



Impact of a Low-Calorie Ration Supplemented with Several Amino Acids for Local Chickens on Ileum Histological Profile and Growth

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Abstract | The adequacy of dietary energy is crucial for optimizing the performance of local chickens, promoting optimal growth while minimizing abdominal fat deposits. Amino acids can influence growth and are linked to energy synthesis through metabolic feedback mechanisms. This study investigated the effects of a low-calorie diet supplemented with various amino acids (valine, serine, tryptophan, and arginine) on local chickens' ileum morphology and growth. Three hundred and fifty day-old Sentul chickens were randomly assigned to 35 experimental groups, each containing ten chickens. The study involved five different dietary treatments: P1: 2417 kcal/kg; P2: 2590 kcal/kg; P3: 2417 kcal/kg with amino acids; P4: 2590 kcal/kg with amino acids; and P5: 3178 kcal/kg. The chickens were fed these diets for eight weeks, from one day to eight weeks old. The results indicated that a low-calorie diet affected ($P < 0.05$) the morphology and growth of the ileum and its cells. However, the group receiving a low-energy diet of 2590 kcal/kg supplemented with amino acids experienced enhanced growth of ileum and villi cells.

Keywords | Energy, Amino acids, Local chicken, Ileum, Growth, Metabolism

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INTRODUCTION

The focus on ration management has become a significant concern for researchers and practitioners in animal nutrition. This is mainly because the essence of a ration lies not just in its nutrient content but also in its role as

a significant production input that constitutes a substantial portion of costs. Currently, standard rations (based on protein and energy iso) for various commercial and local chicken breeds are deemed inefficient (Mushawwir *et al.*, 2011). This inefficiency is particularly evident in accurately determining the ideal metabolic energy requirements (Mu-

hammad *et al.*, 2023; Manin *et al.*, 2024). A key indicator of this issue is the production of abdominal fat in both purebred and non-purebred chickens; under the currently recommended ration, this fat can account for as much as 7% of the slaughter weight (Kharazi *et al.*, 2022; Rahmania *et al.*, 2022).

Excess abdominal fat in chickens is undesirable. While it contributes to body weight, it is lighter than muscle mass in the same area, making it a discarded product for consumers.

The accumulation of abdominal fat indicates excessive energy consumption from the feed. Previous studies have reported several consequences of inappropriate calorie levels in the diet, including premature sexual maturity, which shortens the production period (Muller *et al.*, 2022), as well as increased production of free radicals (Mushawwir *et al.*, 2020, 2021a; Nurfauziah *et al.*, 2024). Additionally, excess fat can lead to increased body heat, making it difficult for poultry to release heat (Pazla *et al.*, 2023; Purwanti *et al.*, 2024) and does not support optimal muscle growth (Rahmania *et al.*, 2022; Mushawwir *et al.*, 2024). Most published studies have primarily focused on the impact of energy on growth (Waniapat *et al.*, 2015), and little research has explored low-energy diets that do not inhibit growth. However, many research reports have publicized that too-low energy levels cannot meet the metabolic energy requirements for maintenance and growth (Wang *et al.*, 2023). Other studies have shown too low growth rates, egg production (Purwanti *et al.*, 2024), and reduced immunity (Abouelezz *et al.*, 2022). Energy deficiency encourages the use of energy deposits (TAG and glycogen). To prevent this catabolism, amino acids can be added.

Amino acid feeding applications are part of a nutrigenomic strategy that involves modulating genes at the molecular level. Research indicates that energy-deficient cattle show a significant increase in the expression of the adenine monophosphate kinase (AMPK) and phosphatidylinositol-3-kinase (PI3K) genes (Aengwanich, 2007). Combining low-energy rations with amino acid supplementation prevents abdominal fat storage while supporting growth. Studies on ileum histology provide strong evidence for the benefits of integrating energy and amino acids to enhance the performance of local chicken breeds.

Formulating a better feed ratio by reducing the total metabolic energy content can achieve an optimal composition without hindering skeletal muscle growth (Hernawan *et al.*, 2027; Kamil *et al.*, 2020). Amino acids can specifically modulate energy signaling to act as nutrigenomic feeds. Amino acids such as valine, serine, and tryptophan can inhibit the signaling of the mTOR gene and prevent the signaling of lipid and carbohydrate catabolism. Utilizing nutrigenomic approaches to feed preparation is an effective

strategy to improve feed efficiency for local chickens. Numerous studies have shown that certain amino acids can influence genes associated with protein synthesis (Dudi *et al.*, 2023) and growth (Firmansyah *et al.*, 2024) and regulate genes involved in lipid and energy metabolism (Abouelezz *et al.*, 2022).

However, the effects of combining energy sources with amino acids on the morphometric histology of the ileum have not yet been reported.

MATERIALS AND METHODS

EXPERIMENTAL LIVESTOCK

The experimental animals used for this study were 350 Sentul chickens *in the starter phase* with an average body weight of 36.74 ± 2.83 g. Sentul chickens began to be treated at 0 to 8 weeks. Sentul chickens were kept in a multi-tiered battery cage measuring 1 m x 0.8 m equipped with feed and drink containers. Ten Sentul chickens were placed into each experimental flock, which was randomly selected. During the study, cage temperature and humidity were recorded at an average of 26°C and 78%, respectively.

EXPERIMENTAL RATION

Feed ingredients used during the study were obtained from a commercial poultry shop and then prepared based on a pre-calculated formulation. The rations were in the form of *crumbles* and were fed *ad libitum*. The table of food substance and metabolic energy content of feed ingredients (Table 1) formulations and experimental rations are shown in Tables 2 and 3.

SAMPLING AND ANALYSIS TECHNIQUE

Sampling was carried out. At the end of the rear, at the age of 8 weeks, the samples were taken randomly, with five chickens from each flock of experimental units, so the total samples taken were 175 chickens. A 3 mL blood sample was taken using a syringe inserted into the flank vein (vena pectoralis externa). Blood samples were placed into a *container containing* EDTA, shaken gently, and stored in the refrigerator before blood analysis to avoid clotting.

Histological observation and analysis were performed using the Hematoxylin-Eosin method. The ileum preparations were cut using a microtome with a thickness of 5 microns and then fixed with NBF 10 for 24 hours. Tissue processing began with dehydration, clearing, embedding, and blocking stages using a Leica TP 1020 tissue processor. Preparations were deparaffinized for 15 minutes using mineral oil and xylol at room temperature. HE staining was performed using standard procedures, followed by clearing the preparations with xylol. Microscopic observation was carried out using a binocular microscope.

Table 1: Food content and metabolic energy of research feedstuffs.

Feed Ingredients	EM	PK	LK	SK	Ca	P	Lysine	Methionine	Cysteine
	Kcal/kg%.....							
Yellow maize	3290	8.7	5.50	2.00	0.02	0.1	0.26	0.19	0.18
Fine bran	1655	11.38	6.65	12	0.12	0.21	0.81	0.26	0.40
Soybean meal	2217	43.55	3.48	6	0.22	0.29	2.70	0.66	0.67
Coconut meal	1591	22.44	12.97	15	0.20	0.20	0.48	0.32	0.30
Fish meal	3068	41.96	9.43	1.00	5.50	2.80	6.10	1.70	0.94
Bone meal	0	0.00	0.00	0.00	24.00	12.00	0.00	0.00	0.00
Coconut oil	8600	0.00	93.97	0.00	0.00	0.00	0.00	0.00	0.00
Premix	0	0.00	0.00	0.00	10.00	5.00	0.30	0.30	0.10

EM: Metabolic Energy; **PK:** Crude Protein; **LK:** Crude Fat; **SK:** Crude Fibre; **Ca:** Calcium; **P:** Phosphorus.

Table 2: Experimental ration formula (%).

Feed Ingredients	Experimental Ration Formula				
	P1	P2	P3	P4	P5
Yellow Corn	45.39	49.02	45.39	49.02	53.70
Rice Bran	14.18	7.55	14.18	7.55	1.50
Soybean Meal	14.18	19.61	14.18	19.61	30.04
Coconut Meal	17.02	15.69	17.02	15.69	0.19
Fish Flour	1.42	1.47	1.42	1.47	9.39
Bone meal	6.38	4.90	6.38	4.90	0.00
Coconut Oil	0.71	1.47	0.71	1.47	4.99
Premix	0.43	0.49	0.43	0.49	0.19
Total	100	100	100	100	100

of the ileum villi of experimental chickens. Based on the results of the current study, it seems that feeding rations with low energy levels (2417 - 2590 kcal/kg) does not seem to be a factor in reducing the morphometric growth of villi if amino acids are added to the rations. Similar results were also shown for villi height and width, indicating that higher doses of chitosan without glutathione induction resulted in lower villi morphometrics. The number, height, and width of villi remained optimal at P3 and P4 with ratio energy levels of 2417 and 2590, respectively, with the addition of amino acids.

Table 3: Nutrient content of food substances in experimental rations n.

Feed Ingredients	Experimental Ration Formula				
	P1	P2	P3	P4	P5
EM (Kcal/kg)	2417	2590	2417	2590	3178
PK (%)	16.16	17.78	16.16	17.78	21.91
LK (%)	6.94	7.42	6.94	7.42	9.7
SK (%)	6.03	5.41	6.03	5.41	3.18
Ca (%)	0.24	0.22	0.24	0.22	0.61
P (%)	0.22	0.22	0.22	0.22	0.42
Lysine (%)	0.79	0.88	0.79	0.88	1.54
Methionine (%)	0.24	0.22	0.24	0.22	0.61
Cysteine (%)	0.22	0.22	0.22	0.22	0.42
Addition:					
Valine (%)	0.00	0.00	0.25	0.25	0.00
Serine (%)	0.00	0.00	0.25	0.25	0.00
Tryptophan (%)	0.00	0.00	0.25	0.25	0.00
Arginine (%)	0.00	0.00	0.25	0.25	0.00

RESULTS AND DISCUSSION

EFFECT OF TREATMENT ON ILEUM MORPHOMETRICS OF LOCAL CHICKEN

Based on the study's results, [Table 4](#) and [Figure 1](#) show the effect of various doses of metabolic energy with the addition of several amino acids on the morphometrics of local chicken ileum, including the length of the ileum and the number, height, and width of ileum villi.

Based on [Table 4](#), the length of the ileum of broiler chickens in P1 (68.93 cm) is significantly different from the other treatment groups (P2 - P5), which are consecutively 71.25, 72.43, 84.23, and 80.15 cm. This result supports the statement of [Rahmania et al. \(2022\)](#) that the administration of chitosan with lower levels shows good anti-inflammatory. [Ahmed-Farid et al. \(2021\)](#) reported the effect of ration energy levels on the morphometric improvement of the ileum of Cihateup ducks. This study showed that the optimal ileum length of local chickens appeared to be 2590 kcal of ration energy with amino acid enhancement.

The analysis of variance also showed that the provision of energy levels with the addition of amino acids had a significant effect ($P < 0.05$) on the number, height, and width

The villi morphometrics of local chickens fed a low-calorie diet without added amino acids tended to be lower than those of chickens that received amino acid supplementation. Research conducted by [El-Attrouny et al. \(2021\)](#) indicated that low-calorie diets reduced lipase activity and fat absorption in the small intestine. Furthermore, other studies demonstrated decreased lipid absorption in the di-

gestive tract due to bile acid binding (Mushawwir *et al.*, 2021b; Tanuwiria *et al.*, 2022a, 2023).

ia *et al.*, 2022a; Purwanti *et al.*, 2024), rearing system and environmental stress (Tanuwiria *et al.*, 2022b).

Table 4: Morphometrics of ileum of local chicken with low energy diet supplemented with several amino acids.

Treat- ment	Parameters			
	Ileum length (cm)	Total Villi*	Villi Height (μm)*	Villi width (μm)*
P1	68.93 \pm 1.35 ^a	34.67 \pm 1.53 ^c	458.38 \pm 4.12 ^c	74.45 \pm 1.68 ^a
P2	71.25 \pm 2.87 ^b	47.00 \pm 2.00 ^a	526.78 \pm 7.11 ^a	81.42 \pm 1.44 ^b
P3	72.43 \pm 1.91 ^b	41.33 \pm 1.53 ^b	549.18 \pm 1.84 ^b	88.39 \pm 1.76 ^c
P4	84.23 \pm 2.88 ^c	43.33 \pm 2.52 ^{bd}	550.58 \pm 2.19 ^c	90.50 \pm 0.93 ^c
P5	80.15 \pm 2.01 ^b	45.33 \pm 1.53 ^{ad}	542.48 \pm 1.84 ^b	88.62 \pm 0.36 ^c

Description: *Occasion field of view with 4x magnification; ^{a,b,c,d} Different letter notations in the same column indicate differences (P<0.05).



Figure 1: Morphometric villi ileum of local chicken is fed a low-calorie diet with added amino acids.

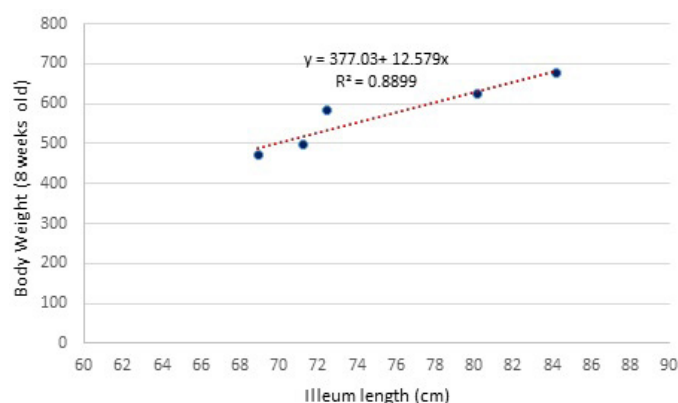


Figure 2: Relationship between ileum length and body weight of eight-week-old experimental chickens.

A study conducted by Wang *et al.* (2023) highlights that several factors influence the performance of small intestinal villi, including the type of feed substances and feed additives. Inconsistent findings regarding small intestine morphology may arise from variations in species and age (Selim *et al.*, 2021; Mushawwir *et al.*, 2023), as well as the metabolic energy of the ration. (Pazla *et al.*, 2023; Tanuwir-

Rations requiring intensive absorption cause the small intestine to expand its surface, expressed by the height and width of the intestinal villi. This causes the growth of the ileum and the growth of the chicken. The results of this study appear to show a close relationship between ileum growth and chicken body boot, with $R^2 = 0.8899$ or $r = 0.943$ (Figure 2).

Ileum growth The role of amino acids as regulators of growth proteins has been shown to increase the expression of proteins associated with gut tissue anabolism, thereby stimulating ileum morphometric improvement. This factor may be why the resulting ileum length and the number, height, and width of ileum villi remain optimal at appropriate doses. Amino acids will increase the intensive work of the ileum, which is the absorption intestine, thus encouraging the growth of the digestive tract to become longer. This is to the view of Petrilla *et al.* (2022) that rations require intensive absorption; the intestine expands the surface by thickening the intestinal wall or lengthening the intestine so that nutrients are absorbed by the intestine optimally. Other researchers have shown a strong relationship between gut length and its volume (Mushawwir *et al.*, 2021b) and its absorption capacity (Mushawwir *et al.*, 2023; Adriani *et al.*, 2024).

The low-calorie diet (P1) showed the lowest average villi growth (P<0.05) among all treatments (Table 4 and Figure 1). This result indicates that the intestine is less active, resulting in villi atrophy. Ration energy without adding amino acids causes lipogenesis inhibition, especially fat synthesis, a precursor to the growth of ileum tissue and villi. Low-energy feed increases digestion viscosity (Adriani *et al.*, 2021), so the feed digestion rate is faster. It will reduce nutrient contact with the small intestine during absorption (El-Attouny *et al.*, 2021), thereby reducing villi development (Adriani and Mushawwir, 2020). Another factor is that early feeding of low-energy diets causes inhibition of the expression of genes or proteins that regulate lipid metabolism (Setiawan *et al.*, 2024). As a result, villi growth and development are reduced (Wang *et al.*, 2023) and suppress the activity of the ileum in performing its function as an absorptive gut (Pertilla *et al.*, 2022).

The role of amino acids in signaling mTOR is crucial in inducing tissue growth in local chickens, even with low-calorie feeding (P3 and P4). The amino acids added in the experimental rations were growth-related amino acids, as reported by Mushawwir *et al.* (2024) that amino acids, especially glutamine, play an essential role in growth-related nutritional pathways and regulation of cell proliferation, preventing apoptosis, and even stimulating the synthesis

of specific proteins related to tissue growth (Adriani and Mushawwir, 2020; Muhammad *et al.*, 2023). In contrast, high-calorie feed (P5) promotes inflammation caused by high reduction-oxidation activity and increased free radicals.

EFFECT OF TREATMENT ON VILLI CELL PROFILE OF LOCAL CHICKEN ILEUM

Based on the study results, Table 5 and Figure 3 show the effect of giving various doses of ration energy with amino acid induction on the local chicken ileum villi cell profile, including the number of goblet cells, normal cells, apoptotic cells, and necrosis cells.

Table 5: Villi ileum cell profile of local chicken with low ration energy and amino acid addition.

Treat-ment	Parameters	Goblet Cell Count*	Normal Cell Count**	Number of Apoptotic Cells**	Number of Necrosis Cells**
P1		22.62±1.03 ^a	785.67±6.51 ^a	97.00±2.00 ^a	131.16±6.11 ^a
P2		23.68±0.62 ^b	815.67±7.09 ^b	74.00±4.36 ^b	100.33±3.06 ^b
P3		25.31±1.11 ^c	859.33±2.52 ^c	70.00±5.57 ^b	100.67±5.08 ^b
P4		27.11±1.01 ^d	877.67±5.51 ^c	42.67±4.04 ^c	69.67±2.69 ^c
P5		26.97±1.21 ^d	852.33±3.51 ^c	53.67±1.53 ^d	97.00±4.58 ^d

Description: *Once the field of view with 100x magnification; **Number of cells in 1000 cells; different letter notations in the same column indicate differences (P<0.05).

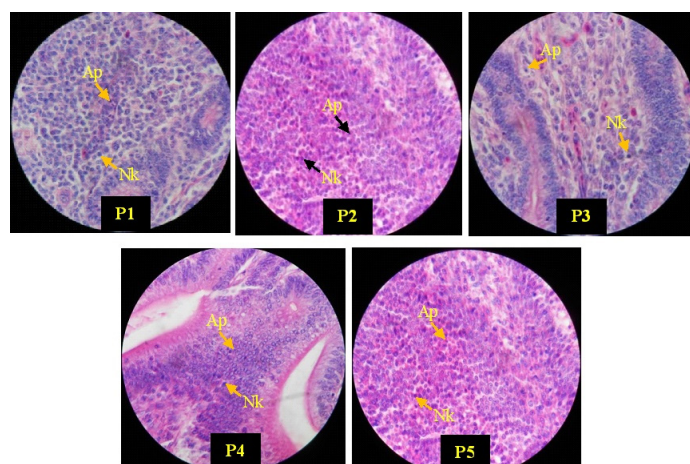


Figure 3: Cell Condition of Villi Ileum of Local Chicken with Low Ration Energy and Amino Acid Addition; Ap: Apoptosis; Nk: Necrosis.

The results of the analysis of variance showed that the provision of low-calorie rations, accompanied by the addition of amino acids, had a significant effect (P < 0.05) on the number of goblet cells, normal cells, apoptotic cells, and necrosis cells of ileum villi of broiler chickens. Based on Table 5 and Figure 3, it appears that the number of goblet cells of ileum villi of local chicken P0 (22.62 cells) was significantly (P<0.05) lower than the other treatments, namely

23.68; 25.31; 27.11; and 26.97 cells, respectively for P2-P5, respectively. Similar results were also observed for the number of normal cells, the number of apoptotic cells, and the number of necrosis cells.

Based on the results of the current study, it can be stated that the number of goblet cells and the number of normal cells do not appear to be influenced by the number of calories in the diet. Still, the role of amino acids is an essential factor for the profile of ileum cells. A decrease in cell death, both apoptosis and necrosis (Chatterjee *et al.*, 2015), even appears to decrease with the provision of amino acids, even with low-calorie rations (Setiawan *et al.*, 2024). Feeding a high-calorie ration without adding amino acids (P5) did not show the capacity for goblet cell growth and standard cell number nor its ability to prevent cell death.

One factor influencing goblet cell growth is the availability of cell growth precursors, especially lipids and amino acids. As shown in the previous discussion, amino acids can stimulate the synthesis of specific proteins, namely proteins that play a role in lipid regulation and tissue-forming proteins. Previous research has been reported by Wang *et al.* (2023) that amino acids can induce the expression of genes that play a role and the regulation of lipid metabolism, namely PPAR λ and SREBP-1 α genes and protein regulation genes, namely mTOR (Aritonang *et al.*, 2024).

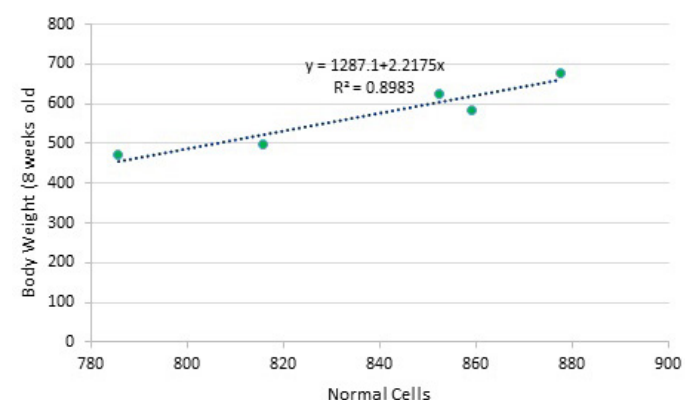


Figure 4: Relationship between the number of normal ileum villi cells and body weight of eight-week-old experimental chickens.

Conversely, low energy levels and amino acid deficiencies inhibit these genes, decreasing the activity of proteins involved in lipogenesis and protein synthesis. This reduces the regulation of endogenous fatty acids and cholesterol, essential precursors for cell growth. The amount of calories in the feed and adding amino acids significantly impact the number of healthy cells and chicken growth. Relationship between the number of normal ileum villi cells and body weight of eight-week-old experimental chickens (see Figure 4), is indicated by the closeness of the effect, R² = 0.8983 and the relationship r = 0.945.

Other studies have shown that low calories cause reactions with functional groups in the thiol group contained in various types of enzymes, including the enzymes acyl-CoA synthetase, phosphotransacetylase-CoA synthetase, and HMG-CoAR by inhibiting their activity (Rahmania *et al.*, 2022; Wang *et al.*, 2023; Mushawwir *et al.*, 2024). Amino acid deficiency accompanied by low energy also inhibits the rate of protein synthesis by reducing the activity of RNA polymerase (Muller *et al.*, 2022; Nurfauziah *et al.*, 2024), inhibition of the rate of protein synthesis triggers apoptotic cell death (Chatterjee *et al.*, 2015; Aritonang *et al.*, 2024) and decreased cell growth in villi and goblet cells (Mushawwir *et al.*, 2023, 2021b; Kamil *et al.*, 2020).

CONCLUSIONS AND RECOMMENDATIONS

The results showed that the addition of amino acids (valine, serine, tryptophan, and arginine), even with a low-calorie diet, was able to stimulate ileum and villous cell development, prevent cell death, and support the growth/body weight of local chickens. This indicates the ability of these amino acids to modulate the inhibition of catabolism of energy deposit sources.

Further investigation of the relationship between calories and lipid-related gene expression is needed to ascertain the genes involved. Similarly, amino acid modulation of growth-related gene expression needs to confirm the relationship between the two (calories and amino acid signaling).

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NOVELTY STATEMENT

Nutrient standards of local chickens are always equated with commercial chickens, whereas different growth characteristics should be an essential consideration for ration application for local chickens. This study shows the impact of low and high-energy diets and the role of amino acid modulation in low-calorie/energy diets on ileum morphometric growth and body weight growth.

AUTHOR'S CONTRIBUTIONS

The research and writing of this article have been undertaken by the authors with equal contribution and equity.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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