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Performance and immune response of broilers born to breeders of different ages and fed different valine levels

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ABSTRACT - Two experiments were carried out to evaluate the effects of digestible valine supplementation in pre-starter and starter diets on the productivity, nutrient metabolizability coefficient (NMC), and immune response of broilers from breeders of different ages. Experiments I and II were conducted with broilers in the pre-starter (1 to 7 days of age) and starter (8 to 21 days of age) phases, respectively. Broilers were fed diets that differed in their digestible valine content. In each trial, 400 male Cobb 500® chicks were randomly housed in a 2 × 4 factorial arrangement with eight treatments and five replicates of 10 birds each. The main effects were breeder age (37 vs. 52 weeks) and the digestible valine level in pre-starter (9.2, 10.2, 11.2, and 12.2 g/kg) and starter (8.3, 9.3, 10.3, and 11.3 g/kg) diets. Productive performance, intestinal histology, and immune response of broilers were evaluated. Supplementation with 11.2 g/kg valine in pre-starter diets improved NMC and increased villus height and villus:crypt ratio in the duodenum, jejunum development, and lymphocyte proliferation in the spleen of broilers at seven days of age, without improving performance (body weight gain, feed intake, and feed conversion ratio). In the starter phase, valine supplementation reduced feed intake but did not affect NMC, intestinal development, or immune response. The present results suggest that the same level of digestible valine should be used in the diet of broilers born to breeders of different ages, and the use of 9.2 and 8.3 g/kg digestible valine in pre-starter and starter diets, respectively, is sufficient to ensure satisfactory broiler performance. However, to improve the duodenum and jejunum development and immune response of broilers in the pre-starter phase, higher digestible valine levels are required.

Keywords: amino acid, immune system, metabolism, poultry

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1. Introduction

It has been reported that broiler breeder age affects the metabolism (Araújo et al., 2016), weight (Iqbal et al., 2016), and quality of day-old broiler chicks as well as their performance in the first week of life (Ipek and Sozcu, 2015). Mohammed and Ali (2019) described that the body weight (BW) at hatching and growth rate of chicks from old breeders were significantly higher than those of chicks born to young breeders. In addition, Beitia et al. (2019) found more over-expressed genes related to fatty acid and carbohydrate metabolism in the yolk sac of embryos from young than in those from older hens.

Because broiler breeder age can affect the performance of their progeny, there is a possible difference in valine metabolism in broilers hatched from eggs of breeders of different ages, which results in different requirements of the amino acid.

Efficient production of broilers entails accurate nutrition. In this respect, dietary amino acids are a major factor (Kidd and Tillman, 2016), and knowledge of the requirements of most amino acids is important. Valine is the fourth limiting amino acid in broiler diets (Corzo et al., 2009). Valine limitation increases particularly with broiler age and in low-crude protein diets (Nascimento et al., 2016). Adjustments in dietary valine are important for adequate broiler development (Tavernari et al., 2013; Ferreira et al., 2016), replacement of lost feathers (De Lima et al., 2016), and improvement of intestinal morphometric characteristics (Allameh and Toghyani, 2019).

Besides incorporating into protein structures, other metabolic functions have been related to branched-chain amino acids (BCAA), such as modulation of hepatic expression of lipogenic genes (Bai et al., 2015; Duan et al., 2016; Duan et al., 2018). Kaplan and Yildiz (2017) reported that valine supplementation increased thymus weight in 21-day-old broilers, suggesting that immune response could be affected by valine. Schedle et al. (2019) reported that valine is a limiting amino acid for performance and must be controlled when the dietary crude protein (CP) is reduced. Information about the requirement of valine varies considerably in its ideal profile because many factors can affect its requirement by poultry, including breeder age.

The present study proposes to examine the effects of digestible valine supplementation in the pre-starter and starter diets on productivity, nutrient metabolizability coefficient, and immune response of broilers from breeders of different ages.

2. Material and Methods

2.1. Experimental design, diets, and management

Two experiments were carried out to evaluate performance, nutrient metabolizability coefficient, and immune response of broilers born to breeders of different ages and fed diets with different digestible valine levels in the pre-starter (1 to 7 days of age) and starter (8 to 21 days of age) phases.

The experiment was conducted in Goiânia, Goiás, Brazil (16°35'33.0" S and 49°16'51.4" W). Research on animals was conducted according to the Ethics Committee on Animal Research (case no. 080/12).

In both experiments (pre-starter and starter phases), 400 one-day-old male Cobb 500® chicks were randomly housed in a 2 × 4 factorial arrangement (breeder age × digestible valine level), in which eight treatments were provided in five replicates, with 10 birds each. The main effects studied were breeder age (37 vs. 52 weeks old) and digestible valine levels in the pre-starter (9.2, 10.2, 11.2, and 12.2 g/kg, equivalent to digestible valine:digestible lysine ratios of 69, 77, 84, and 92) and starter diet (8.3, 9.3, 10.3, and 11.3 g/kg, equivalent to digestible valine:digestible lysine ratios of 68, 76, 84, and 92). The initial body weight (IBW) of chicks from breeders at 37 weeks age was 40.30 g and that of chicks from breeders at 52 weeks was 41.42 g. Diets were iso-nutrient and isoenergetic and based on corn and soybean meal, as recommended by Rostagno et al. (2011), except for the digestible valine levels. Crude protein levels of the diet were reduced by 5 g/kg in the pre-starter diet (Experiment I) and 7 g/kg in the starter (Experiment II) diet. To achieve the proposed nutritional composition of digestible valine levels, experimental diets were obtained by supplementing this amino acid in a basal diet, replacing starch (Table 1). In Experiment II, birds were raised separately and fed a conventional diet from 1 to 8 days of age, prior to experimental period.

From 1 to 7 (Experiment I) and 8 to 21 days of age (Experiment II), chicks were raised in five broiler battery cages equipped with linear feeders and drinkers and metal trays for excreta collection. Each battery contained five floors with 0.33 × 0.50 m divisions and 40 experimental units. To heat the birds, 40-watt incandescent lamps were used for each floor until birds completed seven and 14 days of age,

in experiments I and II, respectively. Broilers were managed according to the management guide of the line. Ambient temperature and relative humidity were recorded daily, and adequate curtain management was adopted. In both experiments, chicks were vaccinated against Marek's disease in the hatchery and received feed and water *ad libitum*.

Table 1 - Composition of basal experimental diets used in the pre-starter and starter phases of broilers fed different L-valine levels

Item	Pre-starter	Starter
Ingredient (g/kg)		
Corn	535.9	582.62
Soybean meal	377.2	327.25
Vegetable oil	27.6	32.86
Dicalcium phosphate	19.1	15.89
Limestone	16	17.44
Salt	5	5
Vitamin-mineral supplement[1]	1.5	1.5
Starch	4.2	4.1
L-lysine HCL	3.2	3.40
DL-methionine	3.7	3.44
L-threonine	1.2	1.22
L-arginine	0.5	0.85
L-isoleucine	0.3	0.48
L-valine	0	0
Avilamycin	0.5	0.5
Anticoccidial	0.5	0.5
Total	1000	1000
Nutritional composition (g/kg)		
Crude protein	219	205
Metabolizable energy (kcal/kg)	2,960	3,050
Calcium	12	11.6
Available phosphorus	4.7	4.01
Sodium	2.2	2.1
Digestible lysine	13.24	12.17
Digestible arginine	14.3	13.15
Digestible methionine + cystine	9.53	8.76
Digestible tryptophan	2.45	2.18
Digestible threonine	8.61	7.91
Digestible valine	9.2	8.3

[1] Vitamin-mineral supplement (per kg): vitamin A, 3,125,000 IU; vitamin D_3, 550,000 IU; vitamin E, 3,750 mg; vitamin K_3, 625 mg; vitamin B_1, 250 mg; vitamin B_2, 1,125 mg; vitamin B6, 250 mg; vitamin B_{12}, 3,750 mg; niacin 9,500 mg; calcium pantothenate, 3,750 mg; folic acid, 125 mg; DL-methionine, 350,000 mg; choline 50%, 150,000 mg; growth promoter, 12,500 mg; coccidiostat, 15,000 mg; Se, 50 mg; antioxidant, 2,500 mg; excipient, 1,000 g; Fe, 100,000 mg; Cu, 16,000 mg; Zn, 100,000 mg; I, 1,500 mg.

2.2. Broiler performance

Broiler performance was measured at seven days of age in Experiment I and at 21 days in Experiment II. Body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) were calculated, and data were corrected for mortality.

Feed intake (g/bird) was calculated by the difference between the amount of feed supplied in first day of the experiment and leftovers on the last day of each experiment. The BWG (g/bird) was estimated by weighing each bird on the first and the last day of each experiment. The FCR was determined as a direct relationship between FI and BWG in each experiment.

2.3. Dry matter and nitrogen metabolizability coefficient

Total excreta were collected from chicks from 4 to 7 (Experiment I) and 17 to 21 (Experiment II) days of age to calculate the metabolizability coefficients of dry matter (DMMC) and nitrogen (NMC). Excreta were collected twice a day. After collection, excreta were packed in labeled plastic bags and frozen. At the end of the experiment, they were thawed and homogenized according to the method proposed by Sakomura and Rostagno (2016). The DM and N contents of diets and excreta were analyzed following the procedures described by Silva and Queiroz (2004).

The metabolizability coefficients were calculated as follows:

$$MC\ (\%) = (\text{Nutrient intake (g)} - \text{Nutrient output (g)}) \div \text{Nutrient intake} \times 100 \quad (1)$$

2.4. Intestinal histomorphometry and lymphocyte count

To examine the development of the small intestine and lymphoid organs and quantify the lymphocytes in lymphoid organs, five birds were euthanized per treatment, by cervical dislocation, at 7 and 21 days of age. Broilers were subjected to a pre-slaughter fast of 6 h (Fukayama et al., 2005). The small intestine, bursa, spleen, and thymus were collected and weighed immediately after collection. Organ relative weight was calculated relative to live body weight. The collection of the small intestine was from the site where the duodenum emerges from the gizzard and the beginning of the ceca. The small intestine was cut in 3-cm segments to obtain samples of the duodenum, jejunum, and ileum. The mesenteric edge was opened and extended by the serous membrane, and intestine portions and lymphoid organs were washed in saline solution and fixed in 10% buffered formaldehyde for 24 h. Then, tissues were washed in running distilled water and dehydrated in increasing concentrations of alcohol (70-95%). Two exchanges of absolute alcohol (75%) were performed, with an interval of 1 h each, and fragments of the small intestine and lymphoid organs were placed in a paraffin bath at 58 °C, in blocks, and cut to a thickness of 5 µm. The sections were then fixed on histological slides and stained by the hematoxylin-eosin technique (Luna, 1968).

Optical microscopy examination for histological evaluation was performed using a bright-field optical microscope (Carl Zeiss®, Model: Jenaval model) linked to Axio Vision 3.0 software (Zeiss® imaging system). The captured images were later investigated using Image J software. Forty measurements of villus height (10X magnification) and crypt depth (10X magnification) of each intestinal region were performed per histological slide. Readings were taken in the basal region of the villus up to its apex, and in the crypt from its base to the crypt:villi transition region (Fukayama et al., 2005). Villus:crypt ratio (V:C) was calculated by dividing the height of villi by the depth of the crypts.

The count of lymphocytes in the lymphoid organs (bursa, spleen, and thymus) was also performed using a bright-field optical microscope (Carl Zeiss®, Model: Jenaval) linked to the Axio Vision 3.0 (Zeiss®) imaging system. The captured images were later investigated using Image J software. Ten readings were taken per slide (400X magnification), and the lymphocyte number was determined by counting lymphocytes located at the intersection points of the grids in Image J software (Barnabé, 2012).

2.5. Statistical analysis

Data were subjected to ANOVA, adopting $P<0.05$ for significant interactions between the main effects and differences between groups. Means were compared by Tukey's test. All data were determined using Statistical Analysis System software (SAS, 2005). The proposed mathematical model was as follows:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \varepsilon_{ijk}, \quad (2)$$

in which Y_{ijk} = observed value at breeder age i (i = 1, 2) and valine level j (j = 1, 2, 3, 4), in replicate k (k = 1, 2, 3, ..., 5); µ = overall mean of the experiment; a_i = fixed effect of breeder age i (i = 1, 2); b_j = fixed

effect of valine level j (j = 1, 2, 3, 4); $(ab)_{ij}$ = fixed effect of the interaction between breeder age i (i = 1, 2) and valine level j (j = 1, 2, 3, 4); and ε_{ijk} = random error at breeder age i (i = 1, 2), valine level j (j = 1, 2, 3, 4), in replicate k (k = 1, 2, 3, ..., 5).

3. Results

There was no interaction effect between breeder age and digestible valine level in the diet for broiler performance in the pre-starter (Experiment I) or starter (Experiment II) phase. Digestible valine levels in the diet and breeder ages resulted in similar FI, BWG, and FCR in the seven-day-old broilers (Table 2). The FI of broilers at 21 days of age decreased when they were fed diets with 10.3 and 11.3 g/kg digestible valine (P<0.05) (Table 2). However, this reduced FI did not translate into decreased FCR or BWG in birds at 21 days of age.

There was no significant interaction between the main factors for DMMC or NMC of broilers in the pre-starter and starter phases (Table 3). At seven days of age, chicks fed 11.2 g/kg digestible valine showed higher NMC (P<0.05) (Table 3). These results demonstrate that the higher amounts of digestible valine improved nitrogen utilization efficiency by broilers. In the starter phase, broilers exhibited similar nutrient metabolism regardless of dietary valine levels (Table 3).

There was a significant interaction effect between broiler breeder age and digestible valine level on villus height and V:C ratio in the jejunum of broilers at pre-starter phase (P<0.05) (Tables 4 and 5). Digestible valine levels influenced villus height and the V:C ratio in the jejunum only in broilers from breeders aged 52 weeks (P<0.05). Broilers fed 12.2 g/kg digestible valine showed greater villus heights and V:C ratios (Table 5). In the pre-starter phase, villus height and V:C ratio in the duodenum were higher in broilers from breeders aged 52 weeks (Table 4). Broilers fed 9.2 g/kg valine exhibited lower villus heights and V:C ratios and higher crypt depths in the duodenum. Breeder age and dietary valine level did not affect the duodenum, jejunum, and ileum morphology of broilers at 21 days of age.

Table 2 - Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter (Experiment I) and starter (Experiment II) phases

Main effect	Experiment I - Pre-starter phase			Main effect	Experiment II - Starter phase		
	FI (g/bird)	BWG (g/bird)	FCR		FI (g/bird)	BWG (g/bird)	FCR
Broiler breeder age (BA, weeks)				Broiler breeder age (weeks)			
37	138.67	124.11	1.118	37	926.80	673.96	1.377
52	138.60	123.48	1.124	52	928.20	681.66	1.364
Digestible valine (VAL, g/kg as fed)				Digestible valine (g/kg as fed)			
9.2	137.97	121.17	1.141	8.3	937.58a	684.46	1.371
10.2	139.37	123.55	1.128	9.3	938.28a	697.76	1.347
11.2	137.69	123.90	1.112	10.3	905.94b	656.04	1.383
12.2	139.44	126.42	1.103	11.3	928.48b	672.98	1.381
				ANOVA			
BA	0.969	0.781	0.672	BA	0.861	0.495	0.527
VAL	0.807	0.319	0.177	Val	0.024	0.072	0.498
VAL × BA	0.351	0.976	0.624	Val × BA	0.638	0.951	0.783
CV (%)	3.62	5.02	3.60	CV (%)	2.707	5.142	4.261

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

There was no interaction effect between the factors on weight of lymphoid organs or intestine of broilers at seven and 21 days of age (Table 6). Valine supplementation did not result in differences in these variables at either evaluated ages. However, intestine weight was higher in broilers from older breeders, in the pre-starter and starter phases (P<0.05).

Table 3 - Metabolizability coefficients of dry matter (DMMC) and nitrogen (NMC) in broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter (Experiment I) and starter (Experiment II) phases

Main effect	Experiment I		Main effect	Experiment II	
	DMMC	NMC		DMMC	NMC
Broiler breeder age (BA, weeks)			Broiler breeder age (weeks)		
37	83.36	77.47a	37	77.77	69.94
52	84.31	76.11b	52	77.98	71.49
Digestible valine (VAL, g/kg as fed)			Digestible valine (g/kg as fed)		
9.2	83.59	74.78b	8.3	78.80	73.51
10.2	85.24	75.95b	9.3	77.94	71.21
11.2	84.11	79.93a	10.3	78.19	69.05
12.2	82.42	76.50b	11.3	76.60	69.10
			ANOVA		
BA	0.225	<0.020	BA	0.883	0.303
VAL	0.092	<0.001	VAL	0.732	0.128
VAL × BA	0.204	0.107	VAL × BA	0.387	0.315
CV (%)	2.89	2.29	CV (%)	5.673	6.636

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

Table 4 - Villus height (µm), crypt depth (µm), and villus height:crypt depth ratio (V:C) in the duodenum, jejunum, and ileum of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter phase

Main effect	Duodenum			Jejunum			Ileum		
	Villus height	Crypt depth	V:C	Villus height	Crypt depth	V:C	Villus height	Crypt depth	V:C
Broiler breeder age (BA, weeks)									
37	1151.16b	264.53	4.53b	763.08	209.60	3.74	604.86	216.13	2.85
52	1273.77a	250.15	5.23a	734.27	193.00	3.81	600.35	207.10	2.94
Digestible valine (VAL, g/kg as fed)									
9.2	1046.95b	303.57a	3.53b	793.75	217.84	3.722	596.76	217.43	2.84
10.2	1215.23ab	258.2ab	4.81a	710.68	202.03	3.586	581.69	214.51	2.77
11.2	1310.92a	240.98b	5.48a	711.48	193.29	3.682	625.62	204.95	3.06
12.2	1276.75a	226.70b	5.70a	778.78	192.06	4.135	606.36	209.59	2.92
				ANOVA					
BA	<0.024	0.243	<0.009	0.271	0.111	0.687	0.862	0.509	0.502
VAL	<0.005	<0.006	<0.001	<0.050	0.269	0.142	0.679	0.919	0.438
VAL × BA	0.318	0.625	0.372	<0.007	0.901	<0.020	0.591	0.726	0.198
CV (%)	13.52	14.87	16.22	10.87	15.93	14.57	13.55	20.22	14.07

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

There was a significant interaction effect between broiler breeder age and digestible valine level on lymphocyte count in the lymphoid organs of broilers at seven days of age (P<0.05; Tables 7 and 8). Valine supplementation of 9.2 and 10.2 g/kg reduced the number of lymphocytes in the spleen of broilers from younger breeders in the pre-starter phase. Valine supplementation of 9.2, 10.2, and 11.2 g/kg reduced the number of lymphocytes in the spleen of broilers from older breeders in the pre-starter phase. It was verified that the number of lymphocytes in the bursa increased with 10.2 g/kg of valine in broilers from younger breeders. However, the number of lymphocytes in the bursa of broiler from older breeders was not affected by valine level. The number of lymphocytes in the thymus of broilers from younger breeders was the same according to valine levels, while supplementation of 11.2 g/kg decreased the number of lymphocytes in the thymus of broilers from older breeders in the pre-starter phase.

There was no interaction effect between the factors on lymphocyte count in the lymphoid organs of broilers at 21 days of age (P>0.05). Valine supplementation did not change the number of lymphocytes in the lymphoid organs in the starter phase. Broilers from older breeder shower a higher lymphocyte count in the bursa at 21 days of age (Table 7).

Table 5 - Decomposition of the interaction between the main factors on the jejunum of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter phase

Broiler breeder age (weeks)	Digestible valine (g/kg)			
	9.2	10.2	11.2	12.2
		Villus height		
37	783.84A	677.60A	806.09A	784.82A
52	803.67Aa	743.74Aab	616.91Bb	772.74Aa
		V:C ratio		
37	3.60A	3.29A	4.13A	3.94A
52	3.83Aab	3.87Aab	3.23Bb	4.32Aa

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

Table 6 - Relative weight of lymphoid organs and intestine of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter (Experiment I) and starter (Experiment II) phases

Main effect	Experiment I - 7 days old				Main effect	Experiment II - 21 days old			
	Spleen (%)	Bursa (%)	Thymus (%)	Intestine (%)		Spleen (%)	Bursa (%)	Thymus (%)	Intestine (%)
Broiler breeder age (BA, weeks)					Broiler breeder age (weeks)				
37	0.08	0.19	0.58	14.21b	37	0.09	0.21	0.64	43.8b
52	0.07	0.17	0.59	15.12a	52	0.1	0.23	0.64	48.4a
Digestible valine (VAL, g/kg as fed)					Digestible valine (g/kg as fed)				
9.2	0.08	0.18	0.57	14.71	8.3	0.1	0.23	0.68	46.4
10.2	0.08	0.19	0.58	14.75	9.3	0.1	0.22	0.61	48.9
11.2	0.08	0.17	0.62	14.49	10.3	0.09	0.19	0.61	45.5
12.2	0.07	0.19	0.57	14.73	11.3	0.1	0.24	0.67	43.6
				ANOVA					
BA	0.705	0.243	0.926	<0.046	BA	0.671	0.483	0.909	<0.025
VAL	0.672	0.682	0.645	0.972	VAL	0.510	0.611	0.401	0.298
VAL × BA	0.261	0.334	0.531	0.641	VAL × BA	0.942	0.400	0.907	0.201
CV (%)	37.791	24.074	17.003	9.430	CV (%)	22.388	39.038	19.143	13.443

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

Table 7 - Lymphocyte count (units) in lymphoid organs of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter (Experiment I) and starter (Experiment II) phases

Main effect	Experiment I			Main effect	Experiment II		
	Spleen	Bursa	Thymus		Spleen	Bursa	Thymus
Broiler breeder age (BA, weeks)				Broiler breeder age (weeks)			
37	17.85	16.74	17.50b	37	69.87	86.24b	114.51
52	22.13	17.34	30.25a	52	75.14	112.67a	104.12
Digestible valine (VAL, g/kg as fed)				Digestible valine (g/kg as fed)			
9.2	14.00	16.22	20.14	8.3	77.04	114.3	109.61
10.2	14.34	20.25	23.78	9.3	59.84	83.95	99.97
11.2	21.76	16.98	29.6	10.3	77.59	105.31	102.75
12.2	30.45	14.7	21.99	11.3	75.55	94.27	124.95
				ANOVA			
BA	0.115	0.668	<0.001	BA	0.421	<0.001	0.204
VAL	<0.001	<0.05	0.121	VAL	0.181	0.245	0.142
VAL × BA	<0.024	<0.037	<0.050	VAL × BA	0.578	0.897	0.347
CV (%)	44.31	25.8	37.51	CV (%)	28.18	20.19	23.19

CV - coefficient of variation.
Means followed by different letters differ by Tukey's test (P<0.05).

Table 8 - Decomposition of the interaction between the main factors on lymphocyte count (units) in lymphoid organs of broilers born to breeders of different ages and fed different L-valine levels, in the pre-starter phase

Broiler breeder age (weeks)	Digestible valine (g/kg)			
	9.2	10.2	11.2	12.2
			Spleen	
37	9.64Ab	8.15Bb	27.39Aa	26.24Aa
52	18.36Ab	20.54Aab	16.13Ab	34.67Aa
			Bursa	
37	13.60Ab	21.16Aa	14.90Aab	17.30Aab
52	18.85Aa	19.35Aa	19.06Aa	12.11Aa
			Thymus	
37	12.84Ba	20.92Aa	16.88Ba	19.36Aa
52	27.44Aab	26.64Ab	42.32Aa	24.62Ab

Means followed by the same lowercase letters in the rows and uppercase letters in the columns do not differ significantly by Tukey's test (P<0.05).

4. Discussion

In the present study, dietary valine levels did not affect FI, BWG, or FCR in the pre-starter phase. Lysine, methionine, and threonine levels were kept equal in all experimental diets. Thus, we may infer that, in acting as a limiting amino acid, valine does not change broiler performance, since limiting amino acids are supplied in an adequate amount to meet their requirements. The increased dietary levels of digestible valine induced a reduction in FI of broilers in the starter phase. Diets containing 10.3 and 11.3 g/kg of valine, corresponding to a valine:lysine ratio of 0.84:1 and 0.92:1, respectively, in the nutritional composition, led to a reduction in FI. Abou-Elkhair et al. (2020) found that amino acid supplementation resulted in low daily FI and concluded that this finding suggested that any excess amino acids may be deaminated, producing more energy. As a consequence, FI would decrease as an animal defense mechanism to limit the absorption and catabolism of excess amino acids. Another possible explanation is that the reduction in FI is likely due the imbalanced supply of BCAA. According to

Gloaguen et al. (2012), the plasma concentration of valine or its concentration relative to the other BCAA during the postprandial period may act as a signal indicating amino acid deficiency. Leucine, another BCAA, is known to regulate FI in chicks. Izumi et al. (2004) reported that L-leucine increased FI of chicks. Therefore, it is possible that, in the present experiment, the higher digestible valine levels resulted in a lower valine:leucine ratio, which in turn induced a reduction in FI.

There was a higher NMC in the animals fed the pre-starter phase diet containing 11.2 g/kg valine, which corresponded to a digestible valine:lysine ratio of 0.84:1. Diets containing more than this amount of valine probably have an excess of the amino acid, which reduces digestibility and absorption in the intestinal lumen. Competition between the BCAA for intestinal uptake may be a contributing factor to their antagonism (Selle et al., 2020). Furthermore, the excess of amino acids is catabolized, and nitrogen is excreted. In the starter phase, nitrogen metabolism was similar in broilers born to breeders of different ages and fed different valine levels.

In the pre-starter phase, villus height and V:C ratio in the duodenum were higher in broilers from breeders aged 52 weeks than in those born to 37-week-old breeders. These results agree with those published by El Sabry et al. (2013), who concluded that chicks from younger breeders had shorter villi than the other groups when hatched at an early stage of incubation. However, the improvement in duodenum development was not sufficient to ensure greater performance in the broilers from older breeders. Yalçin et al. (2013) suggested that not only breeder age differences but also egg weight differences within a breeder age may play an important role in the development of villus surface area in day-old chicks. These authors explained that the larger jejunum villus area of heavy compared with light chicks from old breeders may indicate differences in organ growth rates of chicks when egg weight differences exist in a flock.

In the present experiment, at seven days of age, broilers fed 9.2 g/kg valine in pre-starter phase showed shorter villus height, lower V:C ratios, and deeper crypts in the duodenum, indicating insufficiency of this amino acid to improve duodenum development. According to Zhang et al. (2017), BCAA are necessary to improve intestinal development. These amino acids are catabolized in intestinal cells and are important for intestinal nutrient absorption. Therefore, any changes in intestinal morphometric structure may affect nutrient metabolizability and performance (Adabi et al., 2019), and duodenum weight may be important in facilitating BWG in young broilers (Wijtten et al., 2010). The digestible valine levels increased villus height and V:C ratio in the jejunum only in the broilers from 52-week-old breeders, indicating that breeder age affects intestinal development in broilers in response to the diet. According to Adabi et al. (2019), the observed improvement in the jejunum with the increasing valine levels may be attributed to the utilization of dietary valine as a precursor of nonessential amino acids in the mucosa. Branched-chain amino acids could enhance intestinal development by increasing the local glucose uptake for animals and humans, since they regulate the expression and translocation of intestinal glucose transporters through insulin-dependent or insulin-independent pathways (Zhang et al., 2017).

In the current study, the relative weights of the spleen, bursa, thymus, and intestine of the broilers were similar between the dietary valine supplementation levels. Branched-chain amino acids have been reported to have the greatest potential to modulate immune responses of all amino acids in chickens, and their deficiency to markedly reduce the weights of thymus, spleen, and bursa of Fabricius of chickens (Konashi et al., 2000). However, these authors observed that the weights of lymphoid organs were modified by either the type of essential amino acid or the degree of deficiency, and that the weights of thymus and bursa were more susceptible to dietary amino acid deficiencies than the weight of spleen. We concluded that 9.2 and 8.3 g/kg digestible valine in the pre-starter and starter phases, respectively, were sufficient to prevent a decrease in the weights of lymphoid organs.

Broilers born to older breeders showed a higher lymphocyte count in the bursa at 21 days of age. Leandro et al. (2017) reported that chicks from older breeders exhibited greater immune response than chicks from young breeders, whereas birds from older breeders showed a larger follicular area in the bursa. At seven days of age, valine supplementation above 11.2 and 10.2 g/kg increased the lymphocyte count in the spleen and bursa, respectively, whereas the lymphocyte count in the thymus was not influenced by valine supplementation above 10.2 g/kg in the thymus of older breeders

(Table 8). On the other hand, broilers from older breeders showed a higher lymphocyte number in the spleen and thymus when 12.2 and 11.2 g/kg valine were used, respectively, whereas lymphocyte count in the bursa remained unchanged.

Dietary BCAA deficiency impairs the innate immune function, due to the shortage of lymphocytes and white blood cells, and increases susceptibility to pathogens. Branched-chain amino acids are related to better humoral and cellular immune response in broiler chickens (Abou-Elkhair et al., 2020). Lymphocytes integrate the adaptive acquired immune system, and their immune response is highly specific and affected by BCAA (Li et al., 2007). According to Li et al. (2007), the transport and utilization of BCAA by lymphocytes are dramatically increased in response to mitogens, and a lack of BCAA results in absence of lymphocyte proliferation.

Therefore, valine supplementation in the pre-starter diet improves NMC and jejunum development as well as increases villus height and V:C ratio in the duodenum and lymphocyte proliferation in the spleen of broilers at seven days of age without improving their performance. In the starter phase, valine supplementation reduces feed intake but does not affect NMC, intestinal development, or immune response in broilers hatched from eggs of breeders of different ages. In general, broiler breeder age does not affect valine metabolism in chicks, since the studied factors only interacted for jejunum development and lymphocyte count in the birds at seven days of age, suggesting that the factors act independently. Results suggest that the same digestible valine level should be used in the diet of broilers born to breeders of different ages, and the use of 9.2 and 8.3 g/kg digestible valine in the pre-starter and starter phases, respectively, is sufficient to ensure satisfactory broiler performance. However, to improve the duodenum and jejunum development and immune response of broilers in the pre-starter phase, higher digestible valine levels are required.

5. Conclusions

The use of 9.2 and 8.3 g/kg digestible valine in the pre-starter and starter phases, respectively, is sufficient to ensure satisfactory broiler performance born to breeders at different ages.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: H.H.C. Mello, M.A. Andrade, M.B. Café and J.H. Stringhini. Investigation: G.X. Silva, L.P.S. Gomides and F.B. Carvalho. Funding acquisition: M.B. Café and J.H. Stringhini; Methodology: M.A. Andrade and J.H. Stringhini. Supervision: M.B. Café and J.H. Stringhini. Writing-original draft: G.X. Silva. Writing-review & editing: H.H.C. Mello.

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References

Abou-Elkhair, R.; Ahmed, H.; Ketkat, S. and Selim, S. 2020. Supplementation of a low-protein diet with tryptophan, threonine, and valine and its impact on growth performance, blood biochemical constituents, immune parameters, and carcass traits in broiler chickens. Veterinary World 13:1234-1244. https://doi.org/10.14202/vetworld.2020.1234-1244

Adabi, S. G.; Ceylan, N.; Çiftci, İ. and Ceylan, A. 2019. Response of growing chicks to supplementation of low protein diets with leucine, valine and glycine-glutamic acid. South African Journal Animal Science 49:1047-1062. https://doi.org/10.4314/sajas.v49i6.9

Allameh, S. and Toghyani, M. 2019. Effect of dietary valine supplementation to low protein diets on performance, intestinal morphology and immune responses in broiler chickens. Livestock Science 229:137-144. https://doi.org/10.1016/j.livsci.2019.09.025

Araújo, I. C. S.; Leandro, N. M. S.; Mesquita, M. A.; Café, M. B.; Mello, H. H. C. and Gonzales, E. 2016. Effect of incubator type and broiler breeder age on hatchability and chick quality. Brazilian Journal of Poultry Science 18(SPE2):17-25. https://doi.org/10.1590/1806-9061-2015-0146

Bai, J.; Greene, E.; Li, W.; Kidd, M. T. and Dridi, S. 2015. Branched-chain amino acids modulate the expression of hepatic fatty acid metabolism-related genes in female broiler chickens. Molecular Nutrition Food Research 59:1171-1181. https://doi.org/10.1002/mnfr.201400918

Barnabé, A. C. S. 2012. Aditivos fitogênicos para frangos de corte experimentalmente inoculados com *Salmonella enterica* sorovar Enteritidis. Dissertação (M.Sc.). Universidade Federal de Goiás, Goiânia.

Beitia, A.; Caldas, J. V.; Boonsinchai, N.; Hilton, K. M.; Maharjan, P.; Weil, J. T.; Suesuttajit, N.; England, J. A. and Coon, C. N. 2019. Effect of broiler breeder age and dietary oil sources on metabolism of quality hatched chicks. p.315-316. In: EAAP Scientific Series, 138. Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-891-9_88

Corzo, A.; Loar, R. E. and Kidd, M. T. 2009. Limitations of dietary isoleucine and valine in broiler chick diets. Poultry Science 88:1934-1938. https://doi.org/10.3382/ps.2009-00109

De Lima, M. B.; Sakomura, N. K.; Dorigam, J. C. P.; Da Silva, E. P.; Ferreira, N. T. and Fernandes, J. B. K. 2016. Maintenance valine, isoleucine, and tryptophan requirements for poultry. Poultry Science 95:842-850. https://doi.org/10.3382/ps/pev380

Duan, Y.; Duan, Y.; Li, F.; Li, Y.; Guo, Q.; Ji, Y.; Tan, B.; Li, T. and Yin, Y. 2016. Effects of supplementation with branched-chain amino acids to low-protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids 48:2131-2144. https://doi.org/10.1007/s00726-016-2223-2

Duan, Y.; Li, F.; Guo, Q.; Wang, W.; Zhang, L.; Wen, C. and Yin, Y. 2018. Branched-chain amino acid ratios modulate lipid metabolism in adipose tissues of growing pigs. Journal of Functional Foods 40:614-624. https://doi.org/10.1016/j.jff.2017.12.004

El Sabry, M. I.; Yalçin, S. and Turgay-İzzetoğlu, G. 2013. Interaction between breeder age and hatching time affects intestine development and broiler performance. Livestock Science 157:612-617. https://doi.org/10.1016/j.livsci.2013.07.012

Ferreira, N. T.; Albuquerque, R.; Sakomura, N. K.; Dorigam, J. C. P.; Silva, E. P.; Burbarelli, M. F. C.; Ferreira, J. G. and Gous, R. M. 2016. The response of broilers during three periods of growth to dietary valine. Animal Feed Science and Technology 214:110-120. https://doi.org/10.1016/j.anifeedsci.2016.02.017

Fukayama, E. H.; Bertechini, A. G.; Geraldo, A.; Kato, R. K. and Murgas, L. D. S. 2005. Extrato de orégano como aditivo em rações para frangos de corte. Revista Brasileira de Zootecnia 34:2316-2326. https://doi.org/10.1590/S1516-35982005000700018

Gloaguen, M.; Le Floc'h, N.; Corrent, E.; Primot, Y. and van Milgen, J. 2012. Providing a diet deficient in valine but with excess leucine results in a rapid decrease in feed intake and modifies the postprandial plasma amino acid and α-keto acid concentrations in pigs. Journal of Animal Science 90:3135-3142. https://doi.org/10.2527/jas.2011-4956

Iqbal, J.; Khan, S. H.; Mukhtar, N.; Ahmed, T. and Pasha, R. A. 2016. Effects of egg size (weight) and age on hatching performance and chick quality of broiler breeder. Journal of Applied Animal Research 44:54-64. https://doi.org/10.1080/09712119.2014.987294

Ipek, A. and Sozcu, A. 2015. The effects of broiler breeder age on intestinal development during hatch window, chick quality and first week broiler performance. Journal of Applied Animal Research 43:402-408. https://doi.org/10.1080/09712119.2014.978783

Izumi, T.; Kawamura, K.; Ueda, H. and Bungo, T. 2004. Central administration of leucine, but not isoleucine and valine, stimulates feeding behavior in neonatal chicks. Neuroscience Letters 354:166-168. https://doi.org/10.1016/j.neulet.2003.09.071

Kaplan, M. and Yildiz, G. 2017. The effects of dietary supplementation levels of valine on performance and immune system of broiler chickens. Journal of Agricultural and Crop Research 5:25-31.

Kidd, M. T. and Tillman, P. B. 2016. Key principles concerning dietary amino acid responses in broilers. Animal Feed Science and Technology 221:314-322. https://doi.org/10.1016/j.anifeedsci.2016.05.012

Konashi, S.; Takahashi, K. and Akiba, Y. 2000. Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. British Journal of Nutrition 83:449-456.

Leandro, N. S. M.; Gomes, N. A.; Café, M. B.; Carvalho, F. B.; Stringhini, J. H. and Laboissière, M. 2017. Histomorfometria de orgãos linfoides e desenvolvimento intestinal de pintos de corte originados de matrizes com diferentes idades e submetidos ao estresse por calor na incubação. Ciência Animal Brasileira 18:e-34828. https://doi.org/10.1590/1089-6891v18e-34828

Li, P.; Yin, Y. L.; Li, D.; Kim, S. W. and Wu, G. 2007. Amino acids and immune function. British Journal of Nutrition 98:237-252. https://doi.org/10.1017/S000711450769936X

Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute Pathology. 3rd ed. McGraw-Hill, New York, NY, USA.

Mohammed, H. N. and Ali, K. A. 2019. Broiler performance response to breeder age, egg storage period, egg storage type and sumac powder. Plant Archives 19:1202-1214.

Nascimento, G. R.; Murakami, A. E.; Ospina-Rojas, I. C.; Diaz-Vargas, M.; Picoli K. P. and Garcia, R. G. 2016. Digestible valine requirements in low-protein diets for broilers chicks. Brazilian Journal of Poultry Science 18:381-386. https://doi.org/10.1590/1806-9061-2015-0162

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.

Sakomura, N. K. and Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. 2.ed. FUNEP, São Paulo.

Schedle, K.; Bartelt, J.; Lambert, W. and Corrent, E. 2019. Digestible valine requirements of growing-finishing Ross 308 broilers. Journal of Applied Poultry Research 28:1168-1180. https://doi.org/10.3382/japr/pfz083

Selle, P. H.; Dorigam, J. C. P.; Lemme, A.; Chrystal, P. V. and Liu, S. Y. 2020. Synthetic and crystalline amino acids: Alternatives to soybean meal in chicken-meat production. Animals 10:729. https://doi.org/10.3390/ani10040729

Silva, D. J. and Queiroz, A. C. 2004. Análise de alimentos (Métodos químicos e biológicos). 3.ed. Universidade Federal de Viçosa, Viçosa, MG.

Tavernari, F. C.; Lelis, G. R.; Vieira, R. A.; Rostagno, H. S.; Albino, L. F. T. and Oliveira Neto, A. R. 2013. Valine needs in starting and growing Cobb(500) broilers. Poultry Science 92:151-157. https://doi.org/10.3382/ps.2012-02278

Wijtten, P. J. A.; Hangoor, E.; Sparla, J. K. W. M. and Verstegen, M. W. A. 2010. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. Poultry Science 89:1424-1439. https://doi.org/10.3382/ps.2009-00626

Yalçin, S.; Izzetoğlu, G. T. and Aktaş, A. 2013. Effects of breeder age and egg weight on morphological changes in the small intestine of chicks during the hatch window. British Poultry Science 54:810-817. https://doi.org/10.1080/00071668.2013.860212

Zhang, S.; Zeng, X.; Ren, M.; Mao, X. and Qiao, S. 2017. Novel metabolic and physiological functions of branched chain amino acids: a review. Journal of Animal Science and Biotechnology 8:10. https://doi.org/10.1186/s40104-016-0139-z