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Effects of L-Valine Supplementation in Feed on the Growth and Ileal Morphometry of Grower-Phase Native Chickens

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Abstract

The content of essential amino acids in feed affects the growth rate of grower-phase poultry. In this study, the influence of valine amino acid supplementation (L-valine) in feed on the growth performance and ileal morphology of grower-phase native chickens in Kefamenanu, East Nusa Tenggara Province of Indonesia, was observed from June to August 2022. A total of 200 native chickens of 6 weeks of age were divided into 5 treatment groups with 5 replications based on a completely randomized design. The treatment feeds were T0 (control feed), T1 (T0 + 0.60% L-valine), T2 (T0 + 0.85% L-valine), T3 (T0 + 1.10% L-valine), and T4 (T0 + 1.35% L-valine). The highest body weight was observed in the T3 group (1417.00±16.17 g/bird), with a weight gain of 19.34±0.30 g/bird/day. This was significantly different from that of the other treatments (P<0.05). The lowest body weights observed in the T0, T3 and T4 groups were 1372.00±7.42, 1390.40±16.06 and 1386.60±8.73 g/bird, respectively. The lowest weight gain of 18.53±0.16 g/bird/day was observed in the T0 group. The feed consumption for the T0, T1, T2, T3 and T4 groups was 62.54±0.16, 62.79±0.36, 62.93±0.38, 62.69±0.39, and 62.77±0.34 g/bird/day, respectively, but the differences were not significant. A significantly higher feed conversion of 3.25±0.06 (P<0.05) was observed in the T2 group compared to the T0, T3 and T4 groups. Carcass weight and carcass percentage were significantly higher in the T2 group; these values were 883.18±6.74 g/bird and 62.19±0.93%, respectively (P<0.05). This study showed that the villi height, villi width, and crypt depth of the ileum of the T2 group (1036.60±65.67, 274.00±6.32, and 243.80±6.72, respectively) were significantly different from those of the other treatments. It could be concluded that that feed supplementation with 0.60-0.85% L-valine has a positive influence on the growth and ileal morphometry of native grower-phase chickens.

KEYWORDS
Native chickens, Valine, Growth performance, Ileal morphology, Grower phase

INTRODUCTION

The use of soybean meal and fish meal as sources of protein in native chicken feed is expensive. The use of protein from these two feed ingredients is wasted and inefficient. Lisnahan *et al.* (2022) reported that more than 70% of the cost of the production of native chickens is the cost of feed. As a result, the profits obtained by farmers are low. Another consequence is ammonia pollution in the cage since a large amount of uric acid comes out with feces (excreta).

An alternative to reduce the use of protein from fish meal or soybean meal, which is relatively expensive, is synthetic amino acids. The commercial availability of synthetic amino acids has reduced feed costs without compromising chicken performance. Several studies have modified the feed given to standard cafeteria feed. Lisnahan *et al.* (2017) reported that the use of the amino acids methionine and lysine had a significant impact on the growth of native chickens. Likewise, the use of the amino acids threonine and tryptophan in feed has been reported by Lisnahan and Nahak (2019), and the use of arginine (Lisnahan *et al.*, 2022) increases the productivity of native chickens. Based on the NRC (1994), the next limiting amino acid in broilers and laying hens is valine.

The amino acid valine increases muscle metabolism, which can repair damaged tissue and regulate the immune system (Kidd *et al.*, 2021). In addition, valine is also a precursor in the penicillin biosynthetic pathway and is known to inhibit tryptophan transport across the blood–brain barrier (Refaie *et al.*, 2017). Valine plays a role in stimulating growth and other metabolic functions in livestock animals.

The standard needs for the amino acid valine in broilers and laying hens exist and were reported by the NRC (1994). However, in native chickens, there have been no standards reported thus far. For this reason, it is important to study the needs of this amino acid in native chickens.

MATERIALS AND METHODS

Ethical approval

The research protocol was approved by the Veterinary Com-

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mittee for Animal Science Study Program, Agriculture Faculty, Timor University, Indonesia with 09/UN60.1/SR/2020 number recommendation.

Study duration, location, animals, and feed preparation

This study was conducted from June to August 2022 in the Kefamenanu of Indonesia. Laboratory analyses were performed in the Laboratory of Agriculture Faculty, Timor University and Animal Health Faculty Laboratory of Nusa Cendana University, Kupang, Indonesia. A total of 200 native chickens of 6 weeks of age whose initial weight was 334.28±19.16 g, were housed in 25 (1.5×1.2×0.7 m litter) cages. Feed bins, water dispensers, bulbs, weighing scales, and equipment for the analysis of ileum morphometry were also used. The feed materials included yellow corn, rice bran, soybean meal, fishmeal, calcium carbonate, a vitamin premix, methionine, lysine, arginine, threonine, tryptophan, and valine. Other materials included those needed to analyze ileum morphometry.

Dietary treatment and feeding duration

Native chickens were divided into 5 treatment groups with 5 replications based on a completely randomized design (CRD). The treatment feeds were T0 (control feed), T1 (T0 + 0.60% L-valine), T2 (T0 + 0.85% L-valine), T3 (T0 + 1.10% L-valine), and T4 (T0 + 1.35% L-valine). Control treatment (T0) is based on cafeteria feed (Lisnahan *et al.*, 2017b), and T1 is based on NRC (1994) and the ideal concept of amino acids is for broiler chickens (Samadi, 2012).

The native chickens, aged 14 weeks, were slaughtered (2 chickens/cage) at the Laboratory of the Faculty of Agriculture. The feathers are removed after the chicken is placed in hot water 70°C (scalding water) for 50 seconds. The chickens that have been cleaned of feathers, the legs and neck/head are cut off, and the innards are separated. In a short time, an ileum sample (2 cm) was taken and put into a vial with a 10% formalin concentration. Ileum samples were analyzed at the Faculty of Animal Health Laboratory, Nusa Cendana University, Kupang.

Ileum morphology measurement.

Villus height, villus width and crypt depth were measured in stages.

Chicken ileum sample preparation. The ileum segments were prepared as samples, and 2-cm pieces were fixed in 10% buffer formalin, soaked for 24-48 hours, and then prepared (Lisnahan and Nahak, 2020).

Preparation making. The prepare the samples for hematoxylin-eosin staining, each piece of tissue was hydrated through

Table 1. Composition and content of the nutrients in different native chicken treatments.

Feed material	Treatment				
	T0	T1	T2	T3	T4
Yellow corn (%)	61.1	60.5	60.25	60	59.75
Rice bran (%)	18	18	18	18	18
Soybean meal (%)	10	10	10	10	10
Fish meal (%)	5	5	5	5	5
Mineral Premix (%)	0.4	0.4	0.4	0.4	0.4
Vitamin premix (%)	0.4	0.4	0.4	0.4	0.4
Dl-methionine (%)	0.4	0.4	0.4	0.4	0.4
L-lysine HCl (%)	1	1	1	1	1
L-threonine (%)	0.85	0.85	0.85	0.85	0.85
L-tryptophan (%)	0.5	0.5	0.5	0.5	0.5
L-arginine (%)	0.85	0.85	0.85	0.85	0.85
L-valine (%)	0	0.6	0.85	1.1	1.35
Calcium (%)	1	1	1	1	1
Phosphor (%)	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Composition of nutrients					
Metabolized energy (kcal/kg)*	3027.37	3005.18	2995.93	2986.68	2977.43
Crude protein (%)*	16.17	16.11	16.09	16.07	16.04
Ether extract (%)*	5.21	5.18	5.16	5.15	5.14
Ash (%)*	7.08	7.07	7.06	7.05	7.05
Crude fiber (%)*	4.83	4.82	4.82	4.81	4.81
Methionine	0.43	0.43	0.43	0.43	0.43
Lysine	1.1	1.1	1.1	1.1	1.1
Threonine	1	1	1	1	1
Tryptophan	0.56	0.56	0.56	0.56	0.56
Arginine	0.96	0.96	0.96	0.96	0.96
Valine	0.1	0.7	0.95	1.2	1.45
Calcium	1.78	1.77	1.77	1.77	1.76
Available Phosphor	0.86	0.86	0.86	0.86	0.86

*Analysis result of Biochemical Laboratory of the Faculty of Animal Husbandry UGM Yogyakarta, 2017 a series of alcohols at increasing concentrations (70, 80, 90 and 95%). The samples were individually transferred to each alcohol concentration and allowed to soak for approximately 10 seconds. Then, the sample was placed in xylol and finally immersed in paraffin. The sample was sliced thin using a microtome for hematoxylin-eosin staining. The histological preparations that were ready in the glaze object were observed and measured using a computer microscope (Lisnahan and Nahak, 2020; Lisnahan *et al*., 2022).

Shooting. The object of the sample was viewed and determined using an Olympus BX 51 microscope equipped with an Olympus DP 12 projector adjusted to 10 times magnification. Morphological images appeared on the JVC TMH 1750 C monitor. After finding the ileum morphology as expected, all the preparations to be measured were taken. A minimum of 3 measurements per slide were made for each parameter.

Measurement steps for villus height, villus width and depth of Lieberkuhn crypts. The measurements of villi height, width, and depth of Lieberkuhn crypts were performed using a flat screen computer with the Microsoft Office Picture Manager program at 40% magnification. First, the standard size of μm was determined with the help of a computer, namely, how much the magnification value used or desired is converted into units of length (μm). The μm unit number obtained was then used as a standard in measuring the villi height, villi width and depth of the crypt displayed on the monitor screen.

Data collection and analysis

In addition to ileal morphometry data, other data, including weight gain, feed consumption, feed conversion, carcass weight and percentage, were collected. Weight gain was defined as the difference between the final body weight and initial body weight. Feed conversion was defined as feed consumption divided by weight gain. Carcass weight was obtained after deducting the weight of blood, feathers, head, neck, legs, internal organs, and abdominal fat. The collected data were analyzed using analysis of variance (ANOVA) based on a completely randomized design and Duncan's test using IBM SPSS Statistics 25 (IBM Corp., NY, USA).

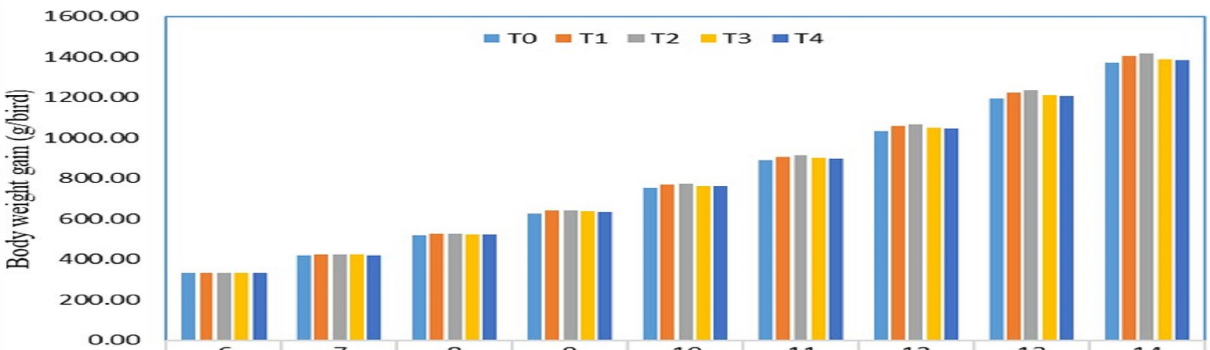
RESULTS

This study determined the amount of L-valine needed to obtain optimum performance of weight gain, feed consumption, feed conversion, carcass weight and the villi height, villi width and crypt depth of the ileum of native chickens. The average body weight and weight gain of grower-phase native chickens (6-14 weeks) in the five treatment groups (T0-T4) are presented in Table 2. At 14 weeks of age, T2 and T1 chickens fed 0.85% and 0.60% L-valine, respectively, had the highest body weight among the groups (1417.00±16.17 g and 1404.20±24.61 g). Likewise, these two groups had the highest body weight gain (19.34±0.30 g/bird/day and 19.12±0.44 g/bird/day). ANOVA showed that L-valine supplementation had a significant influence on the body weight and body weight gain of native chickens ($p<0.05$). The body weight of the 0.60% L-valine (T1) group was increased by 2.35% compared to treatment without L-valine supplementation (T0). The body weight of the 0.85% L-valine (T2) group was increased by 0.91% compared to T1. The body weight of the 1.10%

Table 2. Native chicken performance aged 6-14 weeks (grower phase).

Parameter	Treatments				
	T0	T1	T2	T3	T4
Body weight (g/bird)	1372.00±7.42^{c}	1404.20±24.61^{ab}	1417.00±16.17^{a}	1390.40±16.06^{bc}	1386.60±8.73^{bc}
Body weight gain (g/bird/day)	18.53±0.16^{c}	19.12±0.44^{ab}	19.34±0.30^{a}	18.85±0.30^{bc}	18.80±0.15^{bc}
Feed intake (g/bird/day)	62.54±0.16	62.79±0.36	62.93±0.38	62.69±0.39	62.77±0.34
Feed conversion	3.38±0.04^{a}	3.28±0.06^{bc}	3.25±0.0^{c}	3.33±0.06^{ab}	3.34±0.03^{ab}
Carcass weight (g)	833.83±2.75^{d}	868.20±10.99^{b}	883.18±6.74^{a}	855.02±6.69^{c}	843.17±7.94^{d}
Carcass percentage	60.78±0.29^{b}	61.83±0.58^{a}	62.19±0.93^{a}	61.50±0.35^{ab}	60.81±0.36^{b}
Villus height (μm)	863.40±35.61^{d}	985.40±19.13^{ab}	1036.60±65.67^{a}	939.20±34.13^{bc}	893.40±27.65^{cd}
Villus width (μm)	245.20±5.89^{d}	264.00±5.10^{b}	274.00±6.32^{a}	252.80±5.22^{c}	253.80±2.59^{c}
Crypt depth (μm)	215.40±13.83^{c}	234.40±4.98^{ab}	243.80±6.72^{a}	228.60±2.30^{b}	226.60±4.67^{b}

Remarks: Superscript was different on the average row showing a significant difference ($p<0.05$); T0 (control feed); T1 (T0 + 0.60% L-valine); T2 (T0 + 0.85% L-valine); T3 (T0 + 1.10% L-valine); T4 (T0 + 1.35% L-valine)



	6	7	8	9	10	11	12	13	14
T0	334.60	420.80	518.00	627.80	753.60	888.60	1035.80	1196.60	1372.00
T1	333.40	424.60	525.40	641.20	769.80	908.20	1060.00	1223.80	1404.20
T2	333.80	425.00	527.60	642.40	773.20	913.80	1068.60	1234.40	1417.00
T3	335.00	424.40	523.40	636.60	764.00	900.80	1049.80	1212.20	1390.40
T4	334.00	421.40	522.60	634.60	761.80	898.80	1047.20	1208.80	1386.60

Figure 1. Body weight gain of native chickens during the study period.

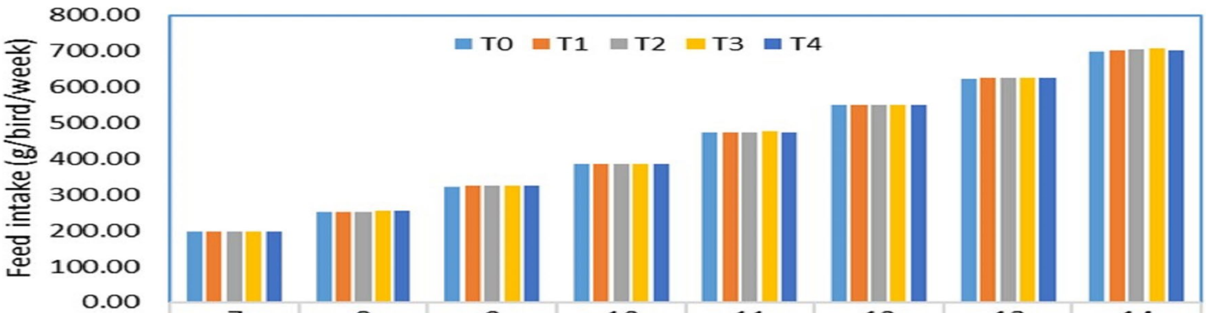
and 1.35% L-valine (T3 and T4) groups trended towards a decrease. This decrease was 1.88% and 2.15% compared to T2 (Fig. 1). The same was true for body weight gain, in which the maximum weight gain was observed in the 0.85% L-valine group.

The highest average feed consumption was in the T3 treatment, but the ANOVA was not significant (Table 2). Feed intake tended to increase in the 0.60% and 0.85% L-valine (T1 and T2) groups and decrease in the 1.10% and 1.35% L-valine groups. The development of feed consumption is shown in Fig. 2.

The T0 (control feed), T3 (control feed + 1.10% L-valine) and T4 (control feed + 0.135% L-valine) groups had the highest feed conversion ratios, which were significantly different from those of the other treatment groups ($p<0.05$). There was no significant difference between the ratios for the T1 and T2 groups. However, when the L-valine content increased from 0.85% (T2) to 1.10% (T3), feed conversion decreased by 2.46% (Fig. 3).

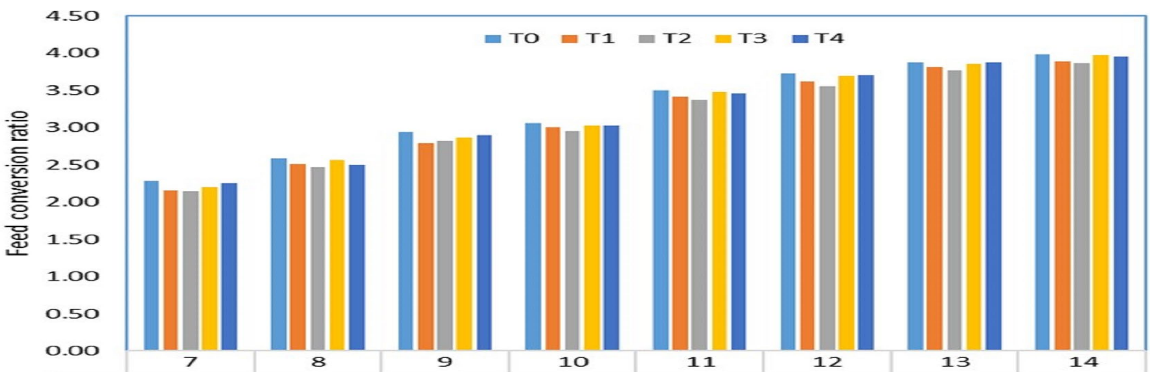
In addition to having the best body weight and feed conversion, the T2 and T1 groups had significantly higher carcass weight and carcass percentage ($p<0.05$) than the other treatment groups. Moreover, supplementation with 1.35% L-valine (T4) and control feed (T0) had no effect on carcass weight and carcass percentage. Carcass weight was increased by 4.12% in the 0.60% L-valine group compared to that in the T0 group. Carcass weight continued to increase by 1.73% in the 0.85% L-valine (T2) group compared to that in the T1 group, and this group demonstrated the highest carcass weight. However, the carcass weight was decreased by 3.19% in the 1.10% L-valine (T3) group compared to that in the T2 group. The carcass percentage also increased by 1.73-2.32% in the T1 and T2 groups compared to that in the T0 group.

The average villi height in the ileum of native chickens of 14 weeks of age was significantly higher ($p<0.05$) in the T2 and T1 groups compared to other treatment groups (Table 2). The villi height was increased by 14.13% in the 0.60% L-valine (T1) group compared to that in the T0 group. At 0.85% L-valine, villi height was increased by 5.20%, and this was the highest villi. At 1.10% L-valine (T3), villi height was decreased by 9.40% compared to that in the T2 group. Chickens in the T2 and T1 group also had the largest villi widths, and this difference was significant ($p<0.05$) compared to the other groups (Table 2). The villus width was increased by 7.67% in the 0.60% L-valine (T1) group. In the 0.85% L-valine (T2) group, the villi width was increased by 3.79% compared to that in the T1 group, and this was the widest villi. The villi width was decreased by 7.74% in the 1.10% L-valine (T3) group compared to that in the T2 group. The highest average crypt depth was observed in the T2 group (Table 2), and ANOVA showed that the treatment influenced this variable significantly ($p<0.05$). The crypt depth was significantly increased by 8.82% in the 0.60% L-valine (T1) group compared to that in the T0 group, and the crypt depth continued to increase by 4.01% in the T2 group compared to the T1 group. In the T3 group, the crypt depth was decreased by 6.23% compared to that in the T2 group.



	7	8	9	10	11	12	13	14
T0	197.09	251.38	323.02	385.26	473.53	549.01	623.66	699.41
T1	196.99	253.09	323.51	386.28	473.42	549.81	625.52	702.09
T2	196.12	253.52	323.77	386.11	473.84	551.47	625.45	706.25
T3	197.12	254.44	324.48	385.55	475.89	550.47	626.41	709.59
T4	197.04	253.77	324.90	385.14	473.37	550.98	626.79	703.20

Figure 2. Feed intake of native chickens over the study period.



	7	8	9	10	11	12	13	14
T0	2.29	2.59	2.94	3.06	3.51	3.73	3.88	3.99
T1	2.16	2.51	2.79	3.00	3.42	3.62	3.82	3.89
T2	2.15	2.47	2.82	2.95	3.37	3.56	3.77	3.87
T3	2.20	2.57	2.87	3.03	3.48	3.69	3.86	3.98
T4	2.25	2.51	2.90	3.03	3.46	3.71	3.88	3.95

Figure 3. Feed conversion ratio of native chickens over the study period.

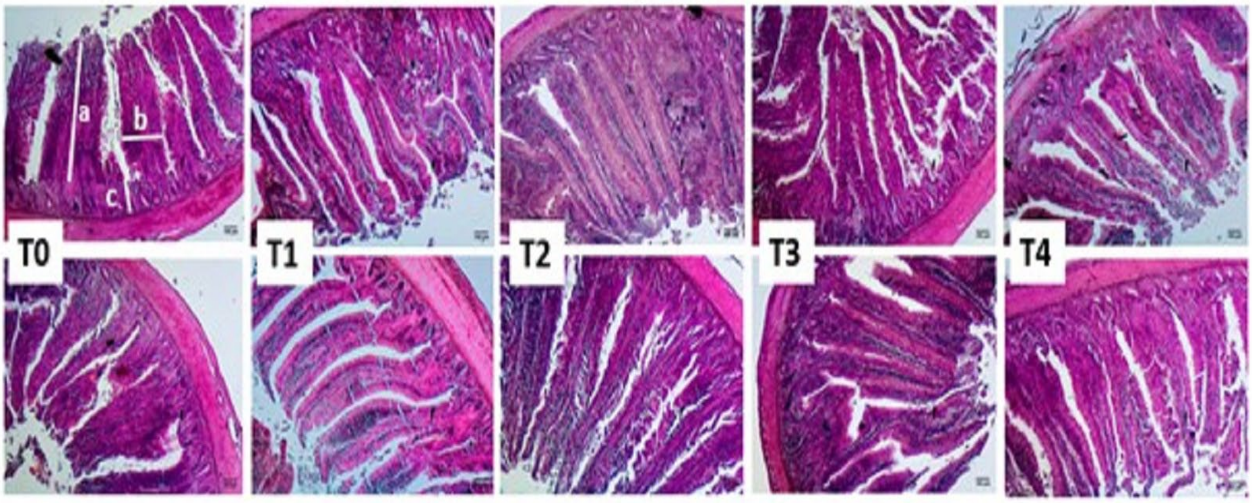


Figure 4. Ileum morphometrics of native chickens over the study period.
a: Villus height; b: Villus width; c: Crypt depth

DISCUSSION

During the grower period, L-valine supplementation increased the body weight of native chickens. However, if the amount is excessive, it will linearly reduce the weight of the chicken. The results showed that the supplementation of up to 0.85% L-valine indicated optimum performance. Weight gain linearly increased from the T0 to T2 groups and then decreased in the T3 and T4 group.

Based on the ideal amino acids, the valine-lysine ratio is 0.81% in broilers (Samadi, 2012). Excess valine in the feed can result in weight loss, as observed in the T3 and T4 groups (1.10-1.35%). The balance of micronutrients such as amino acids affects the growth of native chickens (Lisnahan *et al.*, 2017). In a previous study, Lisnahan *et al.* (2022) reported that the weight gain of native chickens was 49.35 g/bird/week in the starter phase when supplemented with methionine, lysine, tryptophan, threonine and arginine but less valine. Valine has an important role in muscle metabolism and the repair of damaged tissue (Ospina-Rojas *et al.*, 2017). Valine supplementation not only increased body weight but also accelerated growth (Fig. 1). This shows that nutrient balance is influenced not only by methionine, lysine, threonine, tryptophan, and arginine but also by valine. Valine deficiency has always been associated with weight loss, and its use is balanced with other essential amino acids, especially lysine. Likewise, excess valine has a negative effect on plasma and muscle amino acid concentrations as well as on growth performance, as observed in the T3 and T4 groups. However, these supplementation levels demonstrated better effects than the valine-deficient feed in the T0 group (Refaie *et al.*, 2017; Kidd *et al.*, 2021).

Weight loss was seen at L-valine levels above 0.85%. It is suspected that the utilization of valine must be followed by that of the amino acids leucine and isoleucine, which are also branched-chain amino acids (BCAAs). These three BCAAs are antagonistic to each other when administered in excess (Ospina-Rojas *et al.*, 2017). Valine is required for the maintenance and growth of tissues. If the leucine content in the feed is high, it increases the optimal levels of valine and isoleucine or vice versa (Holecek, 2018). This is because these amino acids stimulate branched-chain ketoacid dehydrogenase, which is a complex enzyme involved in the oxidative deamination of these three BCAAs (Ospina-Rojas *et al.*, 2020). Thus, an increase in valine leads to an increase in the oxidation of the other 2 BCAAs.

In contrast to weight gain, feed intake was not significantly different among the groups (Fig. 2). Feed consumption may be affected by energy adequacy. As shown in Table 1, the metabolic energy was between 2977.43 and 3027.37 kcal/kg, which meets the needs of chickens. In plant-based diets, valine is the limiting amino acid after methionine, lysine and threonine and before arginine and isoleucine. L-valine supplementation allows a further reduction of one percentage point of dietary crude protein. Agostini *et al.* (2019) explained that valine can suppress appetite but increase body weight and feed efficiency. Nascimento *et al.* (2016) reported that there was no significant feed consumption in broiler rearing supplemented with 0.85% valine.

Feed consumption values that are not different and significant changes in weight gain have an impact on low feed conversion. Valine supplementation decreased feed conversion (Fig. 3). Feed conversion is the ratio between feed consumed and weight gain. If the feed conversion is high, the feed consumed is less efficient. It is better if the feed conversion is low, which indicates high feed efficiency. In this study, the difference in feed conversion began to be significant at week 7. The T1 and T2 groups had lower feed conversions than the T0, T3 and T4 groups despite the increasing valine levels. This indicates that up to 0.85% L-valine is recommended for native chickens. If the concentration is increased further, it will increase feed conversion. The possible use of valine in feed needs to consider its ratio to other limiting amino acids, such as lysine, and the other 2 BCAAs. The valine-lysine ratio in broilers was reported by Agostini *et al.* (2019) to be 0.88. In this study, a valine-lysine ratio of 0.60-0.85 gave the best results, but high valine ratios (T3 and T4) did not. Based on the ideal amino acid ratios in broilers, the valine ratio in the T1 and T2 groups were similar to this ratio. In the T3 and T4 groups, excessive valine resulted in low body weights and high feed conversion. Likewise, the ratio of valine to leucine and isoleucine is important. These three essential amino acids must be in balance in the feed. If the amount of valine is increased but leucine and isoleucine are low, it will have an impact on growth. It can even become an antagonist if one of these BCAAs is found in excess. Tavernari *et al.* (2013) explained that broilers of 30-43 days of age require valine, isoleucine, and leucine at a ratio of 0.80%:72%:142%.

Carcass weight is the weight of organs without blood, feathers, head, neck, shanks and claws, viscera and abdominal fat. The carcass weight and carcass percentage in this study were positively correlated with body weight. This was influenced by the amount of valine administered. The carcass weight linearly increased in the T2 group but then decreased in the T3 and T4 groups. Likewise, the carcass percentage increased from the T1 group to the T2 group and then was decreased in the T3 and T4 groups. Based on this response, valine is needed for carcass formation but should not be at excessive levels in the feed. At levels above 0.60% L-valine, carcass weight began to increase and peaked at 0.85% L-valine. At higher concentrations, the carcass weight began to decrease. Valine concentrations must be balanced with that of other amino acids, such as lysine or 2 BCAAs. The largest contributors to carcass weight in chickens are the breast and thigh. Agostini *et al.* (2019) stated that BCAAs are important regulators of muscle protein synthesis, and Ospina-Rojas *et al.* (2020) previously reported that BCAAs contribute 35% of the dietary essential amino acids in body protein. Supplementation of feed with this amino acid can affect the diameter of chicken muscle fibers. Berres *et al.* (2011) and Tavernari *et al.* (2013) determined valine levels of 0.81 and 0.80%, respectively, for broilers during the growth phase. Valine plays a role in helping prevent muscle breakdown, as it supplies muscles with extra glucose responsible for energy production during physical activity (Corzo *et al.*, 2011).

The ileal morphometry observed in this study were villi height, villi width and crypt depth (Fig. 4). This section serves as an indicator of health and relates to the digestion and absorption of nutrients. Dietary valine levels affect the ileal mucosa. The villi height was higher in the 0.85% L-valine group than in the other groups. Likewise, for the villi width and the crypt depth, the highest values were observed in the T2 group. This may be related to body weight and feed conversion. Improved ileal morphometrics translate into improved feed conversion ratios, reflecting more efficient feed utilization. Maiorka *et al.* (2003) showed that livestock with increased intestinal mucosal cell regeneration had more villi arising from increased mitotic activity and hyperplasia. The intestinal mucosa grows continuously due to the desquamation of cells in the intestinal lumen, and cell regeneration can be encouraged by increased nutrient intake (Baurhoo *et al.*, 2007).

Valine along with other essential amino acids for chickens may be required for the maintenance and development of the ileal mucosa. The villi become higher and wider and allow for digestive enzymes to work more efficiently. Consequently, nutrient absorption is higher. Likewise, the crypt depth indicates that more nutrients are transported through the ileal wall for further metabolism. Murakami *et al.* (2012) have previously reported that the development of intestinal villi increases the efficiency of digestion and nutrient absorption, and the size of these ileal villi is an indicator of animal health (Jian *et al.*, 2021). Valine and branched chain amino acids (BCAAs), including leucine and isoleucine, are essential amino acids for the growth and development of livestock, and they participate in the synthesis of proteins and other amino acid precursors (Ospina-Rojas *et al.*, 2020). Valine deficiency results in reduced feed intake and growth, and high leucine (Leu) levels further exacerbate the consequences of valine deficiency. In contrast, excess valine, such as at that in the T3 (1.10%) and T4 (1.35%) groups, can cause antagonism with leucine and isoleucine (Agostini *et al.*, 2019). Valine participates in protein synthesis. It is a precursor to other amino acids or takes part in glucose metabolism as a glucogenic amino acid (Wu, 2009). Lisnahan and Nahak (2020) reported that villi height, crypt depth, and villi width contribute to the area of the intestinal mucosa that is available for digestion and absorption of nutrients for use in the development and maintenance of body tissues.

There was a relationship between villi height and ileal crypt depth in native chickens (Table 2). An increase in villi height and crypt depth is associated with more villous surface area to absorb nutrients into the bloodstream (Monavvar *et al.*, 2020). Moreover, it was also stated that the ratio of villi height to crypt depth showed an increase in nutrient absorption (Yu *et al.*, 2018; Abdulkarimi *et al.*, 2019). Lisnahan and Nahak (2020) stated in more detail that an increase in villi height and crypt depth in the ileum of native chickens parallels an increase in digestive and absorption functions due to the expansion of the absorption area. This is a representation of the nutrient transport system throughout the body. Villi height is also likely to have a positive relationship with body weight gain and feed consumption and feed efficiency (Lisnahan and Nahak., 2020; Lisnahan *et al.*, 2022). The regulatory mechanisms exerted on the intestinal mucosa are still poorly understood. There is evidence that amino acids can influence interactions between intrinsic afferent neurons and primary extrinsic neurons and central nervous system by regulating the transcription and expression of genes involved in amino acid metabolism. This has been reported for several amino acids (Wu, 2014; Toprak *et al.*, 2021).

CONCLUSION

Based on the results and discussion, it can be concluded that the feed for native chickens needs to be supplemented with 0.60–0.85% L-valine to achieve optimal growth. The optimal growth indicators are body weight, feed conversion, carcass weight and a wider ileal villi surface.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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