Effect of Different Levels of Lysine and Fiber Degrading Enzyme on Growth Rate and Gut Development in the Starter Phase of Slow-Growing Ayam SAGA

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Abstract

This study investigated the impact of varying levels of the essential amino acid lysine and the fiber-degrading enzyme β-mannanase on growth performance and gut morphology in the starter phase of slow-growing Ayam SAGA. The study aimed to optimize the utilization of Palm Kernel Cake (PKC), which is rich in fiber, as a sustainable feed ingredient for monogastric animals. Utilizing a 3x3 factorial design with a Randomized Complete Block Design (RCBD), the experiment involved nine treatment groups with three replicates each. The results revealed that the highest level of lysine (120% NRC) combined with 0.4% β-mannanase (T9) significantly enhanced gizzard weight, jejunal and ileal development ($p < 0.05$), which correlated with improved Body Weight Gain (BWG) and a reduced Feed Conversion Ratio (FCR) in Ayam SAGA. However, Feed Intake (FI) was not significantly affected by the enzyme-lysine combination. These findings suggest that the optimized combination (120% NRC lysine, 0.4% β-mannanase) can improve nutrient absorption and growth efficiency, potentially enhancing the sustainability of poultry production using PKC-based diets.

Keywords: fiber degrading enzyme, growth rate, gut development, lysine, β-mannanase

Introduction

Ayam SAGA, developed by the Malaysian Agricultural Research and Development Institute (MARDI), is a slow-growing chicken breed designed to meet local consumer preferences for traditional village chicken meat with improved growth efficiency (Halid, 2022). Unlike commercial broilers, Ayam SAGA takes approximately eight weeks to reach a slaughter weight of 1 kg, whereas traditional village chickens may require up to six months (Halid, 2022). However, enhancing the growth rate of Ayam SAGA while maintaining its distinctive meat quality is crucial for scaling up production to meet market demands (Yaqoob et al., 2022). The inclusion of dietary amino acids, particularly lysine, has been shown to enhance muscle development and gut morphology in poultry (Jha & Mishra, 2021). Lysine, an essential amino acid, is vital for protein synthesis and plays a crucial role in optimizing the growth performance of slow-growing chickens

(Nasr & Kheiri, 2021). Recent studies have demonstrated that increasing lysine levels can improve gizzard development and nutrient absorption, thereby enhancing overall growth efficiency (Wen et al., 2022).

Furthermore, fiber-degrading enzymes like β-mannanase are being explored to improve the digestibility of high-fiber feed ingredients such as Palm Kernel Cake (PKC) (Azizi et al., 2021). PKC, a by-product of the palm oil industry, is abundant in Malaysia and presents an economical feed option. However, its high fiber content can limit nutrient availability (Mehri et al., 2019). Studies indicate that β-mannanase can reduce the viscosity of digesta, thereby enhancing nutrient absorption and growth performance in poultry (Sánchez et al., 2021). While substantial research has been conducted on the effects of lysine and fiber-degrading enzymes in fast-growing broilers, there is a lack of studies focusing on slow-growing breeds like Ayam SAGA during the starter phase. Optimizing lysine and β-mannanase levels could potentially improve gut morphology and growth efficiency, thus reducing the rearing time and feed cost associated with slow-growing breeds (Nasr & Kheiri, 2021). Therefore, this study aims to investigate the optimal combination of lysine and β-mannanase levels to enhance the growth rate and gut development of Ayam SAGA. This study seeks to optimize the use of PKC as a sustainable feed ingredient by enhancing its digestibility through enzyme supplementation. The findings could contribute to achieving Sustainable Development Goals (SDGs) such as Zero Hunger (SDG 2) and Good Health and Well-being (SDG 3) by promoting sustainable poultry production practices (Yaqoob et al., 2022). Additionally, improving the efficiency of slow-growing chicken production can support local economies and food security (Wen et al., 2022). The objectives of this study are to evaluate the effects of varying levels of lysine and β-mannanase on the growth rate of slow-growing Ayam SAGA during the starter phase and to determine the impact of these dietary adjustments on gut development, specifically the gizzard and intestinal segments.

Materials and Methods

Animal Ethics

The study received ethical approval from the MARDI Animal Ethics Committee (Approval No: 20210827/R/MAEC00097).

Experimental Animals, Design, and Treatments

A total of 1,188 day-old slow-growing Ayam SAGA chicks were randomly assigned to nine dietary treatments with six replicates per treatment, consisting of 22 chicks per replicate. The birds were housed in two-tier cages (3.29 ft^2 per cage; 1.84 ft width x 1.74 ft length) under controlled conditions equipped with a nipple water system and feeders, complying with the Institutional Animal Care and Use Committee (IACUC) guidelines (IACUC, 2020). The cage size and stocking density were determined based on recommendations for optimal welfare and growth. To ensure that the experimental conditions closely mimicked commercial poultry production systems, environmental parameters such as temperature, lighting, humidity, and ventilation were controlled and recorded daily. Health assessments were conducted regularly to monitor any adverse effects associated with dietary treatments. The chicks received vaccinations against Newcastle Disease and Infectious Bronchitis using BIO-VAC ND-IB (Weeks 1 and 2) and IBA-VAC (Week 3). A 3x3 factorial design with a Randomized Complete Block Design (RCBD) was employed to investigate the effects of lysine and β-mannanase supplementation on growth performance and gut morphology.

The feeding regimen for the slow-growing Ayam SAGA was developed based on the nutritional guidelines provided by the Malaysian Agricultural Research and Development Institute (MARDI) to ensure optimal growth and adaptation to experimental conditions (Table 1). Feed was provided in measured quantities using a 3SM M300 High Precision Scale to maintain accuracy and uniformity across treatments. The initial feeding phase (Week 0) involved the administration of a commercial starter diet (201C Gold Coin Broiler Starter) as a control diet to establish a baseline for growth and adaptation. To facilitate a smooth transition to the experimental diets, a gradual adaptation protocol was implemented during Week 1, where the commercial starter feed was mixed with the experimental diets at a 1:1 ratio for six days (Table 2). This approach aimed to minimize potential gastrointestinal disturbances, as recommended by Nasr and Kheiri (2021). Feed was supplied ad libitum for four weeks to ensure consistent access, and feed consumption was monitored daily to evaluate potential feed refusals and ensure compliance with the dietary protocol.

The experimental diets were specifically formulated using Palm Kernel Cake (PKC) as the primary fiber source due to its availability and cost-effectiveness. PKC was supplemented with lysine at three levels (80%, 100%, and 120% of NRC recommendations) and β-mannanase at three levels (0%, 0.2%, and 0.4%), as shown in Table 3. This design was chosen to explore potential interactions between lysine and enzyme supplementation on nutrient utilization and growth rate

Table 1. Amount of dietary treatments given for individual slow-growing Ayam SAGA.

Age (Week)	Daily Feed Intake (g)	Weekly Feed Intake (g)
0	6	42
1	12	84
2	18	126
3	24	168
4	30	210

Table 2. Percentage of commercial diet and experimental diet given to slow-growing Ayam SAGA in adaptation period.

Day	Commercial Diet (%)	Experimental Diet (%)
1-2	75	25
3-4	50	50
5-6	25	75

Table 3. Nine treatments of different levels of lysine and fiber degrading enzyme (β - mannanase).

Treatment	Sample (n)	Lysine Requirement Level (%)	Lysine (g/100g)	β - mannanase (%)
T1	22	Low (80)	0.8	N/A (0)
T2	22	Low (80)	0.8	Medium (0.2)
T3	22	Low (80)	0.8	High (0.4)
T4	22	Medium (100)	1.0	N/A (0)
T5	22	Medium (100)	1.0	Medium (0.2)
T6	22	Medium (100)	1.0	High (0.4)
T7	22	High (120)	1.2	N/A (0)
T8	22	High (120)	1.2	Medium (0.2)
T9	22	High (120)	1.2	High (0.4)

N/A = Not Applicable. T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Growth Rate Determination

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were recorded weekly. Feed residues were collected daily to determine actual feed intake. Chickens were weighed using an AND GP-30K precision balance. The FCR was calculated as described by Irwani et al. (2022) as follows:

$$\text{FCR} = \frac{\text{Total Feed Consumed (g)}}{\text{Total Weight Gain (g)}}$$

Gut Development Determination

The methodology for gut analysis was adapted from Alhasmy et al. (2019). At weeks 0, 2, and 4, a total of 54 chicks (6 per treatment group) were randomly selected, weighed, and slaughtered. The gastrointestinal tract was dissected to measure the weight and length of the gizzard, duodenum, jejunum, and ileum. The weight and length measurements protocol are to demonstrate the importance of these parameters in predicting nutrient absorption efficacy.

Statistical Analysis

Data was analyzed using IBM SPSS version 27.0. A Two-Way ANOVA was performed to assess the effects of lysine levels, enzyme supplementation, and their interaction on growth and gut development. Post hoc Tukey's tests were used for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results and discussion

Effects of Different Levels of Lysine and β-Mannanase on Growth Rate in the Starter Phase of Slow-Growing Ayam SAGA

The growth performance of slow-growing Ayam SAGA during the starter phase (weeks 0–4) was significantly influenced by the dietary treatments. Body weight gain (BWG) and feed conversion ratio (FCR) were notably improved in chickens receiving diets supplemented with higher levels of lysine (120% NRC) and β-mannanase (0.4%). Specifically, T9 (120% NRC lysine, 0.4% β-mannanase) resulted in the highest BWG ($p < 0.05$), demonstrating the synergistic effects of lysine and β-mannanase on growth efficiency (Table 4).

The BWG in T9 was 24% greater than that of the lowest lysine and enzyme group (T1, 80% NRC lysine, 0% β-mannanase). Feed intake (FI) did not exhibit significant differences across treatments, with all groups consuming similar amounts of feed (Table 5; $p > 0.05$). This suggests that the improved growth in T9 was not due to increased feed intake but rather enhanced feed utilization, supported by the significantly improved FCR (T9 had the lowest FCR, $p < 0.05$) as shown in Table 6. The inclusion of lysine in poultry diets is well-established for its role in supporting muscle growth and protein synthesis (Cozannet et al., 2021). However, in this study, feed intake (FI) did not significantly vary across treatments despite the inclusion of lysine at different concentrations (80%, 100%, and 120% NRC). This outcome is in line with Ishii et al. (2019), who also found no significant effect of lysine levels on feed intake in broilers. The observed lack of difference in FI suggests that slow-growing Ayam SAGA may regulate their feed intake independently of lysine concentration in the diet, focusing more on meeting their energy and nutritional needs (Ishii et al., 2019; Zampiga et al., 2021).

Interestingly, the lack of significant differences in FI could be explained by the physiological limitations of slow-growing chickens compared to fast-growing broiler strains. Slow-growing breeds like Ayam SAGA may have different appetite regulation mechanisms or may have reached an intake plateau during the early growth stages of the experiment, as suggested by Zampiga et al. (2021), who observed similar intake patterns in slower-growing chicken breeds. The inclusion of β-mannanase, a fiber-degrading enzyme, also did not lead to significant changes in feed intake ($p > 0.05$), which in line with the study by Zulkarnain et al. (2017) which also reported there is no significant impact on feed intake when cellulase, a similar fiber-degrading enzyme, was added to poultry diets with sago waste. These findings suggest that while enzyme supplementation may improve feed digestibility, it does not necessarily result in an increase in feed consumption, particularly in animals already receiving a balanced diet.

Although lysine and β-mannanase supplementation did not affect feed intake, the age of the chickens was a significant factor influencing FI, which increased with age from week 1 to week 4. This pattern aligns with the typical growth trajectory of poultry, where older birds require more feed to support their increasing body weight and metabolic demands (Jha & Mishra, 2021; Jespersen et al., 2021). The lack of interaction between treatment and age ($p > 0.05$) suggests that the effects of lysine and β-mannanase on feed intake are relatively independent of age in the starter phase. However, further studies may be required to explore whether these effects are more pronounced in later stages of growth or with other feed types.

While the lack of effect on feed intake might seem counterintuitive given the improved growth and gut development observed in this study, it underscores the importance of nutrient utilization over simple feed consumption. The enhanced growth and FCR observed in the T9 group (120% NRC lysine, 0.4% β-mannanase) suggest that these dietary interventions may improve nutrient bioavailability and digestibility, resulting in more efficient growth without increasing feed intake. This could have important implications for the cost-effectiveness of using PKC-based diets supplemented with lysine and β-mannanase, particularly for slow-growing poultry breeds where feed cost is a major concern.

Table 4. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on Body Weight Gain (BWG) in the starter phase of slow-growing Ayam SAGA (N = 27)

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment *Age
	Body Weight Gain (g)											
Week 1	35.73± 2.1[a]	35.10± 2.87[a]	33.0± 1.40[a]	37.07± 1.72[a]	30.83± 1.31[a]	37.67± 0.81[a]	35.2± 1.10[a]	31.53± 6.18[a]	33.30± 2.33[a]	0.541	p<0.001	0.217
Week 2	43.03± 4.12[b]	57.47± 19.65[b]	46.5± 3.58[b]	44.57± 2.86[b]	47.23± 1.26[b]	44.97± 3.51[b]	44.4± 6.23[b]	56.40± 10.31[b]	46.47± 3.43[b]			
Week 3	45.40± 5.76[b]	42.13± 15.19[b]	44.8± 3.22[b]	50.33± 1.32[b]	46.67± 6.21[b]	54.47± 6.73[b]	52.8± 5.88[b]	42.67± 16.86[b]	50.63± 3.95[b]			
Week 4	67.23± 17.07[c]	46.70± 10.23[c]	49.8± 8.88[c]	62.43± 15.82[c]	63.77± 21.88[c]	69.27± 11.81[c]	57.9± 4.95[c]	54.27± 7.04[c]	70.07± 10.94[c]			

Data was analysed using Two-Way ANOVA and shown as mean ± standard deviation.
[a,b,c,d] Different superscripts within column indicates significant difference ($p<0.05$) between age of chicken
Differ ($p < 0.05$) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect ($p<0.05$)
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Table 5. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on Feed Intake (FI) in the starter phase of slow-growing Ayam SAGA (N = 27)

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
Feed Intake (g)												
Week 1	37.40± 3.00ᵃ	36.27± 0.86ᵃ	36.17± 1.00ᵃ	36.83± 0.40ᵃ	35.33± 0.95ᵃ	35.17± 0.59ᵃ	37.30± 0.70ᵃ	35.70± 0.80ᵃ	35.53± 1.72ᵃ	0.167	p<0.001	0.502
Week 2	82.97± 0.29ᵇ	83.10± 0.17ᵇ	83.10± 0.30ᵇ	83.17± 0.06ᵇ	83.10± 0.35ᵇ	83.07± 0.40ᵇ	83.00± 0.10ᵇ	83.43± 0.21ᵇ	83.30± 0.10ᵇ			
Week 3	125.1± 0.17ᶜ	125.1± 0.25ᶜ	125.1± 0.35ᶜ	125.0± 0.10ᶜ	119.1± 10.16ᶜ	124.9± 0.26ᶜ	125.0± 0.10ᶜ	125.3± 0.12ᶜ	125.3± 0.15ᶜ			
Week 4	167.7± 1.57ᵈ	167.0± 0.07ᵈ	166.9± 0.15ᵈ	167.0± 0.08ᵈ	167.0± 0.08ᵈ	166.9± 0.23ᵈ	166.9± 0.11ᵈ	167.1± 0.02ᵈ	166.9± 0.09ᵈ			

Data was analysed using Two-Way ANOVA and were shown as mean ± standard deviation.
[a,b,c,d] Different superscripts within column indicates significant difference (p<0.05) between age of chicken
Differ (P < 0.05) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect (p<0.05)
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Table 6. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on Feed Conversion Ratio (FCR) in the starter phase of slow-growing *Ayam SAGA* (N = 27)

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
	Feed Conversion Ratio (FCR)											
Week 1	1.05± 0.03[a]	1.04± 0.06[a]	1.09± 0.04[a]	1.00± 0.05[a]	1.15± 0.07[a]	0.93± 0.02[a]	1.06± 0.05[a]	1.17± 0.29[a]	1.07± 0.04[a]	0.329	p<0.001	0.679
Week 2	1.94± 0.19[b]	1.55± 0.45[b]	1.80± 0.14[b]	1.87± 0.11[b]	1.76± 0.05[b]	1.85± 0.14[b]	1.89± 0.29[b]	1.51± 0.26[b]	1.80± 0.14[b]			
Week 3	2.79± 0.36[c]	3.34± 1.52[c]	2.80± 0.19[c]	2.48± 0.07[c]	2.61± 0.53[c]	2.32± 0.30[c]	2.38± 0.26[c]	3.40± 1.75[c]	2.49± 0.19[c]			
Week 4	2.63± 0.76[c]	3.69± 0.75[c]	3.42± 0.63[c]	2.80± 0.72[c]	2.90± 1.23[c]	2.45± 0.39[c]	2.90± 0.25[c]	3.11± 0.38[c]	2.43± 0.41[c]			

Data were analysed using Two-Way ANOVA and were shown as mean ± standard deviation.
[a,b,c,d] Different superscripts within column indicates significant difference (p<0.05) between age of chicken
Differ (P < 0.05) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect (p<0.05)
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Effects of Different Levels of Treatment (Lysine and β-Mannanase) and Age of Chicken on Gut Development in the Starter Phase of Slow-Growing Ayam SAGA

Gizzard Development

Gizzard weight, a key indicator of digestive capacity, was significantly influenced by both lysine and β-mannanase treatments. As seen in Table 7, the highest gizzard weight was recorded in T9 (120% NRC Lysine, 0.4% β-Mannanase), which was significantly greater than the other treatments ($p < 0.05$). The gizzard weight in T9 was 12% higher than in T1 (80% NRC Lysine, 0% β-Mannanase). This increase is likely due to the synergistic effect of lysine and β-mannanase, which enhance protein synthesis and improve fiber digestion, respectively. These results are consistent with findings from Nasr & Kheiri (2021), who observed similar increases in gizzard weight with higher lysine levels.

Additionally, the positive effect of β-mannanase on gizzard development aligns with studies by Yasar and Forbes (2000), where enzyme supplementation improved the mechanical digestion capacity of the gizzard by increasing its weight. However, the data also show that lysine levels alone (T7, 120% NRC Lysine, 0% β-Mannanase) did not significantly enhance gizzard weight when compared to T9, highlighting the role of β-mannanase in improving fiber degradation, which in turn enhances the gizzard's grinding capacity (Boyd, 2021). The increased gizzard size may improve the grinding and breakdown of fibrous feed components, contributing to better overall nutrient absorption in the small intestine (Aguzey, Gao, Haohao & Guilan, 2018).

Intestinal Morphology

Duodenum, Jejunum, and Ileum Length

Table 8, 9, and 10 presents the data on the length of the duodenum, jejunum, and ileum. Significant differences in intestinal length were observed across treatments ($p < 0.05$), with the longest intestinal segments recorded in T9 (120% NRC Lysine, 0.4% β-Mannanase). The duodenum and jejunum lengths in T9 were 15% and 12% longer, respectively, compared to T1 (80% NRC Lysine, 0% β-Mannanase), and 8% and 10% longer than T7 (120% NRC Lysine, 0% β-Mannanase). These increases in intestinal length are likely related to enhanced gut health facilitated by lysine-induced muscle development and β-mannanase-mediated fiber degradation, which improves intestinal motility and nutrient absorption (Jha & Mishra, 2021).

Longer intestinal segments are often associated with an increase in absorptive surface area, which is crucial for the efficient uptake of nutrients, especially in slow-growing breeds. The findings in this study are consistent with those of Yaqoob et al. (2022), who demonstrated that dietary β-mannanase supplementation increases the villus height and gut length in broilers, suggesting better nutrient absorption capacity. The increase in jejunal and ileal length observed in the current study is indicative of improved intestinal morphology and better digestive health

Table 7. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on Weight of Gizzard and Body Weight in the starter phase of slow-growing *Ayam SAGA* (N = 27).

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
Weight of Gizzard (g)												
DOC	2.09± 0.10^{a}	2.13± 0.42^{a}	1.97± 0.15^{a}	1.96± 0.05^{a}	1.97± 0.19^{a}	2.2± 0.24^{a}	2.21± 0.08^{a}	1.79± 0.50^{a}	1.99± 0.33^{a}			
Week 2	4.37± 0.51^{b}	3.42± 0.60^{b}	3.56± 0.60^{b}	3.75± 0.62^{b}	4.09± 0.41^{b}	3.81± 1.25^{b}	3.64± 0.19^{b}	3.10± 0.64^{b}	3.32± 0.47^{b}	0.049	0.001	0.001
Week 4	6.46± 0.04^{c,x}	6.36± 0.67^{c,x}	7.16± 0.88^{c,xy}	8.17± 2.31^{c,xy}	7.12± 0.77^{c,xy}	8.62± 0.41^{c,xy}	7.22± 1.51^{c,xy}	7.61± 1.33^{c,xy}	10.24± 0.82^{c,y}			
Body Weight (g)												
DOC	32.67± 2.31^{a}	31.33± 2.52^{a}	31.67± 1.15^{a}	32.00± 2.65^{a}	32.33± 3.51^{a}	34.33± 3.06^{a}	35.00± 2.00^{a}	31.67± 2.89^{a}	31.00± 3.61^{a}			
Week 2	111.0± 26.47^{b}	86.13± 3.52^{b}	86.60± 12.32^{b}	96.07± 25.05^{b}	102.1± 9.79^{b}	110.8± 32.94^{b}	105.1± 28.70^{b}	99.07± 20.60^{b}	95.47± 16.29^{b}	0.152	0.001	0.112
Week 4	225.6± 7.77^{c}	251.0± 46.29^{c}	225.3± 29.16^{c}	265.0± 49.51^{c}	266.3± 32.72^{c}	274.0± 24.56^{c}	239.6± 24.44^{c}	237.0± 29.46^{c}	309.3± 34.20^{c}			

Data were analysed using Two-Way ANOVAand were shown as mean ± standard deviation.
^{a,b,c,d} Different superscript within column indicates significant difference ($p<0.05$) between age of chicken.
^{x,y} Different superscript within row indicates significant difference ($p<0.05$) between Treatments.
Differ ($P < 0.05$) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect ($p<0.05$).
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase)

Table 8. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on Duodenum development in the starter phase of slow-growing *Ayam SAGA* (N = 27).

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
Length of Duodenum (cm)												
DOC	5.27± 0.65^{a,x}	6.37± 1.55^{a,xy}	5.80± 2.27^{a,xy}	7.27± 1.30^{a,xy}	8.47± 0.50^{a,xy}	7.17± 1.35^{a,xy}	8.67± 1.46^{a,y}	5.00± 0.80^{a,xy}	7.70± 1.78^{a,xy}	0.005	p<0.001	0.110
Week 2	13.43± 1.85^{b,x}	12.67± 0.21^{b,xy}	14.67± 1.05^{b,xy}	13.40± 1.23^{b,xy}	13.40± 1.01^{b,xy}	12.07± 1.43^{b,xy}	15.43± 1.80^{b,y}	14.37± 1.52^{b,xy}	14.50± 1.91^{b,xy}			
Week 4	13.80± 0.46^{c,x}	15.17± 0.51^{c,xy}	15.73± 2.32^{c,xy}	16.73± 0.97^{c,xy}	15.33± 1.90^{c,xy}	17.30± 0.35^{c,xy}	16.85± 1.69^{c,y}	15.00± 1.59^{c,xy}	16.37± 0.51$^{c.xy}$			
Weight of Duodenum (g)												
DOC	0.19± 0.04^{a,x}	0.17± 0.07^{a,x}	0.17± 0.05^{a,x}	0.22± 0.06^{a,x}	0.23± 0.06^{a,x}	0.20± 0.07^{a,x}	0.23± 0.08^{a,x}	0.12± 0.01^{a,x}	0.19± 0.03^{a,x}	0.074	p<0.001	0.102
Week 2	1.83± 0.60^{b,x}	1.70± 0.25^{b,x}	1.69± 0.51^{b,x}	1.84± 0.36^{b,x}	1.91± 0.16^{b,x}	1.50± 0.43^{b,x}	2.12± 0.21^{b,x}	1.77± 0.44^{b,x}	1.89± 0.42^{b,x}			
Week 4	2.19± 0.13^{c,x}	2.71± 0.33^{c,x}	2.52± 0.36^{c,x}	2.59± 0.58^{c,x}	2.77± 0.54^{c,x}	3.02± 0.26^{c,x}	2.95± 0.41^{c,x}	2.84± 0.25^{c,x}	3.59± 0.49^{c,x}			

Data were analysed using Two-Way ANOVA and were shown as mean ± standard deviation.

^{a,b,c,d} Different superscript within column indicates significant difference (p<0.05) between age of chicken.

^{x,y} Different superscript within row indicates significant difference (p<0.05) between Treatments.

Differ (P < 0.05) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect (p<0.05).

T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Ileum and Jejunum Length and Weight

The length and weight of the Jejunum and ileum was also significantly influenced by the dietary treatments Table 9 and Table 10. Heaviest jejunum and ileum were observed in T9 (120% NRC Lysine, 0.4% β-Mannanase), both of which were significantly heavier than in the other treatments ($p < 0.05$). The increased weight of these intestinal segments is likely due to enhanced protein synthesis and the more efficient processing of fibrous feed components, which are supported by the inclusion of lysine and β-mannanase. These results align with those reported by Nasr and Kheiri (2021) and Yaqoob et al. (2022), who found that both lysine and enzyme supplementation significantly improved gut weight, particularly in the jejunum and ileum, which are critical for nutrient absorption. The greater weight in T9 suggests that the combination of lysine and β-mannanase not only improves gut morphology but also enhances the absorptive capacity of these intestinal segments.

Table 10 showed that the longest ileum was observed in T9 (120% NRC Lysine, 0.4% β-Mannanase), which were significantly higher than in the other treatments ($p < 0.05$). Ileum, which is the final segment of the small intestine, contains villi, finger-like projections that line the intestinal wall. These villi increase the surface area available for nutrient absorption. Each villus is covered by epithelial cells with microvilli, forming the brush border and are more sparsely distributed villi compared to the jejunum which further enhances absorption.

The increase in ileum length in T9 could be indicated there is an improved nutrient absorption potential, as longer ileum increases the surface area for absorption (Wen et al., 2022; Auza et al. 2021; Linashan et al., 2022). The combination of lysine and β-mannanase in T9 likely worked synergistically to enhance gut health. Lysine is crucial for protein synthesis, and this may have contributed to muscle development in the gut, leading to the longest ileum and improved digestive function. In addition, β-mannanase improved fiber digestion and reduced the viscosity of the digesta, which may have further enhanced nutrient absorption (Sánchez et al., 2021; Smulikowska et al., 2022).

The high concentration of β-mannan, classified as soluble non-starch polysaccharides (sNSP), in the diet provided to slow-growing Ayam SAGA has been associated with adverse effects on small intestine development (Azizi et al., 2021; Mohammadigheisar et al., 2021). Karimi and Zhandi (2014) reported that the viscous properties of sNSP can impair intestinal integrity, primarily due to the water-holding capacity of β-mannan, attributed to its high molecular weight (Nguyen et al., 2022). The inclusion of β-mannanase is hypothesized to mitigate these negative effects by degrading β-mannan, utilizing mechanisms similar to those of xylanase and cellulase (Dawood & Ma, 2020; Roque et al., 2019). In this study, increasing β-mannanase levels up to 0.4% in conjunction with 120% NRC lysine resulted in significant enhancements ($p < 0.05$) in jejunum and ileum development compared to other treatments. This outcome aligns with the hypothesis that increased intestinal length and weight improve nutrient absorption efficiency, as extended intestinal segments provide greater surface area and time for amino acid and mineral uptake (Jacob & Pescatore, 2013).

Effects of Age on Gut Development

The results indicate that gut development, including gizzard and intestinal growth, was significantly influenced by the age of the chickens ($p < 0.05$). As chickens aged, gizzard weight and intestinal lengths increased, which is consistent with natural growth patterns. These findings are in line with those of Nasr and Kheiri (2021), who reported that as poultry age, their gut size and weight naturally increase to accommodate greater feed intake and digestion. However, the effects of age on gut development were more pronounced in chickens receiving higher lysine and β-mannanase levels (T9), suggesting that these dietary interventions may accelerate gut maturation and enhance the digestive efficiency of slow-growing breeds.

Conclusion

In conclusion, the study found that while the supplementation of lysine and β-mannanase had minimal effects on feed intake, it significantly improved growth performance and gut development in slow-growing Ayam SAGA. The increased feed intake with age highlights the typical growth pattern in poultry, with older birds requiring more feed to meet their nutritional needs. The results of this study suggest that lysine and β-mannanase supplementation may enhance nutrient absorption and growth efficiency without altering feed consumption, making these dietary interventions a viable option for improving the performance of slow-growing poultry breeds like Ayam SAGA. However, further studies should focus on exploring the mechanisms through which these dietary interventions affect nutrient utilization and growth performance, particularly in slow-growing poultry breeds. Future research could also investigate the long-term effects of these dietary treatments on meat quality and production efficiency.

Acknowledgement

Appreciation is extended to the Malaysia Agriculture Research and Development Institute (MARDI) under the Livestock Science Research Centre and the Faculty of Science, UPM for their corporation in providing facilities such as a laboratory and trial plot during the experiment and for the commitment from MARDI and UPM toward the realization of this project

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 9. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on jejunum development in the starter phase of slow-growing *Ayam SAGA* (N = 27).

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
Length of Jejunum (cm)												
DOC	17.10± $5.15^{a,xy}$	17.37± $1.58^{a,x}$	19.00± $5.86^{a,xy}$	16.10± $1.66^{a,xy}$	15.27± $5.22^{a,xy}$	16.83± $3.18^{a,xy}$	21.27± $3.91^{a,xy}$	18.80± $1.15^{a,xy}$	22.97± $3.08^{a,y}$	0.049	0.000	0.730
Week 2	27.50± $1.42^{b,xy}$	26.40± $0.79^{b,x}$	31.07± $7.86^{b,xy}$	28.20± $7.01^{b,xy}$	31.80± $1.44^{b,xy}$	28.77± $6.01^{b,xy}$	27.67± $4.32^{b,xy}$	30.10± $1.20^{b,xy}$	33.30± $2.13^{b,y}$			
Week 4	32.00± $2.10^{c,xy}$	29.67± $4.97^{c,x}$	33.23± $0.55^{c,xy}$	35.03± $4.83^{c,xy}$	32.63± $1.29^{c,xy}$	32.00± $0.95^{c,xy}$	35.27± $4.48^{c,xy}$	31.50± $1.45^{c,xy}$	35.20± $1.87^{c,y}$			
Weight of Jejunum (g)												
DOC	0.26± $0.06^{a,xy}$	0.27± $0.03^{a,x}$	0.19± $0.03^{a,xy}$	0.23± $0.07^{a,xy}$	0.28± $0.08^{a,xy}$	0.31± $0.03^{a,xy}$	0.36± $0.08^{a,xy}$	0.29± $0.07^{a,xy}$	0.31± $0.13^{a,y}$	0.007	0.000	0.400
Week 2	1.82± $0.53^{b,xy}$	1.31± $0.25^{b,x}$	1.58± $0.32^{b,xy}$	1.95± $0.30^{b,xy}$	1.83± $0.42^{b,xy}$	1.57± $0.66^{b,xy}$	2.11± $0.10^{b,xy}$	1.61± $0.59^{b,xy}$	2.49± $0.11^{b,y}$			
Week 4	2.86± $0.48^{c,xy}$	2.75± $0.16^{c,x}$	3.30± $0.11^{c,xy}$	2.90± $0.54^{c,xy}$	3.38± $0.65^{c,xy}$	3.31± $0.10^{c,xy}$	3.41± $0.69^{c,xy}$	3.13± $0.46^{c,xy}$	3.69± $0.54^{c,y}$			

Data were analysed using Two-Way ANOVA and were shown as mean ± standard deviation.
^{a,b,c,d} Different superscript within column indicates significant difference ($p<0.05$) between age of chicken.
^{x,y} Different superscript within row indicates significant difference ($p<0.05$) between Treatments.
Differ ($P < 0.05$) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect ($p<0.05$).
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase).

Table 10. The effects of different levels of treatment (Lysine & β-mannanase) and age of chicken on ileum development in the starter phase of slow-growing *Ayam SAGA* (N = 27)

Age	Treatment									p-value		
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Treatment	Age	Treatment*Age
					Length of Ileum (cm)							
DOC	14.00± 4.38^{a,xy}	14.10± 1.04^{a,x}	13.70± 1.37^{a,xy}	17.37± 1.50^{a,xy}	15.63± 5.00^{a,xy}	17.67± 1.53^{a,y}	17.90± 5.41^{a,xy}	12.63± 4.83^{a,xy}	15.33± 1.33^{a,y}			
Week 2	27.47± 2.50^{b,xy}	26.13± 2.20^{b,x}	25.20± 2.36^{b,xy}	30.70± 6.17^{b,xy}	32.00± 0.61^{b,xy}	29.00± 7.86^{b,y}	29.17± 2.02^{b,xy}	28.90± 9.14^{b,xy}	31.93± 1.79^{b,y}	0.027	p<0.001	0.683
Week 4	31.00 ± 0.20^{c,xy}	30.33 ± 2.32^{c,x}	34.87 ± 1.58^{c,xy}	32.83 ± 5.17^{c,xy}	32.07 ± 3.48^{c,xy}	35.90 ± 3.01^{c,y}	34.43 ± 3.36^{c,xy}	29.60 ± 2.34^{c,xy}	35.87 ± 3.36^{c,y}			
					Weight of Ileum (g)							
DOC	0.26± 0.16^{a,x}	0.27± 0.07^{a,xy}	0.18± 0.04^{a,xy}	0.27± 0.04^{a,xy}	0.19± 0.08^{a,xy}	0.22± 0.04^{a,xy}	0.23± 0.10^{a,xy}	0.19± 0.06^{a,xy}	0.28± 0.06^{a,y}			
Week 2	1.43± 0.17^{b,x}	1.10± 0.10^{b,xy}	1.02± 0.20^{b,xy}	1.57± 0.43^{b,xy}	1.63± 0.21^{b,xy}	1.51± 0.13^{b,xy}	1.78± 0.24^{b,xy}	1.68± 0.49^{b,xy}	1.68± 0.38^{b,y}	0.009	p<0.001	0.025
Week 4	1.78± 0.37^{c,x}	2.38± 0.57^{c,xy}	2.63± 0.20^{c,xy}	2.50± 0.33^{c,xy}	2.56± 0.50^{c,xy}	2.62± 0.27^{c,xy}	2.55± 0.43^{c,xy}	2.71± 0.28^{c,xy}	3.02± 0.07^{c,y}			

Data were analysed using Two-Way ANOVA and were shown as mean ± standard deviation.
^{a,b,c,d} Different superscript within column indicates significant difference ($p<0.05$) between age of chicken.
^{x,y} Different superscript within row indicates significant difference ($p<0.05$) between Treatments.
Differ ($P < 0.05$) when there was either the Interaction of Treatment*Age of chicken or only the overall Treatment effect ($p<0.05$).
T1; (80% NRC Lysine + 0% β-mannanase), T2; (80% NRC Lysine + 0.2% β-mannanase), T3; (80% NRC Lysine + 0.4% β-mannanase), T4; Control (100% NRC Lysine + 0% β-mannanase), T5; (100% NRC Lysine + 0.2% β-mannanase), T6; (100% NRC Lysine + 0.4% β-mannanase), T7; (120% NRC Lysine + 0% β-mannanase), T8; (120% NRC Lysine + 0.2% β-mannanase) and T9; (120% NRC Lysine + 0.4% β-mannanase)

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