Research Note: The influence of different isoleucine: lysine ratios on the growth performance of broiler chickens fed low-protein diets

Carlos H. Oliveira,*,[1] Romário D. Bernardes,* Kelly M. M. Dias,* Artur M. Ribeiro,* Ramalho J. B. Rodrigueiro,† B. K. Koo,‡ Jisoo Tak,‡ Chorong Park,‡ Arele A. Calderano,* and Luiz F. T. Albino*

*Department of Animal Science, Universidade Federal de Viçosa, 36570-900, Viçosa, MG, Brazil; †CJ Corporation, Av. Engenheiro Luís Carlos Berrini, 04571-010, São Paulo, SP, Brazil; and ‡CJ Bio, 04560, Jung-gu, Seoul, Korea

ABSTRACT Two trials were carried out to assess the effects of different ratios of standardized ileal digestible isoleucine:lysine (SID Ile:Lys) on the growth performance of broiler chickens fed low-protein diets. A total of 1,320 male chickens were distributed in each trial into 6 treatments, with 10 replicates with 22 birds each. A control diet was formulated that satisfied the nutritional requirements of the broilers, and a low-protein diet was formulated with reduced protein content, meeting broiler nutritional requirements, except for the SID Ile levels. Five SID Ile:Lys ratios (56%, 61%, 66%, 71%, and 76%) were obtained by adding L-isoleucine to the low-protein diet. The body weight (**BW**), body weight gain (**BWG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were evaluated from day 1 to day 21 in trial 1, and from day 22 to day 44 in trial 2. ANOVA was performed on the data, and the treatments were compared to the control group using Dunnett's test ($P \leq 0.05$). Regression analyses were performed for modeling the variables assessed and the ratios of SID Ile: Lys. There was no significant difference between the treatments on ADFI of birds ($P > 0.05$). The BW, BWG, and FCR showed a quadratic effect as the SID Ile:Lys ratio increased in low-protein diets in trials 1 and 2 ($P \leq 0.05$). In conclusion, the recommended ratio of SID Ile:Lys in low-protein diets for growth performance is around 66% for broiler chickens from 1 to 21 d old and is around 65% for broiler chickens from 22 to 44 d old.

Key words: isoleucine, lysine, low-protein diets, performance, broiler

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INTRODUCTION

The development of unbound amino acids (**AA**) (crystalline or synthetic) has allowed a considerable reduction in the protein content of diets and had entailed many benefits related to feeding cost, gut health, nitrogen excretion and environmental pollution (Maia et al., 2021). However, it is crucial to understand the dietary amount of crude protein (**CP**) that could be decreased to avoid AA imbalance and antagonism, especially for branched-chain amino acids (**BCAA**).

BCAA are a group of three essential AA Ile, Leu, and Val which are powerful stimulators of protein synthesis since it has been reported to be a major activator of the target of rapamycin (**TOR**) complex and regulates mRNA binding to the ribosome and promotes translation initiation (Chen et al., 2016).

Due to the important role that Ile plays on protein synthesis, the ratio of Ile to Lys, the AA reference, should be clarified in low-protein diets. Therefore, the objective of this study was to assess the different ratios of standardized ileal digestible (**SID**) isoleucine:lysine (**Ile:Lys**) on the performance of broilers fed low-protein diets.

MATERIAL AND METHODS

Ethics Committee

All procedures adopted in this study were approved by the Ethics Committee in the Use of Farming Animals at the Federal University of Viçosa (CEUAP-UFV) under protocol 01/2021, in compliance with the National Council for Experimentation Animal Control.

Common Procedures and Experimental Design

The experiment was carried out at the Research & Extension Sector for Poultry Production and Nutrition

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[1]Corresponding author: carlos.oliveira2@ufv.br

Table 1. Control and low-protein diets formulations in trial 1 from 01 to 21 days old, and in trial 2 from 22 to 44 days old.

Ingredients (%)	Trial 1 (1−21 d)		Trial 2 (22−44 d)	
	Control	Low-protein diet	Control	Low-protein diet
Corn	56.03	65.83	64.00	72.48
Soybean meal	27.55	16.94	21.02	11.63
Corn gluten meal	3.00	3.00	3.00	3.00
Meat and bone meal	5.00	5.00	5.00	5.00
Plasma	2.50	2.50	2.50	2.50
Dicalcium phosphate	0.44	0.52	0.05	0.12
Limestone	0.50	0.54	0.25	0.28
Salt	0.05	0.05	0.02	0.02
Soybean oil	3.58	1.54	2.78	1.00
L-Glutamic acid, 99%	0.00	1.00	0.00	1.00
Choline chloride, 60%	0.10	0.10	0.10	0.10
Starch	0.00	0.40	0.00	0.40
L-Lysine HCl	0.24	0.43	0.27	0.39
L-Methionine	0.29	0.38	0.24	0.33
Potassium carbonate	0.00	0.25	0.00	0.28
Sodium bicarbonate	0.30	0.30	0.30	0.30
L-Glycine, 98,5%	0.00	0.17	0.00	0.08
L-Threonine, 98,5%	0.04	0.18	0.03	0.15
L-Isoleucine, 98,5%	0.02	0.00	0.04	0.00
L-Valine, 96,5%	0.00	0.18	0.00	0.16
L-Tryptophan, 98%	0.00	0.04	0.00	0.05
L-Arginine, 98,5%	0.02	0.32	0.05	0.38
Mineral premix[1]	0.15	0.15	0.15	0.15
Vitamin premix[2]	0.13	0.13	0.13	0.13
Salinomycin, 12%	0.06	0.06	0.06	0.06
BHT[3]	0.01	0.01	0.01	0.01
Composition				
Crude protein, %	23.22	20.44	20.95	18.50
Metabolizable energy, kcal/kg	3,150	3,150	3,200	3,200
Calcium, %	0.970	0.970	0.760	0.760
Phosphorus available, %	0.460	0.460	0.380	0.380
SID Isoleucine, %	0.841	0.644	0.760	0.560
SID Lysine, %	1.256	1.150	1.124	1.000
SID Methionine, %	0.609	0.656	0.538	0.580
SID Met. + Cys., %	0.929	0.929	0.832	0.832
SID Threonine, %	0.829	0.829	0.742	0.742
SID Tryptophan	0.236	0.236	0.204	0.204
SID Histidine, %	0.529	0.437	0.477	0.400
SID Leucine, %	1.943	1.702	1.813	1.597
SID Valine, %	0.967	0.967	0.865	0.865

[1]Trace mineral premix provided: Mn, 58.36 g; Fe, 41.68 g; Zn, 54.21 g; Cu, 8.31 g; I, 0.84 g; Se, 0.25 g per kg of diet.
[2]Vitamin premix provided: vitamin A, 9,638,000 IU; vitamin D3, 2,410,000 IU; vitamin E, 36,100 IU; vitamin B1, 2.60 g; vitamin B2, 6.45 g; vitamin B6, 3.61 g; vitamin B12, 15.9 mg; vitamin K3, 1.94 g; pantothenic acid, 12.95 g; nicotinic acid, 39.20 g; folic acid, 0.90 g; biotin, 89.80 mg per kg of diet. Mineral e vitamin premixes (COGRAN, Pará de Minas, Minas Gerais, Brazil).Salinomycin (Shandong Qilu King-Phar Pharmaceutical Co., Ltd., Jinan, China).
[3]Antioxidant butylhydroxytoluene (UAB Chemijos Industrija, Klaipėda, Lithuania).

of the Animal Science Department, Federal University of Viçosa, State of Minas Gerais, Brazil.

The experiment was divided into 2 trials according to the growing phase of the broilers: trial 1 (from 1 to 21 d old) and trial 2 (22−44 d old). In each trial, a total of 1,320 male Cobb500 broiler chickens (Cobb-Vantress, Inc., East Siloam Springs, AK) with initial body weights of 37.25 ± 0.014 g in trial 1, and 955.89 ± 8.32 g in trial 2, were reared in a completely randomized design into 6 treatments, with 10 replicates with 22 birds each. In trial 2, before day 22, broiler chicks were reared in houses under conditions established by the Cobb500 guidelines and fed with diets formulated according to Rostagno et al. (2017). During the experimental period, the temperature and light program was set according to the Cobb500 guidelines.

Experimental Diets

The formulation and composition of diets in trials 1 and 2 are presented in Table 1. Throughout the experiment, diets and water were provided ad libitum. In trial 1, a control diet was formulated to satisfy the nutritional requirements of broilers for the starter phase according to Rostagno et al. (2017), which met the CP content of 23.22% and the SID Ile:Lys ratio of 67% (0.841% SID Ile and 1.256% SID Lys). Additionally, a low-protein diet (20.44% CP) was formulated to meet the nutritional requirements of broiler chickens, according to Rostagno et al. (2017), except for Ile levels. To achieve the lowest SID Ile:Lys ratio of 56% in the low-protein diet, the SID Lys was reduced by 0.106%. Then, 5 different levels of L-isoleucine (0, 0.06, 0.12, 0.18, and 0.23%)

Table 2. Means of average daily feed intake (ADFI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) of broilers fed diets containing different SID Ile:Lys ratios and the control diet in trials 1 and 2.

SID Ile:Lys ratios (%)	ADFI (kg)	BW (kg)	BWG (kg)	FCR (kg/kg)
Trial 1 (1−21 d)				
Control (67%)	1.168	1.013	0.976	1.197
56	1.148	0.909*	0.872*	1.317*
61	1.132	0.942*	0.905*	1.252*
66	1.132	0.949*	0.911*	1.242
71	1.130	0.955*	0.918*	1.230
76	1.131	0.942*	0.905*	1.250*
SEM	0.006	0.005	0.005	0.007
Regression Analysis				
Linear	0.401	0.003	0.002	<0.001
Quadratic	0.584	0.003	0.003	0.001
Trial 2 (22−44 d)				
Control (68%)	4.258	3.657	2.701	1.577
56	4.042*	3.242*	2.286*	1.768*
61	4.078*	3.351*	2.395*	1.703*
66	4.094*	3.355*	2.399*	1.707*
71	4.082*	3.346*	2.391*	1.709*
76	4.098*	3.343*	2.387*	1.718*
SEM	0.015	0.019	0.019	0.009
Regression Analysis				
Linear	0.078	0.007	0.006	0.030
Quadratic	0.144	0.005	0.005	0.006

*Means followed by an asterisk on the same line differ from the control group by Dunnett's test ($P \leq 0.05$).
SEM = Standard Error of the Mean.

(CJ Corporation, Jung-gu, Seoul, South Korea) were added to the low-protein diet, in replacement of starch, to meet the experimental SID Ile:Lys ratios of 56% (0.644% SID Ile and 1.150% SID Lys), 61% (0.702% SID Ile and 1.150% SID Lys), 66% (0.759% SID Ile and 1.150% SID Lys), 71% (0.817% SID Ile and 1.150% SID Lys), and 76% (0.874% SID Ile and 1.150% SID Lys). The experimental diets in trial 2 were obtained in a similar way to trial 1, in which a control diet was formulated to meet nutritional requirements of broilers for the finisher phase according to Rostagno et al. (2017) with CP content of 20.95%, and SID Ile:Lys ratio of 68% (0.760% SID Ile and 1.124% SID Lys). The low-protein diet (18.50% CP) was formulated with Ile deficiency and SID Lys reduction of 0.124%, then 5 levels of L-isoleucine were added to low-protein diet in replacement to starch (0, 0.05, 0.10, 0.15, and 0.20%) to meet the experimental SID Ile:Lys ratios of 56% (0.560% SID Ile and 1.000% SID Lys), 61% (0.610% SID Ile and 1.000% SID Lys), 66% (0.660% SID Ile and 1.000% SID Lys), 71% (0.710% SID Ile and 1.000% SID Lys), and 76% (0.760% SID Ile and 1.000% SID Lys).

Assessment of Parameters

All birds were individually weighed on days 1 and 21 in trial 1, and on days 22 and 44 in trial 2, to determine the mean body weight (**BW**) and calculate the mean BW gain (**BWG**). The added and remaining feed were weighed to calculate the average daily feed intake (**ADFI**). The feed conversion ratio (**FCR**) was calculated using BWG and ADFI. Every day, mortality was recorded to correct the ADFI and FCR.

Statistical Analysis

The package ExpDes.pt from R Software (v. 4.0.4) was used to perform a one-way ANOVA on the data. Dunnett's test ($P \leq 0.05$) was used to compare each treatment to the control group. In addition, linear and quadratic regressions were used to model the relationship between the variables studied and the SID Ile:Lys ratios. When quadratic responses were detected, the optimal SID Ile:Lys ratio was calculated by taking the first derivative of the quadratic equation and then estimating the ratio using 95% of the maximum responses. The control treatment was not considered in the regression analysis.

RESULTS AND DISCUSSION

The growth performance results of broilers in trials 1 and 2 are presented in Table 2. The ADFI of the chickens did not differ with different ratios of SID Ile: Lys in low-protein diets in trial 1 ($P = 0.584$) or trial 2 ($P = 0.144$). In addition, there were no differences between the treatments with low-protein diets and the control diet on the ADFI of the birds in trial 1 ($P > 0.05$). However, in trial 2, birds fed low-protein diets, regardless of the SID Ile:Lys ratio, showed lower ADFI than birds in the control group ($P \leq 0.05$).

A positive quadratic effect was observed for BW and BWG in trial 1 ($P = 0.003$) and trial 2 ($P = 0.005$), with increasing ratios of SID Ile: Lys in low-protein diets. The SID Ile:Lys ratio requirement was estimated using 95% of the maximum value of the first derivate (quadratic regression). Thus, the estimated required ratio of SID Ile: Lys for BW was

65.77% in trial 1, and 65.35% in trial 2. In addition, the estimated SID Ile:Lys ratio requirement for BWG was 65.99% in trial 1, and 65.24% in trial 2. The quadratic equations were as follows: BW (1−21 d) = −0.2876 + 0.03602 * Ile:Lys − 0.0002611 * Ile:Lys^2; R^2 = 0.9739; BW (22−44 d) = 0.1281131 + 0.09405611 * Ile:Lys − 0.0006829 * Ile:Lys^2; R^2 = 0.8592; BWG (01 to 21 d) = − 0.3312 + 0.03619 * Ile:Lys − 0.0002623 * Ile:Lys^2; R^2 = 0.9756; BWG (22−44 d) = − 0.8202651 + 0.0938349 * Ile:Lys − 0.0006811 * Ile:Lys^2; R^2 = 0.8586.

Similarly, the FCR of broilers quadratically decreased in trial 1 ($P = 0.001$) and trial 2 ($P = 0.006$) when the birds were fed increasing ratios of SID Ile:Lys in diets reduced in CP. Therefore, the estimated required SID Ile:Lys ratio for FCR was 65.77% in trial 1, and 64.78% in trial 2. The quadratic equations were as follows: FCR (1 to 21 d) = 3.5105834 − 0.0658646 * Ile:Lys + 0.0004754 * Ile: Lys^2; R^2 = 0.9635; FCR (22−44 d) = 3.6316 − 0.0566214 * Ile: Lys + 0.0004146 * Ile:Lys^2; R^2 = 0.8314.

By comparing the ratios of SID Ile: Lys in low-protein diets to the control group, the birds fed diets with protein reduction, regardless of the SID Ile:Lys ratio, showed lower BW and BWG than the birds fed the control diet ($P \leq 0.05$) in both trials. The FCR of the broilers in trial 1 was similar to the FCR from the control group ($P > 0.05$), which were fed diets containing ratios of 66% and 71% in low-protein diets, whereas the other ratios resulted in broilers with worse FCR ($P \leq 0.05$). In trial 2, all treatments with low-protein diets resulted in birds with worse FCR than the control group ($P \leq 0.05$).

In the current study, the optimum SID Ile:Lys ratio for growth performance of birds was estimated in 66 and 65% in trial 1 and 2, respectively. The Ile, as well as Leu and Val plays an important role on growth of chickens through skeletal muscle hypertrophy (Zeitz et al., 2019). Protein synthesis and degradation are interlinked by AKT/TOR and AKT/FOXO pathways in which the former mechanism stimulate protein synthesis through activation of its downstream targets, and the latter mechanism inhibits the ubiquitin−proteasome system, the major mechanism for protein degradation in vivo (Bodine et al., 2001; Glass, 2010). In a study with hybrid bagrid catfish, Jiang et al. (2021) have reported that Ile acts in both mechanisms AKT/TOR and AKT/FOXO, which improved muscle growth of fish either by improving protein synthesis or inhibiting protein degradation.

It is well-documented that the same enzymes catabolize BCAA, and the common catabolism pathways of these amino acids may result in detrimental effects on broiler performance if they are not supplied at the correct levels. Therefore, the interactions between BCAA are an important consideration in low-protein diets. Brosnan and Brosnan (2006) have reported that Leu, Val, and Ile are transferred to α-keto acids (α-ketoisocaproate, α-ketoisovalerate, and α-keto-β-methylvalerate, respectively) by the enzyme BCAA aminotransferase. These α-keto acids are degraded by a multienzyme BCKD complex, which is inactivated by the enzyme BCKD kinase and activated by the enzyme BCKD phosphatase. When the AA of the BCAA group is excessively supplied, there is an increase in its respective α-keto acid, which stimulates the catabolism of the other AAs since α-keto acid inhibits BCKD kinase, resulting in activation of BCKD phosphatase, and consequently the BCKD complex activity. In the present study, the optimum ratio was estimated at 66% for 1- to 21-day-old broilers and 65% for broilers from 22 to 44 d old. These ratios are in agreement with the recommendations in the existing literature, which vary from 61% to 70% SID Ile: Lys in the starter phase (1−21 d old) and from 63% to 73% SID Ile: Lys in the finisher phase (22−42 d old) (Wise et al., 2021; Brown et al., 2022). The range of the BCAA recommendations is due to the difficulty in fixing their ratios to lysine while at the same time maintaining their rations between themselves constant. Therefore, our findings indicate that the ratios 66 and 65%, in trial 1 and 2, respectively, respect the proportion of Ile:Leu:Val, even with the CP reduction.

When birds fed low-protein diets were compared to those from the control group, birds fed diets reduced in CP showed worse performance, regardless of the SID Ile:Lys ratio. The low-protein diets were formulated with a considerable reduction in Lys compared to the control diet. Lysine is an anabolic AA and are key on muscle deposition. Therefore, isoleucine supplementation could not compensate for the reduction in the lysine level of 0.106 and 0.124%, in trial 1 and 2, respectively.

In conclusion, data from these two experiments indicated that the recommended ratio of SID Ile: Lys for growth performance is around 66% for broiler chickens 1 to 21 d old and is around 65% for broiler chickens 22 to 44 d old. Although our findings agree with previous research, more studies should be conducted to determine the optimum ratio of Ile to Lys for broiler chickens fed low-protein diets.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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