

Understanding the interactive effects of dietary leucine with isoleucine and valine in the modern commercial broiler

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ABSTRACT A study was conducted to understand the relationship among dietary branched-chain amino acids (BCAA) on the performance of Ross 344 × 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 (1,332 g; $P < 0.001$; $R^2 = 0.93$) and FCR (1.54; $P = 0.002$; $R^2 = 0.88$) 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 (71.5%; $P = 0.031$; $R^2 = 0.76$) and breast yield (26.7%; $P < 0.001$; $R^2 = 0.96$) 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. Dietary BCAA had little effect on altering the composition of feather protein and amino acid ($P > 0.10$). 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.

Key words: Isoleucine, Leucine, Valine, branched chain amino acids, central composite design

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INTRODUCTION

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, 1992a,b; Waldroup et al., 2002). Recent developments in feed grade manufacturing of L-Val, and to a lesser extent L-Ile, have resulted in prices that allow 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).

Using semipurified diets and New Hampshire × Columbian chicks, 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 either corn or wheat, 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. For this reason, it is necessary to consider the influence of surplus dietary Leu on the needs of Val and Ile. While the interrelationship of these 3 amino acids have been demonstrated previously, their effects on growth performance of broilers are variable

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(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). Furthermore, all recent BCAA interaction works have employed in one genotype (Waldroup et al., 2002; Ospina-Rojas et al., 2017, 2018, 2020; Zeitz et al., 2019). Therefore, it is crucial to uncover how this interaction affects a different genotype with different growth rates, feed conversion, and yielding capabilities. For that purpose, 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 × 708 broilers.

MATERIALS AND METHODS

The poultry experiment reported herein was reviewed and approved by the Department of Veterinary Services of Aviagen North America. The birds were cared for according to the National Chicken Council Animal Welfare for Broiler Chickens Standards (NCC, 2005) and the Poultry Science Association Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Experimental Diets

Samples of major protein-contributing ingredients (corn, wheat, soybean meal, and peanut meal) were collected and sent to a commercial lab (Eurofins, Des Moines, IA) for analyses of proximate and total amino acid composition. Upon receiving the nutrient analyses of the raw materials, the formulation matrix for each ingredient was updated using analyzed values to formulate the experimental feeds (Table 1). Digestibility coefficients used for the major ingredients corresponded to Evonik AminoDat 5. The starter and grower feeds were common to all birds, provided in crumbles and pellets, respectively, and formulated to meet Aviagen nutrient specifications (Aviagen, 2019). The experimental feed was provided between 20 and 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. This feed served as a common baseline for all of the treatments and was accomplished by mixing 1-ton batch of this basal feed 7 times. Each of the different treatments employed equal aliquots from each of the 7 basal batches, and 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). Feed samples from the center point treatment along with the lowest and highest level supplementations of L-Ile, L-Val, and L-Leu were collected and analyzed (Eurofins, Des Moines, IA) for total amino acid

Table 1. Ingredient and calculated nutrient composition of common and experimental phase basal feed fed to Ross 344 × 708 male broilers from 0 to 35 d of age.

Item	Common feeds		Experimental feed
	Starter ¹	Grower ¹	Finisher
Ingredient, % "as-fed"	0 to 10 d	10 to 20 d	20 to 35 d
Corn	52.24	45.50	36.14
Soybean meal	35.25	28.36	14.37
Wheat	5.00	15.00	33.28
Peanut meal	2.00	5.00	7.07
Dicalcium phosphate	1.96	1.75	1.77
Soy oil	1.26	2.08	2.76
Calcium carbonate	0.94	0.90	0.79
Sodium bicarbonate	0.30	0.35	0.59
L-Methionine	0.27	0.25	0.34
Salt	0.24	0.21	0.04
L-Threonine	0.15	0.15	0.32
L-Lysine HCl	0.12	0.18	0.54
Vitamin premix ²	0.10	0.10	0.10
Mineral premix ³	0.10	0.10	0.10
Choline chloride 60%	0.03	0.05	0.09
Xylanase ⁴	0.03	0.03	0.03
Sand	-	-	1.34
Glycine	-	-	0.13
L-Tryptophan	-	-	0.02
Calculated analysis ⁵			
AMEn, kcal/kg	3,000	3,075	3,150
Crude Protein, %	22.89	21.60	18.00
digestible Lys, %	1.28	1.17	1.10
digestible TSAA, %	0.95	0.88	0.84
digestible Thr, %	0.86	0.78	0.74
digestible Ile, %	0.86	0.78	0.57
digestible Leu, %	1.76	1.61	1.21
digestible Val, %	1.02	0.93	0.70
digestible Arg, %	1.44	0.39	1.24
digestible Trp, %	0.27	0.24	0.20
Total Gly + Ser	2.11	2.00	1.71
Calcium, %	0.96	0.88	0.84
Av. Phosphorus, %	0.48	0.44	0.42

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

²Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; 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 (pyridoxine hydrochloride), 7.7 mg.

³Trace mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

⁴Added 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).

⁵Digestible amino acid values were determined from digestible coefficients and analyzed total amino acid content of the ingredients.

concentration in order to legitimize the proximity to target levels (Table 3). Amino acid concentration of raw materials and finished feeds was determined using acid hydrolysis (AOAC 982.30) for most amino acids. Methionine and cysteine were determined by AOAC 994.12 and tryptophan by AOAC 988.15 methodologies.

Experimental Design and Bird Management

A 2³ full factorial central composite design (CCD) with 3 factors (Ile, Val, and Leu) was used in this study aimed at better understanding the interactive

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.¹

Treatment	Replications	Ile:Lys	Val:Lys	Leu:Lys	BWG, g	FI, g	FCR, g:g	Carcass Yield, %	Breast Yield, %
1	8	57	68	125	1,223	2,002	1.636	71.37	25.92
2	8	57	82	125	1,251	2,011	1.608	70.85	25.26
3	8	57	68	170	1,142	1,950	1.709	70.97	25.18
4	8	57	82	170	1,222	2,010	1.645	70.91	24.72
5	8	70	68	125	1,240	1,995	1.610	71.29	26.31
6	8	70	82	125	1,293	2,029	1.568	71.09	25.86
7	8	70	68	170	1,218	1,976	1.629	71.42	26.56
8	8	70	82	170	1,288	2,015	1.565	71.24	26.24
9	8	64	64	148	1,067	1,827	1.714	70.97	25.95
10	8	64	87	148	1,276	2,019	1.581	71.11	25.76
11	8	64	75	110	1,315	2,037	1.550	71.25	25.97
12	8	64	75	185	1,261	2,019	1.603	71.34	25.97
13	8	52	75	148	1,139	1,964	1.722	70.04	24.11
14	8	75	75	148	1,280	2,039	1.594	71.30	26.23
15	6	64	75	148	1,280	2,110	1.619	71.28	26.17
16	6	64	75	148	1,290	2,029	1.573	71.68	26.07
17	5	64	75	148	1,281	2,043	1.580	71.26	26.25
18	5	64	75	148	1,278	2,017	1.579	71.48	26.15
19	5	64	75	148	1,294	2,028	1.568	71.53	26.12
20	5	64	75	148	1,283	2,022	1.576	71.54	26.31
SE center points ²					23	32	0.018	0.18	0.24
SE factorial and star points					20	25	0.014	0.14	0.19

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

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. At hatch, each chick was administered a subcutaneous injection of the following viral vaccines: a full dose of Marek's with an antibiotic (Gentamicin), one-half dose of reovirus, a full dose of infectious laryngotracheitis, and a full dose of fowlpox vaccines. In addition, one-half dose of a Newcastle and bronchitis vaccine and a full dose of coccidiosis vaccine were administered to each chick via a spray cabinet.

Birds were allocated in an environmentally controlled facility. Bird density was provided at 0.12 m² per bird and was less than 41.5 kg/m² as stipulated by the National Chicken Council welfare guidelines (NCC, 2005) for the duration of the trial. Throughout the first 7 d after hatch, birds were subjected to 23 h of light (26.9 lux, 2.5 ft. candles) and then 18 h of light (10.8 lux, 1.0 ft. candles) for the remainder of the study. Ambient temperatures were controlled as follows: 31 to 29°C from 1 to 7 d, 29 to 27°C from 7 to 14 d, 27 to 24°C from 14 to 21 d, 24 to 22°C from 21 to 28 d, 22 to 21°C from 28 to 35 d, and 21 to 20°C thereafter. Ambient temperature was measured at 15 cm above the head of the chickens. Ad libitum access to water and feed was provided throughout the trial. Individual nipple drinker lines were provided throughout the experiment with additional mini-drinkers for the first 7 d post-hatch. Feed was available throughout the study from a single 33-cm tube feeder located in the center of each pen. Additionally, supplemental chick feeder trays were placed in each pen throughout the first 7 d after hatch. Prior to the initiation of the experimental feed phase on d 20, all pens were standardized to contain 15 birds per

Table 3. Total amino acid concentration analyses (%) of selected experimental diets fed to Ross 344 × 708 male broilers from 20 to 35 d of age

Treatment	Valine		Isoleucine		Leucine		Lysine ¹
	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Analyzed
9	0.80	0.84	0.77	0.77	1.78	1.78	1.28
10	1.03	0.98	0.77	0.73	1.78	1.76	1.29
11	0.90	0.97	0.77	0.80	1.35	1.31	1.28
12	0.90	0.95	0.77	0.76	2.22	2.06	1.28
13	0.90	0.95	0.67	0.69	1.78	1.81	1.28
14	0.90	0.90	0.90	0.84	1.78	1.75	1.27
15 to 20	0.90	0.94	0.77	0.78	1.78	1.78	1.31
Basal	0.80	0.86	0.67	0.72	1.35	1.47	1.33

¹Calculated total lysine content in all diet is 1.22%.

pen by removing those birds visually showing abnormalities and suboptimal or excessive size.

At the end of the experimental feed phase, all birds and feed from each pen were weighed to determine body weight gain (**BWG**), feed intake, and feed conversion ratio (**FCR**). Body weight gain, feed intake, and FCR were calculated from 20 to 34 d of age. Mortality weight was used to adjust the experimental period FCR. All birds but 1 from each pen was processed for measurements of carcass traits at Aviagen's Product Development Center processing plant (between 13 and 15 birds per pen depending on mortality). Each bird was individually wing banded approximately 24 h prior to processing, and feed was removed from all pens approximately 12 h before processing. Birds had access to water during the feed removal period. During processing, birds were stunned, exsanguinated, scalded, and plucked. Then, the carcasses were eviscerated and air chilled for approximately 24 h prior to deboning. Leg quarters, *Pectoralis major*, and *Pectoralis minor* were removed from the carcass and their weights were recorded. Total breast weight was measured by adding the total weight of boneless-skinless *Pectoralis major* and *minor* muscles. All data collected on each bird at the processing plant were recorded to an individual wing band number. 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 5 primary wing feathers collected and sent to a commercial laboratory (ATC Scientific, Little Rock, AR) for analysis of amino acids and dry matter as described by [Farraan and Thomas \(1992a\)](#).

Statistical Analysis

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), which can be described as follows:

$$y = \beta_0 + \beta_1 Val + \beta_2 Leu + \beta_3 Ile + Val(Val \times \beta_4) \\ + Val(Leu \times \beta_5) + Leu(Leu \times \beta_6) \\ + Val(Ile \times \beta_7) + Leu(Ile \times \beta_8) + Ile(Ile \times \beta_9)$$

where y represents the parameter evaluated, β_0 denotes the intercept, β_1 through β_9 denote the coefficients of the regression equation, Val denotes the dietary Val:Lys ratio, Leu denotes the dietary Leu:Lys ratio, and Ile denotes the dietary Ile:Lys ratio. 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.

RESULTS

Data are presented using contour and surface graphs with Val:Lys and Ile:Lys composing the X and Y axes, respectively, for each selected Leu:Lys ratio. The Leu:Lys ratios selected range from 110 to 190 with 20 point increments. These ratios were selected for the contour graphs as they represent Leu:Lys ratios in common broiler feed formulated with different primary ingredients.

Analyzed values of Leu, Ile, and Val from specific diets were in close agreement with the calculated values ([Table 3](#)). Models for BWG, FCR, and feed intake were significant ($P < 0.10$; [Table 4](#)), 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 ([Table 5](#)). 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 ($P = 0.001$) and Ile:Lys ratios ($P = 0.004$) at each Leu:Lys ratio ([Table 4](#)).

Table 4. Regression coefficients of the surface response model for body weight gain (g/bird), feed intake (g/bird), and mortality corrected feed conversion (g/g) of Ross 344 × 708 male broilers from 20 to 34 d of age fed experimental treatments from 20 to 35 d of age

Variable	Body weight gain		Feed conversion ¹		Feed intake	
	Estimates	P-value	Estimates	P-value	Estimates	P-value
Intercept	−4047.4640	0.027	5.1612	0.001	−3134.3190	0.221
Val:Lys	110.4447	0.001	−0.0615	0.006	117.2222	0.010
Leu:Lys	−12.1288	0.095	0.0115	0.037	−4.2343	0.685
Ile:Lys	54.7743	0.040	−0.0580	0.006	27.0125	0.467
Val:Lys × Val:Lys	−0.7645	< 0.001	0.0004	0.001	−0.7943	0.003
Val:Lys × Leu:Lys	0.0568	0.323	−0.00005	0.265	0.0449	0.605
Leu:Lys × Leu:Lys	0.0091	0.486	−0.00001	0.404	−0.0020	0.920
Val:Lys × Ile:Lys	0.0402	0.826	−0.00004	0.781	0.0121	0.966
Leu:Lys × Ile:Lys	0.0695	0.238	−0.0001	0.084	0.0166	0.849
Ile:Lys × Ile:Lys	−0.4990	0.004	0.0005	< 0.001	−0.2250	0.302
Model	< 0.001		< 0.001		0.035	
Lack of fit	0.001		0.61		0.41	
R ²	0.93		0.94		0.75	

¹Feed conversion values were corrected for mortality.

Table 5. Regression coefficients of the surface response model for boneless-skinless breast meat and carcass weight (g/bird) and yield (%), and leg-quarter yield (%) relative to live body weight at 35 d of age of Ross 344 × 708 male broilers fed experimental treatments from 20 to 35 d of age.

Variable	Carcass weight		Carcass yield		Breast weight		Breast yield		Leg-quarter yield	
	Estimates	<i>P</i> -value	Estimates	<i>P</i> -value	Estimates	<i>P</i> -value	Estimates	<i>P</i> -value	Estimates	<i>P</i> -value
Intercept	-2,618.2610	0.114	46.4798	0.013	-1,256.0610	0.106	4.2008	0.713	20.7713	0.054
Val:Lys	77.7362	0.008	0.2484	0.324	30.2496	0.020	0.1598	0.374	0.0886	0.561
Leu:Lys	-8.8804	0.194	-0.0481	0.478	-4.2338	0.186	-0.0953	0.069	0.0250	0.546
Ile:Lys	47.4558	0.061	0.5794	0.031	26.2805	0.032	0.6767	0.002	-0.0918	0.531
Val:Lys × Val:Lys	-0.5644	0.001	-0.0023	0.104	-0.2322	0.003	-0.0019	0.072	-0.0005	0.549
Val:Lys × Leu:Lys	0.0751	0.187	0.0004	0.484	0.0306	0.246	0.0003	0.487	0.0002	0.598
Leu:Lys × Leu:Lys	0.0035	0.780	0.00005	0.713	-0.0012	0.841	-0.0001	0.314	0.00001	0.939
Val:Lys × Ile:Lys	0.0048	0.978	0.0006	0.753	0.0210	0.800	0.0009	0.488	-0.0004	0.692
Leu:Lys × Ile:Lys	0.0285	0.607	0.0005	0.368	0.0330	0.218	0.0016	0.003	-0.0006	0.128
Ile:Lys × Ile:Lys	-0.3817	0.015	-0.0052	0.003	-0.2375	0.003	-0.0071	< 0.001	0.0014	0.116
Model	0.002		0.031		0.001		< 0.001		0.018	
Lack of fit	0.008		0.11		0.006		0.032		0.48	
R ²	0.87		0.76		0.88		0.96		0.79	

However, BWG linearly decreased ($P = 0.095$) 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 g 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 g 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 g) 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 ($P = 0.010$) and quadratic manner ($P = 0.003$) by Val:Lys ratio (Table 4). 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 ($P = 0.001$) ratio (Table 4). Moreover, the interaction ($P = 0.084$) 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, respectively.

Carcass weight had a quadratic relationship with increasing Val:Lys ($P = 0.001$) and Ile:Lys ($P = 0.015$) ratios (Table 5). Optimum carcass weight of broilers (1,429 g) 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 ($P = 0.003$) was observed on carcass yield but ratios of Val:Lys, Leu:Lys, and all corresponding interactions had little influence ($P > 0.10$) 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 ($P = 0.003$) was observed on the total weight of boneless-skinless breast meat (Table 5). 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 ($P = 0.003$). 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 ($P = 0.072$). 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. Additionally, dietary BCAA did not affect ($P > 0.10$) leg quarter yield of broilers (Table 5).

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 (Tables 6 and 7). 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 ($P \leq 0.10$) contents of Ile, Leu, Cys, Lys, and Arg in the feather of broilers (Tables 6 and 7). 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.

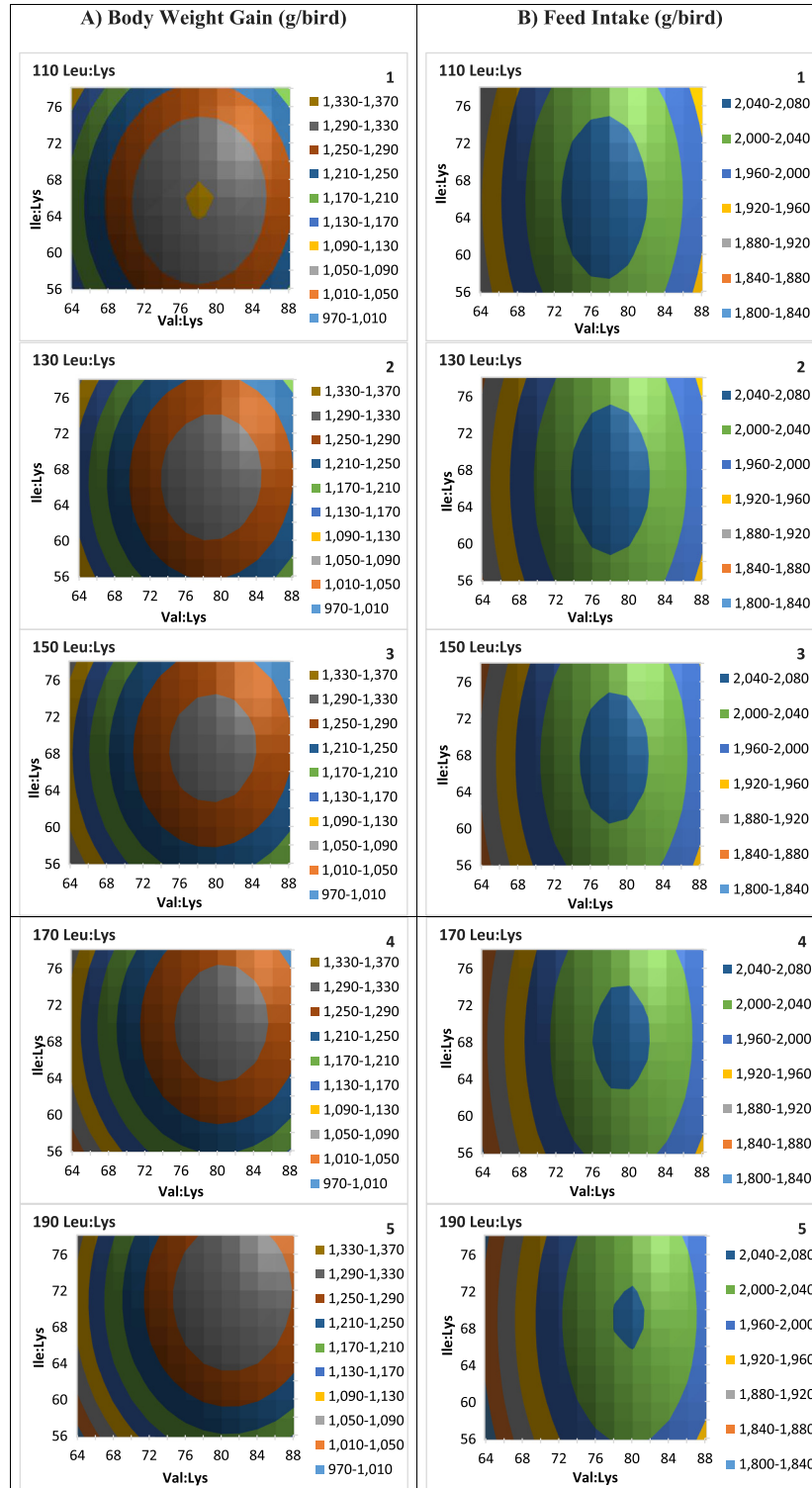


Figure 1. Contour graphs for (A) body weight gain ($P < 0.001$; $R^2 = 0.93$) and (B) feed intake ($P = 0.035$; $R^2 = 0.75$) of Ross 344 $\times$ 708 male broilers from 20 to 34 d of age. Body weight gain was influenced quadratically by Val:Lys ($P < 0.001$) and Ile:Lys ($P = 0.004$) and linearly by 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).

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, as primary ingredients. Furthermore, the inclusion of corn co-product, such as dried-distillers grain with solubles or corn gluten meal

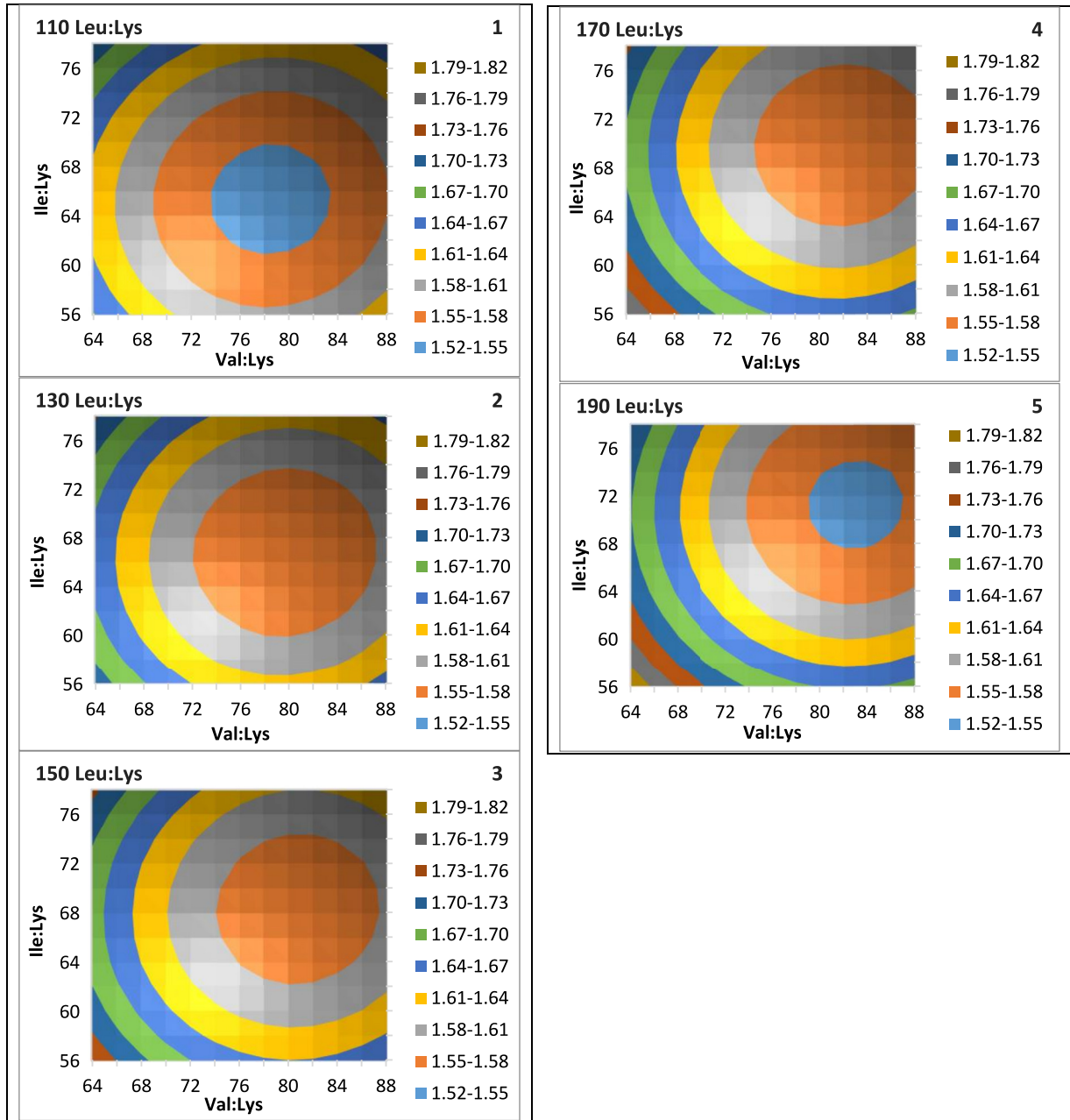


Figure 2. Contour graphs for mortality corrected feed conversion ratio (g/g; $P = 0.002$; $R^2 = 0.88$) of Ross 344 $\times$ 708 male broilers from 20 to 34 d of age. Feed conversion was influenced by Val:Lys ($P = 0.001$) in a quadratic manner and the interaction of Leu:Lys and Ile:Lys ($P = 0.084$). 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).

could increase Leu:Lys ratio up to 170 and 190. Based on the results of this study, it is reasonable to assert 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 although similar broiler performance may not be achieved compared to Leu:Lys at 110. These findings are critical as

achieving Leu:Lys ratio of 110 is unlikely when using either corn or wheat as the primary cereal source or when using any of their byproducts in commercial formulation. The ratio of Leu:Lys may increase up to 140 to 185 depending on the feed ingredients adopted in the formulation, which may impair growth performance of broilers as noted in this present study.

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

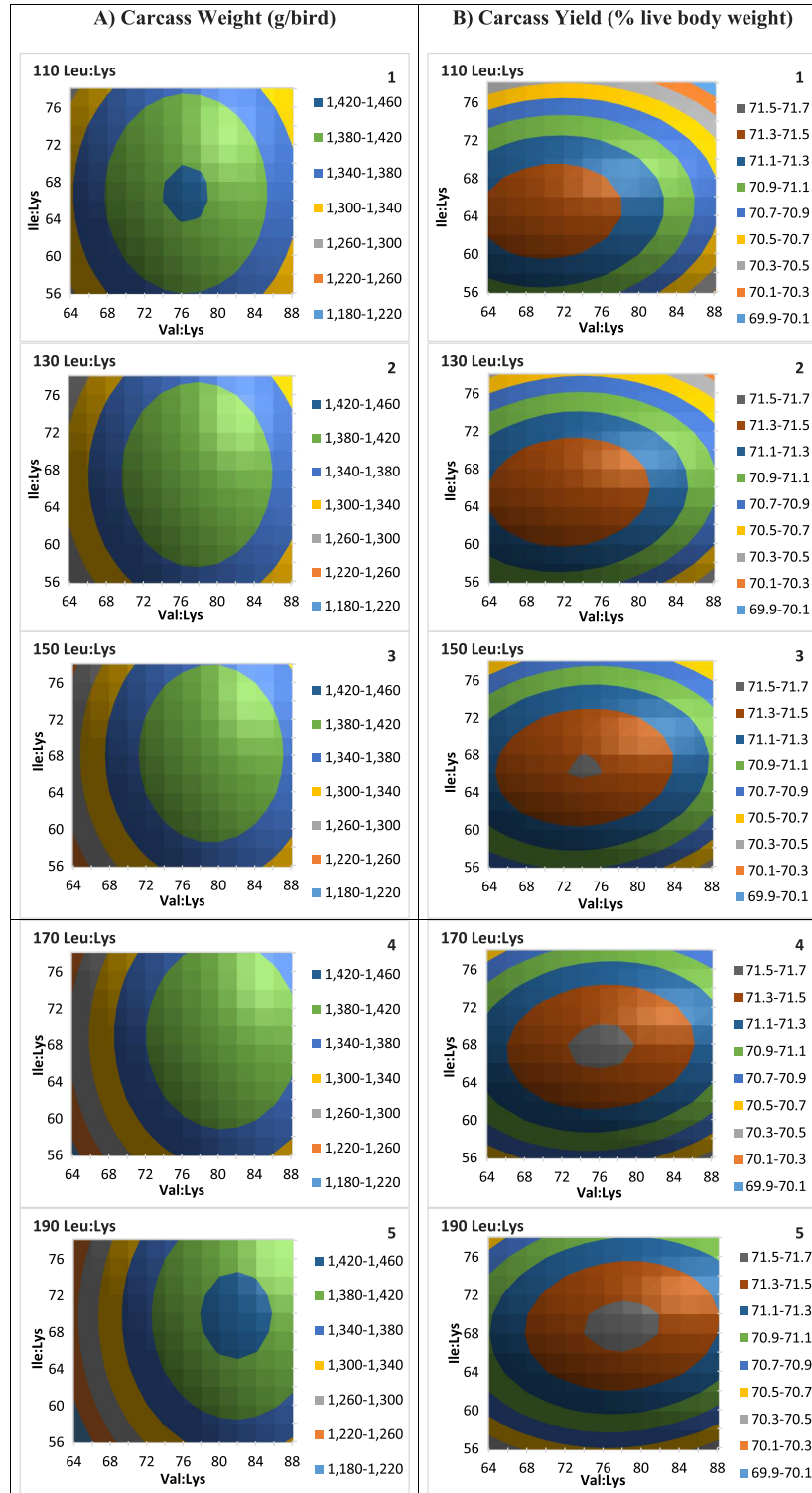


Figure 3. Contour graphs for carcass (A) weight ($P = 0.002$; $R^2 = 0.87$) and (B) yield ($P = 0.031$; $R^2 = 0.76$) of Ross 344 $\times$ 708 male broilers from 20 to 34 d of age. The carcass weight was influenced by Val:Lys ($P = 0.001$) and Ile:Lys ($P = 0.015$) in a quadratic manner. The carcass 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).

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 amino-transferase (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_2 production from labeled Ile and Val, which indicate greater catabolism of Ile and Val

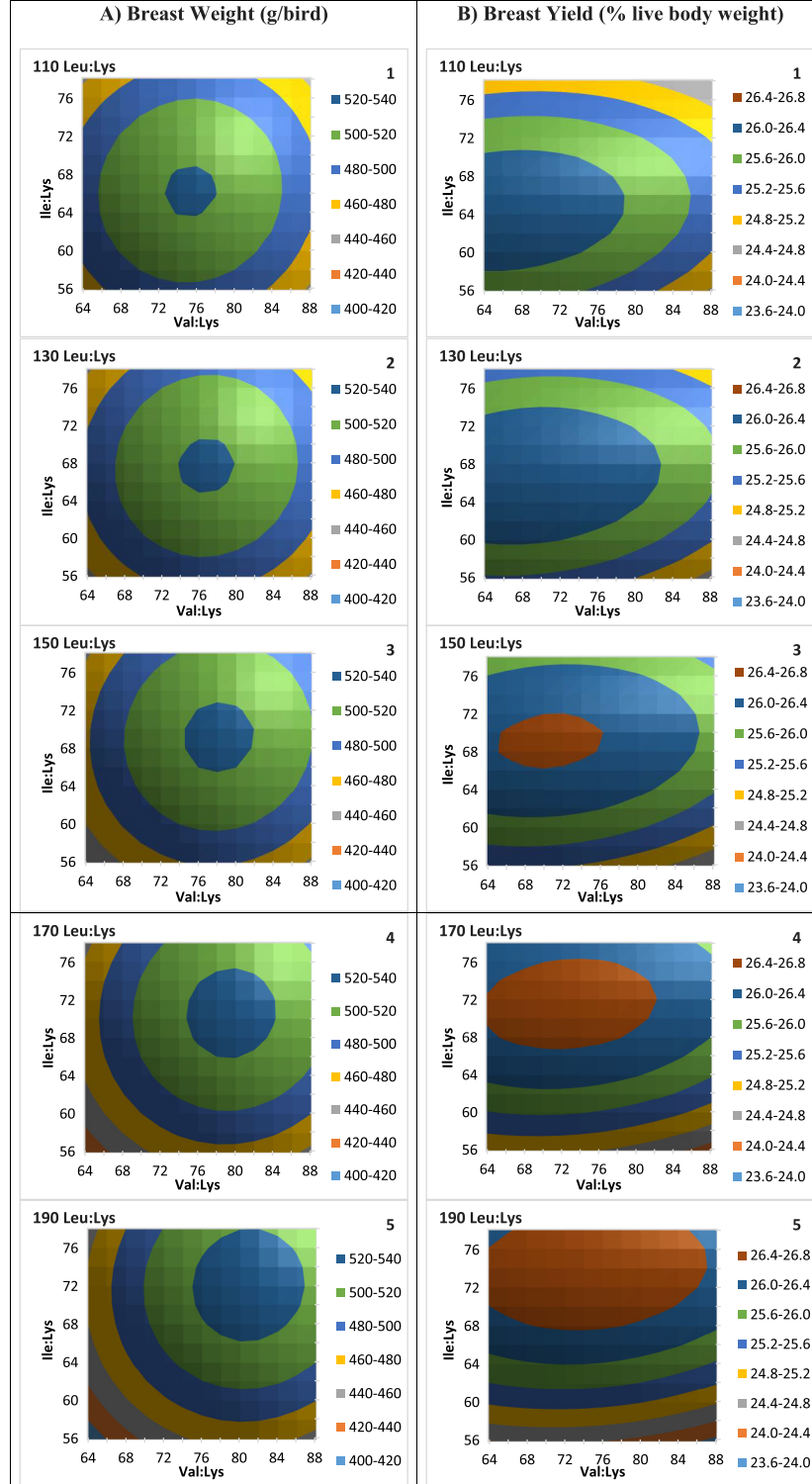


Figure 4. Contour graphs for total breast meat (A) weight ($P = 0.001$; $R^2 = 0.88$) and (B) yield ($P < 0.001$; $R^2 = 0.96$) weights of Ross 344 $\times$ 708 male broilers from 20 to 34 d of age. The total breast meat weight was influenced by Val:Lys ($P = 0.003$) and Ile:Lys ($P = 0.003$) in a quadratic 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).

(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.

Table 6. Regression coefficients of the surface response model for feather dry matter (%), crude protein (%), and branched-chain amino acids (%) at 35 d of age of Ross 344 × 708 male broilers fed experimental treatments from 20 to 35 d of age.

Variable	Dry Matter		Crude Protein		Isoleucine		Leucine		Valine	
	Estimates	P-value	Estimates	P-value	Estimates	P-value	Estimates	P-value	Estimates	P-value
Intercept	137.8336	0.015	-116.7630	0.261	11.0938	0.058	19.8943	0.207	10.2695	0.324
Val:Lys	-0.5558	0.462	2.0244	0.212	-0.1541	0.084	-0.2018	0.398	-0.0857	0.588
Leu:Lys	0.0097	0.962	0.5244	0.234	-0.0224	0.330	-0.0722	0.273	-0.0062	0.886
Ile:Lys	-0.8378	0.258	2.9831	0.068	-0.0089	0.910	0.0159	0.944	-0.0077	0.960
Val:Lys*Val:Lys	0.0071	0.101	-0.0078	0.364	0.0004	0.375	-0.0002	0.888	-0.0004	0.638
Val:Lys*Leu:Lys	-0.0010	0.575	-0.0030	0.407	0.0004	0.081	0.0013	0.029	0.0006	0.115
Leu:Lys*Leu:Lys	0.0002	0.593	-0.0004	0.609	0.0000	0.747	-0.0001	0.431	-0.0001	0.426
Val:Lys*Ile:Lys	-0.0059	0.293	-0.0063	0.584	0.0006	0.317	0.0005	0.779	0.0009	0.459
Leu:Lys*Ile:Lys	0.0002	0.893	-0.0032	0.382	0.0000	0.996	0.0000	0.978	-0.0003	0.389
Ile:Lys*Ile:Lys	0.0099	0.035	-0.0151	0.104	-0.0003	0.556	-0.0004	0.788	-0.0001	0.936
Model	0.29		0.35		0.58		0.49		0.74	
Lack of fit	0.41		0.21		0.29		0.34		0.63	
R ²	0.56		0.54		0.44		0.48		0.37	

A similar response was reported by [Tuttle and Balloun \(1976\)](#) and [Ospina-Rojas et al. \(2020\)](#). Broilers fed diets with high Leu content but marginal Val and Ile had decreased performance. When Val and Ile concentrations were increased, growth performance of broilers was restored ([Tuttle and Balloun, 1976](#); [Ospina-Rojas et al., 2020](#)). The increased need for Val and Ile in a high Leu diet may be necessary to compensate for the degradation of Val and Ile due to excess Leu ([Smith and Austic, 1978](#); [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. In an experiment using pigs, [Suryawan et al. \(2008\)](#) infused overnight-fasted 7-day-old piglets with either saline, saline with rapamycin (inhibitor for mammalian target of rapamycin), Leu, or Leu with rapamycin. The result showed that piglets infused with Leu were the only group with greater protein synthesis. 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.

It is interesting that dietary BCAA did not affect feather amino acid composition in this trial. Perhaps, more than one bird per pen is needed to obtain the statistical support needed to observe differences in feather composition. Furthermore, extending the evaluation beyond the primary feathers may provide stronger evidence to the result. A previous study by [Robel \(1977\)](#) showed that feather abnormality can be visually observed not only in primary feathers, but also the secondary feathers. On the other hand, the change in feather amino acid composition has been reported due to

Table 7. Regression coefficients of the surface response model for feather methionine, cysteine, lysine, and arginine (%) at 35 d of age of Ross 344 × 708 male broilers fed experimental treatments from 20 to 35 d of age.

Variable	Methionine		Cysteine		Lysine		Arginine	
	Estimates	P-value	Estimates	P-value	Estimates	P-value	Estimates	P-value
Intercept	2.3498	0.070	-8.9309	0.329	6.8379	0.040	16.6585	0.079
Val:Lys	-0.0357	0.075	0.2895	0.057	-0.0748	0.127	-0.2282	0.114
Leu:Lys	-0.0050	0.334	0.0119	0.753	-0.0258	0.061	-0.0397	0.294
Ile:Lys	-0.0088	0.620	0.0926	0.491	-0.0239	0.591	0.0190	0.884
Val:Lys*Val:Lys	0.0001	0.212	-0.0017	0.043	0.0001	0.642	0.0006	0.412
Val:Lys*Leu:Lys	0.0000	0.417	0.0005	0.106	0.0003	0.014	0.0007	0.032
Leu:Lys*Leu:Lys	0.0000	0.237	-0.0002	0.030	0.0000	0.833	0.0000	0.498
Val:Lys*Ile:Lys	0.0001	0.311	-0.0017	0.111	0.0001	0.742	0.0004	0.723
Leu:Lys*Ile:Lys	0.0000	0.692	0.0000	0.971	0.0001	0.533	0.0000	0.898
Ile:Lys*Ile:Lys	0.0000	0.986	0.0003	0.692	0.0000	0.869	-0.0003	0.707
Model	0.22		0.14		0.13		0.47	
Lack of fit	0.25		0.54		0.13		0.33	
R ²	0.60		0.65		0.66		0.48	

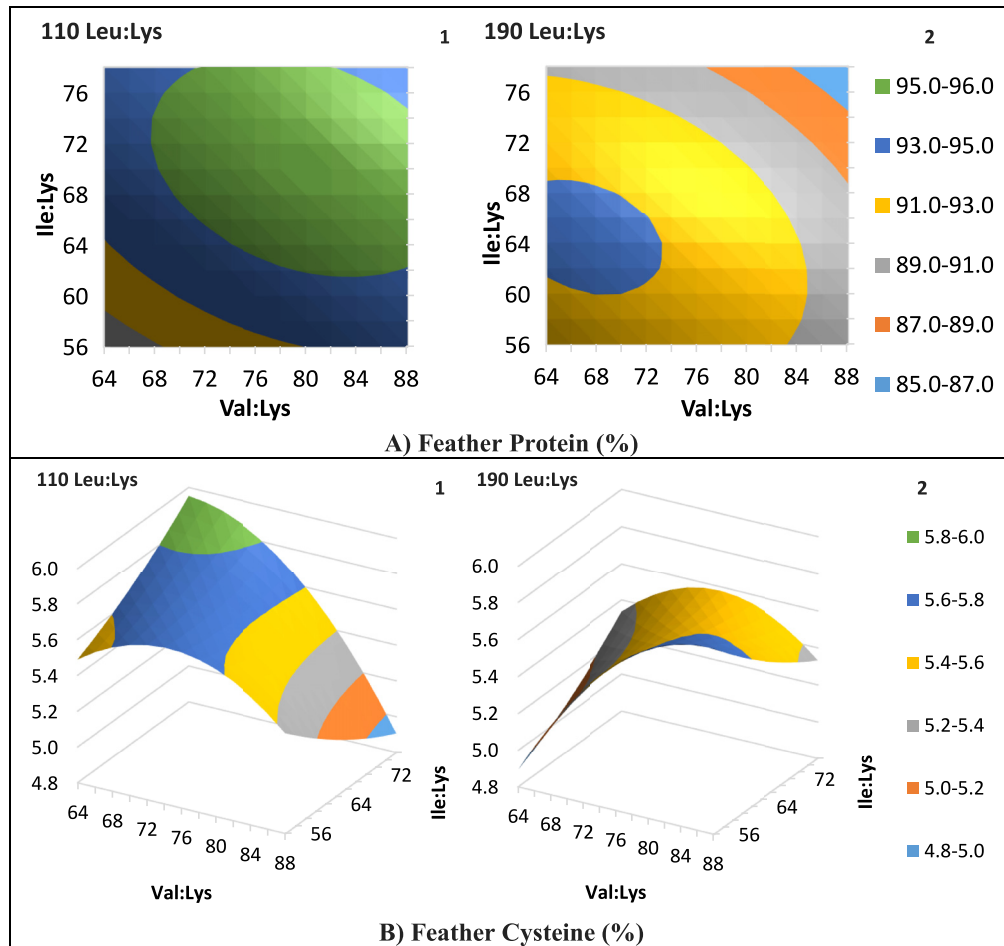


Figure 5. Surface plots of (A) crude protein ($P = 0.35$; $R^2 = 0.54$) and cysteine ($P = 0.14$; $R^2 = 0.65$) contents in feathers of Ross 344 × 708 male broilers at 35 d of age. Graphs were generated using regression coefficients in Tables 6 and 7 by applying various Val:Lys (64 to 88) and Ile:Lys (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.

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. Based on data presented herein, optimum BCAA ratios to Lys vary depending on the desired response criteria. 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. While the

correlation among BCAA is evident in broilers from 20 to 34 d of age, their influence on heavier broilers or chicks may differ, which warrants further investigation.

DISCLOSURES

The authors have no conflicts of interest to declare.

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