Effects of Feeding Reduced Crude Protein Diets while Maintaining Essential Amino Acids and Total Glycine + Serine Concentrations on Growth Performance, Nitrogen Excretion, Plasma Uric Acid Concentration, and Carcass Characteristics of Broilers

by

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ABSTRACT

An excessive reduction of crude protein (**CP**) content in broiler diets has been resulting in poor growth performance. This may be due to sub-optimum concentrations of Val, Ile, Arg, and Trp. Additionally, total Gly + Ser concentration may be below adequacy when broilers were fed reduced CP diets formulated with ingredients of vegetable origin. Glycine supplementation has been reported to ameliorate poor performance of broilers fed reduced CP diets during the starter period. An experiment consisting of 2 trials was conducted to determine the effects of feeding broilers reduced CP diets while maintaining adequate essential amino acids (**AA**) and total Gly + Ser concentrations on growth performance from 1 to 18 (trial 1) and 1 to 21 (trial 2) d of age. In this experiment, AA (trial 1: DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu and trial 2: DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) were sequentially added in the order of limitation to meet their requirements while simultaneously lowering dietary CP content. Total Gly + Ser to digestible Lys ratios and digestible Lys concentrations were formulated at 1.90 and 1.20 and 1.70 and 1.25% in trials 1 and 2, respectively. In trial 1, body weight gain of broilers was maintained when dietary CP was reduced by 4.9 percentage points, but feed conversion increased as dietary CP content was reduced beyond 1.8 percentage points. In trial 2, dietary CP was reduced by 3.9 and 2.2 percentage points from 1 to 14 and 1 to 21 d of age, respectively, without compromising body weight gain and feed conversion of broilers. These data indicated that optimal growth performance of broilers can be obtained without placing a minimum of CP concentration when proper AA ratios are implemented in diet formulation. The lack of Gly research on broilers subsequent to the starter period led to a second experiment evaluating the effects of Gly and L-Gln supplementation to reduced CP diets on growth performance and carcass characteristics of broilers during a 6 wk production period. Glycine and L-Gln (a source of nitrogen) were added to reduced CP diets to increase total Gly + Ser and CP concentrations, respectively at 33 or 66% of the difference between their concentrations in the positive and negative control diets. Glycine had a more pronounced impact than nitrogen contribution on cumulative feed conversion of broilers only when added at 33% of total Gly + Ser concentration difference between the positive and negative control diets to reduced CP diets. Glycine and L-Gln supplementation in reduced CP diets increased total breast meat weight and yield of broilers while reducing abdominal fat weight and percentage compared with birds fed 2.5 percentage points lower CP diets. Hence, the supplementation of Gly and L-Gln provided enhancements in growth performance and carcass characteristics of broilers during a 41 d production period.

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I. INTRODUCTION

Diet cost represents approximately 60 to 70% of total live production cost of broilers (Donohue and Cunningham, 2009). Price volatility of amino acid (**AA**) contributing ingredients may largely impact diet cost and subsequently live production cost. Diet cost can be decreased by supplementing DL-Met, L-Lys, and L-Thr as these feed-grade AA have become increasingly available in the poultry industry. The addition of these AA can provide partial replacement of intact protein sources resulting in a lower dietary cost.

Previous studies have reported that the reduction of crude protein (**CP**) content in broiler diets may negatively depress growth performance of broilers (Kerr and Kidd, 1999; Si et al., 2004; Waldroup et al., 2005; Dean et al., 2006; Hernandez et al., 2012). Attempts to ameliorate poor growth performance of broilers fed reduced CP diets have included supplementing potassium (Han et al., 1992), adding Glu or Asp as a source of nitrogen (Aletor et al., 2000), increasing dietary energy (Hussein et al., 2001), and supplementing essential and non-essential AA (Waldroup et al., 2005), but these methods have produced inconsistent results. When supplementing DL-Met, L-Lys, and L-Thr to decrease CP content in corn-soybean meal based diets, less limiting AA concentrations (Val, Ile, Arg, and Trp) may be lower than diets containing higher CP content resulting in poor growth performance and carcass characteristics of broilers (Corzo et al., 2007). Therefore, maintaining adequate concentrations of these less limiting AA in reduced CP diets may help alleviating poor growth performance of broilers.

Research evaluating broiler responses fed reduced CP diets may have not accounted for Gly + Ser concentrations. However, Gly may be conditionally essential when broilers were fed reduced CP diets formulated with ingredients of vegetable origin during the starter period. Total Gly + Ser concentrations in plant ingredients have been reported to be lower than animal by-products with approximately 4.42% in soybean meal compared with 12.09% in poultry by-product meal (Li et al., 2011). Previous research demonstrated that poor growth performance of broilers fed a low CP diet (16.2%) can be alleviated by supplementing Gly similar to those fed a higher CP diet (22.2%) from 1 to 18 d of age (Dean et al., 2006). However, research evaluating Gly responses have been conducted primarily in the starter period while investigations of Gly effects in the grower and finisher periods are sparse.

The research presented herein consisted of 2 experiments. Experiment 1 (2 trials) was conducted to evaluate the effects of maintaining essential AA as well as total Gly + Ser concentrations on broilers fed reduced CP diets during the starter period. This was accomplished by sequentially adding essential AA and Gly in the order of limitation (trial 1: DL-Met, L-Lys, L-Thr, Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu and trial 2: DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) to provide adequate AA concentrations while simultaneously lowering dietary CP content. Experiment 2 was conducted to investigate the effects of Gly and/or L-Gln (as a source of nitrogen) supplementation to reduced CP diets on broiler growth performance and carcass characteristics during a 6 wk production period.

II. LITERATURE REVIEW

BENEFITS OF LOWERING CRUDE PROTEIN IN BROILER DIETS

The use of feed-grade amino acids (**AA**) such as DL-Met, L-Lys, and L-Thr, has enabled poultry nutritionists to meet the needs of the 3 most essential AA while decreasing both crude protein (**CP**) content and diet cost. This strategy allows diets to contain lower inclusion of AA-contributing ingredients, which are known to play a central role in diet cost. Si et al. (2004) demonstrated that soybean meal inclusion was 10.7% lower when adding DL-Met, L-Lys, and L-Thr compared with only supplementing DL-Met in a starter broiler diet. As a result, dietary CP content was reduced from 22.5 to 20.9% when increasing feed-grade AA inclusion.

In addition to decreasing diet cost, lowering dietary CP content can also be advantageous in reducing nitrogen excretion and ammonia emissions (Ferguson et al., 1998; Kerr and Kidd, 1999; Hernandez et al., 2012). Corzo et al. (2005) reported a 32% reduction in nitrogen excretion of broilers when lowering dietary CP content from 22 to 18% in a starter broiler diet. In addition to decreased nitrogen excretion, Ferguson et al. (1998) noted a reduction in litter ammonia concentration by 30% when lowering CP content in broiler diets approximately 2.1 percentage points during a 6 wk production period. In addition, nitrogen excretion and litter moisture can influence pododermatitis of broilers (Nagaraj et al., 2007). For example, broilers consuming 2.0 percentage points lower CP diets had 2 times lower incidence of severe pododermatitis than those consuming the higher CP diets during a 54 d production period (Nagaraj et al., 2007).

POOR GROWTH PERFORMANCE OF BROILERS FED REDUCED CRUDE PROTEIN DIETS

Despite the benefits from lowering dietary CP content, previous studies have reported sub-optimal growth performance when broilers were provided reduced CP diets (Kerr and Kidd, 1999; Rezaei et al., 2004; Dean et al., 2006; Hernandez et al., 2012). Hernandez et al. (2012) examined the effects of feeding broilers reduced CP diets from 23.0 to 20.0% with increments of 1.5 percentage points from 8 to 21 d of age. These researchers noted that broilers fed the 21.5% CP diet had similar body weight (**BW**) gain and feed conversion ratio (**FCR**) to those consuming the 23.0% CP diet. However, broilers consuming the 20.0% CP diet had decreased BW gain and increased FCR by 9.4 and 9.6%, respectively. Similarly, Dean et al. (2006) observed a linear decrease in gain to feed ratio of broilers from 1 to 18 d of age when gradually decreasing dietary CP content by increments of 1.5 percentage points from 22.2 to 16.2%.

Poor growth performance of broilers fed reduced CP diets may also be observed in older broilers. From 28 to 45 d of age, decreasing CP content in broiler diets from 19.4 to 16.7% reduced average daily gain while also increasing FCR by 2.3 and 3.3%, respectively (Kerr and Kidd, 1999). The magnitude of lowering CP content can also be displayed in carcass characteristics of broilers. Rezaei et al. (2004) conducted a 6 wk trial to evaluate the effects of feeding reduced CP diets on growth performance and carcass characteristics of broilers. From 1 to 3 wk of age and 3 to 6 wk of age, the reduced CP diets were formulated to contain 17.8 and 16.1% CP, while the high CP diets were formulated to contain 20.8 and 18.1% CP, respectively. Results demonstrated that BW gain of broilers consuming the reduced CP diets was 5.5% lower compared with birds provided the high CP diets resulting in a 2.4% decrease in carcass weight. Additionally, breast meat yield decreased and abdominal fat percentage increased by 6.7 and 35.4%, respectively, as dietary CP content was reduced by 3.0 and 2.0 percentage points in broiler diets from 0 to 3 and 3 to 6 wk of age, respectively.

FACTORS AFFECTING POOR PERFORMANCE IN REDUCED PROTEIN DIETS

Poor growth performance of broilers resulting from a reduction of dietary CP content may be influenced by several factors. Dietary electrolyte balance, essential AA concentration, non-specific nitrogen, and the ratio of total essential to non-essential AA nitrogen have been investigated to ameliorate poor performance of broilers fed reduced CP diets. However, causes of depressed growth performance of broilers are not limited to these factors. There may be other aspects that can influence poor growth performance of broilers when fed reduced CP diets that will not be discussed in this review.

Dietary Electrolyte Balance and Potassium

Dietary electrolyte balance is defined as Na + K - Cl. An optimal dietary electrolyte balance can be obtained by dietary manipulation, environmental control, and alteration of broiler metabolic rate (Olanrewaju et al., 2007). Maintaining adequate dietary electrolyte balance is essential to regulate blood and tissue pH. In contrast, changes in dietary electrolyte balance may lead to metabolic acidosis or alkalosis. Sub-optimum dietary electrolyte balance may be encountered when lowering dietary CP content, especially through the reduction of soybean meal inclusion in diets formulated with ingredients of vegetable origin. Soybean meal contains approximately 1.96% potassium, whereas corn consists of approximately 0.32% potassium (NRC, 2012). Hence, decreasing soybean meal inclusion may have a more pronounced effect in reducing potassium concentration compared with other primary ingredients.

An approach to restore inadequate dietary electrolyte balance has mainly focused on potassium supplementation. Si et al. (2004) observed a reduction of 55 meq/kg dietary electrolyte balance when lowering CP content from 20.3 to 18.1%, which led to a 6 point increase in FCR of broilers from 1 to 21 d of age. In order to decrease FCR of broilers fed the 18.1% CP diet, potassium supplementation was utilized to achieve 250 meq/kg dietary electrolyte balance. However, the addition of potassium did not affect FCR of broilers fed the reduced CP diet. The supplementation of potassium to ameliorate poor performance associated with reduced CP diets was also ineffective for broilers beyond 21 d of age. Fancher and Jensen (1989) added potassium to achieve 0.90 % dietary potassium concentration in 19.6 and 16.0% CP diets. However, BW gain and feed efficiency of broilers consuming 19.6 and 16.0% CP diets were lower compared with birds provided a 21.5% CP diet from 21 to 42 d of age. These data indicated that optimizing dietary electrolyte balance may not be an effective strategy to mitigate poor performance of broilers fed reduced CP diets.

Essential Amino Acids

The inclusion of DL-Met, L-Lys, and L-Thr has enabled nutritionists to formulate diets containing lower CP. However, when focusing solely on these AA, less limiting AA concentrations, such as Val, Ile, Arg, and Thr may be below optimum concentrations. Insufficient less limiting AA concentrations may produce confounding effects when research aimed to evaluate the effects of lowering dietary CP content on growth performance of broilers is conducted. Prior research demonstrated that the reduction of dietary CP content from 23 to 20% in diets fed to broilers from 7 to 21 d of age resulted in decreased concentrations of Val, Ile, Arg, and Trp by 15, 16, 10, and 18%, respectively, in the 20% CP diet (Pinchasov et al., 1990). Similarly, Waldroup et al. (2005) noted approximately 10% reductions in Val, Ile, Arg, and Trp concentrations when lowering dietary CP content from 22 to 18% leading to an 8.7% increase in FCR of broilers. Adding a mixture of essential AA (Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to the 18% CP diet to obtain similar concentrations in the 22% CP diet resulted in broilers having similar FCR compared with birds fed the 22% CP diet. However, this strategy did not yield similar results when applied to a 20% or 16% CP diet indicating that other factors may influence poor growth performance of broilers (Waldroup et al., 2005).

Non-specific Nitrogen

Poor performance of broilers fed reduced CP diets may also be affected by non-essential AA concentrations. Nutritionists often do not place a minimum for non-essential AA concentrations in diet formulation because those AA were assumed to be sufficiently synthesized from essential AA or intact protein. However, in reduced CP diets, non-essential AA concentrations may be below adequacy due to low nitrogen concentration or inefficient conversion of essential to non-essential AA. In previous research, Dean et al. (2006) lowered dietary CP content from 22.2 to 16.2% while also maintaining adequate concentrations of essential AA. The reduction in dietary CP content led to an 8.4% lower gain to feed ratio of broilers. In order to ameliorate poor growth performance, broilers consuming the 16.2% CP diet were supplemented with a mixture of either essential (Val, Ile, Arg, Trp, His, Phe, and Leu) or non-essential AA (Gly, Asp, Pro, Ala, Glu) to obtain the concentrations in the 22.2% CP diet. Results demonstrated that the addition of essential AA mixture did not affect growth performance, but broilers receiving a mixture of non-essential AA had similar gain to feed ratio compared with broilers consuming the 22.2% CP diet. Similarly, Awad et al. (2014) did not observe positive effects on BW and FCR of broilers when adding essential AA above requirements in a low CP diet (16.2%). Conversely, BW and FCR of broilers fed a 16.2% CP diet supplemented with non-essential AA were similar to broilers provided a 22.2% CP diet. These studies indicated that non-essential AA may not be sufficient when lowering CP content in diets formulated to meet essential AA specifications (Dean et al., 2006; Awad et al., 2014).

Another strategy to ameliorate poor growth performance of broilers associated with feeding reduced CP diets was through the addition of nitrogen-contributing AA. Several studies have used L-Glu supplementation as a source of nitrogen in reduced CP diets. Moran and Stilborn (1996) observed a 3.1% increase in BW of broilers receiving L-Glu supplementation compared with those without L-Glu addition during a 6 wk production period. Conversely, Kerr and Kidd (1999) did not observe improvements in either BW gain or FCR of broilers when fed reduced CP diets supplemented with L-Glu from 28 to 52 d of age. These studies indicated that the use of L-Glu in reduced CP diets fed to broilers have been inconsistent (Moran and Stilborn, 1996; Kerr and Kidd, 1999). In contrast, Gln can be used as a source of nitrogen because of its role in nitrogen transport between tissues (Bartell and Batal, 2007). The nitrogen content of Gln is also higher compared with other non-essential AA being 19.2 vs. 11.9% (Glu, Asp, and Ala).

Nitrogen from Gln can serve as a source of purine and pyrimidine nucleotides, which can be used for protein synthesis (Cory and Cory, 2006). Previous research indicated that BW gain of broilers increased from 739 to 805 g when broilers received 1% L-Gln in the diet compared with those without L-Gln supplementation from 1 to 21 d of age (Bartell and Batal, 2007).

Ratio of Essential to Non-essential Amino Acid Nitrogen

Due to an inefficient synthesis of non-essential AA from essential AA, it is important to supplement non-essential AA in a reduced CP diet (Heger et al., 1998; Lenis et al., 1999). However, it is also necessary to account for the proportion of essential to non-essential AA on a nitrogen basis (Heger et al., 1998). Maintaining optimal total essential to non-essential AA nitrogen (**EAA_n:$NEAA_n$**) ratio may be useful to reduce catabolism of essential to non-essential AA (Bedford and Summers; 1985) and optimize nitrogen utilization and retention (Heger et al., 1998; Lenis et al., 1999). Previous research indicated that increasing total EAA_n:$NEAA_n$ ratio from 38:62 to 50:50 in an 11.8% CP diet increased nitrogen utilization and retention of pigs by 12 and 14%, respectively (Lenis et al., 1999). A lower nitrogen excretion can also be achieved by increasing nitrogen utilization and retention through optimization of dietary total EAA_n:$NEAA_n$ ratio. Heger et al. (1998) observed a decrease in nitrogen excretion of pigs from 1.00 to 0.56 g/kg$^{0.75}$ when increasing total EAA_n:$NEAA_n$ ratio from 38:62 to 50:50. Optimizing total EAA_n:$NEAA_n$ ratio can also enhance growth performance of broilers. It was reported that formulating diets to a total EAA_n:$NEAA_n$ ratio of 55:45 resulted in a higher BW gain compared with feeding broilers with diets containing 35:65 total EAA_n:$NEAA_n$ ratio (Bedford and Summers, 1985).

Published literature has recommended that ratios of total EAA_n:$NEAA_n$ may vary between 42:58 and 67:33 for maximum growth and nitrogen utilization of broilers and pigs (Stucki and Harper, 1961; Sugahara and Ariyoshi, 1968; Bedford and Summers, 1985; Wang and Fuller, 1989; Heger et al., 1998; Lenis et al., 1999). A wide range of these ratios may be associated with differences in methodology used when assessing these ratios. Traditionally, Gly has been considered as a non-essential AA. However, Gly may conditionally be essential for broilers when fed reduced CP diets during the starter period (Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Siegert et al., 2015a, b). Hence, the optimum ratio of total EAA_n:$NEAA_n$ may be altered depending on total Gly + Ser concentration. Furthermore, results may vary between formulating diets to contain constant nitrogen concentration or essential AA nitrogen (Heger et al., 1998). When formulating diets to contain constant nitrogen concentration, variation in total EAA_n:$NEAA_n$ ratio may cause changes in essential AA nitrogen intake. Hence, altering total nitrogen concentration may have a large impact on the total EAA_n:$NEAA_n$ ratio because essential AA are major factors affecting growth performance. In contrast, formulating diets to contain constant essential AA nitrogen concentration will eliminate the effects of a variation in total essential AA nitrogen intake (Heger et al., 1998).

DIETARY GLYCINE NEEDS FOR BROILERS

Glycine Functions

Glycine is an important AA that is involved in many metabolic and physiological functions, especially for young broilers. It has been reported that Gly may have a role in enterocyte development (Wang et al., 2014a, b). Wang et al. (2014a) suggested that increasing Gly supplementation from 0 to 2% in milk-fed piglets from 14 to 24 d of age increased villus height in duodenum, jejunum, and ileum by approximately 11%. Additionally, Gly and Ser represented approximately 37% of non-essential AA in feathers, which corresponded to 18% of total AA in feathers (Stilborn et al., 1997). Previous research demonstrated that broilers fed diets containing low Gly concentrations (0.32, 0.38, 0.44, and 0.50%) from 8 to 17 d of age had abnormal feather growth (Robel, 1977). Furthermore, Gly was reported to play a critical role in muscle cellular components of broilers (Ngo et al., 1977). Increasing total Gly + Ser concentration from 1.84 to 2.26% increased creatine content from 2.1 to 3.4 mg/g in pectoral muscle of 21 d broilers (Ospina-Rojas et al., 2013b). Glycine is also an important component of uric acid concentration for nitrogen excretion in birds. The formation of one molecule of uric acid requires one molecule of Gly (Leeson and Summers, 2001). Moreover, previous research has also demonstrated that feeding broilers an 18.0% CP diet supplemented with Gly to obtain 2.0% total Gly + Ser concentration increased tibia diameter of broilers by 12.9% compared with birds fed diets containing 1.43% total Gly + Ser concentration (Yuan et al., 2012).

Glycine in Reduced Crude Protein Diets

A constraint in reducing dietary CP content may be encountered when formulating broiler diets with ingredients of vegetable origin. Research demonstrated that AA concentrations in plant ingredients may be lower than the concentrations in animal by-products, especially Gly + Ser (Li et al., 2011). For example, poultry by-product meal contains almost a 3 fold higher total Gly + Ser concentration than soybean meal (12.09 vs. 4.42%). In addition, young chicks may have limited ability to synthesize Gly from other compounds (Almquist and Grau, 1944). Therefore, when lowering CP content in diets formulated with ingredients of vegetable origin without accounting for total Gly + Ser concentration, broilers may not be able to achieve optimal growth performance.

Recent publications indicated poor performance of broilers when lowering dietary CP content from 22 (2.05% total Gly + Ser) to 16% (1.25% total Gly + Ser) without maintaining adequate total Gly + Ser concentration from 1 to 21 d of age (Dean et al., 2006). The addition of Gly in the low CP diet to the concentration in the 22% CP diet resulted in broilers having a 12.5% higher gain to feed ratio compared with those fed the 16% CP diet without Gly supplementation. A similar response was observed when broilers fed the low CP diet were supplemented with a mixture of non-essential AA (Gly, Glu, Ala, Asp, and Pro). However, when non-essential AA other than Gly was individually supplemented, no improvements in growth rate and gain to feed ratio were observed (Corzo et al., 2005; Dean et al., 2006; Awad et al., 2015). Yuan et al. (2012) examined growth responses of broilers fed diets with sequential addition of essential AA (DL-Met to L-His) to decrease CP content from 1 to 18 d of age. Body weight and FCR of broilers were 11.4% lower and 11.2% higher, respectively, when dietary CP content was decreased from 21.4% (DL-Met, L-Lys, and L-Thr) to 18% (DL-Met, L-Lys, L-Thr, L-Val, L-Ile, L-Arg, L-Trp, and L-His). When total Gly + Ser concentration in the 18.0% CP diet was maintained at 2.0%, no difference in 18 d BW or FCR was observed between broilers provided the 21.4% to 18.0% CP diets. These experiments demonstrated that Gly may be conditionally essential in reduced CP diets fed to broilers during the starter period.

Dietary Glycine + Serine Needs for Broilers

Research evaluating an optimum Gly concentration in broiler diets has been conducted along with Ser because of the interconversion between both AA (Baker et al., 1968). Previous research has recommended a minimum concentration of total Gly + Ser from 1.76 to 2.32% (Corzo et al., 2004; Dean et al., 2005; Waguespack et al., 2009; Yuan et al., 2012). Because diets have been formulated using AA ratios to digestible Lys, it may also be useful to determine total Gly + Ser requirement as a ratio to digestible Lys concentration. Our calculation from previous research indicated that total Gly + Ser to digestible Lys ratio for optimal growth performance of broilers ranged from 1.69 to 2.07 in the starter period (Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Yuan et al., 2012). It is important to note that these values were obtained from studies designed to determine optimal total Gly + Ser response and not for a total Gly + Ser to digestible Lys ratio. To our knowledge, research in determining an optimum total Gly + Ser to digestible Lys ratio for broilers is limited. Therefore, additional research in this area is warranted.

Prior studies reported that total Gly + Ser concentration should be expressed as total Gly equivalent (Gly (%) + Ser (%) × 0.7143) because the synthesis of Gly from Ser occurs with a ratio of 0.7143 in equimolar basis (Dean et al., 2006; Siegert et al., 2015a, b). A meta-analysis of 11 previous Gly research studies determined that optimal total Gly equivalent for 95% maximum average daily gain and gain to feed ratio of broilers were 1.61 and 1.58%, respectively, from 1 to 21 d of age (Siegert et al., 2015a). However, these values may be altered depending on Gly precursors or metabolic processes that require Gly. Siegert et al. (2015b) indicated that in a low Thr diet, a higher Gly concentration may be needed to obtain optimal growth performance of broilers. Previous research indicated that Thr can be degraded to Gly by Thr dehydrogenase and Thr aldolase (Kidd and Kerr, 1996). Hence, a higher Gly concentration may prevent Thr degradation. In addition, reductions in dietary Gly and Thr concentrations can be compensated by increasing dietary choline concentration to optimize growth performance of broilers (Siegert et al., 2015b). It has been reported that Glycine can also be synthesized from choline (Baker and Sugahara, 1970). Hence, a 0.84 gain to feed ratio can be achieved by placing 24.9, 1.05, and 10.1 g/kg dry matter of total Gly equivalent, choline, and Thr, respectively in a diet fed to broilers from 7 to 21 d of age (Siegert et al., 2015b). Moreover, Gly is also involved in the conversion of Met to Cys through Ser. In a low Cys diet (0.35%), increasing total Gly + Ser concentration from 1.95 to 2.32% increased gain to feed ratio of broilers from 0.79 to 0.82 (Powell et al., 2011).

Research evaluating Gly responses beyond the starter period is limited. Rostagno et al. (2011) suggested minimum total Gly + Ser concentrations of 1.63 and 1.53% for broilers from 22 to 33 and 34 to 42 d of age. Additionally, a total Gly + Ser concentration of 1.57% was reported to optimize gain to feed ratio of broilers from 21 to 35 d of age (Ospina-Rojas et al., 2013a). However, Corzo et al. (2009) did not observe improvements in growth performance and carcass characteristics of broilers when varying total Gly + Ser concentrations (1.55 vs. 1.65%) from 21 to 42 d of age. These studies demonstrated that there are inconsistencies in Gly responses subsequent to the starter period. Therefore, future research is required to address this issue.

KNOWLEDGE GAPS IN THE LITERATURE

A strategy to lower CP content in broiler diets has been emphasized on supplementing feed-grade AA, such as DL-Met, L-Lys, and L-Thr. Prior research has often focused solely on the concentration of these essential AA to gradually reduce CP content without accounting for less limiting AA concentrations (Val, Ile, Arg, and Trp). Additionally, published literature has not placed a minimum on total Gly + Ser concentration, which has been reported to be conditionally essential when broilers were fed reduced CP diets during the starter period. Incremental reductions in dietary CP content without maintaining adequate essential AA and total Gly + Ser concentrations have led to poor growth performance of broilers. Hence, rather than incrementally decreasing CP content, a strategy may utilize sequential additions of essential AA in their order of limitation while maintaining a minimum total Gly + Ser concentration in broiler diets. This strategy will allow diets to contain adequate AA concentrations while simultaneously decreasing CP content.

To the best of our knowledge, research evaluating dietary Gly responses has been conducted primarily in the starter period while investigations of Gly effects subsequent to the starter phase are sparse. Previous research evaluating dietary Gly needs of broilers have not determined if the positive response on broilers was due to Gly requirement per se or nitrogen contribution. In order to address these knowledge gaps in the literature, 2 experiments were conducted to evaluate the effects of feeding broilers reduced CP diets while maintaining adequate essential AA and total Gly + Ser concentrations on growth performance of broilers. In the first experiment, essential AA were added sequentially in the order of limitation to meet their requirements while simultaneously reducing dietary

CP content. A minimum of total Gly + Ser concentration of 2.10% was also maintained in the experimental diets (Waguespack et al., 2009). The second experiment was conducted to examine the effects of Gly and/or L-Gln (as a nitrogen source) supplementation in reduced CP diets fed to broilers on growth performance and carcass characteristics during a 6 wk production period.

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III. EFFECTS OF FEEDING BROILER CHICKS REDUCED CRUDE PROTEIN DIETS ON GROWTH PERFORMANCE, NITROGEN EXCRETION, AND PLASMA URIC ACID CONCENTRATION DURING THE STARTER PERIOD

ABSTRACT

An experiment (2 trials) was conducted to determine the effects of feeding Ross × Ross 708 male broilers reduced crude protein (**CP**) diets formulated to maintain adequate essential amino acid (**AA**) concentrations on growth performance, nitrogen excretion, and plasma uric acid (**UA)** concentration during the starter period. In trial 1, 11 dietary treatments were fed from 1 to 18 d of age consisting of corn and soybean meal as the primary ingredients. Diet 1 (24.7% CP) was formulated to contain DL-Met, L-Lys, and L-Thr with a 1.20% digestible Lys and a 1.70 total Gly + Ser to digestible Lys ratio. Other dietary treatments were formulated with additional Gly to contain a 1.20% digestible Lys and a 1.90 total Gly + Ser to digestible Lys ratio. Other free AA were added sequentially in the order of limitation (L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) from diets 3 to 10 to decrease CP content from 24.1 to 20.4%. In diet 11, L-Gln was added to increase the CP content to 25.0%. Feed conversion of broilers fed diet 2 was lower ($P < 0.05$) than those consuming diets 6 to 11 from 1 to 17 d of age. Nitrogen excretion (mg/b/d) decreased ($P = 0.035$) by 12.9% when broilers were fed diet 6 compared with birds fed diet 2 from 15 to 16 d of age. Broilers fed diet 4 had lower ($P = 0.011$) plasma UA than birds fed diet 2 at 18 d of age. Therefore, DL-Met, L-Lys, L-Thr, Gly, L-Val, L-Ile, and L-Arg can be used to lower dietary CP content without compromising FCR of broilers from 1 to 17 d of age. In trial 2, 8 dietary treatments containing a 1.25% digestible Lys and a 1.70 total Gly + Ser to digestible Lys ratio were fed from 1 to 21 d of age. Diet 1 (24.0% CP) was supplemented with DL-Met, L-Lys, and L-Thr. Additional AA (L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) were sequentially supplemented in the order of limitation to decrease CP concentration in diets 2 to 8 from 23.8 to 20.3%. Higher ($P < 0.05$) body weight gain and lower ($P < 0.05$) feed conversion were observed when broilers were fed diet 1 compared with diet 7 or 8 from 1 to 21 d of age. Decreasing CP beyond 1.6% (diets 4 to 8) resulted in lower ($P < 0.05$) plasma UA concentrations compared with diet 1 at 21 d of age. Optimal growth performance of broilers can be obtained by supplementing DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, and L-Trp when lowering dietary CP by 3.9 and 2.2 percentage points from 1 to 14 and 1 to 21 d of age, respectively.

INTRODUCTION

Reducing dietary crude protein (**CP**) in broiler diets has been advantageous in decreasing dietary cost, nitrogen excretion (Hernandez et al., 2012), ammonia emissions (Ferguson et al., 1998), and the incidence of pododermatitis (Nagaraj et al., 2007). However, previous research reported that lowering dietary CP content resulted in sub-optimum body weight (**BW**) gain and feed conversion ratio (**FCR**) of broilers (Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008; Hernandez et al., 2012). Strategies to mitigate poor growth performance of broilers include potassium supplementation (Han et al., 1992), an inclusion of Glu or Asp as a source of nitrogen (Aletor et al., 2000), and increasing dietary energy (Hussein et al., 2001). However, these approaches have produced inconsistent results (Han et al., 1992; Aletor et al., 2000; Hussein et al., 2001).

Adverse growth responses of broilers fed reduced CP diets may be attributed to sub-optimum essential amino acid (**AA**) concentrations (Aftab et al., 2006). When reducing dietary CP content in gradient increments, less limiting AA concentrations beyond Met, Lys, and Thr may be lower than diets with a higher CP content. Pinchasov et al. (1990) determined that reducing dietary CP content from 23 to 20% resulted in Val, Ile, Arg, and Trp concentrations approximately 15% below the values in the diet containing 23% CP. The reduction of less limiting AA in the 20% CP diet translated to 4.4% lower gain to feed ratio of broilers from 7 to 21 d of age. Waldroup et al. (2005) reported that reducing CP content incrementally from 22 to 20% decreased Val, Ile, Arg and Trp concentrations by 3, 4, 4, and 16%, respectively, compared with the 22% CP diet. However, the addition of a mixture of essential AA (Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to the 20% CP diet could not ameliorate FCR compared with broilers fed the 22% CP diet.

Recent studies showed that in addition to meeting requirements for essential AA, broilers receiving reduced CP diets may require a minimum total Gly + Ser concentration in order to achieve growth performance similar to those fed high CP diets (Corzo et al., 2004; Dean et al., 2006; Waguespack et al., 2009; Awad et al., 2015). Dean et al. (2006) reported that supplementing various individual non-essential AA (Glu, Pro, Ala, Asp) in a low CP diet (16%) did not affect FCR of broilers, but Gly addition was able to ameliorate poor FCR comparable to birds receiving a high CP diet (22%) from 1 to 18 d of age. Therefore, inadequate Gly concentration may limit growth when broilers consumed reduced CP diets formulated during the starter period.

Previous studies evaluating growth responses in broilers fed reduced CP diets have not implemented a minimum on total Gly + Ser concentration (Aletor et al., 2000; Bregendahl et al., 2002; Si et al., 2004), which could allow for a larger reduction in dietary CP content without compromising growth performance. Published research evaluating the addition of sequential limiting AA and maintaining an adequate total Gly + Ser concentration while simultaneously reducing dietary CP content is sparse (Yuan et al., 2012). Therefore, an experiment consisting of 2 trials was conducted to determine the effects of feeding reduced CP diets to broilers while maintaining adequate essential AA concentrations by sequentially adding essential AA in their order of limitation beyond Thr and a sufficient total Gly + Ser concentration on growth performance, nitrogen excretion, and plasma uric acid concentration during the starter period.

MATERIALS AND METHODS

All procedures involving live birds were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2014-2579).

Bird Husbandry

In both trials, Ross × Ross 708 male chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery at 1 d of age. All birds received vaccination for Marek's disease, Newcastle disease, and infectious bronchitis at the hatchery. In trial 1, 792 broiler chicks were randomly placed into 88 battery cages (9 birds/cage; 0.05 m^2/bird) (Petersime, Gettysburg, OH). Cages were placed in 2 solid-sided rooms equipped with forced-air heaters and cooling pads to control room temperature. Each cage had 1 trough feeder and 1 trough waterer. Feed and water were provided ad libitum from 1 to 18 d of age. Room temperature at chick placement was set at 33^{o}C and was gradually decreased to ensure broiler comfort until 27^{o}C at 18 d of age. Photoperiod was set at 23L:1D with an intensity of 30 lux throughout the experimental period. Birds and feed were weighed to determine BW gain, feed intake (**FI**), and FCR at 1 and 17 d of age. The incidence of mortality was recorded daily.

In trial 2, 1,600 broiler chicks were placed in a solid-sided house with a negative-pressure ventilation system equipped with vent boards, exhaust fans, cooling pads, and an electronic controller to adjust house temperature. Chicks were randomly distributed into 64 floor pens (25 birds/pen; 0.09 m^2/bird) equipped with a tube feeder, a nipple drinker line, and used litter. Feed and water were provided ad libitum throughout the experimental period. House temperature at chick placement was maintained at 33^{o}C and was gradually decreased to 25^{o}C at 21 d of age. Photoperiod was set at 23L:1D for the first 7 d post-hatching and 20L:4D was maintained from 8 to 21 d of age. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 to 21 d of age, respectively. Birds and feed were weighed at 1, 7, 14, and 21 d of age to determine BW gain, FI, and FCR. The incidence of mortality was recorded daily.

Dietary Treatments

In trial 1, 11 dietary treatments were fed to broiler chicks from 1 to 18 d of age consisting of corn and soybean meal as the primary ingredients (Table 3.1). Digestible Lys in all diets were formulated at 1.20% with ratios of digestible TSAA, Thr, Val, Ile, Arg, Trp, His, Phe, and Leu at 0.76, 0.69, 0.77, 0.67, 1.05, 0.17, 0.37, 0.63, and 1.07, respectively. Diets 1 (24.6% CP) and 2 (24.9% CP) served as the control diets containing DL-Met, L-Lys, and L-Thr supplementation with diet 2 containing supplemental Gly. Diet 1 was formulated to contain 1.70 total Gly + Ser to digestible Lys ratio, while Gly addition in diets 2 to 11 increased total Gly + Ser to digestible Lys ratio to 1.90. Based on our calculations from previous studies, optimum ratios of total Gly + Ser to digestible Lys ranged from 1.69 to 1.90 for broilers in the starter period (Corzo et al., 2004; Waguespack et al., 2009; Yuan et al., 2012). However, it is also important to note that these values were obtained from studies designed to determine optimal total Gly + Ser response and not for a total Gly + Ser to digestible Lys ratio. Data are limited in defining an optimum total Gly + Ser to digestible Lys ratio for broilers during the starter period. The range of 1.69 to 1.90 of total Gly + Ser to digestible Lys ratio was used as a basis to formulate diets in the current research with 1.70 and 1.90 total Gly + Ser to digestible Lys ratios. Free AA were sequentially supplemented in the order of limitation (L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to maintain adequate essential AA concentrations while simultaneously decreasing dietary CP contents in diets 3 to 10. Diet 3 was formulated with L-Val; diet 4 with L-Val and L-Ile; diet 5 with L-Val, L-Ile, and L-Arg; diet 6 with L-Val, L-Ile, L-Arg, and L-Trp; diet 7 with L-Val, L-Ile, L-Arg, L-Trp, and L-His; diet 8 with L-Val, L-Ile, L-Arg, L-Trp, L-His, and L-Phe; and diet 9 with L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu. Diet 10 contained a similar AA addition as diet 9 but with a higher inclusion of L-Leu to allow a larger CP reduction. Diet 11 was formulated with additional L-Gln as a source of nitrogen to increase dietary CP content. The resulting CP content in each experimental diet was 24.1, 23.4, 23.3, 22.9, 22.0, 21.1, 20.8, 20.4%, and 25.0%, respectively, in diets 3 to 11.

In trial 2, 8 dietary treatments were formulated with corn and soybean meal as the primary ingredients from 1 to 21 d of age (Table 3.2). All diets were formulated to contain digestible Lys at 1.25% with optimum ratios of digestible TSAA, Thr, Val, Ile, Arg, Trp, His, Phe, and Leu of 0.76, 0.69, 0.77, 0.67, 1.05, 0.17, 0.37, 0.64, and 1.10, respectively. Additionally, a total Gly + Ser to digestible Lys ratio of 1.70 was formulated across all diets. The ratio corresponded to 2.13% total Gly + Ser concentration, which is consistent with the total Gly + Ser concentration used in trial 1 of the present research and Waguespack et al. (2009). Diet 1 was supplemented with DL-Met, L-Lys, and L-Thr to contain 24.0% CP. Free AA were sequentially supplemented in the order of limitation (L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) to maintain adequate essential AA concentrations while decreasing dietary CP contents from diets 2 to 8. Diet 2 was formulated with L-Val; diet 3 with L-Val and Gly; diet 4 with L-Val, Gly, and L-Ile; diet 5 with L-Val, Gly, L-Ile, and L-Arg; diet 6 with L-Val, Gly, L-Ile, L-Arg, and L-Trp; diet 7 with L-Val, Gly, L-Ile, L-Arg, L-Trp, and L-His; and diet 8 with L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe. The resulting CP content in each experimental diet was 23.8, 22.7, 22.4, 22.0, 21.8, 20.5, and 20.3%, respectively, in diets 2 to 8. Digestible AA values in both trials were calculated by multiplying digestible AA coefficients of the corn and soybean meal with the total AA concentrations in these 2 ingredients. In trial 1, experimental diets were provided in mash form, whereas in trial 2, diets were provided in crumble form. Experimental diets of both trials were analyzed for CP (method 968.06; AOAC International, 2006) and total AA concentrations (method 994.12; AOAC International, 2006).

Blood Measures

In trials 1 and 2, blood samples were collected from 3 birds per cage/pen of 6 replicates at d 18 (trial 1) and 21 (trial 2) to determine total blood protein and plasma uric acid (**UA**) concentrations. At the day of blood collection, birds were fasted for 2 hours and re-fed for 2 hours to ensure feed consumption before blood collection (Donsbough et al., 2010). Birds were bled via brachial vein using a 22 gauge needle and approximately 3 mL of blood from each bird was collected into a heparinized tube. Blood samples were held on ice until centrifugation. In order to separate plasma from whole blood, blood samples were fractionated by centrifugation at 3,000 rpm for 5 min. Plasma was transferred to a 1.5 mL tube and was stored in -20^{o}C until analysis. Samples were sent to Auburn University College of Veterinary Medicine for analyses of total blood protein and plasma UA concentrations. Total blood protein and plasma UA concentrations were analyzed according to methods developed by Weichselbaum (1946) and Town et al. (1985), respectively. Each sample was analyzed in duplicate using Hitachi Cobas C311 (Roche Diagnostic, Indianapolis, IN). Reagent kits used in both analyses were provided by Roche Diagnostic (Roche Diagnostic, Indianapolis, IN).

Nitrogen Balance

In trial 1, a 24 hour nitrogen balance assay was conducted from 15 to 16 d of age to determined nitrogen intake and nitrogen excretion. Nitrogen intake represented the amount of nitrogen consumed by each bird per day (mg/b/d), whereas nitrogen excretion was expressed as the amount of nitrogen excreted by each bird per day (mg/b/d) or as the percent of nitrogen intake. Nitrogen excretion as a percent of nitrogen intake was calculated using the following equation:

$$Nitrogen\ Excretion\ (\%) = \frac{Excreta\ Nitrogen\ (mg/b/d)}{Nitrogen\ Intake\ (mg/b/d)} \times 100$$

Total excreta and feed were weighed from each pen. Excreta sub-samples were collected from 6 different locations in each cage to obtain a representative pooled sample. Sub-samples from each pen were mixed to obtain a pooled sample. Pooled excreta samples were stored at -20°C until analysis. Frozen excreta samples were thawed at room temperature and homogenized before lyophilization. Feed and excreta samples were lyophilized using VirTis Genesis 25ES freeze dryer (SP Industries, Inc., Warminster, PA). Duplicate samples of 250 mg dried feed and excreta from each cage were placed into aluminum foil for nitrogen analysis. The analysis was performed using combustion analyzer rapid N cube (Elementar, Hanau, Germany) according to Dumas method (method 968.06; AOAC International, 2006).

Statistical Analyses

Dietary treatments were completely randomized in each block. Cage or pen location served as the blocking factor. Each treatment was represented by 8 replicate cages (trial 1) or pens (trial 2) with cage/pen being the experimental unit. Analysis of variance was performed using the MIXED procedure of SAS (2011) by the following mixed-effects model:

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

Where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance ${\sigma^2}_\rho$; the τ_j are fixed factor level effects corresponding to the j^{th} dietary treatment (diets 1 to 11 in trial 1 or diets 1 to 8 in trial 2) such that $\sum\tau_j = 0$; and the ε_{ij} are identically and independently normally distributed random errors with mean 0 and a variance σ^2. Pre-planned orthogonal contrasts were used to detect differences on growth performance, blood measures, and nitrogen balance between each dietary treatment and the control diet. In trial 1, the effect of each dietary treatment (diets 3 to 11) was individually compared with diet 2. Diet 2 was used as the reference diet because it was formulated to contain a similar ratio of total

Gly + Ser to digestible Lys of 1.90 with diets 3 to 11. In trial 2, diet 1 was used as the reference diet to detect differences with diets 2 to 8. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

In trial 1, nitrogen analysis of experimental diets indicated a slightly higher CP content than the calculated values. This was due to corn being 2.0 percentage points higher in CP content than the value used in the formulation. Analyzed CP values for experimental diets 1 to 11 in trial 1 were 25.0, 25.8, 24.2, 24.0, 24.5, 23.2, 23.0, 21.8, 21.8, 20.9, and 24.9%, respectively. In trial 2, the analysis of CP contents from diets 1 to 8 were in agreement with the calculated values. Analyzed dietary CP values of the experimental diets were 23.9, 22.9, 22.5, 21.8, 22.2, 21.7, 20.0, and 20.2%, respectively, in diets 1 to 8. Due to the variation in dietary CP content in trial 1, results from both trials are presented using analyzed CP values (Tables 3.1 and 3.2).

Growth Performance

In trial 1, reducing dietary CP content up to 4.9 percentage points while maintaining adequate essential AA concentrations (diet 10, 20.9% CP) did not influence ($P > 0.05$) BW gain of broilers compared with those fed the control diet (diet 2, 25.8% CP) from 1 to 17 d of age (Table 3.3). However, reductions in dietary CP content of 2.6 percentage points or greater (diets 6 to 10) increased ($P < 0.05$) FCR compared with feeding broilers the control diet (diet 2). This indicated that supplemental L-Val, L-Ile, and L-Arg (diets 3 to 5) could be used to lower dietary CP content while maintaining similar FCR of broilers compared with the control-fed birds (diet 2). The addition of L-Gln in diet 11 increased dietary CP content to 24.9%, but a 10% decrease ($P < 0.0001$) in

BW gain was observed compared with birds fed the control diet (diet 2). Broilers fed diet 11 also had lower FI ($P = 0.006$) and BW gain ($P = 0.007$) when compared with birds fed diet 10. Feed intake of birds fed diet 8 was higher ($P = 0.043$) compared with broilers consuming the control diet (diet 2). The incidence of mortality was not affected by dietary treatments during the 17 d experimental period. These results indicated that the addition of free AA (DL-Met, L-Lys, L-Thr, Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to reduce dietary CP content did not alter BW gain of broilers. In contrast, supplementation of AA beyond L-Val, L-Ile, and L-Arg to decrease CP content increased FCR of broilers.

In trial 2, BW gain and FCR of broilers were not affected by dietary treatments from 1 to 7 and 1 to 14 d of age as dietary CP content decreased by 3.9 percentage points (Tables 3.4 and 3.5). From 1 to 21 d of age, birds fed diets containing supplemental L-His (diet 7, 20.0% CP) and L-Phe (diet 8, 20.2% CP) had decreased ($P < 0.05$) BW gain and increased ($P < 0.05$) FCR compared with those fed the control diet (diet 1, 23.0% CP). This indicated that lowering dietary CP content beyond 2.2 percentage points led to depressed growth performance of broilers from 1 to 21 d of age. Diverse responses with age may be related to differences in growth rate (g/d) of broilers. From 1 to 7 and 8 to 14 d of age, broilers grew 14.4 and 37.2 g/d, respectively, whereas growth rate of broilers from 15 to 21 d of age increased to 65.5 g/d. A faster growth rate of broilers from 15 to 21 d of age may indicate a higher demand of non-essential AA for protein accretion. Feed intake of birds consuming diet 7 was lower ($P = 0.023$) than those receiving the control diet (diet 1). Mortality was unaffected by dietary treatments except broilers fed diet 4 had a lower ($P = 0.032$) incidence of mortality compared with feeding the control diet (diet 1) from 1 to 21 d of age. Therefore, these data demonstrated that growth performance of broilers was maintained when supplementing DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, and L-Trp to a reduced CP diet from 1 to 21 d of age.

In previous research, incremental reductions of dietary CP content led to poor growth performance of broilers (Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008; Hernandez et al., 2012). These negative effects may be attributed to lower essential AA concentrations in reduced CP diets, especially those AA beyond Met, Lys, and Thr. Si et al. (2004) reported that broilers fed a diet formulated to contain 18.1% CP had lower Val, Ile, Arg, and Trp concentrations by 7, 10, 12, and 31%, respectively, than the diet containing 22.5% CP from 1 to 21 d of age. Broilers fed the 18.1% CP diet also had 8.8% lower BW and 8.4% higher FCR compared with those receiving the 22.5% CP diet (Si et al., 2004). Moreover, Waldroup et al. (2005) observed 3, 4, 4, and 16% decrease in Val, Ile, Arg, and Trp concentrations, respectively, when lowering CP content from 22 to 20% in broiler diets from 1 to 21 d of age. As a result, BW and FCR of broilers fed the 20% CP diet were 6% lower and 3% higher, respectively, compared with those fed the 22% CP diet. Adding a mixture of less limiting AA (L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu) to broilers receiving the 20% CP diet led to a similar BW gain to birds consuming the 22% CP diet, but FCR was not restored when supplementing the AA mixture to a 20% CP diet compared with birds fed the 22% CP diet. These studies indicated that maintaining adequate less limiting AA concentrations may help minimize poor growth performance of broilers (Si et al., 2004; Waldroup et al., 2005).

In the current research, despite maintaining growth performance by sequentially adding free AA (trial 1: DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, and L-Arg and trial 2:

DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, and L-Trp) to reduced CP diets, broilers receiving supplemental L-His, L-Phe, and L-Leu to obtain lower dietary CP content (diets 6 to 10 in trial 1 from 1 to 17 d of age and diets 7 and 8 in trial 2 from 1 to 21 d of age) had inferior growth performance compared with birds fed the control diets. Presumably, depressed performance may be related to the AA ratios used with digestible His, Phe, and Leu to Lys. Minimum ratios of digestible His, Phe, and Leu to Lys were maintained at 0.37, 0.63, and 1.07, respectively (Rostagno et al., 2011). However, diets 6 to 10 had 4, 4, and 3% lower ratio of digestible His, Phe, and Leu to Lys than diet 5 in trial 1. In trial 2, digestible Phe and Leu to Lys ratios in diets 7 and 8 were 10 and 12% lower than their ratios in diet 6. Recommended digestible His, Phe, and Leu to Lys ratios may need to be reevaluated to determine the optimum values for growth performance. Hence, higher ratios of digestible His, Phe, and Leu to Lys may allow further reduction in dietary CP content without compromising growth performance of broilers.

In addition to providing diets with adequate essential AA, maintaining an optimum total Gly + Ser concentration may be needed in reduced CP diets fed to broilers during the starter period. Corzo et al. (2005) reported that poor FCR of broilers fed a reduced CP diet (18%) containing adequate essential AA concentrations from 5 to 21 d of age could be ameliorated by supplementing Gly or a mixture of non-essential AA (L-Glu, Gly, L-Asp, L-Pro, and L-Ala). However, when non-essential AA other than Gly were individually supplemented to the 18% CP diet, FCR of broilers was higher compared with birds fed the control diet (22% CP). This indicated that Gly may be conditionally essential for broilers fed reduced CP diets during the starter period (Corzo et al., 2005; Dean et al., 2006; Awad et al., 2015). Yuan et al. (2012) examined growth responses of broilers fed reduced CP diets containing a 2.0% total Gly + Ser concentration from 1 to 18 d of age. Essential AA (DL-Met to L-His) were sequentially added to decrease CP content. No differences in 18 d BW or FCR were observed as dietary CP decreased from 21.4% (DL-Met, L-Lys, and L-Thr) to 18.0% (DL-Met, L-Lys, L-Thr, L-Val, L-Ile, L-Arg, L-Trp, and L-His). However, a 5 point numerical difference in FCR (1.35 vs. 1.40) was noted between adding L-Val (20.7% CP) compared with the L-His-supplemented diet (18.0% CP). Additionally, this study only utilized 6 replicate pens with 5 chicks per cage. Hence, additional replications may have resulted in treatment differences.

Glycine is central for feather development (Hegsted et al., 1941; Robel, 1977; Stilborn et al., 1997), creatine formation (Ngo et al., 1977), mucin synthesis (Lien et al., 2001; Montagne et al., 2004; Ospina-Rojas et al., 2013), enterocyte development (Wang et al., 2014a, b), uric acid synthesis (Leeson and Summers, 2001), and bone formation (Yuan et al., 2012). Hence, it is important to maintain adequate total Gly + Ser concentrations in diets fed during the starter period. The minimum concentration of total Gly + Ser was determined to be 2.10% to obtain optimal growth performance of broilers fed reduced CP diets from 1 to 21 d of age (Waguespack et al., 2009). In parallel to published research, experimental diets in the current study were formulated to contain a minimum value of 2.10% total Gly + Ser which corresponded to approximately 1.90 and 1.70 total Gly + Ser to digestible Lys ratios, respectively, in trials 1 and 2. Maintaining an adequate total Gly + Ser to digestible Lys ratio and essential AA concentrations in the present research could allow reduction in dietary CP content of 4.9 and 3.9 percentage points, respectively, in trials 1 (from 1 to 17 d of age) and 2 (from 1 to 14 d of age) without compromising BW gain of broilers.

In trial 1 of the present research, L-Gln supplementation was intended to determine if broilers would respond to non-specific nitrogen. L-Glutamine was used due to its high nitrogen content compared with other non-essential AA (Glu, Asp, or Ala), which can contribute to dietary CP content (NRC, 1994). In trial 1, broilers receiving diets with added L-Gln (4.0%) had depressed BW gain compared with those fed the positive control diet. Although birds receiving L-Gln supplementation did not have a depressed FI when compared with those provided positive control diet, these broilers had lower FI than broilers fed diet 10. According to Bartell and Batal (2007), the high inclusion of L-Gln (4.0%) may have been unpalatable to birds resulting in depressed FI and BW gain. Hence, a high inclusion of L-Gln in diet 11 of trial 1 may decrease palatability, which led to a reduction in BW gain of broilers. Other researchers have used L-Glu as the source of non-specific nitrogen, but results have been inconsistent (Pinchasov et al., 1990; Han et al., 1992; Kerr and Kidd, 1999; Aletor et al., 2000; Hussein et al., 2001).

Heger et al. (1998) suggested that non-essential AA must be provided as a proportion to the essential AA to obtain optimum protein utilization. This proportion should be assessed on a nitrogen basis because non-essential AA are primarily utilized to provide non-specific nitrogen (Lenis et al., 1999; Heger, 2003). Previous researchers have reported that optimum ratios of total essential to non-essential AA nitrogen (**EAA_n:$NEAA_n$**) for maximum growth and nitrogen utilization of broilers and pigs ranged from 42:58 to 67:33 (Stucki and Harper, 1961; Sugahara and Ariyoshi, 1968; Bedford and Summers, 1985; Wang and Fuller, 1989; Heger et al., 1998; Lenis et al., 1999). However, these recommendations were assessed decades ago using broilers having a lower rate of lean tissue deposition. Therefore, future studies in evaluating the optimum EAA_n:$NEAA_n$ ratio are warranted for current broilers strains. In the present research, total EAA_n:$NEAA_n$ ratios were calculated using total nitrogen content from each AA without including Gly + Ser as essential AA. In trial 1, experimental diets contained total EAA_n:$NEAA_n$ ratios ranging from 54:46 to 58:42. In trial 2, total EAA_n:$NEAA_n$ ratio in diets 1 to 8 ranged from 55:45 to 57:43. Although depressed growth performance of broilers was observed in both trials, the response may not be influenced by total EAA_n:$NEAA_n$ ratios because of a small variation in total EAA_n:$NEAA_n$ ratio among dietary treatments in both trials.

Blood Measures

In trial 1, broilers fed diets with reduced CP content of 1.8 percentage points or greater (diets 4 to 10) had decreased plasma UA concentration ($P < 0.05$) compared with feeding broilers diet 2 (Table 3.7). When L-Gln was added in diet 11 to increase dietary CP content, plasma UA concentration of broilers increased ($P < 0.0001$) compared with diet 10 and did not differ ($P = 0.09$) from diet 2. Total blood protein concentration of broilers consuming diet 2 was higher ($P < 0.05$) than those receiving diet 8 or 11. In trial 2, plasma UA concentration of broilers was reduced ($P < 0.05$) when dietary CP content was lowered beyond 2.0 percentage points (diets 4 to 8) compared with the plasma UA concentration in broilers fed diet 1 (Table 3.8). Additionally, broilers fed diet 1 (23.9% CP) had higher ($P = 0.006$) total blood protein concentration compared with birds fed diet 8 (20.2% CP). These results demonstrated that reductions in dietary CP content decreased plasma UA concentrations of broilers in a consistent manner that may also indicate a reduction in nitrogen excretion (Corzo et al., 2005; Ospina-Rojas et al., 2012; Awad et al., 2015). Corzo et al. (2005) determined that broilers fed 18% CP diet had reduced plasma UA concentration (mg/dL) and nitrogen excretion (g/b) by 36% and 32%, respectively, compared with those fed 22% CP diet from 5 to 21 d of age. However, total blood protein concentration of broilers did not decrease consistently with the reduction in dietary CP content.

Nitrogen Balance

Nitrogen balance results from trial 1 are presented in Table 3.7. Nitrogen intake and excretion (mg/b/d) were reduced ($P < 0.05$) when dietary CP content was lowered by 1.8 percentage points or greater (diet 2 vs. 4 to 10). However, no differences ($P > 0.05$) were observed in nitrogen intake and excretion (mg/b/d) between birds consuming diets 11 and 2. Broilers fed diet 11 consumed and excreted higher ($P < 0.0001$) amounts of nitrogen (mg/b/d) compared with those fed diet 10. These results demonstrated that nitrogen excretion was reduced up to 14.1% from 994 to 836 mg/b/d translating to a 7.9% reduction in nitrogen excretion per 1 percentage point decrease in CP content from 25.8 to 24.0% (diets 2 to 4) without compromising BW gain and FCR of broilers. Nitrogen excretion as a percent of nitrogen intake was decreased ($P < 0.05$) when broilers were fed diets containing supplemental L-Phe and L-Leu (diets 8 to 10) compared with those provided diet 2. Broilers fed diet 11 excreted a similar ($P = 0.32$) amount of nitrogen (% of N intake) compared with birds consuming diet 2. However, nitrogen excretion (% of N intake) of birds provided diet 11 was higher ($P < 0.0001$) compared with those fed diet 10. Broilers fed diet 8 exhibited a 16.2% reduction in nitrogen excretion compared with birds consuming diet 2, but FCR increased by 10 points with feeding diet 8.

Kerr and Kidd (1999) observed a reduction of nitrogen excretion from 1,300 to 950 mg/b/d when lowering dietary CP content from 19.4 to 18.2% (from 28 to 42 d of age). This resulted in a 22.8% decrease of nitrogen excretion per unit reduction in dietary CP content, which is a much larger decrease than results presented herein. Additionally, Bregendahl et al. (2002) reported a reduction in nitrogen excretion (g/b) of 6.3% for every unit decrease in CP content from 23 to 18.5%. However, the 4.5 percentage points reduction in dietary CP content from 7 to 21 d of age depressed BW gain and gain to feed ratio of broilers by 23 g/bird and 58 g/kg, respectively. Si et al. (2004) reported that lowering dietary CP content from 20 to 18% decreased nitrogen content in the excreta (%) by 13% without compromising BW and FCR of broilers from 1 to 21 d of age. Additionally, Ferguson et al. (1998) reported that lowering dietary CP content approximately 2.1 percentage points during a 42 d production period reduced nitrogen excretion in the litter (g/kg litter) by 17% while maintaining similar BW gain.

In conclusion, the sequential additions of DL-Met, L-Lys, L-Thr, L-Val, Gly (conditional), L-Ile, L-Arg, and L-Trp can be used to meet essential AA requirements of broilers while allowing dietary CP content reduction by 4.0 percentage points without compromising growth performance from 1 to 14 d of age, but only a 2.2 percentage point reduction of CP content can be achieved from 1 to 21 d of age. The use of AA supplementation to lower dietary CP content was also effective in decreasing nitrogen excretion (mg/b/d) by 14.1% without compromising performance objectives. Therefore, optimum broiler performance can be obtained without placing a minimum of CP concentration when proper AA ratios are used in diet formulation.

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Table 3.1 Ingredient and nutrient composition of dietary treatments fed to Ross × Ross 708 male broilers from 1 to 18 d of age, trial 1

	Dietary Treatment										
Ingredient, % "as-fed"	1	2	3	4	5	6	7	8	9	10	11
Corn	50.60	50.24	53.36	55.96	56.49	58.47	62.92	67.22	69.25	71.28	66.04
Soybean meal	41.02	41.07	38.24	35.82	35.32	33.40	29.04	24.82	22.78	20.74	21.26
Vegetable oil	4.43	4.52	3.96	3.50	3.40	3.02	2.18	1.35	0.93	0.51	1.19
Calcium carbonate	0.78	0.78	0.79	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.78
Defluorinated phosphate	1.89	1.89	1.86	1.93	1.93	1.95	1.98	2.02	2.03	2.05	2.07
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.50	0.50	0.45	0.45	0.45	0.45	0.44	0.33	0.27	0.20	0.20
Sodium bicarbonate	---	---	---	---	---	---	---	0.09	0.14	0.20	0.20
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
DL-Met	0.34	0.34	0.36	0.38	0.38	0.40	0.44	0.47	0.49	0.51	0.51
L-Lys•HCl	0.07	0.07	0.16	0.23	0.25	0.31	0.44	0.57	0.63	0.70	0.69
L-Thr	0.09	0.09	0.13	0.16	0.17	0.19	0.25	0.31	0.34	0.37	0.37
Gly	---	0.22	0.33	0.41	0.43	0.50	0.66	0.82	0.89	0.97	0.98
L-Val	---	---	0.05	0.09	0.10	0.13	0.20	0.27	0.31	0.34	0.35
L-Ile	---	---	---	0.01	0.02	0.05	0.12	0.20	0.23	0.27	0.27
L-Arg	---	---	---	---	0.01	0.06	0.18	0.30	0.36	0.42	0.42
L-Trp	---	---	---	---	---	0.01	0.03	0.05	0.06	0.07	0.07
L-His	---	---	---	---	---	---	0.04	0.08	0.09	0.11	0.12
L-Phe	---	---	---	---	---	---	---	0.03	0.07	0.10	0.11
L-Leu	---	---	---	---	---	---	---	---	0.05	0.10	0.12
L-Gln	---	---	---	---	---	---	---	---	---	---	3.99
Calculated analysis, % (unless otherwise noted)											
AME, kcal/kg	3,053	3,053	3,053	3,053	3,053	3,053	3,053	3,053	3,053	3,053	3,053
Crude protein[3]	23.19	23.45	22.62	21.92	21.79	21.36	20.44	19.57	19.19	18.81	23.45
Digestible Lys	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Digestible Met	0.63	0.63	0.64	0.65	0.65	0.66	0.68	0.70	0.70	0.71	0.72

Digestible TSAA[4]	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Digestible Thr	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Digestible Val	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Digestible Ile	0.88	0.88	0.84	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Digestible Arg	1.41	1.41	1.34	1.27	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Digestible Trp	0.26	0.26	0.25	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Digestible His	0.51	0.51	0.49	0.47	0.46	0.44	0.44	0.44	0.44	0.44	0.44
Digestible Phe	1.03	1.03	0.98	0.93	0.92	0.89	0.81	0.76	0.76	0.76	0.76
Digestible Leu	1.68	1.68	1.61	1.55	1.54	1.49	1.39	1.28	1.28	1.28	1.28
Total Gly	0.94	1.16	1.21	1.26	1.27	1.31	1.39	1.48	1.52	1.56	1.57
Total Gly + Ser	2.06	2.28	2.28	2.28	2.28	2.28	2.28	2.28	2.28	2.28	2.28
Total Gly equivalent[5]	1.74	1.96	1.88	1.99	1.99	1.82	2.03	2.05	2.06	2.07	2.08
Total Gly + Ser to digestible Lys ratio	1.70	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90
Total EAA_n:$NEAA_n$[6]	55:45	54:46	54:46	54:46	54:46	54:46	55:45	56:44	57:43	58:42	58:42
Ca	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Non-phytate P	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Na	0.23	0.23	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

[1]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate),1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.

[2]Mineral premix include per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

[3]Analyzed crude protein content in diets 1 to 11 was 25.0, 25.8, 24.2, 24.0, 24.5, 23.2, 23.0, 21.8, 21.8, 20.9, and 24.9%, respectively (method 968.06; AOAC International, 2006).

[4]TSAA = Total sulfuric amino acids

[5]Total Gly equivalent (%) = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[6]Ratio of total essential to non-essential amino acid nitrogen

Table 3.2 Ingredient and nutrient composition of dietary treatments fed to Ross × Ross 708 male broilers from 1 to 21 d of age, trial 2

	Dietary Treatment							
Ingredient, % “as-fed”	1	2	3	4	5	6	7	8
Corn	50.81	51.27	55.50	56.66	58.43	59.15	65.31	66.19
Soybean meal	40.88	40.48	36.54	35.46	33.75	33.05	27.03	26.09
Vegetable oil	4.38	4.30	3.55	3.33	3.00	2.86	1.67	1.51
Calcium carbonate	0.78	0.78	0.79	0.79	0.79	0.79	0.79	0.79
Defluorinated phosphate	1.89	1.89	1.92	1.93	1.94	1.95	2.00	2.00
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.46	0.46	0.40	0.37	0.31	0.29	0.10	0.09
Sodium bicarbonate	---	---	0.04	0.07	0.12	0.14	0.30	0.34
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
DL-Met	0.34	0.35	0.38	0.39	0.41	0.41	0.46	0.47
L-Lys•HCl	0.08	0.09	0.22	0.25	0.31	0.33	0.52	0.55
L-Thr	0.10	0.10	0.16	0.17	0.20	0.21	0.29	0.31
L-Val	---	0.01	0.08	0.09	0.12	0.14	0.24	0.26
Gly	---	---	0.15	0.19	0.25	0.28	0.51	0.54
L-Ile	---	---	---	0.02	0.05	0.06	0.17	0.18
L-Arg	---	---	---	---	0.05	0.07	0.25	0.27
L-Trp	---	---	---	---	---	0.004	0.03	0.04
L-His	---	---	---	---	---	---	0.06	0.06
L-Phe	---	---	---	---	---	---	---	0.02
Calculated analysis, % (unless otherwise noted)								
AME, kcal/kg	3,053	3,053	3,053	3,053	3,053	3,053	3,053	3,053
Crude protein[3]	23.98	23.84	22.66	22.35	21.95	21.79	20.50	20.31
Digestible Lys	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Digestible Met	0.66	0.66	0.67	0.68	0.68	0.69	0.71	0.72
Digestible TSAA[4]	0.95	0.95	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	0.86	0.86
Digestible Val	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Digestible Ile	0.91	0.91	0.84	0.84	0.84	0.84	0.84	0.84
Digestible Arg	1.47	1.46	1.34	1.31	1.31	1.31	1.31	1.31
Digestible Trp	0.25	0.25	0.23	0.22	0.21	0.21	0.21	0.21
Digestible His	0.53	0.53	0.49	0.48	0.47	0.46	0.46	0.46
Digestible Phe	1.06	1.05	0.98	0.69	0.93	0.91	0.80	0.80
Digestible Leu	1.74	0.73	0.63	0.61	0.57	1.55	1.40	1.38
Total Gly	0.97	0.97	1.05	1.07	1.10	1.12	1.24	1.26
Total Gly + Ser	2.14	2.13	2.13	2.13	2.13	2.13	2.13	2.13
Total Gly equivalent[5]	1.81	1.79	1.82	1.82	1.83	1.84	1.87	1.88
Total Gly + Ser to digestible Lys ratio	1.71	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Total EAA_n:$NEAA_n$[6]	55:45	55:45	55:45	55:45	55:45	55:45	57:43	57:43
Calcium	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Non-phytate phosphorus	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Sodium	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

[1]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate),1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.

[2]Mineral premix include per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

[3]Analyzed crude protein content in diets 1 to 8 was 23.9, 22.9, 22.5, 21.8, 22.2, 21.7, 20.0, and 20.2%, respectively (method 968.06; AOAC International, 2006).

[4]TSAA = Total sulfuric amino acids

[5]Total Gly equivalent = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[6]Ratio of total essential to non-essential amino acid nitrogen

Table 3.3 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 17 d of age, trial 1[1]

Item	Analyzed CP, %	BW gain, g/bird[2]	Feed intake, g/bird	FCR, g:g[3]	Mortality, %[4]
Dietary Treatment[5]					
1) Control diet 1 (DL-Met, L-Lys, and L-Thr)	25.0	505	591	1.192	4.2
2) Control diet 2 (DL-Met, L-Lys, L-Thr, and Gly)	25.8	512	587	1.173	2.8
3) Control diet 2 + L-Val	24.2	510	594	1.175	4.2
4) Control diet 2 + L-Val and L-Ile	24.0	510	602	1.203	4.8
5) Control diet 2 + L-Val, L-Ile, and L-Arg	24.5	511	601	1.196	2.8
6) Control diet 2 + L-Val, L-Ile, L-Arg, and L-Trp	23.2	511	608	1.221	4.8
7) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, and L-His	23.0	500	600	1.235	7.9
8) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	21.8	493	617	1.276	6.3
9) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu	21.8	501	601	1.237	5.6
10) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu[6]	20.9	489	603	1.251	4.2
11) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, L-Leu, and L-Gln	24.9	451	563	1.267	5.6
SEM		11	11	0.017	2.7
Pre-planned orthogonal contrasts		*Probabilities*			
Diet 2 vs. 3		0.93	0.61	0.90	0.70
Diet 2 vs. 4		0.91	0.31	0.21	0.60
Diet 2 vs. 5		0.99	0.33	0.31	1.00
Diet 2 vs. 6		1.00	0.14	0.043	0.60
Diet 2 vs. 7		0.44	0.36	0.010	0.17
Diet 2 vs. 8		0.22	0.043	< 0.0001	0.35
Diet 2 vs. 9		0.46	0.31	0.006	0.45

Diet 2 vs. 10	0.11	0.26	0.0009	0.70
Diet 2 vs. 11	< 0.0001	0.09	< 0.0001	0.45
Diet 10 vs. 11	0.007	0.006	0.49	0.70

[1]Values are least-square means of 8 replicate pens, with each pen having 9 chicks at placement.
[2]BW = Body Weight
[3]Feed conversion ratio was corrected for mortality.
[4]Mortality values were arcsine transformed.
[5]Diet 1 was formulated to contain 1.20% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio. Diets 2 to 11 were formulated to contain 1.20% digestible Lys and 1.90 total Gly + Ser to digestible Lys ratio.
[6]Diet 10 was formulated with higher inclusion of L-Leu to contain lower crude protein content than diet 9.

Table 3.4 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 7 d of age, trial 2[1]

Item	Analyzed CP, %	BW Gain, g/bird[2]	Feed Intake, g/bird	FCR, g:g[3]	Mortality, %[4]
Dietary Treatment[5]					
1) Control (DL-Met, L-Lys, and L-Thr)	23.9	100	108	1.085	1.0
2) Control + L-Val	22.9	100	108	1.087	0.0
3) Control + L-Val and Gly	22.5	104	113	1.087	1.0
4) Control + L-Val, Gly, and L-Ile	21.8	99	109	1.110	0.5
5) Control + L-Val, Gly, L-Ile, and L-Arg	22.2	102	110	1.076	0.0
6) Control + L-Val, Gly, L-Ile, L-Arg, and L-Trp	21.7	102	110	1.081	0.5
7) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, and L-His	20.0	100	110	1.095	0.5
8) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	20.2	102	113	1.117	1.5
SEM		2	2	0.020	0.5
Pre-planned orthogonal contrasts		*Probabilities*			
Diet 1 vs. 2		0.90	0.96	0.93	0.18
Diet 1 vs. 3		0.16	0.10	0.94	1.00
Diet 1 vs. 4		0.54	0.80	0.38	0.50
Diet 1 vs. 5		0.54	0.64	0.76	0.18
Diet 1 vs. 6		0.43	0.43	0.89	0.50
Diet 1 vs. 7		0.94	0.60	0.72	0.50
Diet 1 vs. 8		0.52	0.11	0.25	0.50

[1]Values are least-square means of 8 replicate cages, with each cage having 25 chicks at placement.
[2]BW = Body Weight
[3]Feed conversion ratio was corrected for mortality.
[4]Mortality values were arcsine transformed.
[5]All dietary treatments were formulated to contain 1.25% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio.

Table 3.5 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 14 d of age, trial 2[1]

Item	Analyzed CP, %	BW Gain, g/bird[2]	Feed Intake, g/bird	FCR, g:g[3]	Mortality, %[4]
Dietary Treatment[5]					
1) Control (DL-Met, L-Lys, and L-Thr)	23.9	359	428	1.195	1.5
2) Control + L-Val	22.9	360	429	1.194	1.0
3) Control + L-Val and Gly	22.5	369	438	1.189	1.0
4) Control + L-Val, Gly, and L-Ile	21.8	369	439	1.191	0.5
5) Control + L-Val, Gly, L-Ile, and L-Arg	22.2	366	434	1.194	1.5
6) Control + L-Val, Gly, L-Ile, L-Arg, and L-Trp	21.7	371	437	1.181	0.5
7) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, and L-His	20.0	350	427	1.222	1.5
8) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	20.2	362	434	1.210	2.5
SEM		5	4	0.014	0.8
Pre-planned orthogonal contrasts		*Probabilities*			
Diet 1 vs. 2		0.85	0.82	0.94	0.57
Diet 1 vs. 3		0.14	0.054	0.74	0.57
Diet 1 vs. 4		0.14	0.04	0.84	0.26
Diet 1 vs. 5		0.34	0.21	0.94	0.74
Diet 1 vs. 6		0.09	0.08	0.49	0.26
Diet 1 vs. 7		0.21	0.94	0.18	0.74
Diet 1 vs. 8		0.69	0.23	0.44	0.26

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]BW = Body Weight
[3]Feed conversion ratio was corrected for mortality.
[4]Mortality values were arcsine transformed.
[5]All dietary treatments were formulated to contain 1.25% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio.

Table 3.6 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 21 d of age, trial 2[1]

Item	Analyzed CP, %	BW Gain, g/bird[2]	Feed Intake, g/bird	FCR, g:g[3]	Mortality, %[4]
Dietary Treatment[5]					
1) Control (DL-Met, L-Lys, and L-Thr)	23.9	841	1,057	1.269	4.5
2) Control + L-Val	22.9	838	1,056	1.270	2.0
3) Control + L-Val and Gly	22.5	847	1,068	1.267	2.5
4) Control + L-Val, Gly, and L-Ile	21.8	840	1,079	1.286	1.0
5) Control + L-Val, Gly, L-Ile, and L-Arg	22.2	845	1,067	1.271	2.5
6) Control + L-Val, Gly, L-Ile, L-Arg, and L-Trp	21.7	845	1,070	1.274	2.0
7) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, and L-His	20.0	789	1,027	1.307	2.0
8) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	20.2	813	1,045	1.302	4.0
SEM		10	9	0.012	1.1
Pre-planned orthogonal contrasts		*Probabilities*			
Diet 1 vs. 2		0.86	0.95	0.96	0.18
Diet 1 vs. 3		0.63	0.37	0.88	0.16
Diet 1 vs. 4		0.99	0.08	0.28	0.032
Diet 1 vs. 5		0.74	0.42	0.91	0.16
Diet 1 vs. 6		0.74	0.28	0.76	0.18
Diet 1 vs. 7		0.0002	0.023	0.019	0.12
Diet 1 vs. 8		0.033	0.36	0.044	0.89

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]BW = Body Weight
[3]Feed conversion ratio was corrected for mortality.
[4]Mortality values were arcsine transformed.
[5]All dietary treatments were formulated to contain 1.25% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio.

Table 3.7 Nitrogen balance, total blood protein, and plasma uric acid concentrations of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 18 d of age, trial 1

Item	Analyzed CP, %	N intake, mg/bird/d[1]	N excretion, mg/bird/d[1]	N excretion, %[1]	Total blood protein, g/dL[2]	Plasma uric acid, mg/dL[2]
Dietary Treatment[3]						
1) Control diet 1 (DL-Met, L-Lys, and L-Thr)	25.0	2,565	994	38.8	2.58	10.93
2) Control diet 2 (DL-Met, L-Lys, L-Thr, and Gly)	25.8	2,513	974	38.9	2.63	10.85
3) Control diet 2 + L-Val	24.2	2,422	857	35.1	2.64	10.50
4) Control diet 2 + L-Val and L-Ile	24.0	2,314	836	36.2	2.64	9.03
5) Control diet 2 + L-Val, L-Ile, and L-Arg	24.5	2,087	875	42.8	2.64	9.48
6) Control diet 2 + L-Val, L-Ile, L-Arg, and L-Trp	23.2	2,317	848	36.6	2.64	8.96
7) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, and L-His	23.0	2,181	764	35.0	2.53	8.19
8) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	21.8	2,202	716	32.6	2.47	7.48
9) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu	21.8	2,163	673	31.4	2.60	7.84
10) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu[4]	20.9	1,944	626	32.2	2.53	7.47
11) Control diet 2 + L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, L-Leu, and L-Gln	24.9	2,466	1,008	40.9	2.48	12.02
SEM		65	29	1.4	0.07	0.50
Pre-planned orthogonal contrasts		*Probabilities*				
Diet 2 vs. 3		0.32	0.005	0.07	0.82	0.62
Diet 2 vs. 4		0.032	0.0009	0.20	0.89	0.011
Diet 2 vs. 5		< 0.0001	0.015	0.06	0.91	0.053
Diet 2 vs. 6		0.035	0.002	0.28	0.83	0.008
Diet 2 vs. 7		0.0005	< 0.0001	0.07	0.17	0.0002

Diet 2 vs. 8	0.001	< 0.0001	0.003	0.029	< 0.0001
Diet 2 vs. 9	0.0003	< 0.0001	0.0005	0.70	< 0.0001
Diet 2 vs. 10	< 0.0001	< 0.0001	0.002	0.16	< 0.0001
Diet 2 vs. 11	0.61	0.41	0.32	0.041	0.09
Diet 10 vs. 11	< 0.0001	< 0.0001	< 0.0001	0.52	< 0.0001

[1] Nitrogen (N) values are least-square means of 8 replicate cages obtained from 24 hour nitrogen collection period with each cage having 9 chicks at placement.
[2]Values are least-square means of 6 replicate cages using 3 birds per cage.
[3]Diet 1 was formulated to contain 1.20% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio. Diets 2 to 11 were formulated to contain 1.20% digestible Lys and 1.90 total Gly + Ser to digestible Lys ratio.
[4]Diet 10 was formulated with higher inclusion of L-Leu to contain lower crude protein content than diet 9.

Table 3.8 Total blood protein and plasma uric acid concentrations of Ross × Ross 708 male broilers fed reduced crude protein diets with sequential additions of essential amino acids from 1 to 21 d of age in trial 2[1]

Item	Analyzed CP, %	Total Blood Protein, g/dL	Plasma Uric Acid, mg/dL
Dietary Treatment[2]			
1) Control (DL-Met, L-Lys, and L-Thr)	23.9	2.53	9.81
2) Control + L-Val	22.9	2.43	9.22
3) Control + L-Val and Gly	22.5	2.44	9.76
4) Control + L-Val, Gly, and L-Ile	21.8	2.58	8.54
5) Control + L-Val, Gly, L-Ile, and L-Arg	22.2	2.53	7.85
6) Control + L-Val, Gly, L-Ile, L-Arg, and L-Trp	21.7	2.44	8.58
7) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, and L-His	20.0	2.43	7.49
8) Control + L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe	20.2	2.34	7.41
SEM		0.05	0.45
Pre-planned orthogonal contrasts		*Probabilities*	
Diet 1 vs. 2		0.13	0.30
Diet 1 vs. 3		0.19	0.93
Diet 1 vs. 4		0.39	0.027
Diet 1 vs. 5		0.91	0.0006
Diet 1 vs. 6		0.21	0.033
Diet 1 vs. 7		0.13	< 0.0001
Diet 1 vs. 8		0.006	< 0.0001

[1]Values are least-square means of 6 replicate pens using 3 birds per pen.

[2]All dietary treatments were formulated to contain 1.25% digestible Lys and 1.70 total Gly + Ser to digestible Lys ratio.

IV. EFFECTS OF GLYCINE AND GLUTAMINE SUPPLEMENTATION TO REDUCED CRUDE PROTEIN DIETS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF MALE BROILERS DURING A 41 DAY PRODUCTION PERIOD

ABSTRACT

An experiment was conducted to determine the effects of feeding reduced crude protein (**CP**) diets supplemented with Gly and/or L-Gln on growth performance and carcass characteristics of broilers during a 41 d production period. One thousand six hundred Ross × Ross 708 male chicks (25 birds/pen; 0.09 m^2/bird) were fed 1 of 8 dietary treatments. Diets 1 and 8 served as the negative (**NC**) and positive control (**PC**), respectively, with the NC diet formulated to contain 2.5 and 0.29 percentage points lower CP and total Gly + Ser concentrations than the PC diet, respectively. The 6 other diets were formulated to contain intermediate concentrations of total Gly + Ser and/or CP. Diets 2 and 3 were formulated by adding Gly to the NC diets to increase total Gly + Ser concentrations by 33 and 66%, respectively, of the difference between the PC and NC diets. Diets 4 and 5 were formulated by supplementing L-Gln to the NC diets to increase CP concentrations by 33 and 66%, respectively, of the difference between the PC and NC diets. Diets 6 and 7 were formulated by adding Gly and L-Gln to the NC diets, which resulted in 33 and 66% increase in total Gly + Ser and CP concentrations, respectively, of the difference between the PC and NC diets. Broilers provided diet 6 had a lower (P = 0.018) feed conversion and a higher (P = 0.024) body weight gain than those fed diet 4

from 1 to 28 d of age. From 1 to 40 d of age, feed conversion of broilers fed diet 6 was lower (*P* = 0.017) than birds receiving diet 4, but body weight gain was not affected (*P* = 0.37). Total breast meat weight of broilers fed diet 7 was higher (*P* = 0.040) than birds provided the PC diets. Broilers receiving diet 7 also had higher (*P* < 0.05) total breast meat weight and yield than birds consuming diets 3 and 5. These results indicated that Gly had more pronounced impacts on growth performance during the early growth periods than as broilers approached marketing. Providing adequate total Gly + Ser and non-essential nitrogen concentrations to reduced CP diets may be necessary to increase total breast meat weight and yield of broilers.

INTRODUCTION

The supplementation of feed-grade amino acids (**AA)** to broiler diets has been used as a strategy to lower live production costs by reducing soybean meal inclusion that translates to lower dietary crude protein (**CP**) content. Broilers fed reduced CP diets have decreased nitrogen excretion (Bregendahl et al., 2002; Waldroup et al., 2005; Dean et al., 2006; Namroud et al., 2008; Hernandez et al., 2012), ammonia emissions (Ferguson et al., 1998), and the incidence of pododermatitis (Nagaraj et al., 2007) compared with those fed higher CP diets. However, prior studies have reported that reducing dietary CP content may depress growth performance of broilers even with the supplementation of DL-Met, L-Lys, and L-Thr (Dean et al., 2006; Namroud et al., 2008; Hernandez et al., 2012). Published research have investigated several possible reasons for poor performance in broilers fed reduced CP diets, such as insufficient dietary potassium (Han et al., 1992), essential AA deficiencies (Bregendahl et al., 2002), lack of non-specific nitrogen (Aletor et al., 2000), and low dietary energy (Hussein et al., 2001), but variable results were reported.

Supplementing Gly to reduced CP diets formulated using ingredients of vegetable origin has enhanced growth performance of broilers during the first few weeks post-hatching (Corzo et al., 2005; Dean et al., 2006; Waguespack et al., 2009; Yuan et al., 2012). Dean et al. (2006) determined that a combination of Gly, L-Glu, L-Pro, L-Ala, and L-Asp supplementation in a low CP diet (16% CP) restored poor growth performance of broilers when compared with those fed a higher CP diet (22% CP) from 1 to 18 d of age. However, when those non-essential AA were individually supplemented in the 16% CP, Gly was the only AA that could ameliorate growth performance of broilers similar to birds fed the 22% CP diet (Dean et al., 2006). This indicated that Gly may be conditionally essential in broilers fed reduced CP diets during the starter period.

Research is limited in assessing growth and meat yield responses of broilers fed reduced CP diets varying in total Gly + Ser concentrations beyond the starter period. Additionally, it is uncertain if the positive growth responses of broilers associated with Gly supplementation in reduced CP diets were due to a Gly requirement per se or a nitrogen contribution. Therefore, an experiment was conducted to determine the effects of feeding reduced CP diets supplemented with Gly and/or L-Gln (nitrogen source) on growth performance and carcass characteristics of broilers during a 41 d production period.

MATERIALS AND METHODS

The experimental protocol regarding the use of live birds has been approved by the Institutional Animal Care and Use Committee at Auburn University (PRN 2014–2551).

Bird Husbandry

One thousand six hundred Ross × Ross 708 (Aviagen North America, Huntsville, AL) male chicks were obtained from a commercial hatchery and received vaccination for Marek's disease, Newcastle disease, and infectious bronchitis. At 1 d of age, chicks were placed into 64 floor pens (25 birds/pen; 0.09 m^2/bird) in a solid-sided house with a negative-pressure ventilation system equipped with vent boards, exhaust fans, cooling pads, and an electronic controller to adjust house temperature. Each pen was equipped with a nipple drinker line, a tube feeder, and used litter. Feed and water were provided ad libitum throughout the experimental period. Temperature was set at 33^{o}C at the beginning of the experiment and was gradually decreased to 21^{o}C at the end of the experiment. Photoperiod was set at 23L:1D from 1 to 7 d of age and 20L:4D was maintained from 8 to 41 d of age. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 to 41 d of age, respectively. Light intensity was verified at bird level (30 cm) using a photometric sensor (LI-250A Light Meter, LI-COR Bioscience, Lincoln, NE). The incidence of mortality was recorded daily.

Dietary Treatments

Broilers were fed 1 of 8 dietary treatments during the starter (from 1 to 14 d, Table 4.1), grower (from 15 to 28 d, Table 4.2), and finisher (from 29 to 40 d, Table 4.3) periods. Dietary treatments were formulated with corn and soybean meal as the primary ingredients. Diet 8 served as the positive control (**PC**) diet and was formulated to contain adequate essential AA concentrations. Diet 1, the negative control (**NC**) diet, was formulated to contain approximately 2.5 and 0.29 percentage points less CP and total Gly + Ser concentrations, respectively, than the PC diets while maintaining adequate essential

AA concentrations. The 6 other dietary treatments were formulated to contain intermediate concentrations of total Gly + Ser, CP, and the combination of total Gly + Ser and CP. This was accomplished by adding Gly and/or L-Gln to the NC diets. Glycine was supplemented to increase total Gly + Ser concentration while L-Gln was added as a source of nitrogen to increase CP concentration. Diets 2 and 3 were formulated by adding Gly to the NC diets to increase total Gly + Ser concentrations by 33 and 66%, respectively, of the difference between the PC and NC diets. Diets 4 and 5 were formulated by supplementing L-Gln to the NC diets to increase CP concentrations by 33 and 66%, respectively, of the difference between the PC and NC diets. Diets 6 and 7 were formulated by adding Gly and L-Gln to the NC diets, which resulted in 33 and 66% increase in total Gly + Ser and CP concentrations, respectively, of the difference between the PC and NC diets.

Experimental diets were formulated to contain 1.25, 1.10, and 1.00% digestible Lys, respectively, in the starter, grower, and finisher phases with minimum ratios of digestible TSAA, Thr, Val, Ile, Arg, Trp, His, Phe, and Leu at 0.75, 0.69, 0.76, 0.67, 1.04, 0.18, 0.38, 0.74, and 1.28, respectively. Digestible AA values were calculated by multiplying digestibility AA coefficients of corn and soybean meal with the total AA concentrations in these 2 ingredients. In the starter phase, diets were offered in crumble form, whereas subsequent feeds were provided in whole pellet form. Representative diet samples were analyzed for CP content (method 968.06; AOAC International, 2006) and total AA concentrations (method 994.12; AOAC International, 2006).

Measurements

Broilers and feed were weighed at the beginning and the end of each experimental period (1, 14, 28, and 40 d of age) to determine body weight (**BW**) gain, feed intake (**FI**), and feed conversion ratio (**FCR**) as a pen basis and the incidence of mortality was recorded daily. At 41 d of age, 12 birds per pen were selected for processing. Feed was removed from each pen 10 hours prior to processing. On the day of processing, the selected birds were placed in coops and transported to the Auburn University Pilot Processing Plant. Broilers were hung on shackles, electrically stunned, slaughtered, scalded, picked, and manually eviscerated. Following 3 hours of chilling in an ice bath, carcass (without abdominal fat) and abdominal fat weights were recorded. The whole carcass was cut into front and back half portions and was packed in ice for 18 hours. Pectoralis major (boneless breast) and minor (tenders) muscles were deboned from the front half portion of the carcass and were weighed to determine total breast meat yield. Carcass and total breast meat yields, as well as abdominal fat percentage, were calculated relative to the 40 d live BW.

Statistical Analyses

This experiment was designed as a randomized complete block with pen location as the blocking factor. Each treatment was represented by 8 replicate pens with pen being the experimental unit. Data were analyzed by ANOVA using the MIXED procedure of SAS (2011) with the following mixed-effects model:

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

Where $\mu_{..}$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance ${\sigma^2}_\rho$; the τ_j are fixed factor level effects corresponding to the j^{th} dietary treatment (diets 1 to 8) such that $\sum\tau_j = 0$; and the ε_{ij} are identically and independently normally distributed random errors with mean 0 and a variance σ^2. Pre-planned orthogonal contrasts were used to determine if the effects of dietary treatments on growth performance and carcass characteristics of broilers were due to Gly or nitrogen contribution. Hence, contrasts were arranged by comparing the effects of diet 8 vs. diets 1, 6 and 7, diet 6 vs. diets 2 and 4, and diet 7 vs. diets 3 and 5. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Calculated and analyzed values of dietary CP, total Gly, and total Gly + Ser in the starter, grower, and finisher diets are presented in Table 4.4. Nitrogen analysis of the starter diets indicated that CP content was approximately 1.5 percentage points higher than the calculated values. However, CP contents in the grower and finisher diets did not vary substantially between the calculated and analyzed values. Analyzed total Gly + Ser concentrations were slightly higher than the calculated values in the starter, grower, and finisher diets, but variation in total Gly + Ser concentration among dietary treatments was within acceptable range.

During the starter, grower and finisher periods, NC diets were formulated to contain approximately 2.5 and 0.29 percentage points lower CP and total Gly + Ser concentrations, respectively, compared with PC diets (Tables 4.1, 4.2, and 4.3). The addition of Gly in diets 2 and 3 was intended to increase total Gly + Ser concentrations by 33 and 66%, respectively, of the difference between PC and NC diets. L-Glutamine addition in diets 4 and 5 increased CP content by 33 and 66%, respectively, of the difference between PC and NC diets. In diets 6 and 7, both Gly and L-Gln were added to increase both total Gly + Ser and CP concentrations by 33 and 66% of the difference between PC and NC diets.

Growth Performance

Starter Period. From 1 to 14 d of age, feeding broilers diet 6 (2.03% total Gly + Ser and 22.6% CP) resulted in a higher (P = 0.043) FCR of broilers compared with those fed the PC diet (2.24% total Gly + Ser and 24.5% CP, Table 4.5). However, broilers consuming diet 7 (2.14% total Gly + Ser and 23.5% CP) had a similar (P = 0.72) FCR to broilers fed the PC diet (2.24% total Gly + Ser and 24.5% CP). Broilers provided diet 6 (2.03% total Gly + Ser and 22.6% CP) had a lower (P = 0.039) FCR than birds consuming diet 2 (2.03% total Gly + Ser and 21.8% CP), but when compared with birds fed diet 4 (1.92% total Gly + Ser and 22.6% CP), no difference (P = 0.16) in FCR was observed. Feed conversion ratio of broilers consuming diet 5 (1.92% total Gly + Ser and 23.5% CP) was 3.3 points higher (P = 0.003) than the FCR of birds receiving diet 7 (2.14% total Gly + Ser and 23.5% CP) indicating that the positive effect on FCR was due to a Gly contribution. Broilers receiving the PC diet (2.24% total Gly + Ser and 24.5% CP) had lower FCR (P < 0.0001) and FI (P = 0.002) than the NC-fed birds (1.92% total Gly + Ser and 21.7% CP). Additionally, dietary treatments did not affect (P > 0.05) BW gain and incidence of mortality of broilers from 1 to 14 d of age.

The positive Gly response on FCR of broilers was achieved by maintaining a total Gly + Ser concentration at 2.14% in a reduced CP diet from 1 to 14 d of age. In agreement, Waguespack et al. (2009) reported a minimum of 2.10% total Gly + Ser concentration for optimal FCR of broilers. In contrast, previous research indicated that a total Gly + Ser concentration should be expressed on an equimolar basis as total Gly equivalent (Gly (%) + Ser (%) × 0.7143) because the conversion of Gly from Ser occurs with a ratio of 0.7143 (Dean et al., 2006; Siegert et al., 2015a,b). In the current research, an optimum total Gly + Ser of 2.14% corresponds to 1.84% Gly equivalent, which is higher compared with recommended values of 1.58% for 95% of maximum gain to feed ratio of broilers from 1 to 21 d of age (Siegert et al., 2015a). The difference may be influenced by Gly precursors or metabolic processes that require Gly, such as digestible Thr, TSAA, and choline concentrations (Powell et al., 2011; Siegert et al., 2015a,b). In the present research, digestible Thr, TSAA, and added choline were maintained at adequate concentrations of 0.86%, 0.94%, and 500 ppm. Thus, sub-optimum concentrations of these nutrients do not contribute to a total Gly + Ser response. Meeting Gly needs for broilers fed reduced CP diets during the starter period may be central for enterocyte development (Wang et al., 2014a, b), feather formation (Robel; 1977; Stilborn et al., 1997), creatine synthesis (Ngo et al., 1977; Ospina-Rojas et al., 2013b), mucin synthesis (Ospina-Rojas et al., 2013a), uric acid formation (Lesson and Summers, 2001), and bone development (Yuan et al., 2012).

Nitrogen effect from Gln was observed to decrease FCR of broilers when added to increase CP content by 33% of the difference between PC and NC diets during the starter period. Glutamine has been reported to be a preferential energy source for enterocyte development in rats (Windmuller and Spaeth, 1974). Research in pigs demonstrated that applying 5 mM L-Gln to jejunal enterocyte cells from 0 to 7 d of age produced 2 fold higher ATP compared with 2 mM glucose, which indicates Gln is a preferential energy source for the small intestine of pigs (Wu et al., 1995). Moreover, Bartell and Batal (2007) observed 32 and 45% increases in duodenal and jejunal villi height of broilers, respectively, when supplementing diets with 1% L-Gln compared with birds fed diets without L-Gln supplementation from 1 to 14 d of age. Thus, the improvement in FCR of broilers fed L-Gln supplementation may be partially due to enterocyte development.

Grower Period. From 15 to 28 d of age, broilers fed diet 6 (1.78% total Gly + Ser and 20.1% CP) had a 40 g lower (P = 0.035) BW gain, but a similar (P = 0.36) FCR compared with birds consuming the PC diet (1.97% total Gly + Ser and 21.6% CP, Table 4.6). Body weight gain (P = 0.051) and FCR (P = 0.42) were similar between broilers provided diet 7 (1.87% total Gly + Ser and 20.9% CP) and the PC diet (1.97% total Gly + Ser and 21.6% CP). Broilers provided diet 4 (1.69% total Gly + Ser and 20.1% CP) had a lower (P = 0.011) BW gain and a higher (P = 0.008) FCR compared with those receiving diet 6 (1.78% total Gly + Ser and 20.1% CP), while feeding broilers diet 2 (1.78% total Gly + Ser and 19.4% CP) resulted in similar BW gain (P = 0.11) and FCR (P = 0.22) of broilers compared with diet 6 (1.78% total Gly + Ser and 20.1% CP). This indicated that maintaining a total Gly + Ser concentration of 1.78% positively affected BW gain and FCR of broilers fed a reduced CP diet. When increasing total Gly + Ser concentration to 1.87% in diet 3 (19.5% CP), broilers had similar BW gain (P = 0.29) and FCR (P =0.11) to birds fed diet 7 (1.87% total Gly + Ser and 20.9% CP). However, a 39 g lower (P = 0.040) BW gain was observed when broilers were provided diet 5 (1.69% total Gly + Ser and 20.9% CP) was compared with diet 7 (1.87% total Gly + Ser and 20.9% CP). Broilers receiving the PC diet (1.97% total Gly + Ser and 21.6% CP) had a higher (P = 0.001) BW gain than the NC-fed birds (1.69% total Gly + Ser and 19.3% CP), which resulted in a lower (P = 0.003) FCR of broilers fed the PC diet (1.97% total Gly + Ser and 21.6% CP). Feed intake and the incidence of mortality of broilers were not affected (P > 0.05) by the dietary treatments.

These results indicated that a 1.78% total Gly + Ser concentration allowed for optimum BW gain and FCR of broilers when fed a reduced CP diet from 15 to 28 d of age. Conversely, Ospina-Rojas et al. (2013a) recommended a minimum of 1.54% total Gly + Ser concentration for broilers from 21 to 35 d of age. The difference in the total Gly + Ser concentration to optimize growth responses between the current research and Ospina-Rojas et al. (2013a) may be influenced by the growth rate of broilers. Ospina-Rojas et al. (2013a) observed a 1.090 kg increase in BW gain whereas the present research noted a 1.234 kg BW gain from 15 to 28 d of age. The faster growth rate in the current study may indicate a higher need of dietary Gly. However, caution must be exercised when interpreting these results as the current study was not designed to determine a total Gly + Ser requirement during the growing period.

From 1 to 28 d of age, broilers fed diets 6 and 7 had similar ($P > 0.05$) BW gain and FCR compared with PC-fed birds (Table 4.7). Body weight gain of broilers fed diets 2 and 4 were 42 and 49 g lower ($P < 0.05$), respectively, than feeding diet 6, which indicated the importance of Gly and non-essential nitrogen in reduced CP diets to increase BW gain of broilers. Feed conversion did not differ ($P = 0.10$) between broilers fed diets 2 and 6. In contrast, FCR increased ($P = 0.018$) when broilers were fed diet 4 compared with diet 6. Providing broilers diet 3 resulted in similar ($P > 0.05$) BW gain and FCR of broilers compared with those fed diet 7. Broilers fed diet 5 had a 49 g lower ($P = 0.030$) BW gain, but a similar ($P = 0.12$) FCR compared with those fed diet 7. The PC-fed birds had a 4.3% higher ($P = 0.002$) BW gain and a 4.6% lower ($P < 0.0001$) FCR than the NC-fed birds. Feed intake and the incidence of mortality were not affected ($P > 0.05$) by dietary treatments. Glycine effects contributed to an increase in BW gain and FCR of broilers from 1 to 28 d of age. However, Gly response on BW gain occurred primarily during the grower period, but the effect on FCR was due to improvements during the starter and grower periods. These data also indicated that the Gln effect appeared to enhance BW gain of broilers from 1 to 28 d of age.

Finisher Period. From 29 to 40 d of age, broilers fed diet 6 (1.62% total Gly + Ser and 18.4% CP) had a 2.5% lower (P = 0.018) FCR, but a similar (P = 0.36) BW gain compared with birds consuming the PC diet (1.79% total Gly + Ser and 19.8% CP, Table 4.8). However, no differences (P > 0.05) were observed in BW gain and FCR of broilers when adding Gly and L-Gln collectively in diet 7 (1.70% total Gly + Ser and 19.1% CP) compared with the PC-fed birds (1.79% total Gly + Ser and 19.8% CP). Maintaining a total Gly + Ser concentration of 1.62% in diet 2 (17.8% CP) or a CP content of 18.4% in diet 4 (1.53% total Gly + Ser) did not alter (P > 0.05) BW gain and FCR of broilers compared with diet 6 (1.62% total Gly + Ser and 18.4% CP). Broilers provided diet 5 (1.53% total Gly + Ser and 19.1% CP) had a 4.2 point lower (P = 0.024) FCR than those fed diet 7 (1.70% total Gly + Ser and 19.1% CP). An explanation for the difference in FCR between broilers provided diets 5 and 7 is not apparent. Feed conversion ratio of broilers fed the PC diet (1.79% total Gly + Ser and 19.8% CP) was 4 point higher (P = 0.033) than the NC-fed birds (1.53% total Gly + Ser and 17.7% CP), which may be due to NC-fed birds (1.53% total Gly + Ser and 17.7% CP) gaining 59 g more (P = 0.025) BW than PC-fed birds (1.79% total Gly + Ser and 19.8% CP).

Cumulative Performance. Broilers receiving Gly and L-Gln supplementation in diets 6 and 7 had similar (P > 0.05) FCR compared with PC-fed birds (Table 4.9). Feeding broilers diet 2 led to a similar (P = 0.26) FCR compared with those provided diet

6. In contrast, FCR of broilers receiving diet 4 was higher ($P = 0.017$) than the birds consuming diet 6. When broilers provided diet 3 or 5, FCR was similar ($P > 0.05$) to birds fed diet 7. These results indicated that increasing total Gly + Ser concentration by 33% of the difference between the PC and NC diets reduced FCR from 1 to 40 d of age when broilers were fed reduced CP diets. However, the effects of dietary treatments were not apparent on BW gain of broilers. Broilers fed the NC diets had a 3 point higher ($P = 0.045$) FCR than those fed the PC diets. This response may be influenced by lower concentrations of non-essential AA in the reduced CP diets. The NC diets approximately contained 14, 10, 16, 10% lower Glu, Ala, Asp, and Pro than the PC diets. In order to compensate for lower non-essential AA concentrations, broilers receiving the NC diets numerically consumed 88 g more feed than the PC-fed birds. Published research has also demonstrated that decreasing AA density/CP in broiler diets increased feed consumption of broilers compared with those receiving higher AA density/CP diets (Dozier et al., 2007; Zhai et al., 2013). Hence, broilers were able to compensate reduced CP content by adjusting FI (Dozier et al., 2007).

Carcass Characteristics

Broilers provided diet 7 had a higher ($P = 0.040$) total breast meat weight than the PC-fed birds (Table 4.10). A lower ($P = 0.037$) carcass yield was observed when broilers were provided diet 4 compared with those fed diet 6. Carcass yield and total breast weight and yield of broilers consuming diet 3 were lower ($P < 0.05$) while abdominal fat weight and yield were higher ($P = 0.001$) than birds fed diet 7. Total breast meat weight and yield were 23 g and 2.1% lower ($P < 0.05$), respectively, of birds receiving diet 5 compared with diet 7. Abdominal fat weight and percentage increased ($P < 0.05$) when broilers were provided diet 4 compared with diet 6. Broilers fed diets 1, 6, and 7 had higher ($P < 0.01$) abdominal fat weight and percentage than those consuming the PC diets.

These data demonstrated that maintaining adequate total Gly + Ser and nitrogen concentrations increased carcass yield and total breast meat weight and yield of broilers fed reduced CP diets. These responses can be associated with Gly role as a precursor for creatine synthesis in muscle (Ngo et al., 1977). Increasing total Gly + Ser concentration from 1.84 to 2.26% has been reported to increase pectoral muscle creatine from 2.1 to 3.4 mg/g while also increasing breast meat weight from 10.7 to 12.1 g/kg of BW (Ospina-Rojas et al., 2013b). In addition, nitrogen from Gln can be used for purine and pyrimidine synthesis (Cory and Cory, 2006), which may increase protein synthesis in pectoralis major and minor muscles of broilers.

In summary, Gly had more pronounced effects on improving BW gain and FCR of broilers from 1 to 28 d of age than Gln, which translated to a decrease in cumulative FCR of broilers when diets were supplemented only with Gly to obtain 33% total Gly + Ser concentration of the difference between PC and NC diets. Supplementing Gly and L-Gln to provide adequate total Gly + Ser and nitrogen concentrations in reduced CP diets throughout a 41 d production period enabled broilers to increase total breast meat weight and yield while reducing abdominal fat weight and percentage compared with birds fed reduced CP diets. Hence, maintaining total Gly + Ser concentrations and providing adequate nitrogen content in reduced CP diets promoted adequate growth performance and carcass characteristics of broilers during a 6 wk production period.

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Table 4.1 Ingredient and nutrient composition of starter diets fed to Ross × Ross 708 male broilers from 1 to 14 d of age

Diet	1	2	3	4	5	6	7	8
Total Gly + Ser[1]	NC[3]	33%	66%	-	-	33%	66%	PC[3]
Crude protein[2]		-	-	33%	66%	33%	66%	
Ingredient, % "as-fed"								
Corn	59.48	59.33	59.18	58.53	57.57	58.49	57.51	49.79
Soybean meal	34.61	34.61	34.61	34.61	34.61	34.61	34.61	43.48
Vegetable oil	1.56	1.60	1.63	1.68	1.79	1.70	1.84	3.13
Calcium carbonate	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.32
Defluorinated phosphate	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.14
L-Lys•HCl	0.29	0.29	0.29	0.29	0.30	0.29	0.30	0.02
DL-Met	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.29
L-Thr	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.05
L-Val	0.12	0.12	0.12	0.12	0.12	0.12	0.12	---
L-Arg	0.02	0.02	0.02	0.02	0.02	0.02	0.02	---
L-Trp	0.01	0.01	0.01	0.01	0.01	0.01	0.01	---
L-Ile	0.05	0.45	0.45	0.45	0.45	0.45	0.45	---
Gly	---	0.11	0.22	0.01	0.01	0.12	0.23	---
L-Gln	---	---	---	0.83	1.65	0.72	1.45	---
Vitamin premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.25
Sodium bicarbonate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
Choline chloride	0.10	0.96	0.96	0.96	0.96	0.96	0.96	0.10
Phytase[6]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Analysis, % (unless otherwise noted)								
AME, kcal/kg	3,031	3,031	3,031	3,031	3,031	3,031	3,031	3,031
Crude protein	21.69	21.81	21.92	22.61	23.53	22.61	23.53	24.45
Digestible Lys	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Digestible Met	0.67	0.67	0.67	0.67	0.66	0.67	0.66	0.63
Digestible TSAA[7]	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94

Digestible Thr	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Digestible Val	0.95	0.95	0.95	0.95	0.94	0.95	0.94	0.98
Digestible Arg	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.54
Digestible Trp	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.26
Digestible Ile	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.94
Total Gly	0.88	0.98	1.09	0.88	0.88	0.99	1.10	1.02
Total Gly + Ser	1.92	2.03	2.14	1.92	1.92	2.03	2.14	2.24
Total Gly equivalent[8]	1.62	1.73	1.84	1.62	1.62	1.73	1.84	1.89
Total EAA_n:$NEAA_n$[9]	55:45	55:45	54:46	55:45	55:45	55:45	54:46	54:46
Calcium	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Non-phytate phosphorus	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Sodium	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

[1]Total Gly + Ser concentrations in diets 2 and 6 were formulated to contain 33%, while total Gly + Ser concentrations in diets 3 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[2]Crude protein contents in diets 4 and 6 were formulated to contain 33%, while crude protein contents in diets 5 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[3]NC = Negative control, PC = Positive control

[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate),1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.

[5]Mineral premix include per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

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

[7]TSAA = Total sulfuric amino acids

[8]Total Gly equivalent (%) = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[9]Ratio of total essential to non-essential amino acid nitrogen

Table 4.2 Ingredient and nutrient composition of grower diets fed to Ross × Ross 708 male broilers from 15 to 28 d of age

Diet	1	2	3	4	5	6	7	8
Total Gly + Ser[1]	NC[3]	33%	66%	-	-	33%	66%	PC[3]
Crude protein[2]		-	-	33%	66%	33%	66%	
Ingredient, % "as-fed"								
Corn	66.35	66.22	66.09	65.55	64.75	65.52	64.69	57.97
Soybean meal	28.20	28.20	28.20	28.20	28.20	28.20	28.20	35.89
Vegetable oil	1.39	1.42	1.45	1.49	1.59	1.51	1.63	2.77
Calcium carbonate	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
Defluorinated phosphate	1.04	1.04	1.04	1.04	1.04	1.04	1.04	0.98
L-Lys•HCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.06
DL-Met	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.25
L-Thr	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.05
L-Val	0.11	0.11	0.11	0.11	0.11	0.11	0.11	---
L-Arg	0.04	0.04	0.04	0.04	0.04	0.04	0.04	---
L-Trp	0.01	0.01	0.01	0.01	0.01	0.01	0.01	---
L-Ile	0.06	0.06	0.06	0.06	0.06	0.06	0.06	---
Gly	---	0.10	0.19	0.01	0.01	0.10	0.20	---
L-Gln	---	---	---	0.70	1.39	0.61	1.21	---
Vitamin premix[4]	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.20
Sodium bicarbonate	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.26
Choline chloride	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Phytase[6]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Analysis, % (unless otherwise noted)								
AME, kcal/kg	3,086	3,086	3,086	3,086	3,086	3,086	3,086	3,086
Crude protein	19.31	19.41	19.51	20.09	20.86	20.09	20.86	21.64
Digestible Lys	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Digestible Met	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.56
Digestible TSAA[7]	0.84	0.84	0.84	0.83	0.83	0.83	0.83	0.84

Digestible Thr	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Digestible Val	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.86
Digestible Arg	1.16	1.16	1.15	1.15	1.15	1.15	1.15	1.33
Digestible Trp	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.22
Digestible Ile	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.81
Total Gly	0.77	0.86	0.96	0.77	0.78	0.87	0.96	0.90
Total Gly + Ser	1.69	1.78	1.87	1.69	1.69	1.78	1.87	1.97
Total Gly equivalent[8]	1.43	1.52	1.61	1.43	1.43	1.52	1.61	1.66
Total EAA_n:$NEAA_n$[9]	55:45	55:45	55:45	55:45	55:45	55:45	55:45	54:46
Calcium	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Non-phytate phosphorus	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21

[1]Total Gly + Ser concentrations in diets 2 and 6 were formulated to contain 33%, while total Gly + Ser concentrations in diets 3 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[2]Crude protein contents in diets 4 and 6 were formulated to contain 33%, while crude protein contents in diets 5 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[3]NC = Negative control, PC = Positive control

[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate),1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.

[5]Mineral premix include per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

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

[7]TSAA = Total sulfuric amino acids

[8]Total Gly equivalent (%) = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[9]Ratio of total essential to non-essential amino acid nitrogen

Table 4.3 Ingredient and nutrient composition of finisher diets fed to Ross × Ross 708 male broilers from 29 to 40 d of age

Diet	1	2	3	4	5	6	7	8
Total Gly + Ser[1]	NC[3]	33%	66%	-	-	33%	66%	PC[3]
Crude protein[2]		-	-	33%	66%	33%	66%	
Ingredient, % "as-fed"								
Corn	70.67	70.55	70.43	69.96	69.25	69.94	69.20	63.12
Soybean meal	23.97	23.97	23.97	23.97	23.97	23.97	23.97	30.93
Vegetable oil	1.58	1.61	1.64	1.67	1.76	1.69	1.80	2.85
Calcium carbonate	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Defluorinated phosphate	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.80
L-Lys•HCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.09
DL-Met	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.23
L-Thr	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.05
L-Val	0.11	0.11	0.11	0.11	0.11	0.11	0.11	---
L-Arg	0.53	0.53	0.53	0.53	0.53	0.53	0.53	---
L-Trp	0.02	0.02	0.02	0.02	0.02	0.02	0.02	---
L-Ile	0.07	0.07	0.07	0.07	0.07	0.07	0.07	---
Gly	---	0.09	0.17	0.00	0.01	0.09	0.18	---
L-Gln	---	---	---	0.61	1.23	0.53	1.07	---
Vitamin premix[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.15
Sodium bicarbonate	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.30
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Phytase[6]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated analysis, % (unless otherwise noted)								
AME, kcal/kg	3,142	3,142	3,142	3,142	3,142	3,142	3,142	3,142
Crude protein	17.72	17.81	17.90	18.41	19.09	18.41	19.09	19.78
Digestible Lys	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Digestible Met	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.51
Digestible TSAA[7]	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77

Digestible Thr	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Digestible Val	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Digestible Arg	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.19
Digestible Trp	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.20
Digestible Ile	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.73
Total Gly	0.70	0.78	0.87	0.70	0.71	0.79	0.87	0.81
Total Gly + Ser	1.53	1.62	1.70	1.53	1.53	1.62	1.70	1.79
Total Gly equivalent[8]	1.29	1.38	1.46	1.30	1.30	1.38	1.46	1.51
Total EAA_n:$NEAA_n$[9]	55:45	55:45	55:45	55:45	55:45	55:45	55:45	54:46
Calcium	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Non-phytate phosphorus	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

[1]Total Gly + Ser concentrations in diets 2 and 6 were formulated to contain 33%, while total Gly + Ser concentrations in diets 3 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[2]Crude protein contents in diets 4 and 6 were formulated to contain 33%, while crude protein contents in diets 5 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[3]NC = Negative control, PC = Positive control

[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate),1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.

[5]Mineral premix include per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

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

[7]TSAA = Total sulfuric amino acids

[8]Total Gly equivalent (%) = total Gly (%) + [total Ser (%) × 0.7143], where 0.7143 is the ratio of the molar weight of Gly and Ser.

[9] Ratio of total essential to non-essential amino acid nitrogen

Table 4.4 Calculated (C) and analyzed (A) crude protein, total Gly, and total Gly + Ser concentrations of experimental starter (from 1 to 14 d), grower (from 15 to 28 d), and finisher (from 29 to 40 d) diets[1]

Diet		1	2	3	4	5	6	7	8
Total Gly + Ser[2]		NC[4]	33%	66%	-	-	33%	66%	PC[4]
Crude protein[3]			-	-	33%	66%	33%	66%	
Nutrient, %									
Starter									
Crude protein	C	21.69	21.81	21.92	22.61	23.53	22.61	23.53	24.45
	A	24.15	23.21	23.75	23.45	24.15	24.23	24.83	26.47
Total Gly	C	0.88	0.98	1.09	0.88	0.88	0.99	1.10	1.02
	A	0.95	0.99	1.11	0.91	0.90	1.02	1.12	1.08
Total Gly + Ser	C	1.92	2.03	2.14	1.92	1.92	2.03	2.14	2.24
	A	2.01	2.06	2.22	1.97	1.96	2.12	2.22	2.33
Grower									
Crude protein	C	19.31	19.41	19.51	20.09	20.86	20.09	20.86	21.64
	A	19.79	20.50	20.99	21.44	21.93	21.52	22.60	22.79
Total Gly	C	0.77	0.86	0.96	0.77	0.78	0.87	0.96	0.90
	A	0.80	0.89	0.97	0.85	0.82	0.90	0.99	0.94
Total Gly + Ser	C	1.69	1.78	1.87	1.69	1.69	1.78	1.87	1.97
	A	1.74	1.84	1.95	1.83	1.77	1.87	1.97	2.07
Finisher									
Crude protein	C	17.72	17.81	17.90	18.41	19.09	18.41	19.09	19.78
	A	18.78	19.34	18.82	19.11	19.72	19.16	19.78	21.43
Total Gly	C	0.70	0.78	0.87	0.70	0.71	0.79	0.87	0.81
	A	0.74	0.83	0.89	0.73	0.73	0.82	0.89	0.88
Total Gly + Ser	C	1.53	1.62	1.70	1.53	1.53	1.62	1.70	1.79
	A	1.61	1.72	1.77	1.58	1.59	1.71	1.75	1.91

[1]Mean values were based upon duplicate analysis (methods 968.06 and 994.12; AOAC International, 2006).
[2]Total Gly + Ser concentrations in diets 2 and 6 were formulated to contain 33%, while total Gly + Ser concentrations in diets 3 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.

[3]Crude protein contents in diets 4 and 6 were formulated to contain 33%, while crude protein contents in diets 5 and 7 were formulated to contain 66% of the difference between the NC (diet 1) and PC (diet 8) diets.
[4]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.5 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 1 to 14 d of age[1]

Item	Body weight gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary Treatment[4]				
1) NC[5]	0.454	0.566	1.249	2.0
2) 33% total Gly + Ser	0.445	0.544	1.221	1.0
3) 66% total Gly + Ser	0.461	0.550	1.193	2.0
4) 33% CP	0.458	0.555	1.214	2.0
5) 66% CP	0.461	0.556	1.207	2.9
6) 33% total Gly + Ser and CP	0.459	0.551	1.200	0.5
7) 66% total Gly + Ser and CP	0.471	0.553	1.174	1.0
8) PC[5]	0.461	0.543	1.178	1.7
SEM	0.005	0.005	0.008	1.1
Pre-planned orthogonal contrasts	*Probabilities*			
Diet 1 vs. 8	0.33	0.002	< 0.0001	0.78
Diet 6 vs. 8	0.80	0.27	0.043	0.45
Diet 7 vs. 8	0.20	0.18	0.72	0.74
Diet 2 vs. 6	0.06	0.35	0.039	0.66
Diet 4 vs. 6	0.81	0.47	0.16	0.18
Diet 3 vs. 7	0.19	0.76	0.06	0.53
Diet 5 vs. 7	0.19	0.65	0.003	0.48

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality values were arcsine transformed.
[4]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between the NC (diet 1) and PC (diet 8) diets.
[5]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.6 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 15 to 28 d of age[1]

Item	Body weight gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary Treatment[4]				
1) NC[5]	1.237	1.859	1.504	2.5
2) 33% total Gly + Ser	1.234	1.834	1.486	1.5
3) 66% total Gly + Ser	1.247	1.857	1.490	1.0
4) 33% CP	1.216	1.838	1.512	1.0
5) 66% CP	1.227	1.829	1.492	1.2
6) 33% total Gly + Ser and CP	1.263	1.851	1.465	1.5
7) 66% total Gly + Ser and CP	1.266	1.852	1.463	2.5
8) PC[5]	1.303	1.888	1.449	1.2
SEM	0.013	0.016	0.013	1.0
Pre-planned orthogonal contrasts		*Probabilities*		
Diet 1 vs. 8	0.001	0.15	0.003	0.50
Diet 6 vs. 8	0.035	0.07	0.36	0.97
Diet 7 vs. 8	0.051	0.08	0.42	0.36
Diet 2 vs. 6	0.11	0.39	0.22	0.79
Diet 4 vs. 6	0.011	0.51	0.008	0.86
Diet 3 vs. 7	0.29	0.80	0.11	0.18
Diet 5 vs. 7	0.040	0.26	0.10	0.36

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality values were arcsine transformed.
[4]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between the NC (diet 1) and PC (diet 8) diets.
[5]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.7 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 1 to 28 d of age[1]

Item	Body weight gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary Treatment[4]				
1) NC[5]	1.691	2.445	1.447	4.5
2) 33% total Gly + Ser	1.680	2.391	1.424	2.5
3) 66% total Gly + Ser	1.708	2.413	1.413	3.0
4) 33% CP	1.673	2.399	1.433	3.0
5) 66% CP	1.688	2.391	1.417	4.0
6) 33% total Gly + Ser and CP	1.722	2.416	1.403	2.0
7) 66% total Gly + Ser and CP	1.737	2.426	1.367	3.5
8) PC[5]	1.764	2.435	1.381	2.9
SEM	0.016	0.020	0.009	1.5
Pre-planned orthogonal contrasts			*Probabilities*	
Diet 1 vs. 8	0.002	0.69	< 0.0001	0.42
Diet 6 vs. 8	0.06	0.48	0.09	0.76
Diet 7 vs. 8	0.22	0.74	0.22	0.59
Diet 2 vs. 6	0.047	0.33	0.10	0.89
Diet 4 vs. 6	0.024	0.50	0.018	0.47
Diet 3 vs. 7	0.18	0.62	0.19	0.55
Diet 5 vs. 7	0.030	0.19	0.12	0.81

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality values were arcsine transformed.
[4]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between the NC (diet 1) and PC (diet 8) diets.
[5]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.8 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 29 to 40 d of age[1]

Item	Body weight gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary Treatment[4]				
1) NC[5]	1.367	2.419	1.772	1.6
2) 33% total Gly + Ser	1.336	2.382	1.784	1.6
3) 66% total Gly + Ser	1.347	2.400	1.784	0.5
4) 33% CP	1.354	2.370	1.752	4.1
5) 66% CP	1.341	2.345	1.749	3.4
6) 33% total Gly + Ser and CP	1.331	2.353	1.767	2.0
7) 66% total Gly + Ser and CP	1.322	2.367	1.791	1.6
8) PC[5]	1.308	2.368	1.812	1.2
SEM	0.020	0.027	0.016	1.0
Pre-planned orthogonal contrasts		*Probabilities*		
Diet 1 vs. 8	0.025	0.15	0.033	0.74
Diet 6 vs. 8	0.36	0.66	0.018	0.44
Diet 7 vs. 8	0.58	0.98	0.26	0.73
Diet 2 vs. 6	0.85	0.39	0.35	0.65
Diet 4 vs. 6	0.37	0.61	0.38	0.30
Diet 3 vs. 7	0.32	0.33	0.68	0.33
Diet 5 vs. 7	0.46	0.53	0.024	0.28

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality values were arcsine transformed.
[4]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between the NC (diet 1) and PC (diet 8) diets.
[5]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.9 Growth performance of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 1 to 40 d of age[1]

Item	Body weight gain, kg/bird	Feed intake, kg/bird	FCR, kg:kg[2]	Mortality, %[3]
Dietary Treatment[4]				
1) NC[5]	3.057	4.905	1.605	6.0
2) 33% total Gly + Ser	3.016	4.815	1.597	3.9
3) 66% total Gly + Ser	3.055	4.823	1.579	3.5
4) 33% CP	3.027	4.890	1.615	7.0
5) 66% CP	3.029	4.831	1.595	7.4
6) 33% total Gly + Ser and CP	3.054	4.829	1.581	4.0
7) 66% total Gly + Ser and CP	3.059	4.832	1.580	5.0
8) PC[5]	3.072	4.838	1.575	4.0
SEM	0.029	0.052	0.012	1.7
Pre-planned orthogonal contrasts		*Probabilities*		
Diet 1 vs. 8	0.70	0.30	0.045	0.35
Diet 6 vs. 8	0.63	0.89	0.68	0.69
Diet 7 vs. 8	0.74	0.92	0.77	0.53
Diet 2 vs. 6	0.30	0.82	0.26	0.63
Diet 4 vs. 6	0.46	0.62	0.017	0.21
Diet 3 vs. 7	0.91	0.88	0.95	0.38
Diet 5 vs. 7	0.42	0.99	0.29	0.36

[1]Values are least-square means of 8 replicate pens, with each pen having 25 chicks at placement.
[2]Feed conversion ratio was corrected for mortality.
[3]Mortality values were arcsine transformed.
[4]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between NC (diet 1) and PC (diet 8) diets.
[5]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

Table 4.10 Processing yields of Ross × Ross 708 male broilers fed reduced crude protein diets supplemented with Gly and/or L-Gln from 1 to 41 d of age[1]

Item	Carcass		Breast Meat		Abdominal Fat	
	Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Percentage, %
Treatment[2]						
1) NC[3]	2.350	73.02	0.806	25.04	0.038	1.19
2) 33% total Gly + Ser	2.286	72.73	0.786	24.99	0.033	1.06
3) 66% total Gly + Ser	2.318	72.51	0.797	24.91	0.037	1.17
4) 33% CP	2.291	72.50	0.784	24.79	0.035	1.11
5) 66% CP	2.329	72.92	0.797	24.97	0.033	1.02
6) 33% total Gly + Ser and CP	2.322	72.96	0.801	25.15	0.032	1.00
7) 66% total Gly + Ser and CP	2.357	73.35	0.820	25.49	0.033	1.02
8) PC[3]	2.321	73.24	0.797	25.11	0.027	0.85
SEM	0.017	0.18	0.009	0.18	0.001	0.03
Pre-planned orthogonal contrasts			*Probabilities*			
Diet 1 vs. 8	0.19	0.34	0.43	0.75	< 0.0001	< 0.0001
Diet 6 vs. 8	0.99	0.22	0.71	0.88	0.001	0.001
Diet 7 vs. 8	0.10	0.63	0.040	0.13	< 0.0001	< 0.0001
Diet 2 vs. 6	0.08	0.29	0.15	0.52	0.23	0.11
Diet 4 vs. 6	0.13	0.040	0.10	0.13	0.015	0.006
Diet 3 vs. 7	0.06	0.0001	0.028	0.016	0.001	0.001
Diet 5 vs. 7	0.18	0.06	0.034	0.036	0.92	0.94

[1]Values are least-square means of 8 replicate pens, with each pen having 12 birds at processing.
[2]Experimental diets 2 to 7 were formulated with Gly and/or L-Gln supplementation to contain 33 or 66% total Gly + Ser and/or crude protein concentrations, respectively, of the difference between the NC (diet 1) and PC (diet 8) diets.
[3]The negative control (NC) diets were formulated to contain 2.5 and 0.29 percentage points lower crude protein and total Gly + Ser concentrations, respectively, than the positive control (PC) diets.

V. CONCLUSIONS

Lowering dietary CP content in broiler diets has been reported to exhibit several benefits. These advantages consist of decreasing dietary cost, nitrogen excretion, ammonia emissions, and the incidence of pododermatitis. However, the reduction in dietary CP content often negatively affects growth performance of broilers. This may be due to sub-optimum concentrations of less limiting AA (Val, Ile, Arg, and Trp) and total Gly + Ser. Thus, maintaining adequate essential AA and total Gly + Ser concentrations in broiler diets may allow further reduction in dietary CP content while obtaining adequate growth performance.

The first experiment (2 trials) was designed to evaluate growth responses when broilers were fed reduced CP diets formulated to meet optimal essential AA specifications and a total Gly + Ser to digestible Lys ratio. All free AA were sequentially added in the order of limitation (trial 1: DL-Met, L-Lys, L-Thr, Gly, L-Val, L-Ile, L-Arg, L-Trp, L-His, L-Phe, and L-Leu and trial 2: DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, L-Trp, L-His, and L-Phe) to meet their requirements while simultaneously decreasing dietary CP content. In trial 1, FCR of broilers was maintained when reducing dietary CP content from 25.8 (DL-Met, L-Lys, L-Thr, and Gly) to 24.0% (DL-Met, L-Lys, L-Thr, Gly, L-Val, L-Ile, and L-Arg). No difference was observed in BW gain of broilers fed diets containing 4.9 percentage points lower CP content compared with the control-fed birds (25.8% CP) from 1 to 17 d of age. In addition, lowering dietary CP content from 25.8 to 24.0% reduced nitrogen excretion by 7.9% per 1% point decrease in

CP content without compromising BW gain and FCR of broilers. In trial 2, no differences in BW gain and FCR were observed as dietary CP content decreased by 3.9 percentage points from 1 to 7 and 1 to 14 d of age compared with those fed the control diet (23.9% CP). From 1 to 21 d of age, supplementing DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, and L-Trp lowered dietary CP content by 2.2 percentage points without compromising BW gain and FCR of broilers. However, adding L-His and L-Phe to decrease dietary CP content beyond 2.2 percentage points resulted in lower BW gain and higher FCR of broilers than birds fed the control diet (23.9% CP). These results demonstrated that optimal growth performance of broilers can be obtained without placing a minimum on dietary CP content when proper AA concentrations were maintained during the starter period.

Due to limited published research on dietary Gly effects beyond the starter period, the second experiment was conducted to evaluate broiler responses to Gly supplementation during a 6 wk production period. Glycine and/or L-Gln (a nitrogen source) were supplemented to negative control diets (2.5% and 0.29 percentage points lower CP and total Gly + Ser concentrations, respectively) to increase total Gly + Ser and/or CP concentrations, respectively, at 33 or 66% of the difference between the positive and negative control diets. Maintaining a total Gly + Ser concentration of 2.14% in a reduced CP diet led to an optimal FCR of broilers from 1 to 14 d of age. A positive Gly response on FCR was also detected from 1 to 40 d of age, but only when Gly was added to obtain 33% total Gly + Ser concentration of the difference between the PC and NC diets. In contrast, L-Gln supplementation contributed to an increase in BW gain of broilers fed reduced CP diets compared from 1 to 28 d of age. Broilers fed diets with Gly and L-Gln addition to maintaining sufficient total Gly + Ser and non-essential nitrogen concentrations had increased total breast meat weight and yield and decreased abdominal fat weight and percentage compared with those receiving only Gly or L-Gln supplementation. Therefore, maintaining adequate Gly and non-essential nitrogen concentrations provided enhancements in growth performance and carcass characteristics of broilers during a 41 d production period.