# Maintaining a proper branched chain amino acid balance is crucial to optimizing performance

## **Abstract**

A study was conducted to understand the relationship among dietary branched-chain amino acids (BCAA) on the performance of Ross 344  $\times$  708 male broilers. A total of 2,592 d-old male chicks were randomly placed into 144-floor pens according to a 23 full factorial central composite design (CCD) with 20 treatments (14 treatments and 6 center points). Each treatment consisted of varying digestible Ile:Lys (52 to 75), Val:Lys (64 to 87), and Leu:Lys (110 to 185) ratios. Birds and feed were weighed at 20 and 34 d of age to determine body weight gain (BWG), feed intake, and feed conversion ratio (FCR). At 35 d of age, feather amino acid composition and carcass characteristics were evaluated. Data were analyzed as CCD using the surface response option of JMP v. 15. Body weight gain and FCR were optimized at the lowest Leu:Lys ratio (110) with moderate Val:Lys (78 to 79) and Ile:Lys (65 to 66) ratios. Poorer BWG and FCR were observed as Leu:Lys ratio increased while increasing Val:Lys and Ile:Lys ratios alleviated the poor performance. Carcass and breast yield were maximized at the highest Leu:Lys ratio. This effect was complemented by increasing Ile:Lys ratio beyond 68. Lower Ile:Lys and Val:Lys ratios were required to maximize carcass and breast yield at the lowest Leu:Lys ratio. However, this strategy yielded less meat than providing a high Leu:Lys ratio diet. These results suggest that optimum BCAA ratios to Lys may vary depending on response criteria and demonstrate the importance of maintaining proper Val and Ile ratios centered on dietary Leu. Live performance can be optimized in diets with low Leu:Lys ratios; however, meat yield can be enhanced by increasing dietary Leu:Lys along with Ile:Lys ratios.

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## **Background**

The intricacies of the branched-chain amino acid (BCAA) interaction have been elucidated over time in poultry species (D'Mello and Lewis, 1970; D'Mello and Lewis,1971; Barbour and Latshaw, 1992; Farran and Thomas, 1992ab; Waldroup et al., 2002). Recent developments in feed grade amino acid manufacturing have resulted in increased availability and more cost effective feed grade amino acids including L-Valine and L-Isoleucine for more feasible use in feed formulation. Subsequently, interest has grown to better understand how all BCAA interact with one another under commercial conditions, not only in poultry (Ospina-Rojas et al.,2017; Ospina-Rojas et al., 2018; Zeitz et al., 2019; Ospina-Rojas et al., 2020) but other monogastric species as well (Cemin et al., 2019).

Baker and Han (1994) proposed an ideal protein ratio of Leu, Ile, and Val to Lys of 109, 67, and 77, respectively, during the first 21 d post-hatch.

In close agreement, Aviagen (2019) currently recommends a Leu ratio to Lys of 110 across all feed phases. However, commercial feed formulation based on corn, which is rich in Leu, generally exceeds the recommended digestible Leu to Lys ratio to 130 or higher. Moreover, in some regions of the world where soybean meal availability is inconsistent and yellow pigmentation is preferred, higher inclusion of DDGS and corn gluten meal may be observed.

The result of the higher inclusion of corn co-products is a digestible Leu to Lys ratio between 170 and 190. Thus, it is necessary to consider the influence of surplus dietary Leu on the needs of Val and Ile. While the interrelationship of these three amino acids have been demonstrated previously, their effects on growth performance of broilers are variable (D'Mello and Lewis, 1970; Farran and Thomas 1990; Barbour and Latshaw, 1992; Burnham et al., 1992; Waldroup et al., 2002; Zeitz et al., 2019; Ospina-Rojas et al., 2020). Therefore, a study was designed to understand how the interactive effects of dietary Leu, Ile, and Val can impact the live performance, carcass traits, and feather composition in Ross  $344 \times 708$  broilers.

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### **Materials and methods**

### **Experimental Diets**

The starter and grower feeds were common to all birds, provided in crumbles and pellets, respectively, and formulated to meet Aviagen nutrient specifications (Table 1; Aviagen, 2019). The experimental feed was provided between 20 to 35 d of age and was formulated to meet or exceed Aviagen nutrient recommendations (Aviagen, 2019) with exception of Ile, Leu, and Val.

Digestible Ile, Leu, and Val levels of the experimental basal feed were formulated at 0.57, 1.21, and 0.70%, respectively , with a 1.10% digestible lysine, resulting in an Ile to Lys (Ile:Lys) ratio of 52, a Leu to Lys (Leu:Lys) ratio of 110, and a Val to Lys (Val:Lys) ratio of 64, then L-Ile, L-Leu, and/or L-Val were supplemented at the expense of an inert filler to generate the dietary treatments. Resulting ratios for Ile:Lys were 52, 57, 64, 70, and 75, for Val:Lys 64, 68, 75, 82, and 87, and for Leu:Lys 110, 125, 148, 170, and 185 (Table 2).

Table 1. Composition and nutrient content of the basal diet for yellow-feathered chickens (as-fed basis, %)



1 Common starter and grower diets were provided from 1 to 10 d and 10 to 20 d of age, respectively.

<sup>2</sup> Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,7390 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), folacin (folic acid), 2.6 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine <sup>3</sup>Trace mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 m

4 Added as Hostazym X (Huvepharma, Sofia, Bulgaria) provides 6,000 EPU/g of xylanase activity in the form of endo-1,4-beta-xylanase (EC 3.2.1.8)

 $\,$  Digestible amino acid values were determined from digestible coefficients and analyzed total amino acid content of the ingredients.

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### **Materials and methods**

### **Experimental Design and Bird Management Statistical Analysis**

A 23 full factorial central composite design (CCD) with three factors (Ile, Val, and Leu) was used in this study aimed at better understanding the interactive relationship of BCAA and to determine optimal biological performance of broilers under varying levels of dietary Ile, Val, and Leu (Box and Wilson, 1951). The three factors studied were assigned at 5 different levels resulting in 20 treatments (Table 2). Treatments 1 to 14 (8 replications per treatment) composed of 8 factorial points and 6 star points whereas treatments 15 to 20, which are the dietary treatments representing the center point of the design had a total of 32 replications (Val:Lys 75, Leu:Lys 148, and Ile:Lys 64).

A total of 2,592 d-old male chicks was obtained from a parent stock flock (Ross 344 × 708) at 36 wk of age. Immediately after hatch, chicks were sorted by quality, vent-sexed, and randomly placed in 144 floor pens (approximately 1.40 m2/pen) resulting in 18 birds per pen. Prior to the initiation of the experimental feed phase on d 20, all pens were standardized to contain 15 birds per pen by removing those birds visually showing abnormalities and suboptimal or excessive size. Body weight gain, feed intake, and FCR were calculated from 20 to 34 d of age. All birds but 1 from each pen were processed for measurements of carcass traits (between 13 and 15 birds per pen depending on mortality). Yields were calculated relative to an individual live BW measured upon arrival at the processing plant. The one bird from each pen not processed had the last five primary wing feathers collected for analysis of amino acids and dry matter as described by Farran and Thomas (1992a).

The surface response option of JMP v. 15 (SAS Institute, Cary, NC) was used to analyze the central composite design. Linear and quadratic effects of Leu:Lys, Ile:Lys and Val:Lys and their respective interactions were tested using treatment means and considered significant at P < 0.10. These means were used to generate polynomial regression equations using JMP v. 15 (SAS Institute, Cary, NC).

Contour graphics and surface plots were generated from the polynomial regression equation described previously. Optimum response of each category was obtained using the desirability function within the prediction profiler of JMP v. 15.



1 8 57 68 125 1223 Treatment Replications Ile:Lys Val:Lys Leu:Lys BWG, g FI, g FCR, g:g Carcass Yield(%) Breast Yield(%) 2002 1.636 71.37 25.92 2 8 57 82 125 1251 2011 1.608 70.85 25.26 3 8 57 68 170 1142 1950 1.709 70.97 25.18 4 8 57 82 170 1222 2010 1.645 70.91 24.72 5 8 70 68 125 1240 1995 1.610 71.29 26.31 6 8 70 82 125 1293 2029 1.568 71.09 25.86 7 8 70 68 170 1218 1976 1.629 71.42 26.56 8 8 70 82 170 1288 2015 1.565 71.24 26.24 9 8 64 64 148 1067 1827 1.714 70.97 25.95 10 8 64 87 148 1276 2019 1.581 71.11 25.76 11 8 64 75 110 1315 2037 1.550 71.25 25.97 12 8 64 75 185 1261 Treatment Replications Ile:Lys Val:Lys Leu:Lys BWG, g FI, g FCR, g:g Carcass Yield(%) Breast Yield(%) 2019 1.603 71.34 25.97 13 8 52 75 148 1139 1964 1.722 70.04 24.11 14 8 75 75 148 1280 2039 1.594 71.30 26.23 15 6 64 75 148 1280 2110 1.619 71.28 26.17 16 6 64 75 148 1290 2029 1.573 71.68 26.07 17 5 64 75 148 1281 2043 1.580 71.26 26.25 18 5 64 75 148 1278 2017 1.579 71.48 26.15 19 5 64 75 148 1294 2028 1.568 71.53 26.12 20 5 64 75 148 1283 2022 1.576 71.54 26.31 23 32 0.018 0.18 0.24 20 25 0.014 0.14 0.19 SE Center Point<sup>2</sup> SE factorial and star point

Table 2. Dietary treatment design and predicted means for body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) from 20 to 34 d of age and for carcass and breast yield at 35 d of age<sup>1</sup>

2 Standard error for center point means represents treatments 15 to 20 <sup>1</sup> Digestible Lys was formulated to 1.10% in all dietary treatments.

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### **Results**

Models for BWG, FCR, and feed intake were significant (P < 0.10), as were strong coefficients of determination of 0.93, 0.94, and 0.75, respectively. Models for breast weight, breast yield, carcass weight, carcass yield, and leg-quarter yield were also significant (P < 0.10) with corresponding coefficients of determination of 0.88, 0.96, 0.87, 0.76, and 0.79. Lack of fit for BWG, breast weight, and yield had a P-value of less than 0.10.

Body weight gain of broilers responded in a quadratic manner to increasing Val:Lys and Ile:Lys ratios at each Leu:Lys ratio. However, BWG linearly decreased with increasing Leu:Lys ratio regardless of Val:Lys and Ile:Lys ratios. The contour graphs in Figure 1A illustrate that optimum BWG from 20 to 34 d of age of 1,332 grams was obtained when feeding diets formulated to 78 Val:Lys, 66 Ile:Lys, and 110 Leu:Lys ratios. This maximum BWG decreased by approximately 30 grams when Leu:Lys ratio was increased beyond 150 without any change in Val:Lys and Ile:Lys ratios. Incremental Val:Lys and Ile:Lys ratios at high Leu:Lys ratio alleviated the BWG reduction. In contrast, regardless of the Val:Lys and Ile:Lys ratios, BWG of broilers at higher Leu:Lys was lower than BWG at 110 Leu:Lys ratio.

Broilers had the greatest feed intake (2,058 grams) when provided diets with Val:Lys, Ile:Lys, and Leu:Lys ratios of 77, 66, and 110, respectively (Figure 1B). The feed intake response of broilers from 20 to 34 d of age was influenced in both linear and quadratic manner by Val:Lys ratio. This was demonstrated by the vertical oval shape of the contour graphs at Leu:Lys of 110. However, at a high Leu:Lys ratio, changes in feed intake of broilers were also apparent when varying the ratio of Ile:Lys.

Feed conversion of broilers from 20 to 34 d of age responded in quadratic manners with increasing Val:Lys ratio. Moreover, the interaction between Ile:Lys and Leu:Lys ratios impacted FCR of broilers. Optimum FCR (1.54) of broilers from 20 to 34 d of age could be obtained at 110 Leu:Lys ratio with 78 and 66 for Val:Lys and Ile:Lys ratios, respectively (Figure 2). When Leu:Lys ratio was increased from 110 to 150, an increase of 2 points in minimum attainable FCR was observed despite maintaining optimum Val:Lys and Ile:Lys ratios. In order to obtain a similar FCR (1.54) at 190 Leu:Lys ratio, feeds must be formulated to 84 and 72 Val:Lys and Ile:Lys ratios, respctively.



Figure 1. Contour graphs for A) body weight gain (P < 0.001; R² = 0.93) and B) feed intake (P = 0.035; R² = 0.75) of Ross 344  $\times$  708 male broilers from 20 to 34 days of age.

Body weight gain was influenced quadratically by Val:Lys (P < 0.001) and Ile:Lys (P = 0.004) and linearly by Leu:Lys (P = 0.095).Feed intake was influenced by Val:Lys (P = 0.003) in a quadratic manner. Graphs were generated using regression coefficients in Table 4 by applying various Val:Lys (64 to 88) and Ile:Lys (56 to 78) ratios with incremental Leu:Lys (110 to 190).

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Carcass weight had a quadratic relationship with increasing Val:Lys and Ile:Lys ratios. Optimum carcass weight of broilers (1,429g) was obtained by feeding diets containing 82, 70, and 190 Val:Lys, Ile:Lys, and Leu:Lys ratios, respectively (Figure 3A). A similar weight of carcass can be obtained at 110 Leu:Lys ratio but with 76 and 66 Val:Lys and Ile:Lys ratios. A quadratic effect of increasing Ile:Lys ratio was observed on carcass yield but ratios of Val:Lys, Leu:Lys, and all corresponding interactions had little influence on carcass yield. Maximum carcass yield of broilers (71.5%) was obtained by formulating diets to contain 78, 68, and 190, Val:Lys, Ile:Lys, and Leu:Lys respectively (Figure 3B).

Quadratic effects of Val:Lys and Ile:Lys was observed on the total weight of boneless-skinless breast meat. Total breast weight was maximized (528 g) by feeding diets formulated to contain 81, 72, and 190 Val:Lys, Ile:Lys, and Leu:Lys ratios, respectively (Figure 4A). Reducing Val:Lys ratio from 81 to 71 while keeping Ile:Lys and Leu:Lys at optimum value, decreased total breast weight by 20 g. Similarly, a 20 g (3.8%) reduction in the weight of total breast meat was observed when reducing Ile:Lys ratio from 72 to 62. Total breast yield was influenced by the interactive effect of Leu:Lys and Ile:Lys ratios. At a high Leu:Lys ratio, total breast meat yield was enhanced by increasing Ile:Lys ratio (Figure 4B).

In contrast, it is beneficial to lower Ile:Lys ratio when Leu:Lys ratio is low in order to increase total breast meat yield. Total breast meat yield was influenced by Val:Lys ratio in a quadratic manner. Total breast meat yield of broilers was optimized (26.68%) by formulating diets to 75 Val:Lys, 74 Ile:Lys, and 190 Leu:Lys ratios.

The current experiment also evaluated the influence of dietary BCAA on feather protein and amino acid compositions. Surprisingly, the P-values for feather protein and amino acid models were greater than 0.10. However, it is worth noting that individual effects of Val:Lys and Leu:Lys ratios or the interaction of Val and Leu:Lys ratios affected contents of Ile, Leu, Cys, Lys, and Arg in the feather of broilers. For instance, Cys content in the feather was maximized by lowering dietary Val:Lys ratio when Leu:Lys ratio is at 110 (Figure 5B). Conversely, Val:Lys ratio must be increased to maximize feather Cys content at 190 Leu:Lys ratio.



Figure 2. Contour graphs for mortality corrected feed conversion ratio (g/g; P = 0.002; R² = 0.88) of Ross 344  $\times$  708 male broilers from 20 to 34 days of age.

Feed conversion was influenced by Val:Lys (P = 0.001) in a quadratic manner and the interaction of Leu:Lys and Ile:Lys (P = 0.084). Graphs were generated using regression coefficients in Table 4 by applying various Val:Lys (64 to 88) and Ile:Lys (56 to 78) ratios with incremental Leu:Lys (110 to 190).

![](_page_5_Figure_3.jpeg)

The carcass weight was influenced by Val:Lys (P = 0.001) and Ile:Lys (P = 0.015) in a quadratic manner. The carcass yield was influenced by Ile:Lys (P = 0.003) in a quadratic manner. Graphs were generated using regression coefficients in Table 5 by applying various Val:Lys (64 to 88) and Ile:Lys (56 to 78) ratios with incremental Leu:Lys (110 to 190).

The total breast meat weight was influenced by Val:Lys (P = 0.003) and Ile:Lys (P = 0.003) in a quadratic manner. The total breast meat yield was influenced by Val:Lys (P = 0.072) in a quadratic manner and the interaction between Ile:Lys and Leu:Lys (P = 0.003). Graphs were generated using regression coefficients in Table 5 by applying various Val:Lys (64 to 88) and Ile:Lys (56 to 78) ratios with incremental Leu:Lys (110 to 190).

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![](_page_6_Figure_4.jpeg)

Figure 5. Surface plots of A) crude protein (P = 0.35; R<sup>2</sup> = 0.54) and cysteine (P = 0.14; R<sup>2</sup> = 0.65) contents in feathers of Ross  $344 \times 708$  male broilers at 35 days of age.

Graphs were generated using regression coefficients in Tables 6 and 7 by applying various Val:Lys (64 to 88) and Ile:Lys (56 to 78) ratios with incremental Leu:Lys (110 to 190). Only extreme responses at 110 and 190 Leu:Lys are shown in this figure.

![](_page_6_Picture_7.jpeg)

# Maintaining a proper branched chain amino acid balance is crucial to optimizing performance

### **Discussion**

The current study examined how the needs for Val and Ile change with varying Leu levels on live performance, carcass characteristics, and feather composition of broilers from 20 to 35 d of age. The levels of BCAA were evaluated to mimic the use of various feed ingredients in broiler diets. For example, feeds formulated with wheat and peanut meal typically contain 110 Leu:Lys, whereas those having 130 and 150 Leu:Lys ratios are commonly found on feeds formulated using wheat and corn, respectively. Furthermore, the inclusion of corn co-product, such as dried-distillers grain with solubles or corn gluten meal could increase Leu:Lys ratio up to 170 and 190. These data demonstrate that the response of broilers to the dietary level of one BCAA depends on the ratio of the others. This relationship is evident when evaluating ratios to optimize broiler performance. Body weight gain and FCR can be optimized at Val:Lys, Ile:Lys, and Leu:Lys ratios of 77 to 79, 64 to 66, and 110, respectively, which are in close agreement with previous research (Baker and Han, 1994; Corzo et al., 2007; Duarte et al., 2014; Franco et al., 2017; Ospina-Rojas et al., 2017; Kidd et al., 2021). When Leu:Lys ratio increases in feed formulation, the optimum Val:Lys and Ile:Lys ratios then become higher.

The negative effect of excess Leu was explored previously by Smith and Austic (1978). It was reported that increasing excess L-Leu from 0 to 3% linearly decreased the efficacy of Ile to enhance BWG of broilers by 20%. Furthermore, Val efficacy to increase BWG of broilers was reduced to 74% when excess L-Leu reached 5.57%. These negative effects of excess Leu can be explained by the antagonistic relationship of BCAA. Excess Leu in a balanced BCAA diet could impair performance by decreasing feed consumption in broilers (Smith and Austic, 1978). In a marginal Ile and Val diet, excess Leu increased feed intake but depressed efficiency of feed utilization (Smith and Austic, 1978), which was also shown in the current study. The impact of excess Leu on poor feed conversion can be attributed to the increased activity of branched-chain amino acid aminotransferase (the first enzyme in the BCAA catabolism pathway) in the muscle of chicks (Smith and Austic, 1978). The increase of this enzyme

activity resulted in decreased plasma concentrations of Val and Ile as well as increased  $CO<sub>2</sub>$  production from labeled Ile and Val, which indicate greater catabolism of Ile and Val (Smith and Austic, 1978). Moreover, other studies in pigs revealed that serum branched-chain α-keto acid dehydrogenase (second enzyme catalyzing BCAA catabolism) and its products, α-ketoisocaproate, α -ketoisovalerate, and α-keto-β-methylvalerate, increased with the increase of dietary Leu (Wiltafsky et al., 2010), which further implies the catabolism of Val and Ile in the excess of Leu.

Conversely, poor performance associated with excess Leu:Lys may be partially improved by increasing dietary Val:Lys and Ile:Lys ratios although in some instances a small reduction in growth performance was still evident. Broilers fed diets with high Leu content but marginal Val and Ile had decreased performance, however, when Val and Ile concentrations were increased, growth performance of broilers was restored (Tuttle and Balloun, 1976; Ospina-Rojas et al., 2020).

Carcass and breast meat weights and yields in the present study were optimized at higher Ile and Leu:Lys ratios of 68 to 74 and 190, respectively. However, higher Val:Lys ratios of 81 and 82, were only required to optimize carcass and breast meat weights, respectively, and not their yields. The need for high BCAA for optimizing carcass yield may be associated with the role of Leu in stimulating muscle protein synthesis. Piglets infused with Leu have been shown to have greater protein synthesis (Suryawan et al., 2008). This response was attributed to the effect of Leu in activating muscle mammalian target of rapamycin complex (mTOR) 1 and its downstream effectors including S6 kinase 1, eIF4E-binding protein, and active eIF4G•eIF4E complex (Suryawan et al., 2008). This pathway is crucial for cell growth including protein synthesis (Wang and Proud, 2006). In broilers, evidence of upregulation of mTOR and its downstream effector due to dietary Leu supplementation has also been reported previously (Deng et al., 2014; Ospina-Rojas et al., 2020).

Higher mRNA expression of eukaryotic translation elongation factor 2 (a protein that mediates the elongation process in protein synthesis) in the Pectoralis major muscle was observed in broilers fed high dietary Leu, Val, and Ile contents than those provided with a diet low in levels of BCAA (Ospina-Rojas et al., 2020). These findings demonstrated the need for higher Leu, Val, and Ile in promoting muscle accretion.

The change in feather amino acid composition has been reported due to dietary BCAA imbalance (Robel, 1977; Penz et al., 1984; Farran and Thomas, 1992a). Farran and Thomas (1992a) suggested that deficiency in dietary Val lowered feather crude protein content but increased Asp, Glu, Met, Tyr, His, and Lys contents compared to diets supplemented with Val or when all BCAA were deficient. Similarly, excess L-Leu reduced feather protein, Val, Ile, and, Cys, but increased Leu, His, Met, Lys, and Ala, which resulted in the abnormal shape of feathers (Penz et al., 1984).

This study provides compelling evidence of the interrelationship of all three BCAA and their linked influence on broiler performance. Live performance of broilers was optimized at lower ratios, while carcass yields were maximized by providing broilers with feeds containing higher BCAA ratios to Lys. This outcome is critical as currently, optimum Leu:Lys ratio reported herein is not easily achieved when using corn and corn by-products as primary ingredients although optimum ratios for Val:Lys and Ile:Lys can be achieved in diet formulation. These data may benefit nutritionists in identifying the potential loss of performance as diet specification deviates from the optimum BCAA ratios.

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