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Effects of L-Isoleucine Supplementation in the Diet on the Growth Performance of Kampong Chickens at the Starter Phase (1-6 Weeks)

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ABSTRACT

This study aimed to determine the effect of L-isoleucine supplementation in the diet on the growth performance of Kampong chickens at the starter phase (1-6 weeks). This study was conducted at Kefamenanu, East Nusa Tenggara Province, Indonesia, from May to June 2023. A total of two hundred and fifty-week-old Kampong chickens with an average initial weight of 59.03±0.52 g were used in the study. The chickens were divided into five treatments with five replications based on a completely randomized design. The diet treatments were: T0 (control feed); T1 (T0 + 0.20% L-isoleucine, NRC, 1994 for broilers); T2 (T0 + 0.30% L-isoleucine); T3 (T0 + 0.40% L-isoleucine); and T4 (T0 + 0.50% L-isoleucine). The parameters observed were body weight, weight gain, feed intake, feed conversion ratio, carcass weight, and carcass percentage. The data obtained were analyzed using variance analysis and the Duncan test. The study result showed that the groups T3 and T2 had the highest body weight (330.76±1.29; 334.48±1.47 g/bird, respectively). The highest weight gain was in group T2 (275.54±1.39 g/bird/5 weeks). Group T3 had the highest feed intake (613.15±5.79 g/bird/5 weeks) and the lowest feed conversion ratio (2.22±0.02). The highest carcass weight was in groups T2 and T3 (189.95±1.17 and 189.32±0.65 g/bird, respectively), while the highest carcass percentage was in group T2 (57.43±0.47 %/bird). The statistical analysis indicated that body weight, weight gain, feed conversion ratio, and carcass weight were affected by the level of L-isoleucine ($p<0.05$). In contrast, feed intake and carcass percentage were not significant. It was concluded that supplementation with 0.40% L-isoleucine to the diet of Kampong chickens resulted in optimal growth performance at the starter phase (1-6 weeks).

Keywords: Carcass, Growth performance, Isoleucine, Kampong chickens, Starter phase

Introduction

Isoleucine is needed for the maintenance and growth of Kampong chickens. Feed made from yellow corn and soybean meal usually contains low levels of isoleucine, and this has an impact on the production of kampung chickens. The need for isoleucine is associated with increased leucine and valine, which are branched-chain amino acids (BCCA) (Lima *et al*., 2018a). These three amino acids must be in balance. Excess of one of these three amino acids results in antagonism. The isoleucine requirement for starter phase chicks, as recommended by the NRC (1994), is a standard requirement, and to optimize the requirement, it needs to be increased in chicken cultivation practices.

The use of essential amino acids such as isoleucine, which is commercialized, basically aims to reduce the use of feed protein and also reduce the negative impact of ammonia pollution in cages. Likewise, reducing feed protein will decrease the amount of isoleucine. For this reason, it is necessary to supplement with synthetic isoleucine. Isoleucine has the potential to limit low-protein diets for chickens that have been supplemented with lysine, methionine, and tryptophan (Duarte *et al*., 2015). Other previous studies also reported by Erwan *et al*. (2008) found that increasing dietary leucine by 0.5% at different levels of energy did not have a significant effect on feed consumption, weight gain, feed-to-gain ratio, or carcass characteristics but significantly increased carcass weight up to 9% in broiler chickens. Therefore, it is very relevant to determine which isoleucine is most likely to trigger the balance of other essential amino acids and determine the production performance of chickens.

It has previously been demonstrated that in most cases, in practical diets, isoleucine and valine often have the same limiting value as threonine, which is the third limiting amino acid after methionine and lysine (Duarte *et al*., 2015). Several research reports have discussed the need for digestible isoleucine for broiler chickens during the starter and grower periods (Corrent and Bartelt, 2011; Rocha *et al*., 2013; Holecek, 2018), but for Kampong chickens, there is absolutely no standard requirement. In practice, Kampong chicken cultivation has so far only followed the standards for broiler and laying chickens recommended by the NRC (1994). The aim of this research was to determine the need for isoleucine for the growth of Kampong chickens in the starter phase.

Materials and Methods

Experimental birds and management of experimental diets

This experiment was conducted in Kefamenanu, East Nusa Tenggara, from June to July 2023. Two hundred and fifty one-week-old Kampong chickens, with an average initial body weight of 59.03±0.52 g/bird, were used in this experiment. They were randomly assigned to the five diets in a completely randomized design with five replicates. The five treatments were T0 (control feed without supplementation of L-isoleucine); T1 (T0 + 0.20% L-isoleucine, NRC, 1994 for broilers); T2 (T0 + 0.30% L-isoleucine); T3 (T0 + 0.40% L-isoleucine); and T4 (T0 + 0.50% L-isoleucine).

The feed ingredients and nutrient composition of each treatment are shown in Table 1. Feed and water were offered ad libitum during the experiment.

Data collection and analysis

The parameters observed were body weight, weight gain, feed intake, feed conversion ratio, carcass weight, and carcass percentage. The data obtained were analyzed using variance analysis and the Duncan test (Steel and Torrie, 1995).

Results and Discussion

Body weight and body weight gain

The results of this experiment indicated that the body weight and weight gain of Kampong chickens at the starter phase were affected by treatments ($p<0.05$; Table 2). While the feed intake and carcass percentage were not affected, supplementation with 0.40% L-isoleucine (T3) yielded the highest body weight, body weight gain, and carcass weight. Treatment T0 has the highest feed conversion ratio or the lowest feed efficiency.

The average body weight of Kampong chickens at a starter phase fed by L-isoleucine was 326.52±6.63 g/bird in the 6th week, with weight gain at 267.49±6.80 g/bird/5th week. Supplementation with 0.40% L-isoleucine in the feed (T3) increased body weight by 4.87% compared to T0. Likewise, the increase is parallel for weight gain from T1 to T3. Supplementation with L-isoleucine generated better Kampong chicken performance since they are the third-limiting amino acids after lysine and methionine. Isoleucine plays a role in optimizing growth by balancing amino acids and protein in the feed. Isoleucine is a growth hormone stimulant that repairs muscle damage (Lima *et al*., 2018b). Additionally, isoleucine also stimulates growth and other metabolic functions in the chicken body (Corrent and Bartelt, 2011).

Based on the ideal amino acids, broiler chickens' ratio of isoleucine to lysine is 0.69% (Samadi, 2012). Excess isoleucine in feed impacts weight loss, as in T4. The balance of micronutrients, such as amino acids, affects the growth of Kampong chickens (Lisnahan *et al*., 2017).

In previous research, Lisnahan *et al*. (2022) reported that the weight gain of Kampong chickens was 49.35 g/bird/week in the starter phase when given feed supplemented with methionine, lysine, tryptophan, threonine, and arginine but lacking three branched-chain amino acids (BCCA). Isoleucine is important in muscle metabolism and overcoming damaged tissue (Ospina-Rojas *et al*., 2020). Isoleucine supplementation not only increases body weight but accelerates growth. This shows that nutrient balance is influenced not only

Table 1. Composition (%) and nutrient content (% dry matter) of experiment diets

Ingredients	Treatments (%)				
	T0	T1	T2	T3	T4
Yellow corn	65.40	65.20	65.10	65.00	64.90
Rice brand	15.00	15.00	15.00	15.00	15.00
Fish meal	8.00	8.00	8.00	8.00	8.00
Soybean meal	8.00	8.00	8.00	8.00	8.00
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	0.30	0.30	0.30	0.30	0.30
L-isoleucine	0.00	0.20	0.30	0.40	0.50
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})	3,136.33	3,128.93	3,125.23	3,121.53	3,117.83
Crude protein (%)	16.76	16.75	16.74	16.73	16.72
Ether extract (%)	5.23	5.22	5.22	5.21	5.21
Ash (%)	6.71	6.70	6.70	6.70	6.70
Crude fiber (%)	4.63	4.63	4.63	4.63	4.63
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
Isoleucine	0.20	0.40	0.50	0.60	0.70
Calcium	1.50	1.50	1.50	1.50	1.50
Phosphorus	0.60	0.60	0.60	0.60	0.60

Table 2. Performance of Kampong chicken at the starter phase

Parameters	Treatment				
	T0	T1	T2	T3	T4
Body weight (g/bird)	318.96±1.35^{c}	326.68±1.69^{b}	330.76±1.29^{ab}	334.48±1.47^{a}	321.74±2.00^{c}
Gain weight (g/bird/5 wk)	259.90±1.44^{c}	267.86±1.82^{b}	275.54±1.39^{ab}	262.46±1.53^{a}	262.46±2.10^{c}
Gain weight (g/bird/wk)	51.98±0.29^{c}	53.57±0.36^{b}	54.34±0.28^{ab}	55.11±0.31^{a}	52.49±0.42^{c}
Feed intake (g/bird/5 wk)	601.21±4.11	610.25±5.99	609.16±4.99	613.15±5.79	602.75±7.45
Feed intake (g/bird/wk)	120.24±0.82	122.05±1.20	121.83±0.10	122.63±1.16	120.55±1.15
Feed conversion ratio	2.31±0.01^{a}	2.28±0.02^{b}	2.24±0.02^{c}	2.22±0.02^{c}	2.30±0.03^{ab}
Carcass weight (g/bird)	180.22±1.00^{c}	185.84±0.64^{b}	189.95±1.17^{a}	189.32±0.65^{a}	180.48±0.86^{c}
Carcass weight (%/bird)	56.50±0.40	56.89±0.22	57.43±0.47	56.61±0.33	56.10±0.41

^{a,b,c} superscript on the same line indicates a significant difference (p<0.05).

by methionine, lysine, threonine, tryptophan, and arginine but also by isoleucine. Isoleucine deficiency is always associated with weight loss, and its use is balanced with other essential amino acids, especially lysine (Kidd *et al*., 2021; Lima *et al*., 2018).

Weight loss is seen at L-isoleucine levels above 0.35%. In its utilization, it is suspected that isoleucine must be followed by the amino acids valine and leucine, which are included in branched-chain amino acids (BCAA). These three BCCAs are antagonistic to each other if given in excess (Ospina-Rojas *et al*., 2017). Isoleucine is required for the maintenance and growth of tissues. If the isoleucine content in the feed is high, it increases the optimal levels of valine and leucine, or vice versa (Holecek, 2018; Holecek, 2022), because this amino acid stimulates the branched-chain ketoacid dehydrogenase, which is a complex enzyme involved in the oxidative deamination of three BCAAs (Ospina-Rojas *et al*., 2020). Thus, an increase in isoleucine causes an increase in the oxidation of the other two BCAAs.

Feed intake

The results of this experiment indicated that the feed intake of Kampong chickens at the stater phase was not affected by treatments (Table 2). Feed intake may be affected by energy adequacy. In Table 1, the metabolic energy is between 3,063.87 and 3,080.51 kcal/kg, which has met the needs of chickens. In the plant-based diet, isoleucine is the limiting amino acid after methionine, lysine, and threonine, before arginine and valine. L-isoleucine supplementation allowed a further one percentage point reduction in feed crude protein. Duarte *et al*. (2015) explained that isoleucine can suppress appetite but increase body weight and feed efficiency. Rocha *et al*. (2013) reported no significant increase in feed consumption when raising broiler chickens supplemented with 0.58–0.70% isoleucine.

Feed conversion ratio

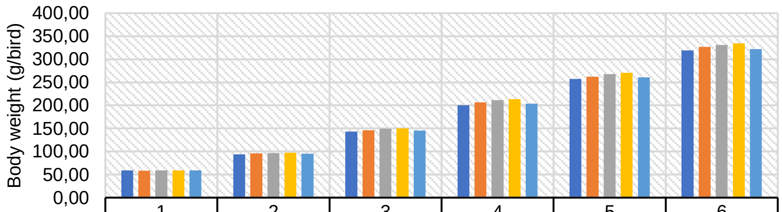
Isoleucine levels significantly affect the feed conversion ratio and are related to weight gain. The T0 and T4 had the highest feed conversion ratios, significantly different from the other treatments (p<0.05). There was no significant difference between the ratios for T2 and T3. However, when the L-isoleucine content increased from 0.20% (T1) to 0.40% (T3), feed conversion decreased by 2.63%. Duarte *et al*. (2015) reported that isoleucine at certain levels affects feed consumption, feed conversion, and the productive efficiency index during the growth phase. Significant body weight and weight gain impact a low feed conversion ratio. The feed conversion ratio is between feed consumed and body weight gain. If the feed conversion ratio is high, the feed consumed is less efficient, preferably with a low feed conversion ratio, indicating high feed efficiency.

The possibility of using isoleucine in feed requires attention to the ratio to other limiting amino acids, such as lysine and two other BCAAs. As reported by Rocha *et al*. (2013), the ratio of isoleucine to lysine in broiler chickens is 0.73. In this study, using 0.30% L-isoleucine or 0.40% isoleucine levels in feed gave the best results, in contrast to high (T4) or low (T0 and T1) isoleucine. Based on the ideal amino acids in broiler chickens, T2 and T3 approach this ratio when added to the feed ingredients' isoleucine content. At T4, isoleucine is excessive, resulting in low body weight and a high feed conversion ratio. Likewise, the ratio of isoleucine to valine and leucine. These three essential amino acids must be in a state of balance in the diet. If the amount of isoleucine is increased but valine and leucine are low, it will impact growth and even become antagonistic if one of these BCAAs is excessive. Corzo et al. (2004) explained that broiler chickens aged 30-43 days needed valine, isoleucine, and leucine with a ratio of 0.88%: 73%: 142%.

Carcasses weight and carcasses percentage

The results of this experiment indicated that the carcass weight of Kampong chickens at the starter phase was affected by treatments (p<0.05; Table 2), while the carcass percentage was not affected.

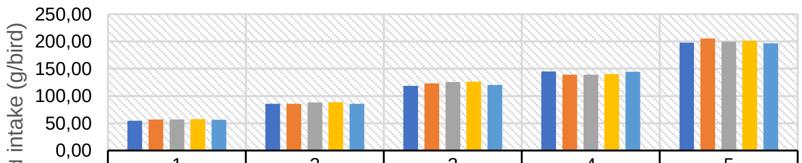
Chicken carcasses, especially breast and thigh muscles, depend on feed consumption, particularly amino acid balance. Isoleucine significantly affected Kampong chicken carcass weight. For the control feed (T0), the carcass weight was lower than that for the T2 (0.30% L-isoleucine) and T3 (0.40% L-isoleucine). This result indicated that dietary isoleucine can substantially affect chicken carcass components. Breast muscle thickness can indeed be increased significantly by increasing the feed isoleucine level. Lima *et al*. (2018a) reported that carcasses increased with an isoleucine diet, which correlated with live weight. Total breast meat and its amount and contribution to the carcasses increased with increasing isoleucine (Tavernari *et al*., 2012; Lima *et al*., 2018a). The use of isoleucine in Kampong chicken



	1	2	3	4	5	6
T0	59,06	93,78	143,40	200,68	257,44	318,96
T1	58,82	95,92	146,40	206,62	262,16	326,68
T2	59,06	97,02	149,32	211,50	267,52	330,76
T3	58,94	97,40	150,30	213,30	270,34	334,48
T4	59,28	95,62	145,42	204,00	260,54	321,74

Week

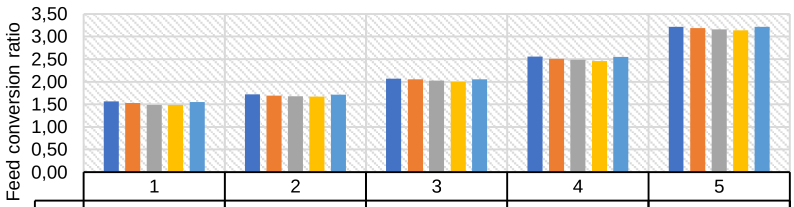
Figure 1. Development of body weight of Kampong chickens at the starter phase.



	1	2	3	4	5
T0	54,32	85,55	118,56	144,97	197,81
T1	56,69	85,32	123,45	139,13	205,66
T2	56,56	87,87	125,73	139,15	199,85
T3	57,23	88,34	126,07	140,22	201,29
T4	56,41	85,35	120,20	144,17	196,61

Week

Figure 2. Development of feed intake of Kampong chickens at the starter phase.



	1	2	3	4	5
T0	1,56	1,72	2,07	2,55	3,22
T1	1,53	1,69	2,05	2,51	3,19
T2	1,49	1,68	2,02	2,48	3,16
T3	1,49	1,67	2,00	2,46	3,14
T4	1,55	1,71	2,05	2,55	3,21

week

Figure 3. Development of feed conversion ratio of Kampong chickens at the starter phase.

feed requires attention to balance with lysine. Berres *et al*. (2010) reported that the ratio of isoleucine and lysine in broiler chickens was 78%.

Conclusion

Based on the results and discussion, it can be concluded that the feed for Kampong chickens needs to be supplemented with 0.40% L-isoleucine to achieve optimal growth. The optimal growth indicators are body weight, body weight gain, feed conversion, and carcass weight.

Conflict of interest

The authors declare that they have no competing interests.

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Author's contribution

CVL: Conceived ideas and designed the study, collected the data, supervised the study, analyzed the data, drafted the manuscript, and oral percentage at the ISTAP X, 2023. ORN and LP: Designed the study, feed preparation, and laboratory work, and supervised the study. All authors revised, read, and approved the final manuscript.

Ethical approval

The experiment protocol was approved by the animal ethics committee of the Animal Husbandry Study Program, Faculty of Agriculture, Science, and Health, University of Timor, Indonesia, on May 29, 2023, with a 15/UN60.1/SR/2023 number recommendation.

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