RESEARCH ARTICLE

Growth Performance and Duodenal Morphometry of 1-6 Week Old Kampung Chickens Supplemented With L-Leucine

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Abstract: This study evaluated the effects of dietary L-leucine supplementation on growth performance and duodenal morphometry of Kampung chickens during the starter phase (1–6 weeks). A total of 600 one-week-old Kampung chickens were randomly assigned to five dietary treatments with six replicates of 20 birds each in a completely randomized design. The treatments consisted of a basal diet without L-leucine (T0) and diets supplemented with 0.50% (T1), 0.75% (T2), 1.00% (T3), and 1.25% (T4) L-leucine, selected to evaluate a graded range of leucine supplementation in relation to growth performance and intestinal development. Growth parameters (body weight, body weight gain, feed intake, and feed conversion ratio), carcass traits, and duodenal morphometry (villus height, villus width, and crypt depth) were measured. Data were analyzed using analysis of variance followed by Duncan's multiple range test. Dietary L-leucine supplementation significantly affected body weight, body weight gain, feed conversion ratio, carcass traits, and duodenal morphometry ($P<0.01$). Chickens receiving 1.00% L-leucine showed the best growth performance, characterized by higher body weight and weight gain, improved feed conversion ratio, and greater carcass yield compared with other treatments. This treatment also resulted in the highest villus height, villus width, and crypt depth in the duodenum, indicating enhanced intestinal development. These findings suggest that dietary supplementation with 1.00% L-leucine may improve growth performance and intestinal development of Kampung chickens during the starter phase and may provide useful information for optimizing feed formulation strategies in local chicken production systems.

Keywords: Growth, Kampung Chickens, Leucine, Intestinal Morphometry, Starter Phase

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Introduction

High protein content in poultry feed can increase feed costs, thereby reducing economic efficiency. Another consequence is environmental pollution resulting from excessive nitrogen excretion in the poultry house. To address this issue, much research has focused on developing low-protein chicken diets supplemented with crystalline or synthetic amino acids. [1, 2].

Previous research by [3] indicates that nutrient adequacy is not necessarily achieved through high crude protein levels in the diet, but rather through a balanced supply of essential amino acids, including Branched Chain Amino Acids (BCAAs). Importantly, the effective use of BCAAs depends not only on their sufficient inclusion but also on maintaining an appropriate balance among leucine, isoleucine, and valine [4].

The balance of essential amino acids, particularly Branched-Chain Amino Acids (BCAAs), namely valine, isoleucine, and leucine, in Kampung chickens' feed is important because an excess of one amino acid will impact the needs of the others. Other impacts include antagonism and impaired metabolism, growth, and feed efficiency. In addition to enhancing growth, the use of BCAAs (synthetic products) aims to reduce the use of expensive fishmeal and soybean meal [5]. Furthermore, the higher amino acid absorption rate reduces protein loss through excreta/feces, which contributes to ammonia pollution in the barn [6, 7].

BCAA supplementation can maintain competitive meat production while minimizing production costs and protein source volatility [8]. Previously, [9] reported that to achieve optimal body weight and feed efficiency in Kampung chickens, it is necessary to supplement the feed with isoleucine and valine at ratios of 0.81, 0.75, and 0.65 during the starter, grower, and pullet phases, respectively. However, the use of leucine in Kampung chickens has never been attempted, unlike in broilers or layers. Leucine functions to repair and build damaged muscles, improve and balance hormones, and play a role in glucose and fat metabolism [10]. Leucine supplementation in feed should be balanced with valine and isoleucine, especially in low-protein feeds.

In general, poultry producers formulate rations based on the recommendations in Nutrient Requirements of Poultry (NRC), particularly regarding the requirements for leucine and other BCAAs for broilers and laying hens. There are no standard recommendations for the use of leucine and the leucine-valine-isoleucine ratio in Kampung chickens. This research aims to improve the growth performance and production of Kampung chickens by developing feed formulations supplemented with varying levels of leucine. We evaluated a graded range of L-leucine supplementation levels (0.50–1.25%) above the basal diet to investigate their effects on growth performance and duodenal morphometry. This range was selected to represent moderate to relatively high levels of supplementation in order to identify the level that may optimize growth and intestinal development in Kampung chickens during the starter phase. We hypothesize that dietary L-leucine supplementation at various levels influences protein synthesis, thereby affecting the growth performance and duodenal development of Kampung chickens. Optimal duodenal growth and development are achieved at an optimal dietary leucine level, where no antagonistic interactions among BCAAs occur due to leucine deficiency or excess.

Materials and Methods

Investigate Duration, Place, Animals, and Feed Preparations

The experiment was performed from June to July 2025 in Kefamenanu, East Nusa Tenggara, Indonesia. The experiment using laboratory facility were conducted at the Laboratory of Agriculture, Science, and Health, University of Timor.

A total of 600 kampung chickens with an average body weight of 59.05 ± 0.41 g were randomly assigned to 30 pens with litter flooring. Each pen measured 1.5 x 1.2 x 0.8 m and was equipped with a drinker, a feeder, and a heating/lighting unit. The rations were formulated using yellow corn and rice bran (energy sources), soybean meal and fish meal (protein sources), calcium-phosphate (mineral source), a vitamin premix, and crystalline essential amino acids (DL-methionine, L-lysine HCl, L-threonine, L-tryptophan, L-arginine, L-valine, L-isoleucine, and L-leucine). All ingredients were mixed according to the treatment formulations and prepared in crumble form. The pens featured concrete floors covered with a 7 cm thick layer of a rice husk and lime mixture. Each pen was equipped with a 60-watt light bulb. To prevent disease, the pens were cleaned weekly. Additionally, the chickens were vaccinated with the Hitchner B1 vaccine at 3 days of age and the La Sota vaccine at 21 days of age.

Dietary Management and Feeding Duration

This study employed a fully randomized design with five treatments, six replicates, and twenty Kampung chickens in each replicate (Table 1). The treatment groups were as follows: T0: Without L-leucine (control); T1: 0.50% L-leucine; T2: 0.75% L-leucine; T3: 1.00% L-leucine; and T4: 1.25% L-leucine. L-leucine supplementation levels were adjusted by proportionally reducing rice bran in order to maintain the total diet at 100%. Because excessive dietary leucine can induce branched-chain

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amino acid antagonism, small adjustments in dietary valine and isoleucine were implemented during formulation to maintain an appropriate BCAA balance and avoid secondary deficiencies as leucine levels increased.

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

Ingredients	Treatments (%)				
	T0	T1	T2	T3	T4
Yellow corn	60.30	60.30	60.30	60.30	60.30
Rice brand	15.00	14.50	14.25	14.00	13.75
Fish meal	9.50	9.50	9.50	9.50	9.50
Soybean meal	9.50	9.50	9.50	9.50	9.50
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	0.40	0.40	0.40	0.40	0.40
L-arginine	1.00	1.00	1.00	1.00	1.00
L-valine	0.60	0.60	0.60	0.60	0.60
L-isoleucine	0.40	0.40	0.40	0.40	0.40
L-leucine	0.00	0.50	0.75	1.00	1.25
Dicalcium-phosphate	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Metabolized energy (kcal/kg-1)	3045.50	3036.40	3027.30	3018.20	3009.10
Crude protein (%)	17.50	17.50	17.50	17.50	17.50
Ether extract (%)	5.40	5.38	5.36	5.34	5.32
Ash (%)	6.72	6.72	6.72	6.72	6.72
Crude fiber (%)	4.65	4.65	4.65	4.65	4.65
Methionine	0.40	0.40	0.40	0.40	0.40
Lysine	1.00	1.00	1.00	1.00	1.00
Tryptophan	0.45	0.45	0.45	0.45	0.45
Threonine	1.00	1.00	1.00	1.00	1.00
Arginine	1.10	1.10	1.10	1.10	1.10
Valine	0.80	0.80	0.80	0.80	0.80
Isoleucine	0.65	0.65	0.65	0.65	0.65
Leucine	0.45	0.95	1.20	1.45	1.90
Calcium	1.50	1.50	1.50	1.50	1.50
Phosphorus	0.60	0.60	0.60	0.60	0.60

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), carcass weight, and villus height, villus width, and depth crypt. Growth performance parameters, including body weight, body weight gain, feed intake, and feed conversion ratio, were calculated using all birds within each replicate (20 birds per replicate; total 600 birds). For carcass and intestinal morphometry analysis, five birds per replicate (25% of the population) were selected based on body weights closest to the replicate mean to ensure representative sampling and minimize variation among individuals. These selected birds were used for carcass evaluation and duodenal histomorphometric measurements (villus height, villus width, and crypt depth). The final body weights of the birds were obtained on the last day after the birds had fasted for 12 hours.

Body weight gain was defined as the difference between the final and initial weights:

$$\text{Weight gain (g/bird)} = \text{final weight} - \text{initial weight}$$

The ratio of feed conversion (FCR) was defined as the feed intake (kg) divided by the total weight gain (kg):

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

Carcass weight is calculated by subtracting the weight of the viscera (excluding the lungs and kidneys), feet, head and neck, blood, feathers, and abdominal fat from the body weight. Carcass percentage is the ratio of carcass weight to body weight:

$$\text{Carcass percentage}(\%/\text{bird}) = \frac{\text{carcass weight}}{\text{body weight}}\ x\ 100\%$$

$$Carcass\ percentage(\%/bird) = \frac{carcass\ weight}{body\ weight}\ x\ 100\%$$

Measurements of Duodenum Morphology

The duodenal morphology was observed using a light microscope (Olympus Corporation Japan) at 40× magnification. The villus height, width and crypt depth of the duodenal from 6-week-old Kampung chickens were determined by employing the method described by [9].

Chicken Intestine Sample Preparation

Duodenal tissue samples were obtained by isolating segments of the small intestine. Approximately 2 cm sections of the duodenum were collected and immediately fixed in 10% buffered formalin for 24–48 h following the procedure described by [11] After fixation, tissues were washed and processed for histological examination [11].

Hematoxylin-Eosin Staining

Fixed tissues were dehydrated through a graded ethanol series (70%, 80%, 90%, and 95%), followed by clearing in xylene, and then embedded in paraffin using standard histological procedures. The paraffin-embedded tissues were sectioned using a microtome to obtain thin sections, which were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) for microscopic observation.

Imaging

Histological sections were examined using a light microscope (Olympus BX51) at 10× magnification, equipped with an Olympus DP12 imaging system. The resulting images were displayed on a JVC TM-H1750C monitor for analysis. Morphometric measurements of villus height, villus width, and crypt depth were obtained from digital micrographs. Prior to measurement, the imaging system was calibrated using a stage micrometer photographed at the same magnification, allowing conversion of pixel distances into micrometer units. For each sample, measurements were performed in triplicate on representative villi, and the mean value was used for statistical analysis.

Villus Height, Villus Width, and Crypt of Lieberkuhn Depth Measurements

The villus height and width and the depth of the crypts of Lieberkuhn were measured using a flat-screen computer with the Microsoft Office Picture Manager program at 40x magnification. To translate magnification values into length units (μm), the standard micrometer size (μm) was measured using a computer. The μm unit number was employed as a standard to measure the villus height and width and the depth of the crypt presented on the screen.

The experimental data were analyzed by employing a one way analysis of variance (ANOVA) based on a completely randomized design and Duncan's test using IBM SPSS Statistics 26 (IBM Corp., NY, USA). The statistical model was $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$. Prior to statistical analysis, the normality of the data (initial body weight) was assessed using the Shapiro Wilk test. The results showed that the data were normally distributed ($p = 0.604$; $p>0.05$). Homogeneity of variance among treatments was evaluated using Levene's test, which indicated that the data were homogeneous ($p>0.05$).

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Results

Body Weight and Weight Gain. The average body weight and weekly weight gain of starter-phase Kampung chickens are presented in Table 2. Statistical analysis showed that dietary L-leucine supplementation significantly affected body weight (P = 0.003). The highest average body weight was observed in treatments T3 (335.73±2.58 g/bird) and T2 (334.52±1.70 g/bird), which were significantly higher than those observed in treatments T1 (328.35±2.37 g/bird), T4 (328.62±2.04 g/bird), and T0 (320.52±3.07 g/bird).

Table 2: Performance of Kampung chickens at the starter phase (1-6 weeks)

Variables	Treatment				
	T0	T1	T2	T3	T4
Initial body weight (g/bird)	59.08±0.48	59.00±0.35	59.05±0.56	59.03±0.45	59.07±0.34
Body weight (g/bird)	320.52±3.07^{c}	328.35±2.37^{b}	334.52±1.70^{a}	335.73±2.58^{a}	328.62±2.04^{b}
Weight gain (g/bird)	261.44±3.41^{c}	269.35±2.54^{b}	275.47±1.62^{a}	276.67±2.25^{a}	269.55±2.01^{b}
Weight gain (g/bird/ week)	52.29±0.68^{c}	53.87±0.51^{b}	55.09±0.32^{a}	55.34±0.45^{a}	53.91±0.40^{b}
Feed intake (g/bird)	611.30±2.48	619.05±1.69	622.55±1.22	621.30±1.99	617.40±1.17
Feed intake (g/bird/ week)	122.26±2.48	123.81±1.69	124.51±1.22	124.26±1.99	123.48±1.17
FCR	2.34±0.02^{a}	2.30±0.01^{b}	2.26±0.01^{c}	2.25±0.02^{c}	2.29±0.01^{b}
Carcass weight (g/bird)	179.60±1.25^{c}	187.99±1.81^{b}	194.71±1.18^{a}	195.78±1.67^{a}	188.89±2.24^{b}
Carcass percentage (%/bird)	56.04±0.62^{c}	57.26±0.82^{b}	58.21±0.46^{a}	58.32±0.47^{a}	57.48±0.60^{b}
Villus height (µm)	1110.50±31.99^{c}	1159.33±36.34^{b}	1194.17±26.44^{ab}	1201.50±33.07^{a}	1170.67±20.87^{ab}
Villus width (µm)	242.67±11.94^{b}	254.50±5.96^{a}	259.33±3.20^{a}	260.00±3.90^{a}	254.67±5.20^{a}
Crypt depth (µm)	259.67±4.50^{b}	262.50±5.96^{b}	277.50±13.91^{a}	278.17±11.09^{a}	263.33±6.44^{b}
VH/CD	4.28±0.12	4.42±0.22	4.31±0.17	4.33±0.21	4.45±0.08

Note: Different superscripts in the same row indicate significant differences (P<0.05). T0: 0.00% L-leucine (control); T1: 0.50% L-leucine; T2: 0.75% L-leucine; T3: 1.00% L-leucine; T4: 1.25% L-leucine

Body weight in the T1 treatment (0.50% L-leucine supplementation) increased by 2.44% compared to the control treatment (T0). Body weight continued to increase in the T2 treatment (0.75% L-leucine supplementation) by 1.88% compared to the T1 treatment. When the L-leucine level was increased to 1.00% (T3), body weight remained relatively similar to that observed in T2. However, when the L-leucine level was increased to 1.25% (T4), body weight decreased by 2.12% compared to T3.

A similar trend was observed for body weight gain (Table 2; Figure 1). Statistical analysis showed that treatment significantly affected body weight gain (P = 0.001). The highest weight gain was observed in treatments T3 (55.34±0.45 g/bird/week) and T2 (55.09±0.32 g/bird/week). Body weight gain increased by 3.02% in T1 compared with T0, and by 2.26% in T2 compared with T1. The response in T3 was similar to that observed in T2. However, weight gain decreased by 2.58% in T4 compared with T3.

Feed Consumption. The average feed intake of starter-phase Kampung chickens is presented in Table 2 and Figure 2. Statistical analysis showed that dietary L-leucine supplementation did not significantly affect feed intake (P = 0.240). Feed intake ranged from 122.26±2.48 g/bird/week in the control treatment (T0) to 124.51±1.22 g/bird/week in treatment T2. Although slight numerical differences were observed among treatments, these differences were not statistically significant.

Feed Conversion Ratio. The Feed Conversion Ratio (FCR) of Kampung chickens supplemented with L-leucine is presented in Table 2 and Figure 3. Statistical analysis indicated that treatment significantly affected FCR (P = 0.009). The lowest FCR values were observed in treatments T3 (2.25±0.02) and T2 (2.26±0.01), which were significantly lower than those observed in treatments T1 (2.30±0.01), T4 (2.29±0.01), and T0 (2.34±0.02). Supplementation of 0.50% L-leucine (T1) reduced FCR by 1.71% compared with the control treatment (T0). Increasing the L-leucine level to 0.75% (T2) resulted in a further reduction in FCR of 1.74% compared with T1. When the L-leucine level was increased to 1.25% (T4), FCR increased by 1.78% compared with T3. The development of feed conversion ratio during the experimental period is presented in Figure 3.

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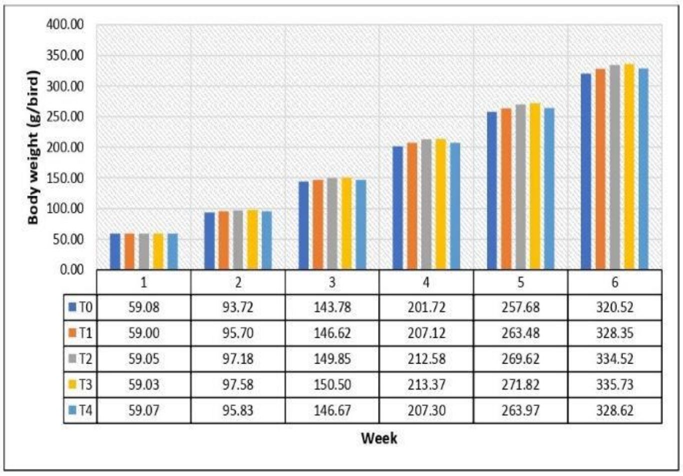

Fig. 1: Weight development of Kampung chickens during the starter phase

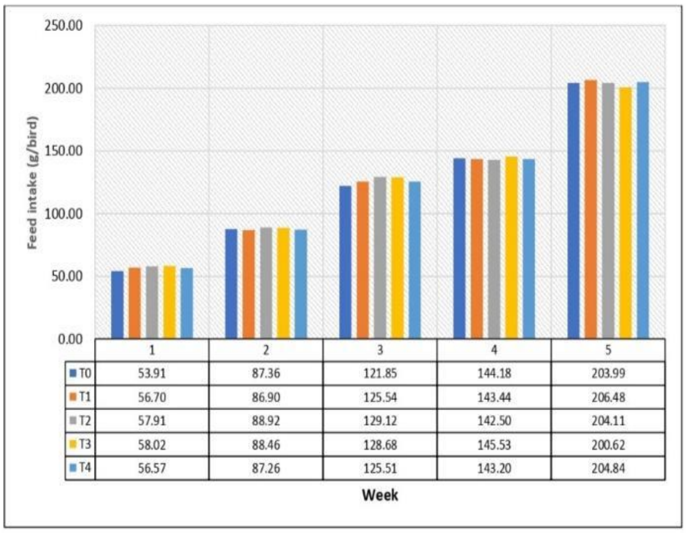

Fig. 2: Development of feed consumption of Kampung chickens in the starter phase

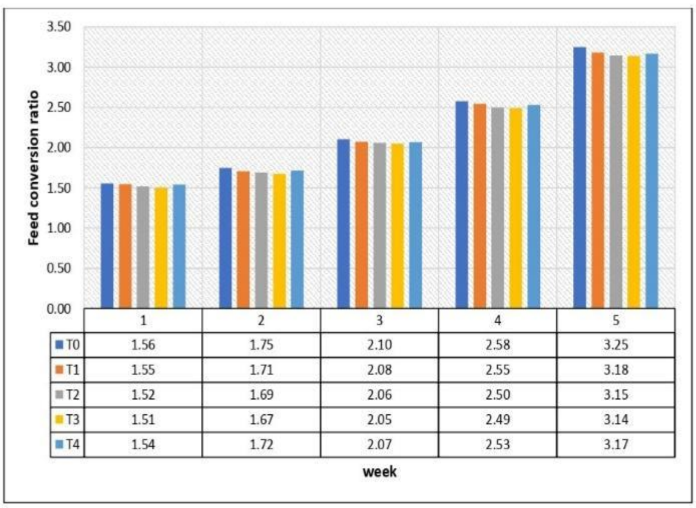

Fig. 3: Development of feed conversion of Kampung chickens in the starter phase

Carcass Weight and Percentage. The average carcass weight and carcass percentage of Kampung chickens at the end of the starter phase are presented in Table 2. Statistical analysis showed that treatment significantly affected carcass weight and carcass percentage (P = 0.003). Supplementation of 0.50% L-leucine (T1) increased carcass weight by 4.76% compared with the control treatment (T0). Increasing the L-leucine level to 0.75% (T2) resulted in an additional increase of 3.57% compared with T1. A similar response was observed at the 1.00% L-leucine level (T3), although this value was not significantly different from T2. When the L-leucine level was increased to 1.25% (T4), carcass weight decreased by 3.52% compared with T3. A similar trend was observed for carcass percentage, with the highest values observed in treatments T2 and T3.

Duodenal Morphometry. Duodenal morphometric parameters observed in this study included villus height, villus width, and crypt depth, as presented in Table 2. Statistical analysis showed that dietary L-leucine supplementation significantly affected villus height, villus width, and crypt depth (P = 0.002). Supplementation with 0.50% L-leucine (T1) increased villus height by 4.40% compared with the control treatment (T0). Increasing the L-leucine level to 0.75% (T2) increased villus height by 3.01% compared with T1. Villus height in T3 was similar to that observed in T2, while villus height in T4 was 2.57% lower than in T3. For villus width, supplementation with 0.50% L-leucine (T1) increased villus width by 4.87% compared with the control treatment (T0). Villus width increased numerically up to treatment T3, and slightly decreased in treatment T4, although these differences were not significant. For crypt depth, treatments T2 and T3 showed higher values than treatments T0, T1, and T4. Crypt depth increased by 5.71% in T2 and 5.97% in T3 compared with T1. Although villus height and crypt depth were significantly affected by dietary treatment, the VH:CD ratio did not differ significantly among treatments (P = 0.337).

Discussion

In this experiment, L-leucine supplementation levels were adjusted by proportionally reducing rice bran in order to maintain the total diet at 100%. This resulted in a slight decrease in calculated metabolizable energy across treatments (approximately 36 kcal/kg). However, this difference represents less than 1.5% of total dietary energy and was considered unlikely to significantly influence growth performance compared with the effect of L-leucine supplementation.

During the starter period, L-leucine supplementation increased the body weight of Kampung chickens. This weight gain occurred at levels of 0.50% to 1.00%. Conversely, excessive amounts (1.25%) resulted in weight loss. This is suspected because the amino acid leucine must be utilized in conjunction with the amino acids isoleucine and valine, which are branched-chain amino acids [12]. These three BCAA amino acids are antagonistic to each other if administered excessively or unbalanced [13].

Branched-Chain Amino Acids (BCAAs) play a crucial role as essential amino acids in poultry diets [14]. Beyond serving as protein building blocks, BCAAs are vital for stimulating protein synthesis to support the maintenance and growth of chicken body tissues [15, 16]. Among the three types of BCAAs, leucine plays a potent role in stimulating protein synthesis by activating the mechanistic target of rapamycin (mTOR) pathway in skeletal muscle [17, 18]. A major negative impact associated with BCAAs is amino acid antagonism; this phenomenon arises because the enzymes involved namely branched-chain aminotransferase (BCAT) and Branched-Chain Alpha-Ketoacid Dehydrogenase (BCKD) are required (in the muscle and liver, respectively) to simultaneously catabolize all three types of BCAAs [19]. Leucine plays a major role in the occurrence of BCAA antagonism, and increased Leucine levels can activate these enzymes, leading to the breakdown of excess. Leucine plays a central role in BCAA antagonism; high leucine levels can activate these enzymes and trigger the breakdown of excess leucine as well as the other two BCAAs, thereby causing valine and isoleucine deficiencies [10, 16]. Branched-chain amino acids imbalance caused by high levels of leucine in the diet can negatively impact muscle growth in birds, and the BCAA ratio may need to be adjusted if Leucine levels are high in diets with low CP [10]. The negative effect of BCAA antagonism caused by excessive Leucine is evident in T4 treatments.

Leucine is essential for the maintenance and growth of chickens [16]. High leucine levels in feed increase the optimal levels of valine and isoleucine, or vice versa [20], because this amino acid stimulates branched-chain ketoacid dehydrogenase, a complex enzyme involved in the oxidative deamination of three BCAAs [13]. Thus, increased leucine leads to increased oxidation of the other two BCAAs. BCAAs account for 35% of the essential amino acids in body protein [21] and are important regulators of muscle protein synthesis [22]. Supplementation with this amino acid can affect the diameter of chicken muscle fibers [23]. Leucine is a branched-chain amino acid that plays a role in physiological functions, including immunity and provides energy for poultry [24]. As a functional amino acid, it plays a role in growth, immunity, protein metabolism, fatty acid metabolism, and glucose transport [25].

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Branched-chain amino acids imbalance caused by high leucine levels in the diet can negatively impact muscle growth in poultry, and BCAA ratios may need to be adjusted if leucine levels are high in low-CP diets [4]. The negative effects of BCAA antagonism caused by excessive leucine were evident in T4 treatments.

Leucine is an essential component for chickens, playing a significant role in optimizing growth rates and strengthening the immune system [26]. Leucine also plays a role in regulating protein synthesis, particularly in muscle tissue [27]. When poultry consume feed supplemented with L-leucine, it directly stimulates the pathways responsible for building and repairing muscle [18]. This results in increased muscle mass, improved feed conversion ratios, and faster overall growth, all indicators of successful poultry farming.

Leucine also plays a crucial role in supporting the poultry immune system. It contributes to the health and function of immune cells, helping to strengthen the birds' natural defenses against common diseases and environmental stressors [28]. A strong immune system means lower morbidity and mortality rates, and a healthier flock [27].

In plant-based poultry diets, the Branched Chain Amino Acids (BCAAs), particularly valine and isoleucine, may become limiting after methionine, lysine, and threonine have been adequately supplied, depending on the dietary ingredient composition [9]. Supplementation with L-leucine, along with valine and leucine, allows for a further reduction of one percentage point in crude protein intake. Feed consumption is more influenced by energy sufficiency [5, 9]. Table 1 shows that metabolizable energy for all treatments has been sufficient for starter-phase chickens. If the feed is lacking energy, chickens will use energy sources from protein and body fat. Conversely, [21, 24] explain that leucine can suppress appetite but increase body weight and feed efficiency.

Feed conversion is the ratio of feed consumption to body weight gain. L-leucine supplementation reduces feed conversion by 0.50-1.00%, compared to feed without L-leucine supplementation (T0). This is expected in chicken farming for the success of the business [11]. As with body weight, a negative correlation also occurs in feed conversion. If L-leucine is excessive, such as in T4 (1.25%), feed conversion is high or inefficient. In broiler chickens, a dietary leucine level of 1.83% has been reported to impair the feed conversion ratio when valine and isoleucine are present at low levels [16]. This finding supports the notion that high dietary leucine concentrations should be accompanied by adequate levels of valine and isoleucine to maintain a proper branched-chain amino acid balance. The use of leucine in feed requires attention to the ratio to other limiting amino acids, such as lysine and the other two BCAAs [4]. These three essential amino acids must be in balance in the feed [2]. If the amount of leucine is increased but valine and leucine are low, it will impact growth and even become antagonistic if one of these BCAAs is excessive [9]. The ratio of valine, isoleucine, and leucine in the diet of Kampung chickens during the starter phase in this study was 0.80: 0.65: 1.45. [29] explained that broiler chickens aged 30-43 days require valine, leucine, and leucine with a ratio of 0.80: 0.72: 1.42.

The chicken breast and thigh are the muscle parts that contribute the most to the chicken carcass [30]. Muscle formation in chickens requires balanced protein or amino acids [16]. Feed supplementation with the amino acid leucine can affect the diameter of chicken muscle fibers [24]. The underlying mechanism is not independent of the roles of valine and isoleucine. Protein synthesis in cells is regulated by the mammalian target of rapamycin (mTOR) signaling pathway. The mTOR complex acts as a central regulator that integrates extracellular signals to either stimulate or inhibit protein synthesis [31]. Amino acid signaling activates mTOR, which subsequently stimulates downstream effectors such as S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-Binding Protein 1 (4EBP1) [32]. High-protein diets have been shown to activate S6K1 and enhance its activity in the breast muscle of broiler chickens [33]. Moreover, S6K1 activation is sensitive to dietary amino acid availability during the early feeding period, which can enhance mRNA translation in skeletal muscle [34]. Therefore, the balance among branched-chain amino acids, including leucine, valine, and isoleucine, plays a crucial role in regulating protein synthesis and growth performance in poultry.

Leucine also plays a role in growth, immunity, protein metabolism, fatty acid metabolism, and glucose transport [17, 35]. In the starter phase of 1.00 and 1.25% L-leucine levels, carcass weight is maximum. After that, carcass weight begins to decline. If the feed is deficient in leucine (T0), carcass weight is low and parallel with the chicken's body weight. The positive effect of leucine supplementation on carcass traits observed in this study may be attributed to its role in stimulating protein synthesis, particularly through the activation of translational machinery that is essential for cellular protein production. Leucine has been shown to enhance protein synthesis in skeletal muscle while simultaneously inhibiting protein degradation [26]. [22] demonstrated that leucine and its metabolites, α-ketoisocaproate and β-hydroxy-β-methylbutyrate, markedly increase muscle protein synthesis in neonatal pigs. However, they also reported that the stimulatory effect of leucine depends on the availability of other amino acids; leucine supplementation in protein- and energy-restricted diets increased mTOR pathway activation but did not enhance muscle protein synthesis. Similarly, [36] showed that dietary leucine levels of 17.3 and 20.3 g/kg stimulated the mTOR signaling pathway in neonatal chicks compared with 14.3 g/kg leucine. They concluded that higher dietary leucine levels increased the mRNA expression of mTOR, S6K1, and 4E-BP1 in breast muscle, thereby promoting protein synthesis.

A study by [25] demonstrated that leucine can shift energy partitioning from adipose tissue to muscle cells, thereby reducing energy storage in adipocytes and enhancing fatty acid utilization in muscle. Similarly, [16] reported that increasing dietary leucine levels (10, 12, 14, 16, or 18 g/kg) linearly decreased serum triglyceride and β-hydroxybutyrate concentrations in male Cobb-500 broiler chickens from 8 to 17 days of age. They concluded that low-protein diets tend to increase fat deposition in poultry carcasses, whereas supplementation with valine and leucine can improve carcass quality by reducing fat accumulation and enhancing lean tissue retention. Conversely, the possibility of excess leucine (T4) must be balanced with other amino acids, such as lysine or 2 BCAAs, namely valine and isoleucine. [37] stated that BCAAs are important regulators of muscle protein synthesis, and previously [38] reported that BCAAs contribute 35% of the essential amino acids in body protein.

Leucine is essential for the formation of body tissue. If a leucine deficiency is present in the feed, it must be supplemented in the form of crystalline or synthetic L-leucine. Conversely, if there is an excess of leucine in the feed, the excess must be balanced with other amino acids such as lysine or two other BCAA amino acids, isoleucine and valine [39]. Leucine, along with Branched Chain Amino Acids (BCAAs), including isoleucine and valine, is an essential amino acid for the growth and development of livestock, participating in the synthesis of proteins and precursors of other amino acids [40]. Leucine deficiency results in decreased feed intake and growth, and high leucine levels further exacerbate the consequences of isoleucine and valine deficiencies [12].

The duodenum, jejunum, and ileum, collectively comprising the small intestine, serve as the primary sites of nutrient digestion and absorption in livestock. [11]. Greater villus height and deeper crypts are essential for improving intestinal function in poultry, as they increase the absorptive surface area and enhance nutrient absorption. These parameters are also widely used as indicators of gut health, since lost villus epithelial cells are continuously replaced by proliferating cells originating from the crypts. An increase in crypt depth is often associated with enhanced cellular turnover and may occur in response to villus atrophy [41].

Digestive enzymes, including amylase, protease, and lipase, are primarily secreted from the pancreas and distributed and activated in the duodenum and jejunum of the small intestine, contributing to nutrient digestion and absorption in poultry [9]. The duodenum and jejunum are the main absorption sites of amino acids, including leucine [25].

In the present study, L-leucine supplementation significantly increased villus height and crypt depth, indicating enhanced intestinal development. The best response was observed at total leucine levels of approximately 1.20–1.45%, which is consistent with findings in broiler chickens. [40] reported that dietary leucine supplementation (1.37–2.20%) promoted intestinal development by increasing villus height, crypt depth, and mTOR gene expression. Similarly, [42] demonstrated that leucine supplementation improved growth performance by enhancing nutrient absorption and utilization, resulting in increased body weight gain and improved feed efficiency.

More recent findings by [3] showed that supplementation of leucine, valine, and isoleucine in low-protein diets increased the villus height to crypt depth ratio and upregulated insulin-like growth factor 1 (IGF-1), thereby enhancing the absorptive surface area of the intestine. In addition, leucine has been reported to strengthen intestinal tight junctions in fish [27] and improve jejunal structure in pigs [15]. However, excessive leucine levels may impair intestinal development, as intestinal growth was reported to be inhibited at high leucine concentrations (2.57%) [40], indicating the importance of maintaining an optimal level.

Branched-chain amino acids also serve as nitrogen donors for the synthesis of other amino acids through transamination, particularly glutamate and aspartate, which are important energy sources for the intestinal mucosa during intracellular protein turnover and nutrient transport [3]. Therefore, an imbalance in the ratio of leucine to isoleucine and valine may disrupt intestinal cell proliferation and overall gut function [43].

As a Branched-Chain Amino Acid (BCAA), leucine controls protein production and immune function in animals [14, 13]. In broiler chickens, leucine supplementation has been shown to improve growth performance by enhancing nutrient absorption and utilization, with proven effects including a 47% increase in Body Weight Gain (BWG) and a 3.1% increase in feed efficiency [42].

Furthermore, leucine has been shown to stimulate intestinal development by increasing villus height in the duodenum, jejunum, and ileum, as well as upregulating mTOR gene expression [40]. However, more recent evidence reported by [42] demonstrated that leucine supplementation significantly increased villus height and crypt depth in the duodenum and jejunum of broiler chickens at 14 and 21 days of age, whereas no significant effect was observed in the ileum, suggesting a segment-specific response to leucine supplementation. Beyond its effects on villus morphology, leucine has also been reported to strengthen intestinal tight junctions in fish [27] and to improve jejunal morphology and cell proliferation, enhance intestinal amino acid absorption through upregulation of amino acid transporter expression, and promote intestinal protein turnover in pigs [15].

Conclusion

Based on the experimental results and discussion, it can be concluded that dietary supplementation with 0.75% L-leucine represents the best-performing treatment level for improving growth performance and duodenal morphometry of Kampung chickens during the starter phase (1–6 weeks), corresponding to a total dietary leucine level of 1.20%. These findings suggest that increasing dietary leucine levels may serve as an effective nutritional strategy to enhance growth performance, improve intestinal development, and increase feed efficiency in Kampung chicken production systems, particularly under low to moderate protein feeding conditions

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Author’s Contributions

Charles Venirius Lisnahan: Conceptualization, funding acquisition, investigation, methodology, supervision, validation, writer original draft.
Steffanie MC Noah and Lukas Pardosi: Investigation, data curation, supervision, validation.
Theresia Nur Indah Koni: Investigation, data curation, validation.

Ethics

This manuscript has not been published elsewhere and is not under consideration by any other journal. We have approved this manuscript and agree to submit it to the American Journal of Animal and Veterinary Sciences. We declare that there is no conflict of interest.

Ethical Approval

The Animal Ethics Committee of the Department of Animal Science, Faculty of Agriculture, Science, and Health, Universitas Timor, has reviewed and approved the research procedures involving experimental animals under reference number 13/UN60.1/SR/2025.

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