase water consumption to excrete excess N (Francesch and Brufau, 2004). Moreover, it is well documented that wet litter conditions play a vital role in pododermatitis (Shepherd and Fairchild, 2010), and the combination of reduced litter moisture and CP reductions can affect FPD responses (Van Harn et al., 2019; Lemme et al., 2019). Despite having similar litter moisture and N content, APB-fed birds had lower FPD scores than CSBM-fed birds. These results coincide with those of Nagaraj et al. (2007) who suggested replacing SBM with an animal protein was a primary factor in reducing FPD. In addition to litter N and moisture, dietary soluble nonstarch polysaccharides may also play a role in the incidence of FPD through increasing viscosity of intestinal digesta and development of sticky excreta that facilitates the adhesions of litter and pathogens to the footpad (Jensen et al., 1970; Shepherd and Fairchild, 2010). Therefore, a reduction in the nonstarch polysaccharide contribution from SBM through its replacement with APB may have attributed to the results of the current study.

## CONCLUSION AND APPLICATIONS

1. Supplementation of L-Val and L-Ile reduced dietary CP by an average of 1.62 percentage units and maintained broilers BWG and FCR, regardless of diet composition.

2. Broilers fed supplemental BCAA had improved breast fillet and total white meat weights and yields. Weights, but not yields, of carcasses, tenders, and leg quarters were reduced for birds fed APB diets.
3. Supplementing BCAA lowered litter N content by 8.5%, resulting in a 5.25% reduction in litter N per 1 percentage unit reduction in dietary CP (averaged across all feeding phases).
4. Footpad dermatitis was improved with the inclusion of APB in CSBM diets but was not affected by BCAA supplementation.

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# TABLES AND FIGURE

**Table 3.1**. Ingredient and calculated nutrient composition (%, unless otherwise noted) of experimental starter diets fed to broilers from 0 to 14 d post-hatch[1]

| Ingredient, as-fed | CSBM | CSBM + BCAA | PM | PM + BCAA | APB | APB + BCAA |
|---|---|---|---|---|---|---|
| Corn | 50.91 | 57.53 | 49.53 | 56.82 | 54.47 | 59.42 |
| Soybean meal (47%) | 42.42 | 36.28 | 38.76 | 31.86 | 36.78 | 32.02 |
| Peanut meal (44%) | - | - | 5.00 | 5.00 | - | - |
| Animal protein blend[2] (56%) | - | - | - | - | 5.00 | 5.00 |
| Poultry fat | 3.28 | 2.09 | 3.22 | 1.93 | 1.89 | 1.03 |
| Limestone | 1.08 | 1.11 | 1.07 | 1.14 | 0.58 | 0.62 |
| Dicalcium phosphate | 1.11 | 1.13 | 1.11 | 1.14 | - | - |
| Sodium chloride | 0.33 | 0.12 | 0.28 | 0.10 | 0.13 | 0.08 |
| L-Met | 0.32 | 0.37 | 0.33 | 0.39 | 0.32 | 0.36 |
| L-Lys·HCl | 0.07 | 0.25 | 0.11 | 0.32 | 0.11 | 0.26 |
| L-Thr | 0.13 | 0.21 | 0.14 | 0.23 | 0.13 | 0.20 |
| L-Val | - | 0.09 | - | 0.11 | - | 0.06 |
| L-Ile | - | 0.08 | - | 0.10 | - | 0.07 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Se premix[5] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.04 | 0.06 | 0.04 | 0.07 | 0.07 | 0.08 |
| Sodium bicarbonate | 0.02 | 0.37 | 0.09 | 0.49 | 0.21 | 0.49 |
| Copper chloride[6] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Coccidiostat[7] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[8] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | |
| $AME_n$, kcal/kg | 3,000 | 3,000 | 3,000 | 3,000 | 3,000 | 3,000 |
| DEB, mEq/kg | 295 | 295 | 295 | 295 | 295 | 295 |
| CP | 24.11 | 22.07 | 24.52 | 22.23 | 24.57 | 22.98 |
| Analyzed CP | 25.18 | 23.31 | 25.71 | 24.28 | 25.78 | 24.54 |
| Digestible Lys | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 |
| Digestible Met | 0.64 | 0.66 | 0.64 | 0.66 | 0.63 | 0.65 |
| Digestible TSAA | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 |
| Digestible Thr | 0.86 | 0.86 | 0.86 | 0.86 | 0.86 | 0.86 |
| Digestible Val | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
| Digestible Ile | 0.88 | 0.86 | 0.86 | 0.86 | 0.86 | 0.86 |
| Digestible Leu | 1.65 | 1.51 | 1.65 | 1.49 | 1.65 | 1.54 |
| Digestible Arg | 1.55 | 1.37 | 1.64 | 1.44 | 1.55 | 1.41 |
| Digestible Trp | 0.24 | 0.21 | 0.24 | 0.20 | 0.23 | 0.20 |
| Total Gly + Ser | 2.17 | 1.95 | 2.22 | 1.97 | 2.46 | 2.29 |
| Analyzed Total Gly + Ser | 2.38 | 2.13 | 2.41 | 2.27 | 2.75 | 2.54 |
| Total Ca | 0.96 | 0.96 | 0.95 | 0.96 | 0.96 | 0.96 |
| Available P | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 |

[1] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; DEB = dietary electrolyte balance; PM= peanut meal.

[2] Pro-Plus™, H. J. Baker & Brothers. Inc., Little Rock, AR.

[3] The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin $D_3$, 4,536 ICU; vitamin E, 45 IU; vitamin $B_{12}$ 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

**Table 3.1 (Cont.)**

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[6] IntelliBond® C, Micronutrients, Indianapolis, IN.

[7] Salinomycin sodium, Bio-Cox®, Huvepharma Inc., Peachtree City, GA.

[8] Optiphos®, Huvepharma Inc., Peachtree City, GA.

**Table 3.2**. Ingredient and calculated nutrient composition (%, unless otherwise noted) of experimental grower diets fed to broilers from 14 to 28 d post-hatch[1]

| Ingredient, as-fed | CSBM | CSBM + BCAA | PM | PM + BCAA | APB | APB + BCAA |
|---|---|---|---|---|---|---|
| Corn | 56.00 | 61.71 | 54.06 | 60.97 | 58.27 | 63.64 |
| Soybean meal (47%) | 36.78 | 31.61 | 33.60 | 27.35 | 32.86 | 28.01 |
| Peanut meal (44%) | - | - | 5.00 | 5.00 | - | - |
| Animal protein blend[2] (56%) | - | - | - | - | 4.00 | 4.00 |
| Poultry fat | 4.04 | 2.97 | 4.08 | 2.79 | 3.00 | 2.01 |
| Limestone | 1.03 | 1.06 | 1.05 | 1.08 | 0.63 | 0.65 |
| Dicalcium phosphate | 0.90 | 0.92 | 0.90 | 0.92 | - | 0.03 |
| Sodium chloride | 0.30 | 0.25 | 0.29 | 0.23 | 0.26 | 0.22 |
| L-Met | 0.30 | 0.34 | 0.30 | 0.35 | 0.29 | 0.33 |
| L-Lys·HCl | 0.07 | 0.22 | 0.11 | 0.29 | 0.09 | 0.24 |
| L-Thr | 0.11 | 0.18 | 0.12 | 0.21 | 0.11 | 0.18 |
| L-Val | - | 0.08 | - | 0.09 | - | 0.05 |
| L-Ile | - | 0.07 | - | 0.10 | - | 0.08 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Se premix[5] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.03 | 0.05 | 0.03 | 0.05 | 0.05 | 0.06 |
| Sodium bicarbonate | 0.15 | 0.24 | 0.17 | 0.27 | 0.13 | 0.21 |
| Copper chloride[6] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Coccidiostat[7] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[8] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | |
| $AME_n$, kcal/kg | 3,100 | 3,100 | 3,100 | 3,100 | 3,100 | 3,100 |
| DEB, mEq/kg | 281 | 256 | 277 | 247 | 264 | 240 |
| CP | 21.80 | 20.10 | 22.38 | 20.32 | 22.38 | 20.78 |
| Analyzed CP | 22.19 | 21.26 | 22.73 | 21.58 | 23.26 | 20.90 |
| Digestible Lys | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 |
| Digestible Met | 0.59 | 0.61 | 0.59 | 0.61 | 0.58 | 0.60 |
| Digestible TSAA | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Digestible Thr | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Digestible Val | 0.87 | 0.87 | 0.88 | 0.87 | 0.90 | 0.87 |
| Digestible Ile | 0.79 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Digestible Leu | 1.52 | 1.40 | 1.52 | 1.38 | 1.53 | 1.42 |
| Digestible Arg | 1.38 | 1.23 | 1.49 | 1.30 | 1.40 | 1.26 |
| Digestible Trp | 0.21 | 0.19 | 0.21 | 0.18 | 0.21 | 0.18 |
| Total Gly + Ser | 1.96 | 1.77 | 2.03 | 1.80 | 2.21 | 2.04 |
| Analyzed Total Gly + Ser | 2.07 | 1.97 | 2.17 | 2.05 | 2.34 | 2.15 |
| Total Ca | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Available P | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 |

[1] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; DEB = dietary electrolyte balance; PM= peanut meal.

[2] Pro-Plus™, H. J. Baker & Brothers. Inc., Little Rock, AR.

[3] The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin $D_3$, 4,536 ICU; vitamin E, 45 IU; vitamin $B_{12}$ 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[6] IntelliBond® C, Micronutrients, Indianapolis, IN.

[7] Salinomycin sodium, Bio-Cox®, Huvepharma Inc., Peachtree City, GA.

[8] Optiphos®, Huvepharma Inc., Peachtree City, GA.

**Table 3.3**. Ingredient and calculated nutrient composition (% unless otherwise noted) of experimental finisher diets fed to broilers from 28 to 39 d post-hatch[1]

| Ingredient, as-fed | CSBM | CSBM + BCAA | PM | PM + BCAA | APB | APB + BCAA |
|---|---|---|---|---|---|---|
| Corn | 60.95 | 65.84 | 58.51 | 64.91 | 62.29 | 67.55 |
| Soybean meal (47%) | 31.37 | 26.95 | 28.64 | 22.86 | 28.77 | 24.03 |
| Peanut meal (44%) | - | - | 5.00 | 5.00 | - | - |
| Animal protein blend[2] (56%) | - | - | - | - | 3.00 | 3.00 |
| Poultry fat | 4.79 | 3.89 | 4.93 | 3.75 | 4.08 | 3.12 |
| Limestone | 0.98 | 1.00 | 0.99 | 1.02 | 0.68 | 0.71 |
| Dicalcium phosphate | 0.68 | 0.70 | 0.68 | 0.70 | - | 0.02 |
| Sodium chloride | 0.30 | 0.25 | 0.29 | 0.24 | 0.28 | 0.23 |
| L-Met | 0.27 | 0.30 | 0.27 | 0.31 | 0.26 | 0.29 |
| L-Lys·HCl | 0.07 | 0.20 | 0.09 | 0.26 | 0.07 | 0.21 |
| L-Thr | 0.10 | 0.16 | 0.10 | 0.18 | 0.09 | 0.16 |
| L-Val | - | 0.05 | - | 0.06 | - | 0.03 |
| L-Ile | - | 0.07 | - | 0.09 | - | 0.07 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Se premix[5] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.03 | 0.04 | 0.03 | 0.05 | 0.04 | 0.06 |
| Sodium bicarbonate | 0.16 | 0.24 | 0.17 | 0.27 | 0.14 | 0.22 |
| Copper chloride[6] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Coccidiostat[7] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[8] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | |
| AME$_n$, kcal/kg | 3,200 | 3,200 | 3,200 | 3,200 | 3,200 | 3,200 |
| DEB, mEq/kg | 254 | 233 | 252 | 224 | 243 | 220 |
| CP | 19.58 | 18.11 | 20.31 | 18.39 | 20.13 | 18.55 |
| Analyzed CP | 20.54 | 18.02 | 20.51 | 18.85 | 20.59 | 19.00 |
| Digestible Lys | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |
| Digestible Met | 0.53 | 0.55 | 0.53 | 0.55 | 0.52 | 0.54 |
| Digestible TSAA | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Digestible Thr | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| Digestible Val | 0.79 | 0.78 | 0.81 | 0.78 | 0.81 | 0.78 |
| Digestible Ile | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Digestible Leu | 1.39 | 1.29 | 1.40 | 1.27 | 1.40 | 1.30 |
| Digestible Arg | 1.22 | 1.09 | 1.34 | 1.17 | 1.24 | 1.11 |
| Digestible Trp | 0.19 | 0.17 | 0.19 | 0.16 | 0.19 | 0.16 |
| Total Gly + Ser | 1.76 | 1.59 | 1.84 | 1.63 | 1.96 | 1.79 |
| Analyzed Total Gly + Ser | 1.83 | 1.52 | 1.75 | 1.64 | 2.02 | 1.81 |
| Total Ca | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Available P | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |

[1] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; DEB = dietary electrolyte balance; PM= peanut meal.

[2] Pro-Plus™, H. J. Baker & Brothers. Inc., Little Rock, AR.

[3] The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin $D_3$, 4,536 ICU; vitamin E, 45 IU; vitamin $B_{12}$ 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[6] IntelliBond® C, Micronutrients, Indianapolis, IN.

[7] Salinomycin sodium, Bio-Cox®, Huvepharma Inc., Peachtree City, GA.

[8] Optiphos®, Huvepharma Inc., Peachtree City, GA.

**Table 3.4**. Ingredient and calculated nutrient composition (%, unless otherwise noted) of experimental withdrawal diets fed to broilers from 39 to 48 d post-hatch[1]

| Ingredient, as-fed | CSBM | CSBM + BCAA | PM | PM + BCAA | APB | APB + BCAA |
|---|---|---|---|---|---|---|
| Corn | 64.52 | 68.46 | 62.08 | 67.80 | 65.74 | 70.42 |
| Soybean meal (47%) | 28.58 | 25.02 | 25.85 | 20.69 | 26.19 | 21.98 |
| Peanut meal (44%) | - | - | 5.00 | 5.00 | - | - |
| Animal protein blend[2] (56%) | - | - | - | - | 2.75 | 2.75 |
| Poultry fat | 4.16 | 3.43 | 4.30 | 3.24 | 3.51 | 2.65 |
| Limestone | 0.97 | 0.99 | 0.98 | 1.01 | 0.69 | 0.72 |
| Dicalcium phosphate | 0.61 | 0.62 | 0.61 | 0.63 | - | - |
| Sodium chloride | 0.30 | 0.26 | 0.29 | 0.24 | 0.28 | 0.23 |
| L-Met | 0.24 | 0.27 | 0.24 | 0.28 | 0.23 | 0.26 |
| L-Lys·HCl | 0.07 | 0.18 | 0.10 | 0.25 | 0.08 | 0.20 |
| L-Thr | 0.09 | 0.14 | 0.10 | 0.16 | 0.09 | 0.14 |
| L-Val | - | 0.05 | - | 0.06 | - | 0.04 |
| L-Ile | - | 0.06 | - | 0.08 | - | 0.07 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Se premix[5] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.02 | 0.04 | 0.03 | 0.04 | 0.04 | 0.05 |
| Sodium bicarbonate | 0.17 | 0.23 | 0.18 | 0.27 | 0.15 | 0.22 |
| Copper chloride[6] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Phytase[7] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | |
| $AME_n$, kcal/kg | 3,200 | 3,200 | 3,200 | 3,200 | 3,200 | 3,200 |
| DEB, mEq/kg | 241 | 224 | 239 | 214 | 231 | 210 |
| CP | 18.51 | 17.34 | 19.24 | 17.53 | 19.02 | 17.62 |
| Analyzed CP | 18.99 | 17.58 | 19.86 | 17.98 | 19.36 | 17.92 |
| Digestible Lys | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
| Digestible Met | 0.49 | 0.51 | 0.49 | 0.51 | 0.49 | 0.50 |
| Digestible TSAA | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Digestible Thr | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 |
| Digestible Val | 0.75 | 0.75 | 0.77 | 0.75 | 0.77 | 0.75 |
| Digestible Ile | 0.66 | 0.66 | 0.66 | 0.66 | 0.66 | 0.66 |
| Digestible Leu | 1.33 | 1.25 | 1.34 | 1.22 | 1.34 | 1.25 |
| Digestible Arg | 1.14 | 1.04 | 1.26 | 1.11 | 1.16 | 1.04 |
| Digestible Trp | 0.18 | 0.16 | 0.18 | 0.15 | 0.17 | 0.15 |
| Total Gly + Ser | 1.66 | 1.53 | 1.74 | 1.55 | 1.84 | 1.69 |
| Analyzed Total Gly + Ser | 1.76 | 1.60 | 1.81 | 1.64 | 1.76 | 1.67 |
| Total Ca | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Available P | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |

[1] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; DEB = dietary electrolyte balance; PM= peanut meal.

[2] Pro-Plus™, H. J. Baker & Brothers. Inc., Little Rock, AR.

[3] The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin $D_3$, 4,536 ICU; vitamin E, 45 IU; vitamin $B_{12}$ 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[6] IntelliBond® C, Micronutrients, Indianapolis, IN.

[7] Optiphos®, Huvepharma Inc., Peachtree City, GA.

**Table 3.5.** Live performance of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 14 d post-hatch[1]

| Item[2] | 0 d BW, kg | 14 d BW, kg | 0 to 14 d BWG, kg | 0 to 14 d FI, kg | 0 to 14 d FCR[3], kg:kg |
|---|---|---|---|---|---|
| Main effect of diet | | | | | |
| CSBM | 0.039 | 0.384 | 0.345 | 0.442[a] | 1.284[a] |
| CSBM + PM | 0.039 | 0.393 | 0.354 | 0.439[ab] | 1.244[b] |
| CSBM + APB | 0.039 | 0.383 | 0.343 | 0.430[b] | 1.258[b] |
| SEM | 0.0001 | 0.0033 | 0.0033 | 0.0033 | 0.0058 |
| Main effect of BCAA | | | | | |
| No BCAA | 0.039 | 0.384 | 0.344 | 0.433 | 1.266 |
| BCAA | 0.039 | 0.389 | 0.350 | 0.440 | 1.262 |
| SEM | 0.0001 | 0.0027 | 0.0027 | 0.0027 | 0.0048 |
| *P*-values | | | | | |
| Diet | 0.695 | 0.066 | 0.064 | 0.032 | < 0.001 |
| BCAA | 0.649 | 0.136 | 0.136 | 0.057 | 0.854 |
| Diet × BCAA | 0.376 | 0.898 | 0.908 | 0.844 | 0.710 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey’s multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; BWG = body weight gain; CSBM = corn and soybean meal; FI = feed intake; PM = peanut meal.

[3] Corrected for mortality.

**Table 3.6.** Live performance of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 28 d post-hatch[1]

| Item[2] | 28 d BW, kg | 0 to 28 d BWG, kg | 0 to 28 d FI, kg | 0 to 28 d FCR[3], kg:kg |
|---|---|---|---|---|
| Main effect of diet | | | | |
| CSBM | $1.406^{ab}$ | $1.367^{ab}$ | $1.880^{a}$ | $1.388^{a}$ |
| CSBM + PM | $1.428^{a}$ | $1.389^{a}$ | $1.875^{a}$ | $1.363^{b}$ |
| CSBM + APB | $1.392^{b}$ | $1.353^{b}$ | $1.820^{b}$ | $1.375^{ab}$ |
| SEM | 0.0084 | 0.0084 | 0.0139 | 0.0046 |
| Main effect of BCAA | | | | |
| No BCAA | 1.407 | 1.368 | $1.840^{b}$ | $1.369^{b}$ |
| BCAA | 1.410 | 1.371 | $1.876^{a}$ | $1.382^{a}$ |
| SEM | 0.0069 | 0.0069 | 0.0114 | 0.0037 |
| *P*-values | | | | |
| Diet | 0.014 | 0.014 | 0.005 | 0.001 |
| BCAA | 0.731 | 0.733 | 0.029 | 0.015 |
| Diet × BCAA | 0.814 | 0.819 | 0.244 | 0.219 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; BWG = body weight gain; CSBM = corn and soybean meal; FI = feed intake; PM = peanut meal.

[3] Corrected for mortality.

**Table 3.7.** Live performance of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 39 d post-hatch[1]

| Item[2] | 39 d BW, kg | 0 to 39 d BWG, kg | 0 to 39 d FI, kg | 0 to 39 d FCR[3], kg:kg |
|---|---|---|---|---|
| Main effect of diet | | | | |
| CSBM | 2.574 | 2.534 | 3.723 | 1.478 |
| CSBM + PM | 2.577 | 2.534 | 3.713 | 1.468 |
| CSBM + APB | 2.538 | 2.499 | 3.670 | 1.478 |
| SEM | 0.0147 | 0.0147 | 0.0187 | 0.0041 |
| Main effect of BCAA | | | | |
| No BCAA | 2.553 | 2.514 | $3.669^{b}$ | 1.470 |
| BCAA | 2.572 | 2.533 | $3.735^{a}$ | 1.478 |
| SEM | 0.0120 | 0.0120 | 0.0152 | 0.0033 |
| *P*-values | | | | |
| Diet | 0.129 | 0.128 | 0.108 | 0.226 |
| BCAA | 0.263 | 0.262 | 0.003 | 0.096 |
| Diet × BCAA | 0.729 | 0.728 | 0.876 | 0.413 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; BWG = body weight gain; CSBM = corn and soybean meal; FI = feed intake; PM = peanut meal.

[3] Corrected for mortality.

**Table 3.8.** Live performance of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d post-hatch[1]

| Item[2] | 48 d BW, kg | 0 to 48 d BWG, kg | 0 to 48 d FI, kg | 0 to 48 d FCR[3], kg:kg | 0 to 48 d N intake, kg |
|---|---|---|---|---|---|
| Main effect of diet | | | | | |
| CSBM | 3.666 | 3.627 | 5.603 | 1.558 | 0.179$^{b}$ |
| CSBM + PM | 3.651 | 3.612 | 5.567 | 1.554 | 0.183$^{a}$ |
| CSBM + APB | 3.629 | 3.590 | 5.334 | 1.557 | 0.181$^{ab}$ |
| SEM | 0.0174 | 0.0173 | 0.0261 | 0.0049 | 0.0008 |
| Main effect of BCAA | | | | | |
| No BCAA | 3.634 | 3.595 | 5.530$^{b}$ | 1.553 | 0.187$^{a}$ |
| BCAA | 3.664 | 3.624 | 5.606$^{a}$ | 1.560 | 0.175$^{b}$ |
| SEM | 0.0142 | 0.0142 | 0.0213 | 0.0040 | 0.0007 |
| *P*-values | | | | | |
| Diet | 0.321 | 0.323 | 0.180 | 0.835 | 0.017 |
| BCAA | 0.150 | 0.150 | 0.014 | 0.220 | < 0.001 |
| Diet × BCAA | 0.448 | 0.442 | 0.992 | 0.166 | 0.898 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; BWG = body weight gain; CSBM = corn and soybean meal; FI = feed intake; PM = peanut meal; N = nitrogen.

[3] Corrected for mortality.

**Table 3.9.** Digestible amino acid (dAA) intake of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d post-hatch[1,2]

| Item[3] | dTSAA, g | dLys, g | dThr, g | dVal, g | dIle, g | dArg, g | dTrp, g | dGly + Ser, g |
|---|---|---|---|---|---|---|---|---|
| Main effect of diet | | | | | | | | |
| CSBM | $57.7^{a}$ | $59.9^{a}$ | $41.0^{ab}$ | $49.4^{c}$ | $44.8^{c}$ | $54.9^{b}$ | $12.6^{a}$ | $96.8^{c}$ |
| CSBM + PM | $56.4^{b}$ | $60.1^{a}$ | $40.9^{b}$ | $50.3^{b}$ | $46.5^{b}$ | $57.7^{a}$ | $12.6^{a}$ | $99.1^{b}$ |
| CSBM + APB | $55.5^{b}$ | $58.3^{b}$ | $41.6^{a}$ | $51.2^{a}$ | $47.3^{a}$ | $55.6^{b}$ | $12.1^{b}$ | $107.4^{a}$ |
| SEM | 0.26 | 0.27 | 0.19 | 0.24 | 0.22 | 0.26 | 0.06 | 0.473 |
| Main effect of BCAA | | | | | | | | |
| No BCAA | 56.6 | $58.9^{b}$ | $40.6^{b}$ | $49.7^{b}$ | $46.8^{a}$ | $58.5^{a}$ | $13.3^{a}$ | $105.4^{a}$ |
| BCAA | 56.5 | $60.0^{a}$ | $41.7^{a}$ | $50.9^{a}$ | $45.6^{b}$ | $53.6^{b}$ | $11.6^{b}$ | $96.8^{b}$ |
| SEM | 0.22 | 0.22 | 0.16 | 0.19 | 0.18 | 0.21 | 0.05 | 0.39 |
| Interactions of the main effect | | | | | | | | |
| CSBM | 57.8 | $59.9^{ab}$ | $41.0^{bc}$ | 48.6 | $44.6^{b}$ | $58.7^{ab}$ | $13.3^{a}$ | 100.7 |
| CSBM + BCAA | 57.7 | $60.0^{ab}$ | $41.0^{bc}$ | 50.1 | $45.0^{b}$ | $51.1^{e}$ | $11.9^{b}$ | 92.8 |
| CSBM + PM | 56.0 | $58.7^{bc}$ | $40.2^{c}$ | 49.5 | $47.7^{a}$ | $59.2^{a}$ | $13.4^{a}$ | 104.3 |
| CSBM + PM + BCAA | 56.8 | $61.5^{a}$ | $41.6^{ab}$ | 51.2 | $45.2^{b}$ | $56.2^{c}$ | $11.9^{b}$ | 93.8 |
| CSBM + APB | 56.0 | $58.0^{c}$ | $40.6^{bc}$ | 51.0 | $47.9^{a}$ | $57.6^{bc}$ | $13.3^{a}$ | 111.2 |
| CSBM + APB + BCAA | 55.1 | $58.6^{bc}$ | $42.6^{a}$ | 51.4 | $46.7^{a}$ | $53.6^{d}$ | $11.0^{c}$ | 103.6 |
| SEM | 0.37 | 0.39 | 0.27 | 0.33 | 0.31 | 0.37 | 0.08 | 0.67 |
| *P*-values | | | | | | | | |
| Diet | < 0.001 | < 0.001 | 0.018 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| BCAA | 0.875 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Diet × BCAA | 0.059 | 0.002 | 0.001 | 0.09 | < 0.001 | < 0.001 | < 0.001 | 0.068 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Digestible AA intake was calculated by multiplying feed intake by the calculated by digestible concentration of each AA, which was determined by multiplying the calculated digestibility coefficient for each AA in the complete by its analyzed content.

[3] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; PM = peanut meal.

**Table 3.10.** Live weight and carcass characteristics of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d[1]

| | | Hot carcass | | Fat pad | | Chilled carcass | |
|---|---|---|---|---|---|---|---|
| Item[2] | Live weight, kg | Weight, kg | Yield, % | Weight, kg | % | Weight, kg | Yield, % |
| Main effect of diet | | | | | | | |
| CSBM | $3.667^{ab}$ | $2.839^{ab}$ | 77.4 | 0.040 | 1.09 | $2.877^{ab}$ | 78.4 |
| CSBM + PM | $3.700^{a}$ | $2.865^{a}$ | 77.5 | 0.042 | 1.13 | $2.904^{a}$ | 78.5 |
| CSBM + APB | $3.610^{b}$ | $2.784^{b}$ | 77.1 | 0.041 | 1.14 | $2.823^{b}$ | 78.2 |
| SEM | 0.0229 | 0.0185 | 0.14 | 0.0009 | 0.026 | 0.019 | 0.15 |
| Main effect of BCAA | | | | | | | |
| No BCAA | 3.643 | 2.814 | 77.2 | $0.039^{b}$ | $1.07^{b}$ | 2.853 | 78.3 |
| BCAA | 3.675 | 2.845 | 77.4 | $0.043^{a}$ | $1.17^{a}$ | 2.883 | 78.5 |
| SEM | 0.0188 | 0.0152 | 0.12 | 0.0007 | 0.021 | 0.0155 | 0.12 |
| *P*-values | | | | | | | |
| Diet | 0.025 | 0.010 | 0.173 | 0.419 | 0.297 | 0.013 | 0.233 |
| BCAA | 0.234 | 0.151 | 0.211 | < 0.001 | 0.002 | 0.183 | 0.365 |
| Diet × BCAA | 0.443 | 0.251 | 0.185 | 0.519 | 0.664 | 0.264 | 0.165 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; PM = peanut meal.

**Table 3.11.** Carcass parts characteristics of Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d[1]

| | Breast fillets | | Tenders | | Total white meat | | Wings | | Leg quarters | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[2] | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % |
| Main effect of diet | | | | | | | | | | |
| CSBM | 0.883 | 24.0 | 0.161$^{a}$ | 4.39 | 1.044 | 28.4 | 0.221 | 7.68 | 0.835$^{ab}$ | 22.8 |
| CSBM + PM | 0.879 | 23.7 | 0.161$^{a}$ | 4.36 | 1.039 | 28.1 | 0.284 | 7.63 | 0.853$^{a}$ | 23.1 |
| CSBM + APB | 0.859 | 23.7 | 0.157$^{b}$ | 4.32 | 1.015 | 28.1 | 0.279 | 7.75 | 0.820$^{b}$ | 22.7 |
| SEM | 0.0091 | 0.14 | 0.0014 | 0.031 | 0.0101 | 0.15 | 0.0020 | 0.053 | 0.0063 | 0.12 |
| Main effect of BCAA | | | | | | | | | | |
| No BCAA | 0.862$^{b}$ | 23.6$^{b}$ | 0.159 | 4.36 | 1.021$^{b}$ | 28.0$^{b}$ | 0.283 | 7.75 | 0.838 | 23.0 |
| BCAA | 0.885$^{a}$ | 24.1$^{a}$ | 0.160 | 4.35 | 1.045$^{a}$ | 28.4$^{a}$ | 0.280 | 7.63 | 0.835 | 22.8 |
| SEM | 0.0074 | 0.12 | 0.0011 | 0.025 | 0.0082 | 0.12 | 0.0016 | 0.043 | 0.0051 | 0.10 |
| *P*-values | | | | | | | | | | |
| Diet | 0.148 | 0.204 | 0.044 | 0.273 | 0.108 | 0.171 | 0.247 | 0.304 | 0.002 | 0.095 |
| BCAA | 0.032 | 0.009 | 0.535 | 0.697 | 0.042 | 0.016 | 0.184 | 0.062 | 0.750 | 0.091 |
| Diet × BCAA | 0.405 | 0.483 | 0.738 | 0.481 | 0.450 | 0.638 | 0.568 | 0.506 | 0.307 | 0.520 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; PM = peanut meal.

**Table 3.12.** Litter characteristics and footpad lesion scores on d 48 for Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d post-hatch[1]

| | Litter characteristics | | | Footpad lesion scores[3] | |
|---|---|---|---|---|---|
| Item[2] | Moisture, % | pH | Nitrogen, % | No lesions, % | Mild lesions, % |
| Main effect of diet | | | | | |
| CSBM | 27.96 | 9.19 | 2.96 | 86.42 (1.24)$^{b}$ | 13.58 (0.33)$^{a}$ |
| CSBM + PM | 28.19 | 9.25 | 3.02 | 88.29 (1.29)$^{ab}$ | 11.71 (0.28)$^{ab}$ |
| CSBM + APB | 27.30 | 9.13 | 3.10 | 94.08 (1.38)$^{a}$ | 5.92 (0.19)$^{b}$ |
| SEM | 0.363 | 0.074 | 0.048 | 1.89 (0.04) | 1.89 (0.04) |
| Main effect of BCAA | | | | | |
| No BCAA | 28.11 | 9.13 | 3.16$^{a}$ | 88.14 (1.28) | 11.86 (0.29) |
| BCAA | 27.52 | 9.24 | 2.89$^{b}$ | 91.06 (1.33) | 8.94 (0.24) |
| SEM | 0.296 | 0.060 | 0.039 | 1.54 (0.03) | 1.54 (0.03) |
| *P*-values | | | | | |
| Diet | 0.200 | 0.543 | 0.153 | 0.041 | 0.041 |
| BCAA | 0.160 | 0.197 | < 0.001 | 0.220 | 0.220 |
| Diet × BCAA | 0.999 | 0.999 | 0.053 | 0.178 | 0.178 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment with n of 24 and 36 for the main effects of diet type and BCAA inclusion, respectively.

[2] Abbreviations: APB = animal protein blend; BCAA = branched chain amino acids; CSBM = corn and soybean meal; PM = peanut meal.

[3] Score of 0 indicated no lesions, score of 1 indicated mild lesions, and a score of 2 indicated sever lesions. A score of 2 was not recorded, therefore removed from the table. Day 48 lesion scores were subjected to square root arcsine transformation for statistical analysis and transformed data are reported in parenthesis.

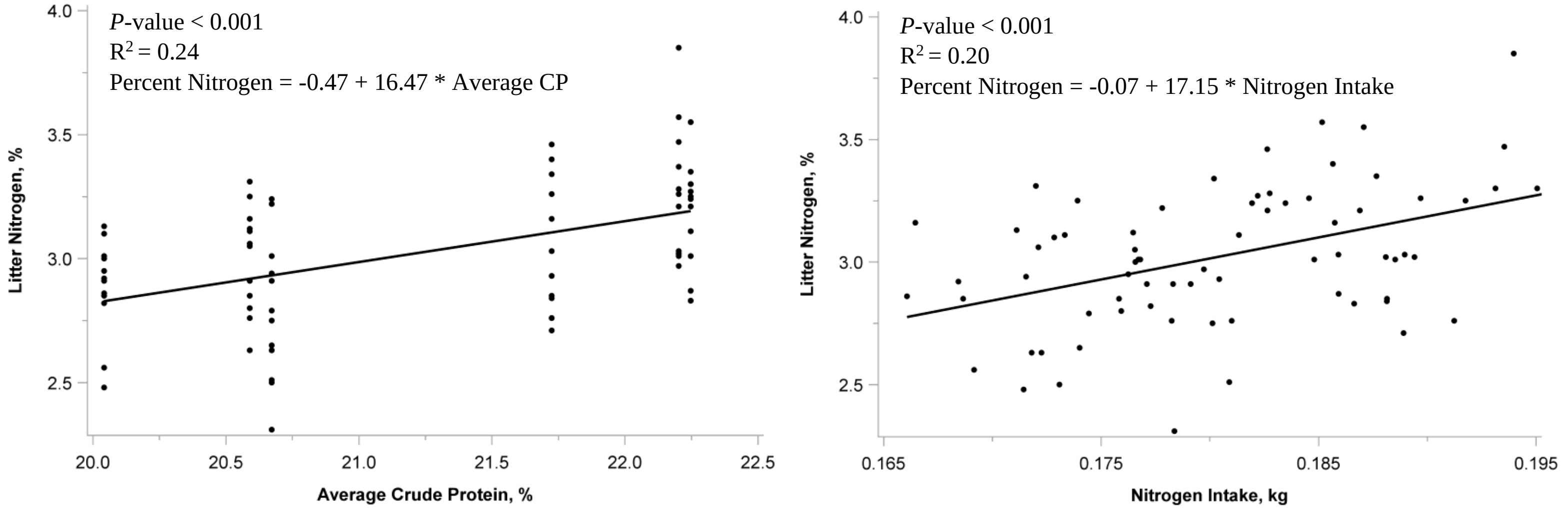

**Figure 3.1.** Linear responses of litter nitrogen concentration (%) to total nitrogen intake (g/bird) or analyzed dietary CP content (%) determined using Ross 708 broilers fed diets varying in ingredient composition with or without BCAA supplementation from 0 to 48 d post-hatch[1]

# CHAPTER IV:

# EVALUATION OF A THREONINE FERMENTATION PRODUCT AS A DIGESTIBLE THREONINE SOURCE IN BROILERS

## ABSTRACT

Residual biomass from bacterial fermentation processes to produce feed-grade amino acids can be viable sources of digestible nutrients. In the present experiment, a Thr fermentation product (**FP**) (75% minimum digestible Thr) was evaluated as a replacement for crystalline L-Thr (98.5% minimum digestible Thr) as a source of digestible Thr and other nutrients to support broiler performance (0 to 48 d) and processing characteristics. Birds were allocated to 1 of 5 diets: 1) a positive control (**PC**) diet containing L-Met, L-Lys, and L-Thr, 2) a reduced protein control (**RPC**) diet with additions of L-Val, L-Ile, and L-Arg, 3) a negative control (**NC**) identical to the RPC except that cellulose was added at the expense of L-Thr, 4) a RPC diet with Thr FP used in place of L-Thr with only accounting for digestible Thr and energy contributions of the FP (**TFP**), or 5) a RPC diet with Thr FP used in place of L-Thr and accounting for the full nutrient matrix of the Thr FP (**TFP-FM**). Overall, broilers fed the RPC diet had increased feed intake, whereas broilers fed the NC diet reduced body weight gain and increased FCR. Birds fed the TFP diet sustained performance; however, birds fed the TFP-FM diet reduced feed intake and body weight gain. At processing, broilers fed the RPC diet had increased relative and absolute breast weights, as well as heavier fat pads compared to broilers fed the PC diet. Debone parts yields for the Thr FP groups and NC group were similar to the RPC group.

## INTRODUCTION

Threonine is generally considered to be the third limiting amino acid (**AA**) for broilers fed corn and soybean meal-based diets (Han et al., 1992; Fernandez et al., 1994; Kidd and Kerr, 1996). Regarding metabolism, Thr is important in the synthesis of proteins (Fisher et al., 1981; Stilborn et al., 2010), mucin glycoproteins (Mantle and Allen, 1981), immunoglobulins (Zhang et al., 2016), and uric acid (Baker et al., 1972; Corzo et al., 2009). The most recent estimates for the dietary ratio of digestible Thr to digestible Lys range from 63 to 69 for optimal broiler FCR, body weight gain (**BWG**), and breast yield (Mejia et al., 2012; Dozier et al., 2015), and these estimates closely agree with current primary breeder recommendations that range from 65 to 68 (Cobb-Vantress, 2018; Aviagen, 2019) across broiler feeding phases. Moderate reductions of the Thr to Lys ratio have been shown to increase broiler FCR, whereas larger reductions can negatively affect BWG, feed intake (**FI**), and breast yield (Mejia et al., 2012; Dozier et al., 2015).

The majority of supplemental AA are produced via fermentation and purified in crystalline form after separation from the fermentation biomass. However, the fermented biomass contains other essential and nonessential AA, as well as vitamins, minerals, and energy, and feeding a fermentation by-product could provide additional nutrients while reducing manufacturing costs associated with separation of the target AA. A previous study showed that a Thr fermentation by-product broth had a relative bioavailability ranging from 98.7 to 100.2% when compared with feed-grade crystalline L-Thr (98.5% digestible Thr) (Dozier et al., 2003). More recently, Wensley et al. (2019) demonstrated that supplementing a Thr fermentation product (**FP**) (75% minimum digestible Thr) in place of feed-grade crystalline L-Thr maintained broiler performance to 28 d post-hatch. However, these authors did not account for the additional nutrients supplied by the Thr FP and only evaluated the performance of young broilers.

It is becoming increasingly common for poultry nutritionists to offer additional AA beyond Met, Lys, and Thr, including L-Val, L-Ile, and L-Arg in formulation, to further reduce dietary CP. These AA are used at the expense of intact proteins to reduce diet cost without impairing live performance or processing yields. Other benefits of reducing dietary CP include improved environmental sustainability and bird welfare (Van Harn et al., 2019; Lemme et al., 2019). However, as additional limiting AA such as L-Val, L-Ile, or L-Arg are included in diets, higher inclusions of Met, Lys, and Thr are needed to meet formulation minimums, placing an importance on confirming the efficacy of novel, alternative AA supplements. Therefore, the objective of this experiment was to evaluate a Thr FP (75% minimum digestible Thr) as a substitute for feed-grade crystalline L-Thr (98.5% minimum digestible Thr), with and without applying the nutrient and energy matrix values of the fermentation biomass fraction, when fed to broilers from 0 to 48 d post-hatch. It was hypothesized that reducing digestible Thr would impair broiler performance and processing characteristics, and that meeting minimum dietary Thr levels by supplementing either Thr FP or crystalline L-Thr would equally restore growth performance and processing yields of broilers.

## MATERIALS AND METHODS

All procedures involving live birds were approved by University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

### *Bird Husbandry*

A total of 1,260 male chicks from a Ross 708 breeder line (Aviagen North America, Sallisaw, OK) were hatched and transported to the University of Arkansas poultry research farm. All chicks were administered a vaccine against Marek’s disease in ovo. Upon arrival, chicks were selected and weighed to ensure that each group weight of 21 chicks fell within 3% of the expected average BW based on a preliminary weight of approximately one-third of the population. Chicks were then allocated to 60 floor pens (0.09 $m^2$/bird; 21 birds/pen) in a solid-sided, tunnel-ventilated research barn. The barn was equipped with exhaust fans, stir fans, radiant tube heaters, vent boards, and an electronic controller to maintain target environmental temperatures. Each pen contained one hanging feeder, a section of the continuous nipple drinker line that extended through each row of pens (4 total rows; 5 nipples/pen), and bedding of de-caked litter with a top dressing of fresh pine shavings. All birds had access to feed and water ad libitum for the duration of the experiment. Ambient temperature at chick placement was set at 33°C and gradually decreased to maintain bird comfort as they aged until a final set point of 20°C was reached. The photoperiod was set at 23L:1D from placement to 7 d of age, 16L:8D from 8 to 28 d of age, and 18L:6D from 29 d until the end of the experiment. Light intensity was set at 27 lux from 1 to 7 d of age, 16 lux from 8 to 14 d of age, and 1 lux from 15 d to the end of the trial.

### *Dietary Treatments*

On d of placement, all birds were provided 1 of 5 corn-soybean meal-based dietary treatments within a block of pens of similar location in the house, with 12 replicate pens per treatment. Dietary treatments were as follows: 1) a positive control (**PC**) diet with feed-grade L-Met, L-Lys·HCl, and L-Thr, 2) a reduced protein control (**RPC**) diet formulated to the same AA specifications as the PC diet but with feed-grade L-Val, L-Ile (all phases) and L-Arg (finisher and withdrawal phases only), 3) a negative control (**NC**) diet identical to the RPC diet except that cellulose was added at the expense of L-Thr, 4) the RPC diet with the Thr FP used in place of L-Thr, accounting for only the digestible Thr and energy contributions of the Thr FP (**TFP**), or 5) a diet similar to the TFP diet but with the full nutrient matrix of the Thr FP applied (**TFP-FM**). Prior to formulation, samples of corn, soybean meal, and the Thr FP were analyzed for total AA and CP content (Eurofins Nutrient Analysis Center, Des Moines, IA), and finished experimental feeds from each phase were analyzed for nutrient content at the same laboratory.

Nutrient and energy specifications of the Thr FP provided by the manufacturer were used in formulation and were (as-is basis) 3,197 kcal/kg $AME_n$, 70% CP, and 75, 0.31, 0.17, 0.48, 0.35, 0.44, and 0.04% digestible Thr, Lys, TSAA, Val, Ile, Arg, and Trp, respectively. All diets, excluding the NC, were formulated to meet or exceed primary breeder recommendations (Aviagen, 2019) and to be isocaloric with the same minimum digestible AA to digestible Lys ratios within each phase. Starter diets were formulated to contain 1.28% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 74, 67, 75, 67, 105, and 21% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 4.1). Grower diets were formulated to contain 1.15% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 76, 67, 76, 68, 105, and 21% for TSAA, Thr, Val, Ile, Arg, and Trp, respectively (Table 4.1). Finisher diets were formulated to contain 1.02% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 78, 67, 76, 69, 107, and 19% for TSAA, Thr, Val, Ile, Arg and Trp, respectively (Table 4.2). Withdrawal diets were formulated to contain 0.96% digestible Lys with minimum ratios of digestible AA relative to Lys (100%) of 78, 67, 78, 69, 108, and 18% for TSAA, Thr, Val, Ile, Arg and Trp, respectively (Table 4.2). The NC diet was formulated to contain ratios of digestible Thr relative to Lys (100%) of 56, 56, 53, and 53% for the starter, grower, finisher, and withdrawal phases, respectively. Additionally, replacement of L-Thr (3,570 kcal/kg) with cellulose (inert space filler; no energy matrix applied) in the NC diet resulted in an approximately 5 kcal/kg reduction in $AME_n$ for each growth phase.

### *Determination of Growth Performance*

All dietary treatments were maintained throughout all broiler growth phases, which consisted of starter (0 to 14 d), grower (14 to 28 d), finisher (28 to 39 d), and withdrawal (39 to 48 d) phases.

### *Processing*

All birds were weighed on d 48 before a total of 360 birds (6 birds/pen) were randomly selected to be wing-banded and processed. On d 49, following a 10 hr feed removal, banded birds were transported to the University of Arkansas Pilot Processing Plant and weighed individually prior to processing. Birds were humanely electrically stunned prior to exsanguination via jugular vein incision. Carcasses were then scalded and defeathered and the neck, head, and feet were removed. After hand-removal of the viscera and lungs, hot carcass and fat pad weights were recorded. Hot carcasses were submerged in an ice water bath for 3 hr, and chilled carcasses were weighed and manually deboned to collect weights of breast, tenders, wings, and leg quarters. Percentage yield of each of these parts was determined relative to individual back dock live BW.

### *Statistical Analysis*

Dietary treatments were arranged in a randomized complete block design with pen location as the blocking factor. Broiler diets were considered fixed effects and replications random. Data were analyzed within growth phase using a one-way ANOVA using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). Following detection of statistically significant ANOVA *P*-values, means were subjected to a Tukey's honestly significant difference test for post-hoc mean separation. In addition to the ANOVA, pre-planned orthogonal contrasts were conducted to investigate 4 primary research questions: PC vs. RPC, RPC vs. NC, RPC vs. TFP, and RPC vs. TRP-FM. Mortality data were subjected to a square root arcsine transformation prior to analysis. Statistical significance was considered at $P \leq 0.05$ in all cases.

## RESULTS AND DISCUSSION

The objective of reducing dietary CP was to facilitate greater contributions of supplemental feed-grade AA to meet minimum digestible AA requirements, which was achieved. The analyzed CP reduction from the PC diet to the RPC diet averaged 0.77 percentage units across all phases, less than the calculated overall average 1.04 percentage units (Table 4.1- 4.2). The NC diet was formulated with cellulose at the expense of supplemental Thr and was calculated to have 17.5, 15.6, 20.6, and 20.3% less digestible Thr for the starter, grower, finisher, and withdrawal phases, respectively. Analyzed total Thr levels of the NC diet were reduced by 11.0, 11.5, 14.1, and 17.1% for the starter, grower, finisher, and withdrawal phases, respectively, when compared to the RPC (Table 4.3). All other analyzed AA contents were in close agreement among dietary treatments. The Thr FP diets were formulated using either its digestible Thr and energy contributions only or its full energy and nutrient matrix (digestible Thr and other digestible AA). Applying the full matrix of the Thr FP resulted in minor changes in corn, soybean meal, and supplemental Lys and Ile inclusions (Table 4.1- 4.2).

Growth performance and processing measurements are shown in Tables 4.4- 4.10. Mortality was unaffected ($P > 0.05$) in every phase and cumulatively from 0 to 48 d (data not shown). From 0 to 14 d, dietary treatments influenced broiler BWG ($P = 0.019$), FI ($P = 0.043$), and FCR ($P = 0.025$). Broilers fed the RPC, which had additions of feed-grade L-Val and L-Ile, had similar ($P > 0.05$) BWG, FI, and FCR compared to broilers fed the PC diet. Broilers offered the NC diet with an 11.0% (analyzed) average reduction in dietary Thr during the starter phase were not affected ($P > 0.05$) compared to the performance of birds fed the RPC diet.

Interestingly, FI and BWG were highest for birds fed TFP, lowest for birds fed TFP-FM, and intermediate for birds fed the PC, RPC, or NC diets. Broiler FCR ($P > 0.05$), however, was not different between birds receiving either of the Thr FP-containing diets. The lower FI and BWG for birds fed the TFP-FM diet compared with those fed the TFP diet is not readily explainable given the slight differences in ingredient composition between these diets. Nevertheless, both Thr FP-fed groups were similar ($P > 0.05$) in performance when compared to the RPC-fed birds.

From 0 to 28 d, broiler BWG ($P = 0.026$) and FCR ($P = 0.041$), but not FI ($P > 0.05$), were impacted by dietary treatments. Similarly to the starter phase, broilers that received the RPC diet continued to have equal ($P > 0.05$) performance compared to those fed the PC diet, with no effects ($P > 0.05$) of digestible Thr reduction on FI, BWG, or FCR for broilers offered the NC diet. Birds fed either of the Thr FP-containing diets had similar ($P > 0.05$) performance to the RPC-fed birds, excluding BWG ($P = 0.032$) of the birds fed the TFP-FM diet. Similarly, Wensley et al. (2019) reported that male Cobb 500 broilers had similar weight gain and feed efficiency from 0 to 28 d when supplementing the Thr FP in place of crystalline L-Thr in diets containing 21 and 19% CP in the starter (0 to 14 d) and grower (14 to 28 d) phases, respectively.

For the finisher (28 to 39 d) and withdrawal (39 to 48 d) phases, the RPC diet was supplemented with L-Arg in addition to L-Val and L-Ile to facilitate a greater reduction in digestible Thr content for the NC diet. The difference in the digestible Thr to Lys ratio between the RPC and NC diets increased from 15.6% in the 14 to 28 d phase to 20.6% in the 28 to 39 d phase. From 0 to 39 d, dietary treatments influenced BWG ($P < 0.001$), FCR ($P < 0.001$), and FI ($P < 0.05$). Unlike 0 to 14 d and 0 to 28 d, broilers that received the RPC diet had greater BWG ($P = 0.006$) and tended to have lower FCR ($P = 0.059$) as well as similar FI ($P > 0.05$) compared to birds fed the PC diet. Additionally, birds provided the NC diet had reduced BWG ($P < 0.001$)

and increased FCR (*P* < 0.001), but similar FI (*P* > 0.05) compared to RPC-fed birds. It is not clear if the effect of Thr reduction in the NC diet on broiler performance was due to the greater reduction of Thr from 28 to 39 d, increasing maintenance needs for Thr in older birds, or a combination of the two. Compared with the RPC group, broilers fed the TFP diet were similar (*P* > 0.05) in performance, whereas broilers fed the TFP-FM had reduced BWG (*P* = 0.002) and FI (*P* = 0.025) and higher FCR (*P* = 0.010).

From 0 to 48 d, broiler BWG (*P* = 0.026), FI (*P* = 0.042), and FCR (*P* < 0.001) varied among dietary treatments. Broilers fed either the PC or RPC diet had similar (*P* > 0.05) BWG and FCR, but those provided the RPC diet had greater FI (*P* = 0.040) than birds fed the PC diet. Increased feed consumption of broilers fed reduced CP diets had been previously reported, possibly to satisfy a limiting essential AA need (Summers et al., 1992). The NC-fed birds had a higher FCR (*P* = 0.001) and lower BWG (*P* = 0.014), but similar FI (*P* > 0.05) compared to the RPC-fed birds. As in earlier phases, broilers fed the TFP were similar (*P* > 0.05) in all performance parameters to the RPC-fed, while birds fed the TFP-FM diet had lower FI (*P* = 0.028) and BWG (*P* = 0.025), but comparable FCR (*P* > 0.05) to those fed the RPC diet.

Analyzed Thr intake was highest (*P* < 0.001) for the RPC (46.1 g) group, intermediate for the TFP-FM (45.3 g) group, moderate for the PC (45.1 g) and TFP (44.6 g) groups, and lowest for the NC (39.6 g) group (Table 4.8). The efficiency of Thr utilization for BWG was highest (*P* < 0.001) for the NC (86%), intermediate for the TFP (78%), and lowest for PC (76%), RPC (75%), and TFP-FM (75%) groups. Broilers fed marginal levels of a single AA are often more efficient in utilization of that particular AA for growth (Park, 2006), as previously demonstrated for Thr specifically (Çiftci and Ceylan, 2004). The Thr efficiency of broilers fed either of the Thr FP diets was either similar or superior to that of RPC-fed birds, indicating that the efficacy of

Thr FP is comparable to that of crystalline L-Thr in broiler diets. However, further research is warranted to quantify the bioavailability of the Thr FP relative to feed grade L-Thr in broilers.

For processed birds, live, hot carcass, and chilled carcass weights, but not yield ($P >$ 0.05), of birds fed the RPC diet were greater ($P < 0.05$) than those of birds fed the PC and TFP-FM diets, but similar ($P > 0.05$) to those fed the TFP and NC diets. The relative ($P = 0.001$) and absolute ($P = 0.002$) weights of fat pads were lowest for the PC group, intermediate for the RPC group, and highest for the NC, TFP, and TFP-FM groups. In agreement, others have reported that feeding reduced CP diets increased fat pad deposition (Fraps, 1943; Moran and Stilborn, 1996; Lee et al., 2020). Birds fed greater protein levels may have lower fat pad deposition due to the increased heat increment involved in the deamination and transamination of surplus AA to other metabolites and finally uric acid (Namroud et al., 2008). It has also been suggested that increasing the calorie to protein ratio will increase fat deposition, and typically broilers will increase FI when fed reduced CP diets and will consume more ME that will be stored as fat (Bartov et al. 1974; Belloir et al. 2017)

Dietary treatments did not affect deboned part yields for tenders ($P > 0.05$), total white meat ($P > 0.05$), and leg quarters ($P > 0.05$). Wing yield ($P < 0.001$) was highest for broilers provided the PC while all other treatments were lowest. Additionally, breast fillet weight ($P =$ 0.008) and yield ($P = 0.029$) and total white meat weight ($P = 0.012$) of the RPC birds were greater than those of the PC birds, with a trend ($P = 0.056$) for greater total white meat yield. Previous work reported similar findings, where broilers provided reduced CP diets with feed-grade L-Met, L-Lys·HCl, L-Thr, L-Val, and L-Ile had greater breast fillet weight and yield than broilers fed a higher CP diet (Lee et al., 2020). Weights and yields of debone parts for both of the Thr FP groups and the NC group were similar ($P > 0.05$) to the RPC group, excluding reduced weights for wings (*P* = 0.011) and leg quarters (*P* = 0.041) of the TFP-FM group. It has been suggested that the Thr requirements for Ross broilers were lowest for weight gain, moderate for breast meat, and highest for FCR (Mack et al., 1999; Kidd, 2000). A 15% reduction of Thr in the diets of Ross PM3 broilers (male and female) from 0 to 42 d only impacted FCR, but a 23% (0 to 21 d: 7.8 to 6.0 g Thr/kg) reduction of Thr evoked responses for FI, BWG, and breast weight (Çiftci and Ceylan, 2004). Ospina-Rojas et al. (2014) fed reduced digestible Thr to Lys ratios from 67.5 to 57 to male Cobb 500 broilers from 22 to 42 d and detected a linear response for FCR, with no effects on BWG or breast yield. Mejia et al. (2012) evaluated male Ross 708 broilers from 35 to 49 d and observed reduced BWG and FCR when the digestible Thr to Lys ratio was lowered from 66 to 43, whereas further reduction of the ratio to 35 negatively affected broiler FI and breast yield.

In the current study, reducing the digestible Thr to Lys ratio from 67 to 56 (starter and grower) to 53 (finisher and withdrawal) in diets fed to male Ross 708 broilers impacted BWG and FCR but not breast fillet yield. An additional consideration for this study is responses to the NC diet might have been greater under more challenging conditions such as disease, stress, or poor environmental hygiene (Corzo et al., 2007; Star et al., 2012; Taghinejad-Roudbaneh et al., 2013). Nonetheless, the Thr levels of the NC were low enough to possibly detect differences in the bioavailability of the Thr FP. Supplementing the Thr FP as a substitute to crystalline L-Thr in reduced CP diets adequately supported broiler performance. However, when formulating diets to the full nutrient matrix of the Thr FP, broiler BWG was reduced by the decrease in FI, the cause of which is unclear. Nonetheless, processing yields were similar among dietary treatments.

## CONCLUSIONS AND APPLICATIONS

1. Inclusion of feed-grade L-Val, L-Ile, and L-Arg reduced dietary CP by an average of 0.77 percentage units from 0 to 48 d and resulted in similar BWG and FCR, as well as improved breast weight and yield to broilers fed the PC diet.
2. Removal of feed-grade L-Thr from the RPC diet, which lowered the digestible Thr to Lys ratio from 67 to 56 (starter and grower) to 53 (finisher and withdrawal), negatively impacted FCR and BWG, but not processing yields.
3. The Thr FP (75% minimum digestible Thr) was efficacious in replacing crystalline L-Thr (98.5% minimum digestible Thr) to restore BWG and FCR when formulating diets to equal digestible Thr levels in reduced CP, corn and soybean meal-based diets from 0 to 48 d.

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**Table 4.1**. Ingredient and calculated nutrient composition (%, unless otherwise noted) of experimental diets fed to broilers from 0 to 14 d and 14 to 28 d post-hatch[1]

| | 0 to 14 d | | | | | 14 to 28 d | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient, as-fed | PC | RPC | NC | TFP | TFP-FM | PC | RPC | NC | TFP | TFP-FM |
| Corn | 55.85 | 58.05 | 58.05 | 58.05 | 58.08 | 60.68 | 62.28 | 62.28 | 62.28 | 62.31 |
| Soybean meal (48%) | 38.03 | 36.00 | 36.00 | 36.00 | 35.97 | 32.72 | 31.23 | 31.23 | 31.23 | 31.21 |
| Poultry fat | 2.50 | 2.10 | 2.10 | 2.10 | 2.10 | 3.28 | 2.98 | 2.98 | 2.98 | 2.98 |
| Limestone | 1.10 | 1.12 | 1.12 | 1.12 | 1.12 | 1.03 | 1.04 | 1.04 | 1.04 | 1.04 |
| Dicalcium phosphate | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 |
| Sodium chloride | 0.28 | 0.25 | 0.25 | 0.25 | 0.25 | 0.27 | 0.26 | 0.26 | 0.26 | 0.26 |
| L-Met | 0.31 | 0.33 | 0.33 | 0.33 | 0.33 | 0.29 | 0.30 | 0.30 | 0.30 | 0.30 |
| L-Lys·HCl | 0.13 | 0.20 | 0.20 | 0.20 | 0.20 | 0.14 | 0.19 | 0.19 | 0.19 | 0.18 |
| L-Thr | 0.12 | 0.15 | - | - | - | 0.11 | 0.13 | - | - | - |
| ThrPro (75% dThr only) | - | - | - | 0.19 | - | - | - | - | 0.16 | - |
| ThrPro-FM (75% dThr) | - | - | - | - | 0.19 | - | - | - | - | 0.16 |
| Cellulose | - | 0.05 | 0.19 | - | - | - | 0.04 | 0.16 | - | - |
| L-Val | - | 0.03 | 0.03 | 0.03 | 0.03 | - | 0.02 | 0.02 | 0.02 | 0.02 |
| L-Ile | - | 0.01 | 0.01 | 0.01 | 0.01 | - | 0.02 | 0.02 | 0.02 | 0.02 |
| Vitamin premix[3] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Se premix[4] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.04 | 0.05 | 0.05 | 0.05 | 0.05 |
| Sodium bicarbonate | 0.18 | 0.21 | 0.21 | 0.21 | 0.21 | 0.19 | 0.22 | 0.22 | 0.22 | 0.22 |
| Copper chloride[5] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Coccidiostat[6] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Phytase[7] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | | | | | |
| $AME_n$, kcal/kg | 3,000 | 3,000 | 2,995 | 3,000 | 3,000 | 3,100 | 3,100 | 3,096 | 3,100 | 3,100 |
| CP | 22.86 | 22.20 | 22.07 | 22.20 | 22.19 | 20.65 | 20.17 | 20.06 | 20.17 | 20.16 |
| Digestible Lys | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 |
| Digestible Met | 0.66 | 0.67 | 0.67 | 0.67 | 0.67 | 0.61 | 0.62 | 0.62 | 0.62 | 0.62 |
| Digestible TSAA | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Digestible Val | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Digestible Ile | 0.88 | 0.86 | 0.86 | 0.86 | 0.86 | 0.79 | 0.78 | 0.78 | 0.78 | 0.78 |
| Digestible Arg | 1.40 | 1.34 | 1.34 | 1.34 | 1.34 | 1.25 | 1.21 | 1.21 | 1.21 | 1.21 |
| Digestible Trp | 0.28 | 0.27 | 0.27 | 0.27 | 0.27 | 0.25 | 0.24 | 0.24 | 0.24 | 0.24 |
| Total Gly+Ser | 2.12 | 2.05 | 2.05 | 2.05 | 2.05 | 1.92 | 1.87 | 1.87 | 1.87 | 1.87 |
| Total Ca | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Available P | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 |
| Analyzed composition | | | | | | | | | | |
| CP | 23.41 | 23.07 | - | - | - | 21.93 | 21.40 | - | - | - |
| EE | 4.66 | 4.44 | - | - | - | 5.56 | 5.50 | - | - | - |
| Total Lys | 1.43 | 1.50 | 1.59 | 1.48 | 1.53 | 1.31 | 1.35 | 1.36 | 1.50 | 1.30 |
| Total TSAA | 0.95 | 0.93 | 0.93 | 0.96 | 0.95 | 0.82 | 0.83 | 0.89 | 0.89 | 0.90 |
| Total Val | 1.11 | 1.13 | 1.16 | 1.14 | 1.09 | 1.03 | 1.06 | 1.05 | 1.07 | 1.01 |
| Total Ile | 0.99 | 0.99 | 1.03 | 1.01 | 0.96 | 0.91 | 0.92 | 0.94 | 0.95 | 0.89 |
| Total Arg | 1.56 | 1.49 | 1.53 | 1.55 | 1.41 | 1.41 | 1.41 | 1.44 | 1.40 | 1.35 |
| Total Trp | 0.73 | 0.69 | 0.74 | 0.76 | 0.68 | 0.67 | 0.68 | 0.71 | 0.68 | 0.67 |

[1] Abbreviations: $AME_n$ = nitrogen-corrected apparent metabolizable energy; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[3] The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin $D_3$, 4,536 ICU; vitamin E, 45 IU; vitamin $B_{12}$ 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[5] IntelliBond® C, Micronutrients, Indianapolis, IN.

[6] Salinomycin sodium, Bio-Cox®, Huvepharma Inc., Sofia, Bulgaria.

[7] Optiphos®, Huvepharma Inc., Sofia, Bulgaria.

**Table 4.2**. Ingredient and calculated nutrient composition (%, unless otherwise noted) of experimental diets fed to broilers from 28 to 39 d and 39 to 48 d post-hatch[1]

| | 28 to 39 d | | | | | 39 to 48 d | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient, as-fed | PC | RPC | NC | TFP | TFP-FM | PC | RPC | NC | TFP | TFP-FM |
| Corn | 65.44 | 70.26 | 70.26 | 70.26 | 70.26 | 68.46 | 73.28 | 73.28 | 73.28 | 73.28 |
| Soybean meal (48%) | 27.43 | 22.88 | 22.88 | 22.88 | 22.89 | 25.18 | 20.63 | 20.63 | 20.63 | 20.64 |
| Poultry fat | 4.08 | 3.16 | 3.16 | 3.16 | 3.16 | 3.53 | 2.61 | 2.61 | 2.61 | 2.61 |
| Limestone | 0.99 | 1.02 | 1.02 | 1.02 | 1.02 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 |
| Dicalcium phosphate | 0.70 | 0.72 | 0.72 | 0.72 | 0.72 | 0.59 | 0.61 | 0.61 | 0.61 | 0.61 |
| Sodium chloride | 0.27 | 0.23 | 0.23 | 0.23 | 0.23 | 0.27 | 0.23 | 0.23 | 0.23 | 0.23 |
| L-Met | 0.26 | 0.30 | 0.30 | 0.30 | 0.30 | 0.23 | 0.27 | 0.27 | 0.27 | 0.27 |
| L-Lys·HCl | 0.14 | 0.29 | 0.29 | 0.29 | 0.28 | 0.14 | 0.28 | 0.28 | 0.28 | 0.28 |
| L-Thr | 0.09 | 0.15 | - | - | - | 0.08 | 0.14 | - | - | - |
| ThrPro (75% dThr only) | - | - | - | 0.20 | - | - | - | - | 0.18 | - |
| ThrPro-FM (75% dThr) | - | - | - | - | 0.20 | - | - | - | - | 0.18 |
| Cellulose | - | 0.05 | 0.20 | - | - | - | 0.04 | 0.18 | - | - |
| L-Val | - | 0.06 | 0.06 | 0.06 | 0.06 | - | 0.07 | 0.07 | 0.07 | 0.07 |
| L-Ile | - | 0.07 | 0.07 | 0.07 | 0.07 | - | 0.07 | 0.07 | 0.07 | 0.06 |
| L-Arg | - | 0.12 | 0.12 | 0.12 | 0.12 | - | 0.13 | 0.13 | 0.13 | 0.13 |
| Vitamin/TM premix[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Se premix[4] (0.06%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Choline chloride (60%) | 0.04 | 0.06 | 0.06 | 0.06 | 0.06 | 0.04 | 0.05 | 0.05 | 0.05 | 0.05 |
| Sodium bicarbonate | 0.21 | 0.29 | 0.29 | 0.29 | 0.29 | 0.21 | 0.29 | 0.29 | 0.29 | 0.29 |
| Copper chloride[5] (54%) | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Coccidiostat[6] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | - | - | - | - | - |
| Phytase[7] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Nutrient composition, calculated unless otherwise noted | | | | | | | | | | |
| $AME_n$, kcal/kg | 3,200 | 3,200 | 3,195 | 3,200 | 3,200 | 3,200 | 3,200 | 3,195 | 3,200 | 3,200 |
| CP | 18.44 | 16.94 | 16.80 | 16.94 | 16.94 | 17.55 | 16.05 | 15.92 | 16.05 | 16.05 |
| Digestible Lys | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
| Digestible Met | 0.55 | 0.57 | 0.57 | 0.57 | 0.57 | 0.51 | 0.53 | 0.53 | 0.53 | 0.53 |
| Digestible TSAA | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Digestible Val | 0.79 | 0.78 | 0.78 | 0.78 | 0.78 | 0.76 | 0.75 | 0.75 | 0.75 | 0.75 |
| Digestible Ile | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.67 | 0.66 | 0.66 | 0.66 | 0.66 |
| Digestible Arg | 1.10 | 1.09 | 1.09 | 1.09 | 1.09 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 |
| Digestible Trp | 0.22 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.18 | 0.18 | 0.18 | 0.18 |
| Total Gly+Ser | 1.72 | 1.55 | 1.55 | 1.55 | 1.55 | 1.64 | 1.47 | 1.47 | 1.47 | 1.48 |
| Total Ca | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 |
| Available P | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.48 | 0.47 | 0.47 | 0.47 | 0.47 |
| Analyzed composition | | | | | | | | | | |
| CP | 18.71 | 17.56 | - | - | - | 18.33 | 17.29 | - | - | - |
| EE | 6.06 | 5.28 | - | - | - | 5.80 | 5.02 | - | - | - |
| Total Lys | 1.18 | 1.14 | 1.15 | 1.20 | 1.25 | 1.08 | 1.07 | 1.06 | 1.05 | 1.08 |
| Total TSAA | 0.77 | 0.79 | 0.78 | 0.79 | 0.79 | 0.74 | 0.72 | 0.71 | 0.73 | 0.76 |
| Total Val | 0.93 | 0.89 | 0.90 | 0.88 | 0.88 | 0.87 | 0.84 | 0.83 | 0.85 | 0.84 |
| Total Ile | 0.80 | 0.80 | 0.79 | 0.78 | 0.78 | 0.76 | 0.74 | 0.72 | 0.72 | 0.72 |
| Total Arg | 1.21 | 1.22 | 1.21 | 1.19 | 1.16 | 1.16 | 1.16 | 1.12 | 1.14 | 1.14 |
| Total Trp | 0.60 | 0.59 | 0.57 | 0.54 | 0.52 | 0.57 | 0.52 | 0.52 | 0.51 | 0.49 |

[1] Abbreviations: $AME_n$ = nitrogen-corrected apparent metabolizable energy; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[3] The vitamin premix contained (per kg of diet):

[4] The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[5] Supplied 0.12 mg of selenium per kg of diet.

[5] IntelliBond® C, Micronutrients, Indianapolis, IN.

[7] Optiphos®, Huvepharma Inc., Sofia, Bulgaria.

**Table 4.3**. Digestible (calculated [**C**]) and total (calculated and analyzed [**A**]) Thr (%, as-fed) concentration of experimental diets[1]

| Item | | PC | RPC | NC | TFP | TFP-FM |
|---|---|---|---|---|---|---|
| *Starter* | | | | | | |
| dThr | C | 0.86 | 0.86 | 0.71 | 0.86 | 0.86 |
| Total Thr | C | 1.00 | 0.99 | 0.85 | 0.99 | 0.99 |
| Total Thr | A | 1.01 | 1.00 | 0.89 | 1.04 | 0.97 |
| *Grower* | | | | | | |
| dThr | C | 0.77 | 0.77 | 0.65 | 0.77 | 0.77 |
| Total Thr | C | 0.89 | 0.89 | 0.77 | 0.89 | 0.89 |
| Total Thr | A | 0.92 | 0.96 | 0.85 | 0.94 | 0.93 |
| *Finisher* | | | | | | |
| dThr | C | 0.68 | 0.68 | 0.54 | 0.68 | 0.68 |
| Total Thr | C | 0.79 | 0.78 | 0.63 | 0.78 | 0.78 |
| Total Thr | A | 0.80 | 0.78 | 0.67 | 0.81 | 0.80 |
| *Withdrawal* | | | | | | |
| dThr | C | 0.64 | 0.64 | 0.51 | 0.64 | 0.64 |
| Total Thr | C | 0.75 | 0.73 | 0.60 | 0.73 | 0.73 |
| Total Thr | A | 0.75 | 0.76 | 0.63 | 0.75 | 0.77 |

[1] Abbreviations:NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix; dThr = digestible threonine.

**Table 4.4.** Live performance of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 14 d post-hatch[1,2]

| Item[3] | d 0 BW, kg | d 14 BW, kg | 0 to 14 d BWG, kg | 0 to 14 d FI, kg | 0 to 14 d FCR[4], kg:kg |
|---|---|---|---|---|---|
| PC | 0.047 | 0.444$^{ab}$ | 0.397$^{ab}$ | 0.489$^{ab}$ | 1.245$^{b}$ |
| RPC | 0.046 | 0.439$^{ab}$ | 0.392$^{ab}$ | 0.494$^{ab}$ | 1.277$^{ab}$ |
| NC | 0.046 | 0.437$^{ab}$ | 0.391$^{ab}$ | 0.498$^{ab}$ | 1.287$^{a}$ |
| TFP | 0.046 | 0.450$^{a}$ | 0.404$^{a}$ | 0.506$^{a}$ | 1.262$^{ab}$ |
| TFP-FM | 0.046 | 0.425$^{b}$ | 0.379$^{b}$ | 0.481$^{b}$ | 1.284$^{ab}$ |
| SEM | 0.0001 | 0.005 | 0.0051 | 0.0055 | 0.0097 |
| | | | *P*-values | | |
| ANOVA | 0.610 | 0.017 | 0.019 | 0.043 | 0.025 |
| PC vs. RPC | 0.548 | 0.491 | 0.526 | 0.502 | 0.511 |
| RPC vs. NC | 0.763 | 0.854 | 0.931 | 0.690 | 0.271 |
| RPC vs. TFP | 0.368 | 0.122 | 0.294 | 0.167 | 0.281 |
| RPC vs. TFP-FM | 0.548 | 0.058 | 0.071 | 0.098 | 0.658 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[3] Abbreviations: BWG = body weight gain; FI = feed intake; NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[4] Corrected for mortality.

**Table 4.5.** Live performance of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 28 d post-hatch[1,2]

| Item[3] | d 28 BW, kg | 0 to 28 d BWG, kg | 0 to 28 d FI, kg | 0 to 28 d FCR[4], kg:kg |
|---|---|---|---|---|
| PC | 1.474$^{ab}$ | 1.427$^{ab}$ | 1.964 | 1.380 |
| RPC | 1.476$^{ab}$ | 1.430$^{ab}$ | 1.976 | 1.385 |
| NC | 1.471$^{ab}$ | 1.425$^{ab}$ | 1.977 | 1.394 |
| TFP | 1.493$^{a}$ | 1.447$^{a}$ | 1.995 | 1.380 |
| TFP-FM | 1.443$^{b}$ | 1.397$^{b}$ | 1.942 | 1.397 |
| SEM | 0.0102 | 0.0102 | 0.0128 | 0.0049 |
| | | *P*-values | | |
| ANOVA | 0.025 | 0.026 | 0.073 | 0.041 |
| PC vs. RPC | 0.859 | 0.863 | 0.526 | 0.408 |
| RPC vs. NC | 0.744 | 0.753 | 0.931 | 0.219 |
| RPC vs. TFP | 0.238 | 0.226 | 0.294 | 0.435 |
| RPC vs. TFP-FM | 0.029 | 0.032 | 0.071 | 0.092 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment

[3] Abbreviations: BWG = body weight gain; FI = feed intake; NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[4] Corrected for mortality.

**Table 4.6.** Live performance of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 39 d post-hatch[1,2]

| Item[3] | d 39 BW, kg | 0 to 39 d BWG, kg | 0 to 39 d FI, kg | 0 to 39 d FCR[4], kg:kg |
|---|---|---|---|---|
| PC | 2.629$^{bc}$ | 2.583$^{bc}$ | 3.825 | 1.483$^{b}$ |
| RPC | 2.704$^{a}$ | 2.658$^{a}$ | 3.885 | 1.469$^{b}$ |
| NC | 2.601$^{c}$ | 2.555$^{c}$ | 3.836 | 1.505$^{a}$ |
| TFP | 2.690$^{ab}$ | 2.644$^{ab}$ | 3.886 | 1.469$^{b}$ |
| TFP-FM | 2.617$^{bc}$ | 2.571$^{bc}$ | 3.806 | 1.488$^{ab}$ |
| SEM | 0.0186 | 0.0186 | 0.0241 | 0.0053 |
| | | *P*-values | | |
| ANOVA | < 0.001 | < 0.001 | 0.077 | < 0.001 |
| PC vs. RPC | 0.006 | 0.006 | 0.085 | 0.059 |
| RPC vs. NC | 0.003 | < 0.001 | 0.156 | < 0.001 |
| RPC vs. TFP | 0.590 | 0.603 | 0.979 | 0.952 |
| RPC vs. TFP-FM | 0.002 | 0.002 | 0.025 | 0.010 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[3] Abbreviations: BWG = body weight gain; FI = feed intake; NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[4] Corrected for mortality.

**Table 4.7.** Live performance of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 48 d post-hatch[1,2]

| Item[3] | d 48 BW, kg | 0 to 48 d BWG, kg | 0 to 48 d FI, kg | 0 to 48 d FCR[4], kg:kg |
|---|---|---|---|---|
| PC | 3.474 | 3.428 | 5.381 | $1.572^{b}$ |
| RPC | 3.518 | 3.472 | 5.469 | $1.577^{b}$ |
| NC | 3.446 | 3.399 | 5.441 | $1.604^{a}$ |
| TFP | 3.518 | 3.472 | 5.474 | $1.575^{b}$ |
| TFP-FM | 3.453 | 3.407 | 5.377 | $1.587^{ab}$ |
| SEM | 0.0198 | 0.0198 | 0.0286 | 0.0052 |
| | | *P*-values | | |
| ANOVA | 0.025 | 0.026 | 0.042 | < 0.001 |
| PC vs. RPC | 0.145 | 0.123 | 0.040 | 0.506 |
| RPC vs. NC | 0.012 | 0.014 | 0.502 | 0.001 |
| RPC vs. TFP | 0.953 | 0.976 | 0.886 | 0.824 |
| RPC vs. TFP-FM | 0.027 | 0.025 | 0.028 | 0.101 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[3] Abbreviations: BWG = body weight gain; FI = feed intake; NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[4] Corrected for mortality.

**Table 4.8.** Analyzed Thr intake and efficiency of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 48 d post-hatch[1,2]

| Item[3] | Intake, g[4] | Efficiency, %[5] |
|---|---|---|
| PC | $45.07^{b}$ | $76.1^{c}$ |
| RPC | $46.10^{a}$ | $75.3^{c}$ |
| NC | $39.57^{c}$ | $85.9^{a}$ |
| TFP | $44.58^{b}$ | $77.9^{b}$ |
| TFP-FM | $45.30^{ab}$ | $75.3^{c}$ |
| SEM | 0.252 | 0.30 |
| | *P*-values | |
| ANOVA | < 0.001 | < 0.001 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[3] Abbreviations: NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

[4] Average feed intake × analyzed Thr per growth phase.

[5] Average 0 to 48 d body weight gain / Thr intake × 100.

**Table 4.9**. Live weight and carcass characteristics of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 48 d[1]

| | | Hot carcass | | Fat pad | | Chilled carcass | |
|---|---|---|---|---|---|---|---|
| Item[2] | Live weight, kg | Weight, kg | Yield, % | Weight, kg | % | Weight, kg | Yield, % |
| PC | 3.409 | 2.620 | 76.80 | 0.039[b] | 1.16[b] | 2.668 | 78.24 |
| RPC | 3.507 | 2.691 | 76.72 | 0.042[ab] | 1.21[ab] | 2.748 | 78.35 |
| NC | 3.439 | 2.645 | 76.86 | 0.046[a] | 1.33[a] | 2.697 | 78.38 |
| TFP | 3.436 | 2.640 | 76.83 | 0.045[a] | 1.30[a] | 2.695 | 78.42 |
| TFP-FM | 3.391 | 2.595 | 76.69 | 0.044[a] | 1.30[a] | 2.649 | 78.10 |
| SEM | 0.0304 | 0.0243 | 0.174 | 1.076 | 0.033 | 0.0253 | 0.203 |
| | ——— | ——— | ——— | *P*-values | ——— | ——— | ——— |
| ANOVA | 0.095 | 0.095 | 0.948 | 0.001 | 0.002 | 0.087 | 0.797 |
| PC vs. RPC | 0.028 | 0.045 | 0.697 | 0.056 | 0.274 | 0.031 | 0.682 |
| RPC vs. NC | 0.122 | 0.188 | 0.557 | 0.039 | 0.012 | 0.161 | 0.929 |
| RPC vs. TFP | 0.104 | 0.152 | 0.630 | 0.119 | 0.045 | 0.145 | 0.819 |
| RPC vs. TFP-FM | 0.010 | 0.008 | 0.926 | 0.215 | 0.045 | 0.008 | 0.378 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[2] Abbreviations: NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix.

**Table 4.10.** Carcass parts characteristics of Ross 708 broilers fed diets with a threonine fermentation product as a source of threonine from 0 to 48 d[1]

| | Breast fillets | | Tenders | | Total white meat | | Wings | | Leg quarters | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[2] | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % | Weight, kg | Yield, % |
| PC | 0.793 | 23.2 | 0.149 | 4.37 | 0.942 | 27.6 | 0.274 | $8.04^{a}$ | 0.770 | 22.6 |
| RPC | 0.839 | 23.9 | 0.151 | 4.31 | 0.990 | 28.2 | 0.273 | $7.79^{b}$ | 0.783 | 22.3 |
| NC | 0.807 | 23.4 | 0.148 | 4.36 | 0.956 | 27.7 | 0.267 | $7.77^{b}$ | 0.777 | 22.6 |
| TFP | 0.814 | 23.9 | 0.147 | 4.27 | 0.961 | 28.2 | 0.268 | $7.81^{b}$ | 0.773 | 22.5 |
| TFP-FM | 0.811 | 23.9 | 0.146 | 4.35 | 0.957 | 28.2 | 0.262 | $7.75^{b}$ | 0.759 | 22.4 |
| SEM | 0.0118 | 0.22 | 0.0020 | 0.036 | 0.0130 | 0.23 | 0.0028 | 0.049 | 0.0079 | 0.11 |
| | | | | | *P*-values | | | | | |
| ANOVA | 0.105 | 0.073 | 0.352 | 0.267 | 0.130 | 0.152 | 0.042 | < 0.001 | 0.313 | 0.320 |
| PC vs. RPC | 0.008 | 0.029 | 0.453 | 0.232 | 0.012 | 0.056 | 0.868 | 0.001 | 0.263 | 0.095 |
| RPC vs. NC | 0.061 | 0.112 | 0.349 | 0.311 | 0.066 | 0.131 | 0.143 | 0.727 | 0.595 | 0.091 |
| RPC vs. TFP | 0.137 | 0.977 | 0.124 | 0.440 | 0.114 | 0.958 | 0.240 | 0.800 | 0.383 | 0.284 |
| RPC vs. TFP-FM | 0.098 | 0.958 | 0.058 | 0.470 | 0.075 | 0.936 | 0.011 | 0.540 | 0.041 | 0.706 |

[a-b] Means within a column that do not share a common superscript were deemed different ($P \leq 0.05$) by a Tukey's multiple comparison test.

[1] Values are least square means of 12 replicate pens per treatment.

[2] Abbreviations: NC = negative control; PC = positive control; RPC = reduced protein control; TFP = threonine fermentation product; TFP-FM = threonine fermentation product full matrix

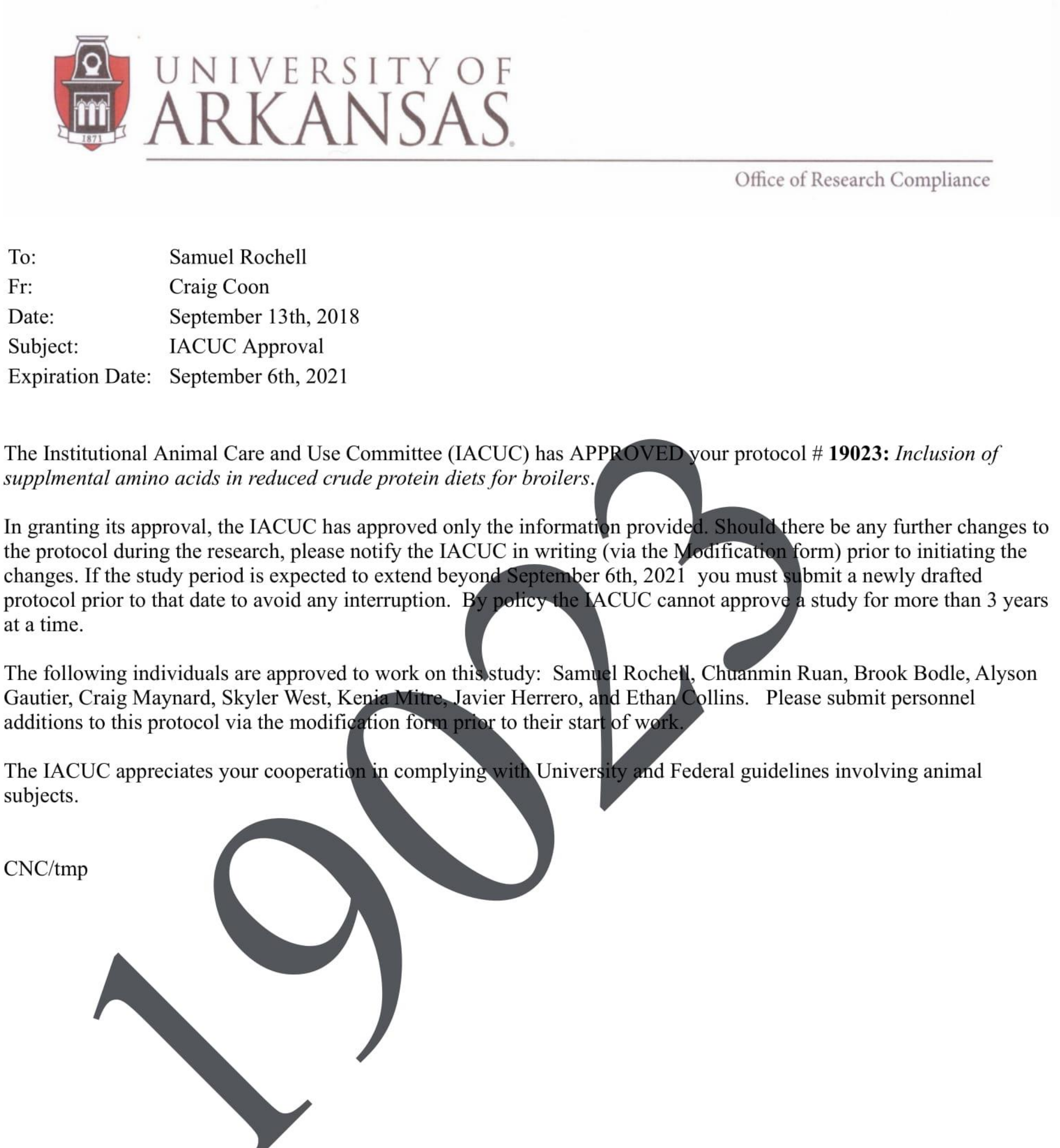
# CHAPTER V: CONCLUSIONS

The overall focus of both of these experiments was to evaluate diets containing individual AA to replace intact proteins thereby reducing dietary CP and to determine if broiler live performance (0 to 48 d) and processing characteristics could be maintained. The first experiment evaluated inclusion of L-Val and L-Ile in addition to L-Met, L-Lys, and L-Thr in CSBM-based diets with or without inclusions of PM or APB. Supplementing L-Val and L-Ile reduced CP by an average of 1.62 percentage units and supported broiler BWG and FCR, independent of diet composition. Broilers fed feed-grade BCAA exhibited improvements in breast meat weight and yield, as well as an 8.5% reduction in litter N. Footpad dermatitis was not affected by feed-grade BCAA but improved with the inclusion of APB at the expense of SBM.

The second experiment investigated a novel Thr biomass as a replacement to crystalline L-Thr in reduced CP diets. Feed-grade L-Val, L-Ile, and L-Arg inclusion reduced dietary CP by an average of 0.77 percentage units and maintained broiler BWG and FCR but improved breast meat weight and yield. The Thr biomass was efficacious in replacing crystalline L-Thr; however, broilers reduced FI and BWG when formulating the Thr biomass on the full nutrient matrix versus formulating the Thr biomass only on the digestible Thr and energy contributions, for which the reason is unclear. In addition, the removal of supplemental Thr, thus lowering dietary Thr levels, negatively affected FCR and BWG of broilers, thereby confirming the need to maintain digestible Thr levels for broilers fed reduced CP, CSBM diets.

Collectively, both experiments indicate that reducing dietary CP with BCAA or using an alternative supplemental Thr, such as the Thr biomass, are valuable options for nutritionists to implement, but also revealed areas for further research. From this work, future investigators should consider the following: the cause of the positive influence of feed-grade L-Val and L-Ile on breast meat accretion, the reduction in FI of broilers when formulating the Thr biomass on the full nutrient matrix, an evaluation of L-Val and L-Ile supplementation or Thr biomass supplementation in broiler diets with greater CP reductions, and finally, the global impact of implementing reduced CP diets.

# APPENDIX



UNIVERSITY OF
ARKANSAS
Office of Research Compliance

To: Samuel Rochell
Fr: Craig Coon
Date: September 13th, 2018
Subject: IACUC Approval
Expiration Date: September 6th, 2021

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **19023:** *Inclusion of supplmental amino acids in reduced crude protein diets for broilers*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond September 6th, 2021 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Samuel Rochell, Chuanmin Ruan, Brook Bodle, Alyson Gautier, Craig Maynard, Skyler West, Kenia Mitre, Javier Herrero, and Ethan Collins. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

UNIVERSITY OF
ARKANSAS
Office of Research Compliance

To: Samuel Rochell
Fr: Craig Coon
Date: February 5th, 2019
Subject: IACUC Approval
Expiration Date: February 1st, 2022

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **19052:** *Evaluation of amino acid and mineral supplements for broilers*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond February 1st, 2022 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Samuel Rochell, Chuanmin Ruan, Brook Bodle, Alyson Gautier, Derrell Lee, Kenia Mitre, Joshua Deines, and Ethan Collins. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp