

EFFECT OF INCREASING ARGININE, VALINE, AND ISOLEUCINE ON PRODUCTION PERFORMANCE, NUTRIENT UTILIZATION EFFICIENCY, EXCRETA QUALITY, AND CARCASS CHARACTERISTICS OF BROILERS FED WITH LOW CRUDE PROTEIN DIETS

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ABSTRACT

The study investigated the effect of arginine (Arg), valine (Val), and isoleucine (Ile) in low crude protein (LCP) diets on production performance, nutrient utilization efficiency, excreta quality, and carcass characteristics of broilers. A total of 650 Cobb 500 broilers were allotted to five dietary treatments in a randomized complete block design. Dietary treatments were: (1) standard CP diet with Arg, Ile and Val:Lys set at 105, 65, and 73%, (2) LCP diet with the same AA:Lys as Diet 1 with reduced CP by around 4%, (3) Diet 2 with Arg:Lys increased to 115%, (4) Diet 3 with Ile and Val:Lys increased to 70 and 78%, and (5) Diet 3 with Ile and Val:Lys increased to 75 and 83%. Overall (d 0 to 35), LCP diet-fed birds had poorer ($P=0.001$) growth performance, nutrient utilization efficiency, and carcass quality than those fed the standard CP diet. Increasing Arg, Val, or Ile did not mitigate the effects of dietary CP reduction. Excreta were drier ($P=0.001$) and contained less ($P=0.001$) nitrogen in birds fed LCP diets. In conclusion, feeding low-CP diets improves excreta quality and reduces nitrogen excretion in broilers; however, supplementing with Arg, Val, and Ile does not mitigate the adverse effects of CP reduction.

Keywords: arginine, valine, isoleucine, crude protein, broiler chickens

INTRODUCTION

Protein is vital for the normal growth and reproduction of animals. It provides the amino acids required for protein synthesis, tissue repair, nucleic acid, enzyme, and hormone metabolism, as well as muscle function. Reducing dietary crude protein (CP) decreases the body's nitrogen supply, which is necessary to produce non-essential amino acids (NEAA) for protein formation, along with other nitrogen-containing compounds. Most broiler producers have lowered dietary CP levels by about 1.0 to 1.5%, as this approach not only reduces feed costs but also significantly decreases intestinal disease incidence and nitrogen excretion. A challenge faced by producers when reducing CP beyond 1 to 1.5% is a decline in feed intake and growth performance. This is likely due to a potential shortage of free nitrogen for endogenous NEAA synthesis. According to Si *et al.* (2004), compensatory increases in key amino acid ratios above established requirements are necessary when formulating low CP diets to counteract the associated negative physiological and performance effects in broilers.

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Some essential amino acids can produce metabolites that play pivotal roles in providing functional benefits to animals. One of the most discussed amino acids is arginine (Arg). Among the ten known essential amino acids in poultry, this amino acid is considered the most metabolically versatile due to its involvement in multiple biochemical pathways. It supports the synthesis of nonessential amino acids such as proline (Pro) and glutamate (Glu) (Albaugh *et al.*, 2017) and serves as a precursor for numerous metabolites, including nitric oxide, guanidinoacetic acid, creatine, polyamines, and ornithine. Arg is well known for its role in the urea cycle, as birds lack the enzymes needed for endogenous synthesis. Brugaletta *et al.* (2023) reported that broilers fed diets with Arg:Lys ratios exceeding established requirements exhibited improved final body weight, growth rate, and cumulative feed conversion ratio. These findings support the hypothesis that elevating dietary Arg levels in low CP formulations may enhance broiler growth performance.

In addition, branched-chain amino acids (BCAA), specifically valine (Val) and isoleucine (Ile), are also being considered for their potential contribution to NEAA synthesis and important role in feed intake and muscle accretion. In standard corn–soybean meal diets, leucine (Leu) is often supplied in excess, which can activate branched-chain α -keto acid dehydrogenase and other BCAA catabolic pathways (Brosnan and Brosnan, 2006). This enzymatic upregulation accelerates the degradation of Ile and Val, thereby reducing their metabolic availability and potentially impairing growth performance and protein accretion in broilers. According to Holecek (2018), BCAA can be metabolized to synthesize NEAAs such as alanine (Ala), glutamine (Gln), and glutamic acid (Glu). However, there is limited data on the effect of increased Val and Ile on broilers fed a low CP diet. Therefore, this study was conducted to determine the effects of Arg, Val, and Ile in low-CP diets on growth performance, nutrient utilization efficiency, carcass characteristics, excreta moisture, and nitrogen excretion in broilers.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños, College, Laguna (IBS-2016-008).

A total of 650 day-old, straight-run Cobb 500 broilers were allotted to five dietary treatments using a randomized complete block design. The cage was considered the experimental unit. The experimental units were blocked according to initial body weight to minimize variability in growth performance among the birds before the start of the experiment. Each treatment was replicated 13 times, with 10 chicks per replicate in a cage. Dietary treatments were (1) standard CP diet with Arg, Ile and Val:Lys set at 105, 65, and 73%, and no restriction on Leu levels (PC), (2) LCP diet with the same AA:Lys as Diet 1 but with Leu level at 100% and reduced CP levels by around 4% (NC), (3) Diet 2 with Arg:Lys increased to 115% (NC+Arg), (4) Diet 3 with Ile and Val:Lys increased to 70 and 78% (NC+Arg+Val/Ile5), and (5) Diet 3 with Ile and Val:Lys increased to 75 and 83% (NC+Arg+Val/Ile10).

All treatment diets were composed of corn, soybean meal, and coconut oil, with crystalline AA supplementation, and the feed formulations for chick booster, broiler starter, and broiler finisher are presented in Tables 1, 2, and 3, respectively. The dietary treatments were formulated based on the SID Lysine (Lys) and metabolizable energy (ME) requirements

of broilers on a per-day basis, calculated as the total SID Lys requirement in grams per Mcal of ME, with no CP restriction except for Treatment 1, which was formulated to meet the breed's required CP. The nutrient levels of all treatment diets were formulated to meet or exceed the recommendations of Cobb 500 broilers. The (3) diets were fed in mash form, and the ratio of Leu relative to Lys was maintained at 100%, except for Treatment 1 with 132% Leu:Lys ratio.

Table 1. Ingredient and nutrient composition of chick booster diets (as-fed basis)^{1,2}

Item	CP Arg:Lys, %	Treatment				
		Standard	Low	Low	Low	Low
	Ile:Lys, %	105	105	115	115	115
	Val:Lys, %	65	65	65	70	75
		73	73	73	78	83
Ingredient, %						
Corn		59.69	79.11	78.15	78.07	77.99
Soybean meal		32.41	14.33	14.33	14.33	14.33
Coconut oil		4.05	0.39	0.35	0.30	0.25
L-methionine		0.33	0.48	0.48	0.48	0.48
L-lysine HCl		0.26	0.83	0.83	0.83	0.83
L-threonine		0.17	0.41	0.41	0.41	0.41
L-tryptophan		--	0.07	0.07	0.07	0.07
L-isoleucine		0.02	0.32	0.32	0.39	0.45
L-valine		0.05	0.34	0.35	0.41	0.48
L-arginine		0.02	0.55	0.68	0.68	0.68
Corn starch		--	--	0.86	0.86	0.86
Monocalcium phosphate		0.79	0.85	0.85	0.85	0.85
Limestone		1.14	1.26	1.26	1.26	1.26
Salt		0.46	0.45	0.45	0.45	0.45
Vitamin premix ³		0.035	0.035	0.035	0.035	0.035
Trace mineral premix ⁴		0.10	0.10	0.10	0.10	0.10
Choline chloride 60%		0.25	0.25	0.25	0.25	0.25
Phytase		0.01	0.01	0.01	0.01	0.01
Mold inhibitor		0.20	0.20	0.20	0.20	0.20
Antioxidant		0.012	0.012	0.012	0.012	0.012
Total		100.00	100.00	100.00	100.00	100.00
Calculated, %						
DM		88.92	88.17	88.19	88.2	88.21
ME, kcal/kg		3,000	3,000	3,000	3,000	3,000
CP		20.68	16.19	16.42	16.5	16.59

Table 1 continued . . .

Item	CP Arg:Lys, %	Standard 105	Treatment			
			Low 105	Low 115	Low 115	Low 115
	Ile:Lys, %	65	65	65	70	75
	Val:Lys, %	73	73	73	78	83
Crude Fiber		2.80	2.45	2.45	2.45	2.45
Crude Fat		7.22	3.58	3.63	3.57	3.52
SID Lys:ME, g/ Mcal		4.07	4.07	4.07	4.07	4.07
SID ⁵ Amino Acids						
Lysine		1.22	1.22	1.22	1.22	1.22
Threonine		0.83	0.83	0.83	0.83	0.83
Methionine		0.61	0.69	0.69	0.69	0.69
Methionine+Cysteine		0.91	0.91	0.91	0.91	0.91
Tryptophan		0.22	0.20	0.20	0.20	0.20
Isoleucine		0.79	0.79	0.79	0.85	0.92
Valine		0.89	0.89	0.89	0.95	1.01
Arginine		1.28	1.28	1.40	1.40	1.40
Leucine ⁶		1.62	1.22	1.22	1.22	1.22
Glycine+Serine		1.33	0.91	0.91	0.91	0.91
Total Lysine		1.32	1.28	1.28	1.28	1.28
Calcium		0.90	0.90	0.90	0.90	0.90
Available Phosphorus		0.47	0.47	0.47	0.47	0.47
Sodium		0.20	0.20	0.20	0.20	0.20
Chlorine		0.32	0.31	0.31	0.31	0.31

¹Diet fed in meal form from day 0 to 10.

²Diet composition was calculated using Rostagno Brazilian Standard (Rostagno *et al.*, 2017) values for ingredients.

³The vitamin premix provided the following quantities of vitamins per kg of the diet: Vitamin A, 12,250 IU/kg; Vitamin D₃, 3,325 IU/kg; Vitamin E, 31.5 g/kg; Vitamin K₃, 2.1 g/kg; thiamine, 2.45 g/kg; riboflavin, 7.7 g/kg; pyridoxine, 4.2 g/kg; niacin, 42 g/kg; pantothenic acid, 12.25 g/kg; vitamin B₁₂, 0.0245 mg/kg; folic acid, 1.05 g/kg; biotin, 0.105 mg/kg.

⁴The trace mineral premix provided the following quantities of micro minerals per kg of the diet: Fe, 30 mg/kg; Cu, 10 mg/kg; Zn, 60 mg/kg; Mn, 100 mg/kg; Co, 0.1 mg/kg; I, 0.5 mg/kg; Se, 0.2 mg/kg.

⁵SID=Standardized ileal digestible.

⁶SID Leu:Lys ratio is maintained at 100% for low CP diets.

Table 2. Ingredient and nutrient composition of broiler starter diets (as-fed basis)^{1,2}

Item	Treatment					
	CP	Standard	Low	Low	Low	Low
	Arg:Lys, %	105	105	115	115	115
	Ile:Lys, %	65	65	65	70	75
	Val:Lys, %	73	73	73	78	83
Ingredient, %						
Corn		66.87	83.97	83.07	82.97	82.81
Soybean meal		26.10	10.15	10.15	10.15	10.15
Coconut oil		3.25	0.03	0.01	--	--
L-methionine		0.33	0.83	0.83	0.83	0.83
L-lysine HCl		0.315	0.44	0.44	0.44	0.44
L-threonine		0.18	0.40	0.40	0.40	0.40
L-tryptophan		--	0.08	0.08	0.08	0.08
L-isoleucine		0.06	0.33	0.33	0.38	0.48
L-valine		0.10	0.34	0.34	0.40	0.46
L-arginine		0.10	0.57	0.68	0.68	0.68
Corn starch		--	--	0.81	0.81	0.81
Monocalcium phosphate		0.68	0.74	0.74	0.74	0.74
Limestone		1.08	1.19	1.19	1.19	1.19
Salt		0.46	0.45	0.45	0.45	0.45
Vitamin premix ³		0.035	0.035	0.035	0.035	0.035
Trace mineral premix ⁴		0.10	0.10	0.10	0.10	0.10
Choline chloride 60%		0.12	0.12	0.12	0.12	0.12
Phytase		0.01	0.01	0.01	0.01	0.01
Mold inhibitor		0.20	0.20	0.20	0.20	0.20
Antioxidant		0.012	0.012	0.012	0.012	0.012
Total		100.00	100.00	100.00	100.00	100.00
Calculated, %						
DM		88.63	87.98	88.00	88.01	88.02
ME, kcal/kg		3,025	3,025	3,025	3,028	3,032
CP		18.62	14.66	14.87	14.94	15.02
Crude Fiber		2.69	2.38	2.37	2.37	2.37
Crude Fat		6.44	3.35	3.33	3.32	3.31
SID Lys:ME, g/ Mcal		3.70	3.70	3.70	3.70	3.69

Table 2 continued . . .

Item	CP Arg:Lys, %	Standard 105	Treatment			
			Low 105	Low 115	Low 115	Low 115
	Ile:Lys, %	65	65	65	70	75
	Val:Lys, %	73	73	73	78	83
SID ⁵ Amino Acids						
Lysine		1.12	1.12	1.12	1.12	1.12
Threonine		0.76	0.76	0.76	0.76	0.76
Methionine		0.57	0.63	0.63	0.63	0.63
Methionine+Cysteine		0.85	0.84	0.84	0.84	0.84
Tryptophan		0.19	0.18	0.18	0.18	0.18
Isoleucine		0.73	0.73	0.73	0.78	0.87
Valine		0.84	0.82	0.82	0.87	0.93
Arginine		1.18	1.18	1.29	1.29	1.29
Leucine ⁶		1.48	1.12	1.12	1.12	1.12
Glycine+Serine		1.19	0.81	0.81	0.81	0.81
Total Lysine		1.21	1.17	1.17	1.17	1.17
Calcium		0.84	0.84	0.84	0.84	0.84
Available Phosphorus		0.44	0.44	0.44	0.44	0.44
Sodium		0.20	0.20	0.20	0.20	0.20
Chlorine		0.32	0.31	0.31	0.31	0.31

¹Diet fed in meal form from day 11 to 24.

²Diet composition was calculated using Rostagno Brazilian Standard (Rostagno *et al.*, 2017) values for ingredients.

³The vitamin premix provided the following quantities of vitamins per kg of the diet: Vitamin A, 12,250 IU/kg; Vitamin D₃, 3,325 IU/kg; Vitamin E, 31.5 g/kg; Vitamin K₃, 2.1 g/kg; thiamine, 2.45 g/kg; riboflavin, 7.7 g/kg; pyridoxine, 4.2 g/kg; niacin, 42 g/kg; pantothenic acid, 12.25 g/kg; vitamin B₁₂, 0.0245 mg/kg; folic acid, 1.05 g/kg; biotin, 0.105 mg/kg.

⁴The trace mineral premix provided the following quantities of micro minerals per kg of the diet: Fe, 30 mg/kg; Cu, 10 mg/kg; Zn, 60 mg/kg; Mn, 100 mg/kg; Co, 0.1 mg/kg; I, 0.5 mg/kg; Se, 0.2 mg/kg.

⁵SID=Standardized ileal digestible.

⁶SID Leu:Lys ratio is maintained at 100% for low CP diets.

Table 3. Ingredient and nutrient composition of broiler finisher diets (as-fed basis)^{1,2}

Item	Treatment					
	CP	Standard	Low	Low	Low	Low
	Arg:Lys, %	105	105	115	115	115
	Ile:Lys, %	65	65	65	70	75
	Val:Lys, %	73	73	73	78	83
Ingredient, %						
Corn		68.27	88.03	87.17	87.20	87.15
Soybean meal		24.40	5.91	5.91	5.85	5.85
Coconut oil		4.25	0.53	0.52	0.45	0.40
L-methionine		0.26	0.84	0.84	0.84	0.84
L-lysine HCl		0.28	0.44	0.44	0.44	0.44
L-threonine		0.11	0.36	0.36	0.36	0.36
L-tryptophan		--	0.10	0.10	0.10	0.10
L-isoleucine		0.02	0.34	0.34	0.39	0.44
L-valine		0.03	0.34	0.34	0.39	0.44
L-arginine		0.04	0.59	0.69	0.69	0.69
Corn starch		--	--	0.77	0.77	0.77
Monocalcium phosphate		0.41	0.47	0.47	0.47	0.47
Limestone		1.01	1.14	1.14	1.14	1.14
Salt		0.46	0.45	0.45	0.45	0.45
Vitamin premix ³		0.035	0.035	0.035	0.035	0.035
Trace mineral premix ⁴		0.10	0.10	0.10	0.10	0.10
Choline chloride 60%		0.10	0.10	0.10	0.10	0.10
Phytase		0.01	0.01	0.01	0.01	0.01
Mold inhibitor		0.20	0.20	0.20	0.20	0.20
Antioxidant		0.012	0.012	0.012	0.012	0.012
Total		100.00	100.00	100.00	100.00	100.00
Calculated, %						
DM		88.63	87.89	87.90	87.90	87.90
ME, kcal/kg		3,100	3,100	3,100	3,100	3,100
CP		17.63	13.06	13.25	13.30	13.36
Crude Fiber		2.64	2.28	2.28	2.28	2.28
Crude Fat		7.44	3.88	3.86	3.79	3.74
SID Lys:ME, g/ Mcal		3.30	3.29	3.29	3.29	3.28

Table 3 continued . . .

Item	CP Arg:Lys, %	Standard 105	Treatment			
			Low 105	Low 115	Low 115	Low 115
	Ile:Lys, %	65	65	65	70	75
	Val:Lys, %	73	73	73	78	83
SID ⁵ Amino Acids						
Lysine		1.02	1.02	1.02	1.02	1.02
Threonine		0.66	0.66	0.66	0.66	0.66
Methionine		0.53	0.61	0.61	0.61	0.61
Methionine+Cysteine		0.80	0.80	0.80	0.80	0.80
Tryptophan		0.18	0.18	0.18	0.18	0.18
Isoleucine		0.66	0.66	0.66	0.71	0.76
Valine		0.75	0.74	0.74	0.79	0.84
Arginine		1.07	1.07	1.17	1.17	1.17
Leucine ⁶		1.44	1.02	1.02	1.02	1.02
Glycine+Serine		1.14	0.71	0.71	0.71	0.71
Total Lysine		1.1	1.07	1.07	1.06	1.06
Calcium		0.76	0.76	0.76	0.76	0.76
Available Phosphorus		0.39	0.39	0.39	0.39	0.39
Sodium		0.20	0.20	0.20	0.20	0.20
Chlorine		0.32	0.32	0.32	0.32	0.31

¹Diet fed in meal form from day 25 to 35.

²Diet composition was calculated using Rostagno Brazilian Standard (**Rostagno *et al.*, 2017**) values for ingredients.

³The vitamin premix provided the following quantities of vitamins per kg of the diet: Vitamin A, 12,250 IU/kg; Vitamin D₃, 3,325 IU/kg; Vitamin E, 31.5 g/kg; Vitamin K₃, 2.1 g/kg; thiamine, 2.45 g/kg; riboflavin, 7.7 g/kg; pyridoxine, 4.2 g/kg; niacin, 42 g/kg; pantothenic acid, 12.25 g/kg; vitamin B₁₂, 0.0245 mg/kg; folic acid, 1.05 g/kg; biotin, 0.105 mg/kg.

⁴The trace mineral premix provided the following quantities of micro minerals per kg of the diet: Fe, 30 mg/kg; Cu, 10 mg/kg; Zn, 60 mg/kg; Mn, 100 mg/kg; Co, 0.1 mg/kg; I, 0.5 mg/kg; Se, 0.2 mg/kg.

⁵SID=Standardized ileal digestible.

⁶SID Leu:Lys ratio is maintained at 100% for low CP diets.

The feeding period lasted for 35 days. Day-old chicks were group-weighted at the start of the experiment and at days 10, 24, and 35. Experimental birds were fed with their corresponding diets based on their designated treatment in the morning and afternoon: booster diet from 0 to 10 days, starter diet from 11 to 24 days, and finisher diet from 25 to 35 days of age. Each cage was equipped with one feeder and a bell-drinker to allow *ad libitum* access to feed and water. Artificial lights were provided to enable the chicks to eat and drink at night. The birds were vaccinated against NCD at 7 and 21 days of age. Daily feed consumption for each treatment was monitored and recorded. Sanitary and management practices were standardized across all treatments. Protective nets were installed on each

cage, and floor pens were cleaned regularly to maintain optimal hygiene and minimize environmental variability.

The body weights of broilers were recorded on days 10, 24, and 35, and their corresponding weight gain was calculated. Feed intake from days 10, 24, and 35 was measured, and the feed-to-gain ratio (F/G) was calculated. Mortality was recorded together with the feed refusal of the animals in the cage when it occurred. The weight and age of birds with extreme deformities sustained through accidents were recorded and removed from the cages. Daily ME intake was calculated by multiplying the ME of the diet by the overall ADFI per bird, and ME efficiency was calculated by dividing the daily ME intake by the average daily gain (ADG). Production efficiency index (PEI) was calculated using the following equation: $PEI = [ADG \times \% \text{ viability} \times 100] / [F/G]$. Lys utilization efficiency was calculated by multiplying the Lys content of the diet by the total feed intake and dividing by the total body weight gain.

The excreta quality score of each pen was assessed visually at day 3, 10, 24, and 35 by two independent evaluators in the morning and afternoon, with the score ranging from 1 to 5 as described in Figure 1 (1 = dry, well-formed excreta with characteristic white uric acid cover, 2 = mostly dry excreta with white uric acid cover, 3 = moist excreta with white uric acid cover, 4 = moist excreta with less white uric acid cover and droppings lose their shape, and 5 = extremely wet excreta with little to no white uric acid cover).



Figure 1. Excreta quality scores. Scores range from 1 to 5 (1 = dry; well-formed excreta with characteristic white uric acid cover, 2 = mostly dry excreta with white uric acid cover, 3 = moist excreta with white uric acid cover, 4 = wet excreta with less white uric acid cover and droppings lose their shape, and 5 = extremely wet excreta with little to no white uric acid cover).

At 33 days of age, excreta were collected by replicates for 4 hours to collect at least 100 grams of pooled samples. The excreta samples were immediately placed in the oven at 70°C to measure the moisture content. After 16 days, the excreta samples were weighed, milled, and transferred to sealed plastic bags. The excreta moisture at 105°C and total nitrogen content of the samples were determined using the AOAC Official Methods of Analysis (Latimer, 2016).

At days 36 and 37, a total of 130 birds were slaughtered, with one male and one female from each replicate of each treatment. The liveweight of the birds was recorded before slaughter. The birds were dressed to evaluate carcass, retail cuts, and deboned breast muscle yield. The birds were manually bled, scalded at 70°C for 2 minutes, and manually defeathered to prevent labels from being removed. Evisceration and rinsing followed, and

then the carcasses were immediately placed in the chiller after weighing. Carcasses were separated, weighed, and recorded into breast muscles with and without bones, wings, legs, thighs, back, and abdominal fat.

All the experimental diets were analyzed for proximate, Ca, P, and individual AAs (cystine, aspartic acid, methionine, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, proline, and tryptophan) using AOAC Official Methods of Analysis (Latimer, 2016) and HPLC.

All data gathered were analyzed using the MIXED procedure of SAS (SAS Institute, 1996). The statistical model included diet as a fixed effect and block as a random effect, with the cage considered as the experimental unit. Least square means (LSMeans) were calculated for each response variable. Orthogonal polynomial contrasts were determined to evaluate the linear and quadratic effects of dietary treatments on broiler performance. When the effect of diet was significant, LSMs were compared using the PDIF option and adjusted with the Tukey-Kramer test. Statistical significance and tendencies were defined at $P \leq 0.05$ and $0.05 < P < 0.10$, respectively.

RESULTS AND DISCUSSION

Birds fed diets with standard CP showed higher BWG and lower FI and F/G ($P < 0.001$) compared to diets with low CP levels from day 0 to 35 (Table 4). On day 10, the NCP+Arg resulted in improved BWG ($P < 0.001$) compared to the NC, and NCP+Arg+Val/Ile5 and NCP+Arg+Val/Ile10 treatments. The F/G on day 10 for birds fed the PC diet was not significantly different from the NC diet, with increased ratios of Arg, Val, and Ile. Broilers fed NC diets with increased ratios of Arg, Val, and Ile showed no effect on BWG, FI, F/G, PEI, ME efficiency, or Lys utilization efficiency at days 24, 35, or over 0 to 35 days. The dietary treatments did not influence livability.

The growth performance data showed that increasing AA levels beyond the required amount did not provide additional benefits for broilers fed the low CP diets. A similar experiment was conducted by Belloir *et al.* (2017), which found that providing extra amounts of Lys, total sulfur AA (TSAA), and threonine (Thr) did not confer further benefits to the animals, except during the early growth stage. This contrasts with the report by Si *et al.* (2004), stating that birds fed 110% of the AA recommendation had better growth rates. Moreover, several researchers have discussed that some factors causing negative performance in poultry after lowering CP may relate to other issues such as inadequate levels of NEAA, imbalances among certain AAs like BCAA and Arg to Lys ratio, tryptophan (Trp) to large neutral amino acids (LNAA) ratio, and potential toxic concentrations of specific AAs (Si *et al.*, 2004; Applegate and Angel, 2008; Namroud *et al.*, 2008). Brestenský *et al.* (2015) explained that BCAA and Trp share the same blood-brain barrier transport system and typically compete for entry into the brain. This indicates that when BCAA concentrations in the blood increase, Trp transport to the brain decreases. Reduced transport of Trp into the brain results in lower serotonin production, leading to poor performance in birds.

Another possible reason for the results showing no significant improvement with increasing BCAA levels was that only Val and Ile were increased, while Leu remained constant. At the time the study was conducted, no specific recommendation for standardized ileal digestible (SID) Leu:Lys ratios was provided in the breeder's nutrition guide. Consequently, constraining SID Leu:Lys to 100% may have resulted in a suboptimal level

Table 4. Effect of increasing arginine, valine, and isoleucine on production performance of broilers fed with low crude protein diets^{1,2}

Item	CP	Treatment						SEM	Treatment	P-value	
		Standard	Low		Low		Linear			Quadratic	
			Arg:Lys, %	Low	Low	Low					
				Ile:Lys, %	Low	Low					Low
	Val:Lys, %	73	73	73	73	78	83				
Day 0 to 10											
BWG, g		170.44 ^a	137.98 ^c	149.53 ^b	142.85 ^{bc}	143.02 ^{bc}	143.02 ^{bc}	2.71	<0.001	<0.001	0.11
FI, g		253.12 ^a	226.83 ^b	236.91 ^b	225.59 ^b	226.92 ^b	226.92 ^b	4.12	<0.001	0.002	0.09
F/G		1.486 ^b	1.650 ^a	1.590 ^{ab}	1.582 ^{ab}	1.591 ^{ab}	1.591 ^{ab}	0.03	0.006	0.46	0.99
Day 11 to 24											
BWG, g		805.89 ^a	445.78 ^b	445.39 ^b	445.96 ^b	447.02 ^b	447.02 ^b	10.97	<0.001	<0.001	0.17
FI, g		1221.35 ^a	831.76 ^b	842.88 ^b	898.05 ^b	850.88 ^b	850.88 ^b	22.70	<0.001	<0.001	0.46
F/G		1.522 ^c	1.870 ^b	1.899 ^{ab}	2.020 ^a	1.903 ^{ab}	1.903 ^{ab}	0.04	<0.001	<0.001	0.59
Day 25 to 35											
BWG, g		949.45 ^a	402.74 ^b	432.69 ^b	402.75 ^b	443.83 ^b	443.83 ^b	19.81	<0.001	<0.001	0.06
FI, g		1585.62 ^a	905.07 ^b	958.87 ^b	937.76 ^b	976.74 ^b	976.74 ^b	23.48	<0.001	<0.001	0.09
F/G		1.676 ^b	2.285 ^a	2.241 ^a	2.362 ^a	2.227 ^a	2.227 ^a	0.06	<0.001	<0.001	0.53
Day 0 to 35											
Day 0 BW, g		42.66	42.68	42.67	42.67	42.67	42.67	0.25	0.97	0.82	0.93
Day 35 BW, g		1,981.68 ^a	1,035.95 ^b	1,083.75 ^b	1,040.27 ^b	1,083.79 ^b	1,083.79 ^b	23.62	<0.001	<0.001	0.11
BWG, g		1,920.45 ^a	979.84 ^b	1,016.12 ^b	985.95 ^b	1,029.10 ^b	1,029.10 ^b	24.26	<0.001	<0.001	0.03
FI, g		3,050.68 ^a	1,947.92 ^b	2,010.92 ^b	2,044.63 ^b	2,042.84 ^b	2,042.84 ^b	34.76	<0.001	<0.001	0.11
F/G		1.590 ^b	1.994 ^a	1.985 ^a	2.085 ^a	1.991 ^a	1.991 ^a	0.03	<0.001	<0.001	0.60

Table 4 continued . . .

Item	CP	Treatment				SEM	Treatment	P-value	Linear	Quadratic
		Standard	Low	Low	Low					
Arg:Lys, %	105	105	115	115	115					
Ile:Lys, %	65	65	65	70	75					
Val:Lys, %	73	73	73	78	83					
Livability, %	98.46	97.69	95.38	97.69	99.23	1.36	0.35	0.69	0.05	
PEI ³	340.89 ^a	138.09 ^b	140.46 ^b	133.98 ^b	147.60 ^b	6.75	<0.001	<0.001	0.02	
ME efficiency, kcal ME/kg BWG	4,883.82 ^b	6,145.89 ^a	6,156.63 ^a	6,431.81 ^a	6,128.28 ^a	114.68	<0.001	<0.001	0.38	
Lys efficiency, g Lys/kg BWG	17.17 ^b	21.83 ^a	21.84 ^a	22.83 ^a	21.69 ^a	0.41	<0.001	<0.001	0.32	

¹Values are the least square means of 13 replicates per treatment with 10 birds per replicate.

²Dietary treatments: (1) standard CP diet with Arg, Ile and Val:Lys set at 105, 65, and 73%, and no restriction on Leu levels (PC), (2) LCP diet with the same AA:Lys as Diet 1 but with Leu level at 100% and reduced CP levels by around 4% (NC), (3) Diet 2 with Arg:Lys increased to 115% (NC+Arg), (4) Diet 3 with Ile and Val:Lys increased to 70 and 78% (NC+Arg+Val/Ile5), and (5) Diet 3 with Ile and Val:Lys increased to 75 and 83% (NC+Arg+Val/Ile10).

³Production Efficiency Index (PEI) = [ADG × % viability] / [F/G × 10].

^{a-c}Least square means within a row lacking a common superscript letter significantly differ ($P \leq 0.05$).

relative to the actual leucine requirement of modern broilers. Leu plays an important role in protein metabolism by stimulating protein synthesis and inhibiting protein breakdown via the mTOR (mammalian target of rapamycin) pathway (Mordier *et al.*, 2000; Bolster *et al.*, 2004; Ospina-Rojas *et al.*, 2020). The results observed in the study by Ospina-Rojas *et al.* (2020), which indicated a higher dietary requirement for Val and Ile to minimize the antagonistic effect of Leu on broiler performance, were not observed in this experiment due to the different ratios of Leu, Ile, and Val used.

No statistical differences were observed in the excreta scores of birds fed either PC or NC diets, with or without increased ratios of Arg, Val, and Ile relative to Lys at days 3, 10, 24, and 35 (Table 5). However, excreta moisture and excreta nitrogen in birds fed NC diets were significantly lower ($P<0.001$) than in birds fed PC diets. Meanwhile, broilers fed NC diets showed similar levels of excreta moisture and nitrogen, regardless of whether the ratios of Arg, Val, and Ile to Lys increased.

The results show a significant reduction in nitrogen excretion when dietary CP levels are lowered. It is expected that treatments with increased Arg, Val, and Ile levels will produce nitrogen excretion similar to that of the PC diets due to imbalances in amino acid levels, specifically from excess levels of supplemented amino acids relative to Lys, which can increase deamination and subsequently elevate nitrogen excretion. The low nitrogen excretion observed in NC+Arg+Val/Ile5 and NC+Arg+Val/Ile10 diets can be attributed to the reduced feed intake of the birds, possibly due to the competition between BCAAs, which are also considered as large neutral amino acids (LNAA), and Trp. A similar explanation was provided by Namroud *et al.* (2008) and Si *et al.* (2004), indicating that the decrease in feed intake of animals fed low CP diets may result from inadequate Trp to LNAA ratios. The inadequate Trp-to-LNAA ratios were highlighted by elevated BCAA levels in low-CP diets.

On the other hand, birds fed the PC diet showed similar dressing percentage with giblets to those fed with the NC diet, and to the NC diets with increased levels of Arg, Val, and Ile (Table 6). The NC+Arg+Val/Ile10 was reported to have the poorest ($P<0.05$) dressing percentage with giblets. After removing the giblets, only the birds fed the NC+Arg diet showed dressing percentages similar to those fed the PC diet. The breast and deboned breast of broilers fed PC were greater ($P<0.001$) compared to those on NC diets, even with increased ratios of Arg, Val, and Ile. The increased ratios of Arg, Ile, and Val relative to Lys could not improve the performance of the birds fed NC diets. Conversely, birds fed NC diets with and without the increase in Arg, Val, and Ile:Lys showed increased ($P<0.001$) leg and back weight compared to those fed the PC diet. A similar trend was observed in wing weight, with birds fed NC diets achieving a better ($P<0.05$) yield compared to those fed the PC diet, except for the NC+Arg treatment. Moreover, abdominal fat was less ($P<0.001$) in birds fed the PC diet. The deposition of fat in birds could be attributed to the increased concentration of cereal starch when CP levels are reduced (Striffler *et al.*, 2023). This rise in starch levels increases the availability of glucose in the small intestinal lumen for absorption. It can lead to reduced amino acid absorption due to competition with glucose for the electrochemical gradient generated by Na⁺/K⁺ ATPase to transport them to the apical membrane of enterocytes (Woyengo *et al.*, 2023). In this regard, amino acid availability for protein accretion will be negatively affected, and the FCR will tend to deteriorate with a high dietary starch:CP ratio (Striffler *et al.*, 2023). Furthermore, Choe *et al.* (2013) and Kamran *et al.* (2010) explained that high levels of dietary ME and CP boost the levels of glucose in the serum by either promoting the de novo lipogenesis, or by decreasing body fat oxidation following the increase in the oxidation of carbohydrates.

Table 5. Effect of increasing arginine, valine, and isoleucine on excreta score, moisture, and nitrogen of broilers fed with low crude protein diets^{1,2}

Item	CP*	Treatment					SEM	P-value		
		Standard	Low	Low	Low	Low		Treatment	Linear	Quadratic
			Low	Low	Low	Low				
	Arg:Lys, %	105	105	115	115	115				
	Ile:Lys, %	65	65	65	70	75				
	Val:Lys, %	73	73	73	78	83				
Excreta Score ³										
Day 3		1.80	2.07	2.19	1.92	2.01	0.14	0.43	0.48	0.40
Day 10		2.09	2.25	2.25	2.11	2.30	0.10	0.50	0.56	0.68
Day 24		1.59	1.86	1.86	1.67	1.84	0.08	0.08	0.42	0.87
Day 35		2.55	2.44	2.42	2.38	2.28	0.07	0.19	0.05	0.27
Excreta Moisture (d 35), %		76.61 ^a	71.72 ^b	71.66 ^b	71.88 ^b	72.25 ^b	0.58	<0.001	<0.001	0.23
Excreta Nitrogen (d 35), %		3.26 ^a	2.41 ^b	2.53 ^b	2.59 ^b	2.64 ^b	0.07	<0.001	<0.001	0.19

¹Values are the least square means of 13 replicates per treatment with 10 birds per replicate.

²Dietary treatments: (1) standard CP diet with Arg, Ile and Val:Lys set at 105, 65, and 73%, and no restriction on Leu levels (PC), (2) LCP diet with the same AA:Lys as Diet 1 but with Leu level at 100% and reduced CP levels by around 4% (NC), (3) Diet 2 with Arg:Lys increased to 115% (NC+Arg), (4) Diet 3 with Ile and Val:Lys increased to 70 and 78% (NC+Arg+Val/Ile5), and (5) Diet 3 with Ile and Val:Lys increased to 75 and 83% (NC+Arg+Val/Ile10).

³Score ranging from 1 to 5: 1 = dry, well-formed excreta with characteristic white uric acid cover, 2 = mostly dry excreta with white uric acid cover, 3 = moist excreta with white uric acid cover, 4 = wet excreta with less white uric acid cover and droppings lose their shape, and 5 = extremely wet excreta with little to no white uric acid cover.

^{a-b}Least square means within a row lacking a common superscript letter significantly differ ($P \leq 0.05$).

Table 6. Effect of increasing arginine, valine, and isoleucine on carcass characteristics of broilers fed with low crude protein diets^{1,2}

Item	CP*	Treatment						SEM	Treatment	P-value	
		Standard	Low	Low	Low	Low	Low			Linear	Quadratic
	Arg:Lys, %	105	105	115	115	115	115				
	Ile:Lys, %	65	65	65	70	70	75				
	Val:Lys, %	73	73	73	73	78	83				
Dressing % with Giblets		79.57 ^a	78.24 ^{ab}	79.01 ^{ab}	78.43 ^{ab}	78.09 ^b		0.38	0.03	0.19	0.09
Dressing % without Giblets		75.51 ^a	73.42 ^b	74.15 ^{ab}	73.37 ^b	73.26 ^b		0.37	<0.001	0.006	0.12
Abdominal Fat %		0.86 ^b	1.16 ^{ab}	1.28 ^a	1.15 ^{ab}	1.25 ^a		0.08	0.007	0.007	0.71
Retail Cuts Yield, %											
Breast		27.37 ^a	22.14 ^b	22.84 ^b	21.95 ^b	22.54 ^b		0.43	<0.001	<0.001	0.92
Deboned breast		19.53 ^a	14.19 ^b	14.63 ^b	13.76 ^b	14.45 ^b		0.37	<0.001	<0.001	0.87
Legs		20.12 ^b	21.83 ^a	21.72 ^a	21.76 ^a	21.76 ^a		0.20	<0.001	<0.001	0.91
Wings		8.99 ^c	9.86 ^a	9.33 ^{bc}	9.61 ^{ab}	9.84 ^{ab}		0.13	<0.001	0.12	0.01
Back		18.36 ^b	19.68 ^a	20.50 ^a	19.74 ^a	19.94 ^a		0.27	<0.001	<0.001	0.09

¹Values are the least square means of 13 replicates per treatment with 10 birds per replicate.²Dietary treatments: (1) standard CP diet with Arg, Ile and Val:Lys set at 105, 65, and 73%, and no restriction on Leu levels (PC), (2) LCP diet with the same AA:Lys as Diet 1 but with Leu level at 100% and reduced CP levels by around 4% (NC), (3) Diet 2 with Arg:Lys increased to 115% (NC+Arg), (4) Diet 3 with Ile and Val:Lys increased to 70 and 78% (NC+Arg+Val/Ile5), and (5) Diet 3 with Ile and Val:Lys increased to 75 and 83% (NC+Arg+Val/Ile10).^{a-c}Least square means within a row lacking a common superscript letter significantly differ ($P \leq 0.05$).

A related trial conducted by Kidd *et al.* (2021) showed a different response of BCAA in broilers from 22 to 35 days of age. The experiment reported an improvement in breast muscle yield with increased dietary valine and isoleucine, but only when the birds were fed the lowest dietary leucine. This differs from this experiment because of the contribution of the low CP levels in the diets used in that study. The increased abdominal fat yield may be related to the antagonism between Lys and Arg in the diet, in which higher levels of Arg could lead to Lys catabolism. The results on abdominal fat yield showed an opposite response to those of Miranda *et al.* (2014), in which fat weight was lowest in birds fed diets without CP restrictions, with expectations of similar results with L-Ile and L-Val supplementation. The decline in breast yield and the increase in legs and back on NC diets with higher levels of Arg, Val, and Ile may indicate a possible reprioritization of protein deposition in response to the available amino acids. Each carcass component likely exhibits distinct amino acid requirements, such that optimizing the yield of a specific cut may necessitate a targeted amino acid profile tailored to the metabolic and growth demands of that tissue. This observation may also indicate that the development of leg muscle in Cobb 500 broilers requires relatively higher levels of Arg and BCAAs to support optimal protein accretion and tissue growth.

Although additional research is warranted, the present findings suggest that elevating Trp levels in NC diets, as well as in diets supplemented with higher concentrations of Val and Ile, may enhance broiler performance to levels comparable with those fed PC diets, particularly during the early phases of growth. On the other hand, elevating the SID Arg:Lys ratio, increasing the SID Lys:ME ratio, and increasing Leu levels in diets containing low CP may help reduce the negative effects of decreased dietary CP.

In conclusion, increasing the Arg:Lys ratio to 115%, Ile from 65% to 75%, and Val from 73% to 83% was not sufficient to compensate for the decline in overall broiler production performance when fed reduced CP from day 0 to 35. However, excreta moisture and nitrogen decreased with lower CP levels despite the adjustments and the AA imbalances caused by the increased Arg, Val, and Ile ratios.

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