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Research Article

Effects of Dietary Isoleucine Supplementation on the Growth Performance, Carcass Characteristics and Ileal Morphology of Native Chickens

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Abstract: The roles of isoleucine in the protein synthesis, metabolisms, and growth of chicken have been widely reported. However, its effect on native chicken has not been previously reported. In this work, we reported the effects of dietary isoleucine supplementation on growth performance, carcass characteristics, and ileal morphology in native chickens during the grower phase (6-12 weeks). Two hundred native chickens with the age of 6 weeks (average initial weight of 319.7±1.81 g) were used. The chickens were divided into five treatments with five replicates of 8 birds each in a fully randomized design. The diet treatments were T0 as the control feed, T1 (T0+0.15% isoleucine), T2 (T0 + 0.25% isoleucine), T3 (T0+0.35% isoleucine), and T4 (T0 + 0.45% isoleucine). In this experiment, we assessed the body weight, body weight gain, feed intake, feed conversion ratio, carcass weight, and carcass percentage. A one-way analysis of variance and Duncan's test were used to examine the data. The greatest body weight and body weight gain in the T3 group were 1032.2±8.70 g/bird and 712.5±8.25 g/bird/6 weeks, respectively. The highest feed intake and feed conversion ratio in the T3 group were 2934.8±45.15 g/bird/6 weeks and 4.12±0.26, respectively. The highest carcass weight and carcass percentage in the T3 group were 625.5±10.81 g/bird and 61.0±1.07%/bird, respectively. The T2 and T3 groups had the greatest villus heights (1378.0±35.54 and 1385.8±39.08 µm, respectively) and greatest crypt depths (311.4±4.66 and 261.6±3.97 µm, respectively). The body weight, weight gain, feed intake, feed conversion ratio, carcass weight, carcass percentage, villus height, and crypt depth were significantly affected by the isoleucine level ($p<0.05$), but villus width was not. Therefore, 0.35% isoleucine supplementation in the diet of native chickens resulted in optimal growth performance at the grower phase.

Keywords: Isoleucine, Native Chickens, Growth Performance, Carcass, Grower Phase

Introduction

Protein is undeniably an essential nutrient for poultry and is an integral part of poultry feed. Protein-rich ingredients, such as fishmeal and soybean meal, constitute 30-40% of the ration and are essential for determining feed costs. It is universally accepted that poultry requires a diet with balanced amino acids rather than crude protein (Attia et al., 2022). In poultry feed formulations, first-line essential amino acids in the synthetic form are supplemented extensively to rationalize amino acid levels and reduce feed costs in corn and soybean feeds (Selle et al., 2020) Pesti and Choct, 2023). Reducing feed crude protein levels and using synthetic amino acids may reduce food costs and nitrogen-induced environmental pollution (Attia et al., 2020). Reducing the protein content of feed has become a practice for raising poultry, especially chickens. The goal is to reduce production costs and ammonia pollution in cages (Cappelaere et al., 2021).

However, a progressive reduction in the protein content of feed may lead to a situation in which a lack of certain amino acids, such as isoleucine, affects bird performance. Therefore, free-form supplementation of this amino acid is necessary in this case. Isoleucine is necessary for the maintenance and growth of chickens (Gu et al., 2019). Feed made from corn and soybean meal contains low isoleucine, especially for the growth of chickens. The need for isoleucine is known to increase in association with two other amino acids, leucine and valine (Kim et al., 2022a). Isoleucine has the potential to limit a low-protein diet for laying hens supplemented with lysine, methionine and tryptophan (Corrent and Bartelt, 2011). Following lysine and methionine, threonine is the third amino acid to become limiting in practical diets, a limitation often shared by isoleucine and valine at similar levels.

Several previous studies have investigated the need for isoleucine in broiler chickens and laying hens. In native chickens, no research has been conducted on the need for this amino acid. It is important to determine the levels of isoleucine required by native chickens as their growth slows with increasing feed conversion. Hence, this study aimed to determine the effect of various levels of isoleucine on the growth performance, carcass traits and ileal morphometry of the grower phase (6-12 weeks) native chickens. We expected that optimum isoleucine levels tailored to the needs of native chickens could enhance growth and production.

Materials and Methods

Ethical Approval

The animal ethics committee located at the Animal Science Department, Faculty of Agriculture, Science and Health, University of Timor, Indonesia, authorized the methodology used in the experiment.

Investigate Duration, Place, Animals and Feed Preparations

The experiment was conducted in Kefamenanu, East Nusa Tenggara, Indonesia, between July and August 2023. The laboratory aspects of the experiment were performed at the Laboratory of Agriculture, Science and Health located at the University of Timor.

In the experiment, we used 6 weeks old of 200 native chickens (average weight = 319.7±1.81 g). The chickens were placed in 25 1.2×1.2×0.8 m litter cages. The cages had feed storage containers, water dispensers and lightbulbs. The raw materials used in the diets contained yellow corn, rice bran, soybean meal, fish meal, dicalcium phosphate, vitamin premix, methionine, lysine, threonine, tryptophan, arginine and isoleucine. The feed was given in the form of pellets. The cage was provided with 75-watt lighting. The cage floor was made of concrete and covered with husk and lime to a thickness of 7 cm. The cage litter was cleaned every week. The chickens were vaccinated with the Newcastle disease Hitcher B1 and LaSota vaccines at 3 days and 21 days of age and with the LaSota vaccine at 3 months of age.

Dietary Management and Feeding Duration

This study employed a fully randomized design with five treatments, five replicates and eight native chickens in each replicate (Table 1). The treatment groups were as follows: T0: control feed; T1: T0+0.15% isoleucine; T2: T0+0.25% isoleucine; T3: T0+0.35% isoleucine; and T4: T0+0.45% isoleucine.

Table 1: Composition (% dry matter) and nutrient content in various native chicken treatments

Feed material	Treatment (%)				
	T0	T1	T2	T3	T4
Yellow corn	64.85	64.70	64.60	64.50	64.40
Rice bran	17.00	17.00	17.00	17.00	17.00
Soybean meal	7.00	7.00	7.00	7.00	7.00
Fish meal	6.00	6.00	6.00	6.00	6.00
Mineral premix	0.30	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.85	0.85	0.85	0.85	0.85
L-threonine	0.85	0.85	0.85	0.85	0.85
L-tryptophan	0.45	0.45	0.45	0.45	0.45
L-arginine	0.60	0.60	0.60	0.60	0.60
L-isoleucine	0.00	0.15	0.25	0.35	0.45
Calcium	1.00	1.00	1.00	1.00	1.00
Phosphorus	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Composition of nutrients					
Metabolized energy (kcal/kg)*	3081	3075	3071	3068	3064
Crude protein (%)*	15.20	15.18	15.17	15.16	15.16
Ether extract (%)*	6.66	6.65	6.65	6.64	6.64
Ash (%)*	6.07	6.07	6.07	6.07	6.06
Crude fiber (%)*	4.68	4.68	4.68	4.67	4.67
Methionine	0.33	0.33	0.33	0.33	0.33
Lysine	1.00	1.00	1.00	1.00	1.00
Threonine	1.00	1.00	1.00	1.00	1.00
Tryptophan	0.50	0.50	0.50	0.50	0.50
Arginine	0.80	0.80	0.80	0.80	0.80
Isoleucine	0.30	0.45	0.55	0.65	0.75
Calcium	1.60	1.60	1.60	1.60	1.60
Available Phosphor	0.80	0.80	0.80	0.80	0.80

** Data from the Biochemical Laboratory, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta, 2017. The optimum digestible isoleucine to lysine ratio was 0.67 for broilers from day-old chicks to 21-42 days of age (NRC, 1994). The digestibility of lysine is 89% and that of isoleucine is 90% for feed based on yellow corn and soybean meal (Samadi, 2012)*

Data Collection and Analysis

The feeding trial lasted for six weeks, after which the animals were slaughtered for carcass characteristic measurements. The parameters assessed were weekly body weight, weight gain, feed intake, Feed Conversion Ratio (FCR) and carcass weight. The final body weights of the birds were obtained on the last day after the birds had fasted for 12 h. Body weight gain was defined as the difference between the final and initial weights:

$$Weight\ gain\,(g/bird) = \frac{final\ weight - initial\ weight}{6\ weeks}$$

The Ratio of Feed Conversion (FCR) was defined as the feed intake (kg) divided by the total weight gain (kg):

$$FCR = \frac{Feed\ intake}{weight\ gain}$$

The weight of the internal organs, feet, head and neck, blood, feathers and belly fat were subtracted to get the weight of the carcass. The carcass percentage was calculated as the ratio between the carcass weight and body weight.

$$Carcass\ percentage\,(\%/bird) = \frac{carcass\ weight}{bodyweight} x100\%$$

Measurements of Ileum Morphology

The ileal morphology was observed using a light microscope (Olympus Corporation Japan) at 40× magnification. The villus height, width and crypt depth of the ileum from 12-week-old native chickens were determined by employing the method described by Lisnahan et al. (2022).

Chicken Intestine Sample Preparation

Ileal tissue was obtained by isolating segments of the small intestine. Ileal pieces 2 cm in size were collected and fixed by soaking in 10% of buffer formalin for 24-48 h (Lisnahan et al., 2022).

Hematoxylin-Eosin Staining

The fixed tissues were immersed in an alcohol with various concentrations (70, 80, 90 and 95%) by soaking in each solution for approximately 10s. After being submerged in xylol, the samples were immersed in paraffin. The paraffin-embedded samples were thinly sliced and placed on glass slides using a microtome.

Imaging

The samples were analyzed under 10x magnification using an Olympus B.X. 51 microscope connected to an Olympus D.P. 12 projector (Olympus Corporation). A JVC TMH 1750 C monitor displayed the resulting images. This process involved scanning the samples to quantify the structural features of the ileum and assess its morphometry. To ensure accuracy, triplicate measurements were taken for every parameter.

Measurements of Villus Height, Width and Crypt of Lieberkuhn Depth

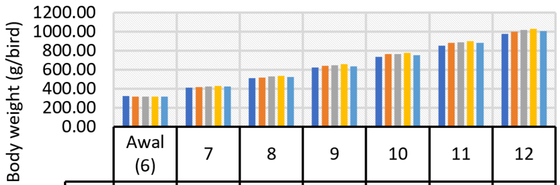
The villus height and width and the depth of the crypts of Lieberkuhn were measured using a Microsoft Office Picture Manager program at 40x magnification. To convert the magnified measurements into micrometers (μm), a standard micrometer was measured on the computer screen. This standard μm value was then used to calculate the actual height, width and crypt depth shown on the screen.

The experimental data were analyzed using IBM SPSS Statistics 26 (IBM Corp., NY, USA) by employing ANOVA based on a completely randomized design (Dean et al., 2017) and Duncan's test. The statistical model was Yij = μ + τi + εij. The normality test of initial body weight was 0.604. The growth parameters of 100% of the native chickens were analyzed, while the carcass and intestinal morphometry parameters in 25% of the chickens were analyzed.

Results

Body Weight

Table (2) illustrates the typical body mass and subsequent weight accretion observed in native chickens from 6-12 weeks of age, categorized by the five distinct intervention groups (T0-T4). Among all treatment groups, the T3 group (0.35% isoleucine) chickens had the largest body weight at 12 weeks of age. Similarly, this group had the greatest weight gain (712.5±8.25 g/bird/6 weeks). Statistical analysis revealed that isoleucine supplementation significantly affected the body weight and weight gain of the native chickens ($p<0.05$). Supplementation with 0.15% isoleucine (T1 treatment) increased body weight by 2.74% compared to the T0 treatment without isoleucine supplementation. When the isoleucine level was increased by 0.25% (T2 treatment), the body weight increased by 1.20% compared to that in the T1 group. After supplementation with 0.35% isoleucine (T3 treatment), the body weight reached a maximum of 1032.2±8.70 g, with an increase in body weight of 1.65% compared to that in the T2 group. The opposite response occurred at the highest level of isoleucine supplementation (0.45%) in the T4 group, in which the body weight decreased by 2.58% compared to that in the T3 group (Figure 1). A similar pattern occurred with weight gain, with the maximum weight gain occurring in the 0.35% isoleucine supplementation group.



	Awal (6)	7	8	9	10	11	12
T0	320.36	412.20	509.00	623.20	734.60	852.40	976.60
T1	319.48	418.00	519.40	641.60	762.40	882.80	1003.4
T2	319.76	423.60	528.80	647.00	765.40	888.80	1015.4
T3	319.68	426.80	534.00	656.60	779.00	899.60	1032.2
T4	319.14	420.60	524.00	634.20	755.40	884.80	1005.6

Fig. 1: Body weight development in native chickens during the grower phase

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Table 2: Productivity metrics for native chickens during the 6-12 week growth stage

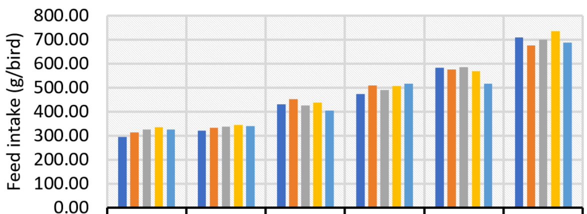
Feed material	Treatment (%)				
	T0	T1	T2	T3	T4
Body weight (g/bird)	976.6±9.07^{c}	1003.4±9.61^{b}	1015.4±9.07^{b}	1032.2±8.70^{a}	1005.6±12.72^{b}
Body weight gain (g/bird/6 weeks)	656.2±8.85^{c}	683.9±8.56^{b}	695.6±10.66^{b}	712.5±8.25^{a}	686.5±15.07^{b}
Feed intake (g/bird/6 weeks)	2815.2±57.48^{b}	2861.6±61.35^{ab}	2870.6±51.62^{ab}	2934.8±45.15^{a}	2915.2±81.23^{a}
FCR	4.29±0.46^{a}	4.18±0.42^{b}	4.13±0.23^{c}	4.12±0.26^{c}	4.25±0.36^{a}
Carcass weight (g/bird)	571.9±6.29^{c}	598.8±8.77^{b}	617.9±13.61^{a}	625.5±10.81^{a}	602.1±12.19^{b}
Carcass percentage (%/bird)	58.6±0.73^{c}	59.7±0.34^{b}	60.9±0.94^{a}	61.0±1.07^{ab}	59.9±0.56^{ab}
Villus height (μm)	1314.2±17.59^{b}	1354.8±40.46^{ab}	1378.0±35.54^{a}	1385.8±39.08^{a}	1351.0±9.72^{ab}
Villus width (μm)	259.0±9.35	262.6±12.12	263.2±4.66	261.6±3.97	262.8±7.73
Crypt depth (μm)	289.0±16.87^{b}	294.4±8.62^{b}	311.4±10.78^{a}	312.0±5.70^{a}	298.6±6.66^{ab}

Note: Superscripts displayed on the average row denote a statistically significant difference (p<0.05). The treatments consisted of a control feed (T0) and the same feed supplemented with increasing levels of isoleucine 0.15% (T1), 0.25% (T2), 0.35% (T3) and 0.45% (T4)

Feed Consumption

Table (2) presents the mean feed consumption of native chickens across the five treatment groups (T0-T4) throughout their grower phase (6-12 weeks). By the end of the 12th week, chickens in the T3 group (receiving 0.35% isoleucine) exhibited the greatest feed intake compared to the other treatment groups. Statistical analysis determined that supplementing the diet with isoleucine had a significant impact on the feed consumption of these native chickens (P<0.05). Moreover, feed intake increased in the groups with feed supplementation of 0.35 and 0.45% isoleucine, whereas feed intake decreased in the groups with 0.15 and 0.25% isoleucine supplementation. The weekly feed intake of the birds is shown in Fig. (2).

In contrast to weight gain, feed intake increased from the T0 group to the T4 group (Figure 2). Feed intake in the T0 group was the same as that in the T1 and T2 groups (0.15 and 0.25% isoleucine supplementation, respectively). However, in the 0.35% isoleucine group (T3 treatment), feed consumption increased by 4.25% compared with T0 group. Similarly, for the T4 group (0.45% isoleucine), there was an increase of 3.55% in the feed intake.



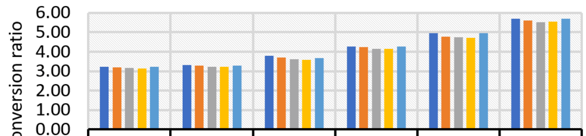
	7	8	9	10	11	12
T0	295.33	321.77	432.05	474.36	582.66	709.02
T1	314.13	332.61	452.25	510.68	576.14	675.82
T2	327.03	339.33	425.99	491.24	586.46	700.53
T3	336.15	346.46	438.54	508.39	569.18	736.11
T4	326.70	339.73	404.94	515.94	515.94	688.48

Week

Fig. 2: Evolution of feed intake in native chickens throughout the grower period

Feed Conversion Ratio (FCR)

As shown in Table (2), the highest feed conversion ratios were observed in the T0 (control feed) and T4 (0.45% isoleucine) groups, which differed significantly from those of the remaining treatments (p<0.05). No statistically significant difference in feed conversion ratio was found between the T2 and T3 groups. However, increasing the isoleucine level from 0.15% (T1 group) to 0.25% (T2 group) and subsequently to 0.35% (T3 group) resulted in a 2.00% improvement (decrease) in the feed conversion ratio (Figure 3). Similarly, between the T0 group (control feed) and the T1 group (0.15% isoleucine), the feed conversion ratio decreased by 2.56%.



	7	8	9	10	11	12
T0	3.22	3.32	3.78	4.26	4.95	5.71
T1	3.19	3.28	3.71	4.23	4.79	5.60
T2	3.15	3.23	3.60	4.15	4.75	5.53
T3	3.14	3.23	3.58	4.15	4.72	5.55
T4	3.22	3.29	3.67	4.26	4.94	5.70

Week

Fig. 3: The development of native chickens feed conversion ratio at the grower phase

Carcass Weight

Beyond achieving the highest body weight and optimal feed conversion ratio, treatments T2 and T3 also resulted in significantly greater carcass weights and percentages compared to the other groups (p<0.05; Table 2). Specifically, carcass weight increased by 4.70% in the T1 group (0.15% isoleucine) relative to the T0 control. This improvement continued, with the T2 group (0.25% isoleucine) showing a further 3.19% increase over T1. The peak carcass weight was observed in the T3 group (0.35% isoleucine) at 625.47±10.81 g/bird, representing a 4.45% increase compared to T1. Conversely, the T4 group (0.45% isoleucine) experienced a decrease in carcass weight of 3.74% when compared to the T3 group. A similar pattern was noted for carcass percentage, which generally rose from T1 through T3 before declining in the T4 treatment.

Ileal Morphometry

Ileum morphometries, specifically villus height, villus width and crypt depth, were measured (Figure 4). These parameters are indicators of health related to nutrient digestion and absorption. For 12-week-old native chickens, the average ileum villus height in the T2 and T3 groups was significantly greater ($P<0.05$) than in the other treatment groups (Table 2). Compared with the T0 group, the T1 group supplemented with 0.15% isoleucine showed a 3.09% increase in villus height. In the T2 group supplemented with 0.25% isoleucine, the villus height increased by 1.71%, which, along with that of the T3 group, was the highest villus height detected. In the 0.45% L-isoleucine supplemented group (T4 treatment), the villus height decreased by 2.56% compared to that in the T3 group.

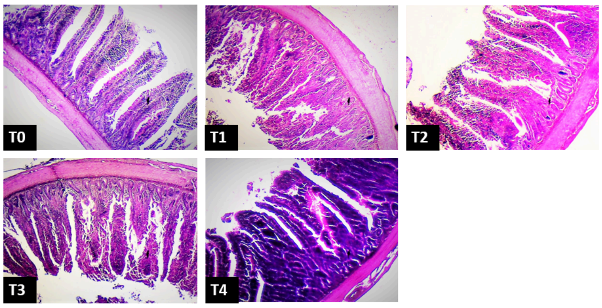


Fig. 4: Ileal morphometrics of native chickens at the grower phase

In contrast to villus height, the villus width of native chickens was not significantly affected by isoleucine levels. However, the differences in crypt depth were significant ($p<0.05$). The greatest crypt depth average was detected in birds in the T2 and T3 groups. Supplementation with 0.15% isoleucine (T1 and T2 groups) did not affect the depth of ileal crypts in native chickens. However, increasing the isoleucine content from 0.25-0.35% significantly increased the crypt depth by 5.77% compared with the T1 group. A similar response occurred in the T3 group (0.35% isoleucine) compared with the T2 group. In group T4, the crypt depth decreased by 4.29% compared with the T2 group.

Discussion

During the growth phase, isoleucine supplementation increased native chicken weight. However, if the amount was excessive, as in the T4 group, the chickens lost weight. The results obtained showed a tendency for the use of isoleucine supplementation to be up to 0.35% to achieve optimum performance. From the T0 group to the T3 group, the weight gain rose linearly; in the T4 group, it then declined.

The ideal ratio of isoleucine-lysine in broiler chickens is 0.69 (Samadi, 2012). The decreased of weight gain in T4 group might be due to the excess isoleucine in feed, thus affected the impacts weight loss. The development of native chickens is influenced by the balance of micronutrients, including amino acids (Lisnahan et al., 2017b). In previous research, Lisnahan et al. (2022) reported that in the starter phase, native chickens gained 49.35 g/bird/week when fed a diet rich in methionine, lysine, tryptophan, threonine and arginine but deficient in three branched-chain amino acids. Isoleucine is essential for muscle metabolism and for repairing damaged tissue (Ospina-Rojas et al., 2020; Attia et al., 2022). As illustrated in Figure (1), supplementing with isoleucine not only leads to increased body weight but also promotes faster growth. The findings suggest that isoleucine plays a role in nutrient balance, similar to the effects observed with methionine, lysine, threonine, tryptophan and arginine. Isoleucine shortage has traditionally been linked to weight loss and its consumption is balanced with that of other necessary amino acids, particularly lysine (Kidd et al., 2021; Lima et al., 2018). Similarly, excess isoleucine, such as in the T4 group, decreases amino acid concentrations in plasma and muscle and affects growth performance (Kop-Bozbay et al., 2021). However, this effect is better compared to a deficiency of isoleucine in the diet, such as in the T0 group.

Supplemented isoleucine levels above 0.35% led to observed weight loss. It is suggested that isoleucine consumption needs to correspond with the body's use of the Branched-Chain Amino Acids (BCAAs), valine and leucine (Kim et al., 2022b; Macelline et al., 2021). When supplied at excessive levels, these three BCAAs can antagonize each other (Ospina-Rojas et al., 2017). Isoleucine is necessary for tissue upkeep and growth (Kidd et al., 2021). When the isoleucine level in the feed is elevated, it raises the optimal dietary concentration needed for valine and leucine and this relationship is reciprocal (Holeček, 2018). This amino acid activates branched-chain ketoacid dehydrogenase, a complex enzyme key to the oxidative deamination of BCAAs (Ospina-Rojas et al., 2020). Therefore, an enhance in isoleucine causes an enhance in the oxidation of the other 2 BCAAs (Kim et al., 2022b; Goo et al., 2024).

Energy sufficiency may alter feed intake (Pesti and Choct, 2023). The metabolic energy was at the range of 3064-3081 kcal/kg, which met the required energy of the chickens (Table 1). In a plant-based diet, isoleucine is the next limiting amino acid after methionine, lysine and threonine, with arginine and valine becoming limiting subsequently (Attia et al., 2020; Ospina-Rojas et al., 2020; Selle et al., 2020)). Supplementing with isoleucine made it possible to further decrease the crude protein content in the feed by one percentage point (Ma et al., 2023; Oluwabiyi and Song, 2024). Ospina-Rojas et al. (2017) reported that isoleucine can reduce hunger while increasing body weight and feed efficiency. Rocha et al. (2013) report indicated that feed consumption did not significantly differ among broiler chickens receiving diets supplemented with 0.58–0.70% isoleucine.

Significant body weight changes and weight gain result in a low feed conversion ratio (Lisnahan et al., 2022). Isoleucine supplementation improved the feed conversion ratio (Figure 3). A high FCR reflects inefficient feed use, whereas a low FCR demonstrates high feed efficiency (Lisnahan et al., 2022; Lisnahan et al., 2023). In the present research, a variance in feed conversion ratio was noticeable in the seventh week. Compared with the other groups, the T2 and T3 groups had the best feed conversion ratios. The results exhibited that isoleucine supplementation of up to 0.35% is recommended for native chickens. If the concentration was further increased, the feed conversion was increased (T4 group).

When using isoleucine in feed, it is important to consider the proportion of other restricting amino acids, including lysine, to the other two BCAAs (Macelline et al., 2021; Ullah et al., 2022; Liu et al., 2023). As reported by Mello et al. (2012), the ratio of isoleucine-lysine in broiler chickens is 0.73. In this study, the use of 0.35 or 0.45% isoleucine in feed gave the best results, in contrast to the use of high (T4 group) or low (T0 and T1 groups) percentages of isoleucine. Based on the optimal amino acid contents in broiler chickens (Kidd et al., 2021; Liu et al., 2023; Ma et al., 2023), the T2 and T3 groups approached this ratio when added to the feed ingredients' isoleucine content. In the T4 group, the isoleucine percentage was excessive, resulting in low body weight, a high feed conversion ratio and a high ratio of isoleucine to valine and leucine. A balanced state of these three essential amino acids must be maintained in the diet (Holeček, 2018; Kop-Bozbay et al., 2021; Kidd et al., 2021; Kim et al., 2022a). Suppose the amount of isoleucine is enhanced, but valine and leucine are small. In that case, this will impact growth and even become antagonistic if one of these BCAAs is present in an excessive amount (Cappelaere et al., 2021; Goo et al., 2024). Mello et al. (2012) reported that broiler chickens aged 21-42 days required valine, isoleucine and leucine in a proportion of 1.00:0.83:1.58%.

Isoleucine is 2-amino-3-methyl-pentanoic acid (Adeva-Andany et al., 2017; Kim et al., 2022b). Isoleucine is susceptible to antagonism and enzymatic degradation in response to the addition of leucine and valine to the diet (Dimou et al., 2022; Kim et al., 2022b). The three BCAAs are structurally similar and are degraded first by Branched-Chain Aminotransferase (BCAT) and then by Branched-Chain A-Keto Acid Dehydrogenase complex (BCKD), which causes irreversible catabolism of coenzyme A compounds (Dimou et al., 2022). Stimulation of enzymatic activity by one BCAA leads to catabolism of other BCAAs (Goo et al., 2024; Holeček, 2018). Thus, excessive levels of individual BCAAs can lead to degradation and deficiency of other BCAAs present in lower concentrations (Brown et al., 2022).

Dimou et al. (2022) and Kim et al. (2022b) observed that excessive levels of leucine and to a lesser extent isoleucine, impaired chick growth and utilization of other BCAAs. Feed ingredients included in poultry diets have disproportionate levels of isoleucine compared to other BCAAs, which can lead to an imbalance in the BCAA ratio (Ospina-Rojas et al., 2020). Feeds with high levels of isoleucine may increase the antagonistic effect if not balanced by valine and leucine (Kidd et al., 2021; Kim et al., 2022b; Macelline et al., 2021). High levels of isoleucine with an imbalance of other AAs, especially lysine, can exacerbate AA degradation and interfere with muscle gain in chickens (Brown et al., 2022).

In this study, carcass weight and carcass percentage were positively linked with body weight and were affected by the isoleucine in the feed. Carcass weight showed an increasing trend from the T0 to T3 groups but fell in the T4 group. Likewise, carcass percentage improved progressively from the T1/T2 groups towards T3, subsequently declining in the T4 group. This response indicated that isoleucine is required at optimum level for carcass formation (Kidd et al., 2021; Liu et al., 2023). At levels above 0.35% isoleucine, the carcass weight reached its peak, after which the carcass weight decreased. The possibility of an excess of isoleucine in the feed must be balanced with other amino acids, including lysine or the 2 other BCAAs (Brown et al., 2022; Tavernari et al., 2012; Wise et al., 2021; Kidd et al., 2021; Attia et al., 2020; Goo et al., 2024).

The greatest muscles in chicken carcasses are the chest and thighs (Kidd et al., 2021; Lisnahan and Nahak, 2020). Agostini et al. (2019) reported that BCAAs are important mediators of muscle protein production. Agostini et al. (2019); Ospina-Rojas et al. (2020) reported that BCAAs account about 35% of the essential amino acids in the body protein. Supplementation of feed with these amino acids can affect muscle development in chickens (Kidd et al., 2021; Liu et al., 2023; Wise et al., 2021; Goo et al., 2024). Kidd et al. (2021) reported an isoleucine level of 0.73% for broilers during the growth phase. Isoleucine prevents muscular injury by providing muscles with more glucose, which is important for energy generation during physical exertion (Oliveira et al., 2023).

Dietary levels of isoleucine influence the ileal mucosa (Rocha et al., 2013; Lima et al., 2018; Liu et al., 2020). Villus height was greater in the 0.25 and 0.35% isoleucine groups than in the other groups. Similarly, crypt depth was greatest in the T2 and T3 groups, which was associated to body weight and feed conversion. Improvements in ileal morphometric characteristics lead to higher feed conversion ratios, indicating more efficient feed consumption (Frikha et al., 2014; Lisnahan and Nahak, 2020; Liu et al., 2020). Animals with higher intestinal mucosal cell renewal have more villi because of greater mitotic activity and hyperplasia (Liu et al., 2020). Cell desquamation in the intestinal lumen causes the intestinal mucosa to develop continually (Lisnahan et al., 2022; Lisnahan et al., 2023; Liu et al., 2020) and increasing nutritional intake can accelerate cell regeneration (Baurhoo et al., 2007; Frikha et al., 2014).

Chickens may need isoleucine and other important amino acids to maintain and build their ileal mucosa (Brown et al., 2022; Kidd et al., 2021; Ma et al., 2023; Macelline et al., 2021; Nie et al., 2018). The result is taller and broader villi, which allows digestive enzymes to work better and subsequently results in greater nutrient absorption. Likewise, the depth of the crypts indicates that a greater amount of nutrients is transported across the ileal walls for subsequent processing. Baurhoo et al. (2007) previously observed that enhanced growth of intestinal villi improves the efficiency of nutrient digestion and absorption and thus the dimensions of the ileal villi serve as a marker of livestock health. Furthermore, isoleucine, along with other BCAAs like valine and leucine, are essential amino acids known to be involved in the synthesis of proteins and other amino acid precursors (Ospina-Rojas et al., 2020; Kidd et al., 2021; Kim et al., 2022b; Kop-Bozbay et al., 2021; Zhao et al., 2024). A deficiency in isoleucine leads to reduced feed intake and growth and these negative effects are compounded by high levels of valine and leucine (Attia et al., 2022; Goo et al., 2024; Kidd et al., 2021; Liu et al., 2023).

Conversely, an excess of isoleucine, such as in the T4 group (0.45%), can cause antagonism with valine and leucine (Ospina-Rojas et al., 2020; Nie et al., 2018). Isoleucine contributes to protein synthesis, serves as a precursor to other amino acids and functions as a glucogenic amino acid in glucose metabolism (Nie et al., 2018; Neinast et al., 2019). Lisnahan and Nahak (2019) found that villus height, villus width and crypt depth influence the amount of intestinal mucosal surface area available for nutrient digestion and absorption, essential for body tissue growth and maintenance.

A correlation was observed between the height of the villus and the depth of the ileum crypt in the native chickens (Table 2). Enhanced villus height and crypt depth are connected with an increased villus surface area that absorbs nutrients into the circulation (Selle et al., 2020; Lisnahan et al., 2022). However, the proportion of villus height to crypt depth facilitates the absorption of nutrients (Yu et al., 2018; Abdulkarimi et al., 2019). Lisnahan and Nahak (2019) provided a more detailed account showing that the increases in villus height and crypt depth in the ileum of native chickens correspond to improved digestion and absorption functions resulting from an expanded absorptive surface. This serves as a manifestation of the body-wide nutrition transport system. Additionally, villus height could potentially be favorably linked to body weight gain, feed intake and feed efficiency (Lisnahan et al., 2017a; Lisnahan and Nahak, 2020; Lisnahan et al., 2022). The regulatory mechanisms of the intestinal mucosa remain poorly characterized. Evidence exists showing that amino acids can affect interactions between intrinsic afferent and primary extrinsic neurons (Liu et al., 2023; Ma et al., 2023; Zhu et al., 2025). They also impact the central nervous system by influencing the transcription and expression of genes related to amino acid metabolism (Wu, 2014; Toprak et al., 2021). Villi are the leading site for the dietary amino acids absorption (Lisnahan and Nahak, 2020). In the small intestine, amino acid transport is primarily mediated by amino acid transporters in intestinal epithelial cells (Frikha et al., 2014; Brown et al., 2022; Zhao et al., 2024; Zhu et al., 2025). This molecular transporter is mainly expressed on cellular membranes and plays an essential role in the cellular uptake of amino acids (Ma et al., 2023; Zhu et al., 2025).

The study has a significant contribution to the growth performance of native chickens. A limitation of this study is that it only obtained the optimum level of isoleucine but did not assess its potential to reduce protein usage in native chicken feed. Further research is needed to explore its impact on minimizing crude protein levels in feed formulations.

Conclusion

Based on the experimental findings and subsequent discussions, it can be concluded that supplementing native chickens' feed with 0.35% isoleucine during their grower phase (6-12 weeks) improved both their growth performance and ileal morphometry. The optimal ratio of isoleucine to lysine for native chickens at this growth stage was found to be 0.65.

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Authors' Contributions

Charles Venirius Lisnahan: Conceived the ideas and designed the study, collected the data, supervised the study, analyzed the data and drafted the manuscript.

Revised the manuscript. All the authors have read and approved the final manuscript.

Oktovianus Rafael Nahak and Lukas Pardosi: Designed the study, prepared the feed, performed the laboratory work and supervised the study. Revised the manuscript. All the authors have read and approved the final manuscript.

Ethics

This article is original and has not been published before. We inform you that all authors (corresponding author and co-authors) have read and approved this manuscript and there are no ethical issues involved.

Competing Interests

The authors declare that they have no competing interests.

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