Research Article

Effects of Probiotic on Performance, Carcass Quality and Intestinal Morphology of Broiler Fed with Different Levels of Palm Kernel Meal

Mirnawati Mirnawat[1]*, Harnentis Harnentis[1], Gita Ciptaan[1], Zurmiati Zurmiati[1], Anifah Srifani[2], Gusri Yanti[3], Annisa Rahma Iryos[4]

[1]Department of Animal Feed and Nutrition, Faculty of Animal Science, Universitas Andalas, Padang 25175, Indonesia; [2]Post-Doctoral of Research Centre for Animal Husbandry, National Research and Innovation Agency (BRIN), Cibinong Science Centre, Cibinong, Bogor, Indonesia; [3]Department of Agricultural Extension, Faculty of Social, Science and Education, Prima Nusantara Bukittinggi University, Bukittinggi 26122, Indonesia; [4]Postgraduate student of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Andalas, 25175, Padang, Indonesia.

Abstract | Palm kernel meal (PKM) is a potential feed for poultry but is limited by its relatively high cellulose and mannan content. Supplementation with probiotics, particularly lactic acid bacteria that produce cellulase and mannanase enzymes, is used to overcome these limitations. The aim of this study was to evaluate the effect of introducing palm kernel meal into the diet of broiler chickens, with probiotics (combination of *Lactobacillus fermentum* and *Bacillus subtilis*) on performance, carcass quality and intestinal morphology of broiler. This study used 162 broilers of the Cobb CP 707 strain. The research was conducted using an experimental Completely Randomized Design (CRD) with a 3×3 factorial arrangement and three replications. Factor A (*L. fermentum* and *B. subtillis* dose) consisted of A1 (No Probiotic), A2 ($1.21x10^{12}$ CFU/ml), and A3 ($1.21x10^{14}$ CFU/ml). Factor B (Percentage of PKM) consisted of B1 (0% PKM), B2 (25% PKM), and B3 (30% PKM). The parameters observed were performance (feed intake, body weight gain and FCR), carcass quality (carcass percentage, abdominal fat percentage and thigh meat cholesterol) and intestinal morphology of broiler. The results show that PKM can be included up to 30% in the diet when supplemented with probiotics at a dose of 1.21×10^{14} CFU/mL, resulting in significant improvements ($P < 0.05$) in performance, carcass quality, and intestinal morphology. In conclusion, the use of PKM up to 30% in broiler feed accompanied by the administration of probiotics at a dose of $1.21x10^{14}$ CFU/mL can improve broiler performance, carcass quality and intestinal morphology.

Keywords | Broiler, Cellulase, Mannanase, Probiotic, Palm Kernel Meal, Performance

Received | January 03, 2026; **Accepted** | April 02, 2026; **Published** | June 29, 2026
***Correspondence** | Mirnawati Mirnawati, Department of Animal Feed and Nutrition, Faculty of Animal Science, Universitas Andalas, Padang 25175, Indonesia;
Email: mirnawati@ansci.unand.ac.id
Citation | Mirnawat M, Harnentis H, Ciptaan G, Zurmiati Z, Srifani A, Yanti G, Iryos AR (2026). Effects of probiotic on performance, carcass quality and intestinal morphology of broiler fed with different levels of palm kernel meal. J. Anim. Health Prod. 14(3): 934-944.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.2.934.944
ISSN (Online) | 2308-2801

INTRODUCTION

Feed constitutes the largest cost component in livestock production systems, accounting for approximately 60–80% of total production expenses. Therefore, strategic selection of feed ingredients is a key determinant of production efficiency and economic sustainability. Cost reduction efforts can be achieved through the incorporation of locally available plant-based resources and nutritionally valuable agro-industrial by-products (Srifani *et al.*, 2024; Djulardi *et al.*, 2023). In this context, palm kernel meal (PKM), an abundant by-product of the palm oil industry

in Indonesia, has gained increasing attention as a potential alternative feed ingredient.

Palm kernel meal is a locally available feed ingredient derived from the solid by-product of the palm oil processing industry and has considerable potential as an alternative feed resource due to its abundant availability in Indonesia and its non-competitive nature with human food consumption. Nutritionally, PKM contains moderate levels of crude protein (17.31%), crude fiber (27.62%), crude fat (7.14%), calcium (0.27%), phosphorus (0.94%), and a relatively high copper concentration of 48.04 ppm (Mirnawati *et al*., 2023). However, its application in broiler diets remains limited, typically restricted to 5–10%, primarily due to its high crude fiber content dominated by β-mannan, which constitutes approximately 57.8% of the total fiber fraction (Gómez-Osorio *et al*., 2022). Broiler chickens lack endogenous enzymes capable of efficiently degrading complex polysaccharides such as β-mannan, resulting in reduced nutrient digestibility and utilization (Mirnawati *et al*., 2025a). Therefore, strategies to improve the inclusion level of PKM in broiler rations are required, particularly through approaches aimed at enhancing fiber degradation, such as probiotic supplementation with enzyme-producing bacteria capable of synthesizing cellulase and mannanase, which may improve nutrient availability and overall feed efficiency.

Probiotics are defined as live microorganisms that confer health benefits to the host by maintaining the balance of intestinal microflora upon colonization of the gastrointestinal tract. Their supplementation has been widely reported to enhance feed digestibility, improve gut health, promote body weight gain, optimize feed conversion ratio (FCR), and increase overall nutrient utilization in poultry (Melia *et al*., 2022; Sabo *et al*., 2020; Iryos *et al*., 2025). In addition, probiotics function as natural and safe alternatives to antibiotic growth promoters, as many probiotic strains are classified as Generally Recognized as Safe (GRAS), thereby supporting sustainable and antibiotic-free livestock production systems (Srifani *et al*., 2024).

Certain probiotic bacteria are known to produce digestive enzymes that enhance nutrient utilization in poultry. In the present study, cellulase- and mannanase-producing bacteria were employed to mitigate the limitation associated with the high β-mannan content of diets containing palm kernel meal (PKM). Among the potential probiotic candidates, *Lactobacillus fermentum* and *Bacillus subtilis* have been widely reported for their ability to produce cellulase and mannanase enzymes. Mirnawati *et al*. (2025b) evaluated a 1:1 consortium of *L. fermentum* and *B. subtilis* and reported strong *in vitro* probiotic characteristics, including high enzyme activities (cellulase 13.71 U/mL, mannanase 17.05 U/mL, and protease 9.32 U/mL), tolerance to acidic conditions (70.6% survival at pH 2.5), bile salts (62.84% at 0.3%), and elevated temperature (83.15% at 42°C), along with high hydrophobicity (83.75%), autoaggregation (71.64–73.32%), coaggregation (78.13%), and pronounced antagonistic activity against *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* (inhibition zones of 15.07–17.12 mm). Despite the promising *in vitro* probiotic performance, the *in vivo* efficacy of this bacterial consortium has not yet been investigated. Therefore, the objective of this study was to biologically evaluate the optimal dosage of the *L. fermentum* and *B. subtilis* consortium in broilers fed PKM-containing diets, with the aim of improving PKM utilization and achieving optimal broiler performance.

MATERIALS AND METHODS

Experimental design

The present study was approved by the Animal Ethics Committee of Andalas University, West Sumatera, Indonesia. This study was conducted at the Poultry Experimental Farm and the Non-Ruminant Nutrition Laboratory, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia. This study employed an experimental method using a Completely Randomized Design (CRD) with a 3×3 factorial arrangement and three replications. The first factor (A) was the probiotic dosage, consisting of three levels: A1 (without probiotic), A2 (1.21×10^{12} CFU/mL), and A3 (1.21×10^{14}CFU/mL). The second factor (B) was the level of palm kernel meal (PKM) inclusion in the diet, with three levels: B1 (0% PKM), B2 (25% PKM), and B3 (30% PKM). The probiotics used in this study were a consortium of *L. fermentum* and *B. subtilis* with a ratio of 1:1.

Birds and diet

The composition of the experimental diets is presented in Table 1, while the nutritional composition (%) and metabolizable energy (kcal/kg) of the treatment diets are shown in Table 2. A total of 162 one-day-old Cobb CP707 broiler chicks, with an average initial body weight of 42 ± 1.2 g were used in this study. The chicks were evenly distributed across 9 treatment groups in a 3×3 factorial design, with 3 replications per treatment and 6 birds per experimental unit.

Birds were housed in floor pens bedded with wood shavings, with a feeder and drinker provided in each pen. All birds were maintained under uniform management and environmental conditions. Probiotics were administered through drinking water. Feed and drinking water were provided *ad libitum* throughout the experimental period. The broilers were reared for 42 days.

Table 1: Composition of treatment rations (%).

Feed ingredients	Treatment feed		
	B1	B2	B3
Corn	45.10	25.10	20.40
Soybean meal	29.50	23.60	22.30
Rice bran	3.55	0.00	0.00
Palm kernel meal	0.00	25.00	30.00
Fish meal	20.00	20.00	20.00
Coconut oil	1.25	5.70	6.70
Mineral mixture	0.60	0.60	0.60
Total	100	100	100

Table 2: Nutrient composition (%) and metabolizable energy (kcal/kg) of experimental feeds.

Food substances	Treatment feed		
	B1	B2	B3
Crude protein (CP)	22.15	22.12	22.08
Crude lipid (CL)	4.02	9.63	10.88
Crude fiber (CF)	4.14	9.72	10.86
Ca	1.37	1.34	1.33
P	0.79	0.91	0.95
Methionine	0.65	0.56	0.54
Lysine	2.27	2.04	1.99
Metabolic Energy (ME)	3000.54	3006.51	3002.10

Parameters

Growth performance

Feed consumption was recorded from reducing the amount of ration given with the remaining feed. Data on body weight gain were obtained by weighing the chickens every week from the second week to the fifth week. Body weight gain was calculated as a result of weight reduction at time t compared to previous body weight. The feed conversion ratio was calculated as ration consumption divided by body weight gain.

Carcass quality

The carcass quality observed in this study were carcass percentage, abdominal fat percentages and cholesterol in broiler thigh meat. The carcass percentage was calculated as the ratio of carcass weight to live weight multiplied by 100%. The abdominal fat percentage was calculated as the weight of fat deposited between the abdominal muscles and the intestines divided by the live body weight, multiplied by 100%.

Cholesterol analysis was conducted using the Burke (1974) method. The principle of this analysis is based on the reaction between cholesterol extracted in chloroform and acetic anhydride followed by concentrated sulfuric acid, resulting in a colored complex whose absorbance is measured at a wavelength of 340 nm. The intensity of absorbance is directly proportional to the cholesterol concentration. Thigh meat samples (50 g) were weighed and homogenized using a blender. The homogenized samples were extracted with chloroform, and the resulting clear extract was divided into two portions: one treated with the Liebermann–Burchard reagent as the test solution and the other treated with the Liebermann–Burchard control solution. The developed color was measured using a spectrophotometer at 340 nm, allowing the determination of cholesterol content in the samples.

Intestinal morphology

Small intestinal morphology was evaluated following the method described by Harimurti and Endang (2009). The observed parameters included villus height, villus width, and crypt depth in the duodenum, jejunum, and ileum. The duodenum was defined as the proximal segment of the small intestine forming parallel loops, the jejunum as the middle segment located between the distal end of the duodenum and Meckel's diverticulum, and the ileum as the segment extending from Meckel's diverticulum to the ileocecal junction. For ileal sampling, a 2 cm segment was collected approximately 4 cm distal to Meckel's diverticulum.

Fresh intestinal samples were excised into 2 cm segments from each intestinal section (duodenum, jejunum, and ileum) and immediately fixed in 10% buffered formalin for 24–48 h prior to histological processing. Tissue samples were then prepared for histological examination using the hematoxylin–eosin (H&E) staining technique. Briefly, tissue sections were dehydrated through a graded series of ethanol solutions with increasing concentrations, immersed sequentially in each concentration for approximately 10 s, cleared in xylol, and subsequently embedded in paraffin. Paraffin-embedded tissues were sectioned using a microtome and stained with hematoxylin and eosin.

Prepared histological slides were examined and measured using a light microscope equipped with computer-assisted imaging. Measurements of villus height, villus width, and crypt depth were performed using an Olympus BX51 microscope fitted with an Olympus DP12 digital camera. Initial observation and field selection were conducted at 4× magnification, and representative histological images were captured once appropriate intestinal morphology was identified. A minimum of six measurements per slide were recorded for each morphological parameter. Quantitative analysis was conducted using Microsoft Office Picture Manager at 40× magnification, following calibration of micrometer scale units (µm) to ensure accurate measurement of villus and crypt dimensions displayed on the monitor.

Data analysis

All data obtained in this study were analyzed using analysis of variance (ANOVA) to determine the significant effect of the treatments (Steel and Torie, 2097). Each experimental unit consisted of six birds, and the experimental unit was used as the basis for statistical analysis. Duncan's multiple range test (DMRT) was used to distinguish significant differences among treatment means at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of treatment on broiler performance

Effect of treatments on feed intake

Data in Table 3 indicate that increasing probiotic (*L. fermentum* and *B. subtilis*) supplementation alongside higher dietary PKM levels progressively increased ($P < 0.01$) broiler feed intake. These findings indicate that supplementation with a high probiotic dose, particularly 1.21×10^{14}CFU/mL administered through drinking water, effectively supported the inclusion of PKM up to 30% in broiler diets without compromising feed consumption. This suggests that probiotic supplementation plays a crucial role in improving broiler tolerance to high-fiber diets.

The increased feed intake observed in treatments A2 and A3 was closely associated with probiotic supplementation at doses of 1.21×10^{12} and 1.21×10^{14} CFU/mL, respectively. Probiotics enhanced the population of beneficial microorganisms in the gastrointestinal tract, thereby promoting a more stable intestinal microbial ecosystem and suppressing pathogenic bacteria. Improved gut health and digestive efficiency accelerated digesta passage and nutrient absorption, leading to earlier onset of hunger signals and increased feeding frequency. These results are consistent with Zhang *et al.* (2021), who reported that probiotic supplementation via drinking water improves intestinal health, enhances nutrient absorption, and accelerates gastrointestinal emptying, ultimately increasing feed intake in broilers.

In contrast, the lower feed intake observed in treatments A1B1, A1B2, and A1B3 (without probiotic supplementation) with increasing PKM levels was primarily attributed to the high crude fiber content of the diets. High-fiber diets tend to reduce palatability and nutrient digestibility while slowing digesta transit, resulting in prolonged satiety and delayed hunger signals, which subsequently reduce feeding frequency and total feed intake. This observation is in agreement with Tejeda and Kim (2021), who reported that elevated dietary fiber increases digesta bulk and slows gastrointestinal passage, thereby prolonging satiety and decreasing feed consumption.

In the present study, treatment A3B3 yielded the highest feed intake, reaching 854 g/bird/week with a diet containing 30% PKM and a probiotic dose of 1.21×10^{14} CFU/mL. This value exceeded the feed intake reported by Mirnawati *et al.* (2025a), who utilized PKM-fermented diets supplemented with a similar bacterial consortium and reported a feed intake of 815.27 g/bird/week. These results demonstrate that high-dose probiotic supplementation via drinking water can effectively enhance feed intake even in diets with high PKM inclusion, offering a practical alternative to feed fermentation for improving PKM utilization in broiler production.

Effect of treatments on body weight gain

Based on the data presented in Table 3, increasing the dosage of *L. fermentum* and *B. subtilis* probiotics in conjunction with higher levels of PKM resulted in a corresponding increase ($P < 0.01$) in body weight gain (BWG) of broilers. These findings indicate that supplementation with a high probiotic dose, particularly 1.21×10^{14} CFU/mL administered through drinking water, effectively enhanced the utilization of PKM up to 30% in broiler diets. This demonstrates that probiotic supplementation can mitigate the limitations associated with high PKM inclusion and support improved growth performance.

Table 3: Effect of treatments on broiler performance.

Parameters	Factor A (Probiotic dose)	Factor B (PKM percentage)			SEM	SE
		B1 (0%)	B2 (25%)	B3 (30%)		
Feed intake (g/bird/week)	A1 (without probiotic)	758 ^{aB}	736 ^{bC}	724 ^{bC}	10.11	2.81
	A2 (1.21x 10^{12} CFU/ml)	770 ^{aB}	774 ^{aB}	779 ^{abB}	2.48	
	A3 (1.21x 10^{14} CFU/ml)	785 ^{cA}	833 ^{aA}	854 ^{aA}	20.33	
Body weight gain (g/bird/week)	A1 (without probiotic)	425 ^{aB}	415 ^{bC}	399 ^{cC}	7.72	2.72
	A2 (1.21x 10^{12} CFU/ml)	433 ^{bB}	443 ^{aB}	447 ^{aB}	3.9	
	A3 (1.21x 10^{14} CFU/ml)	455 ^{cA}	506 ^{bA}	526 ^{aA}	21	
FCR	A1 (without probiotic)	1.83 ^{aA}	1.85 ^{aA}	1.86 ^{aA}	0.01	0.02
	A2 (1.21x 10^{12} CFU/ml)	1.82 ^{aA}	1.75 ^{bB}	1.77 ^{bB}	0.02	
	A3 (1.21x 10^{14} CFU/ml)	1.71 ^{aB}	1.66 ^{bC}	1.66 ^{aC}	0.02	

Description: Different lowercase letters in rows ($P<0.01$) and different uppercase letters in columns ($P<0.01$) indicate a very significant difference.

The increased BWG observed in treatments A2 and A3 was closely associated with probiotic supplementation at doses of 1.21×10^{12} and 1.21×10^{14}CFU/mL, respectively. The presence of *L. fermentum* and *B. subtilis* promotes the proliferation of beneficial intestinal microflora, leading to a more balanced microbial ecosystem in the gastrointestinal tract. Improved microbial balance enhances digestive efficiency and nutrient absorption, allowing broilers to utilize dietary nutrients more effectively for tissue growth. This is consistent with Idowu *et al.* (2025), who reported that probiotics such as *Lactobacillus* and *Bacillus* spp. increase beneficial microbial populations, suppress pathogenic bacteria, and improve digestive efficiency, ultimately resulting in greater body weight gain.

Conversely, the lower BWG observed in treatments A1B1, A1B2, and A1B3 (without probiotic supplementation) was primarily attributed to the high crude fiber content of the diets, which was not accompanied by enzymatic support. Broilers possess a limited ability to digest dietary fiber; as a result, undigested fiber accumulates in the gastrointestinal tract, creating a bulky effect that prolongs satiety and decreases nutrient availability. High fiber content has been reported to significantly decrease nutrient digestibility and impair growth performance due to its swelling properties and reduced efficiency of nutrient absorption (Jha and Mishra, 2021). As a result, nutrient utilization for muscle deposition becomes suboptimal, leading to reduced BWG.

In the present study, treatment A3B3 produced the highest BWG, reaching 526 g/bird/week with a diet containing 30% PKM and a probiotic dose of 1.21×10^{14} CFU/mL. This value exceeded the BWG reported by Mirnawati *et al.* (2025a), who utilized PKM-fermented diets supplemented with a similar bacterial consortium and achieved a BWG of 470.45 g/bird/week. These results indicate that high-dose probiotic supplementation via drinking water can effectively enhance growth performance in broilers fed high-PKM diets, offering a practical and efficient alternative to feed fermentation strategies.

Effect of treatments on FCR

Based on the data presented in Table 3, the reduction ($P < 0.01$) in feed conversion ratio (FCR) observed in treatments A2 and A3 was attributed to probiotic supplementation of *L. fermentum* and *B. subtilis* administered through drinking water. A lower FCR indicates that less feed is required to achieve a given level of BWG, reflecting more efficient feed utilization. This finding is consistent with Ciptaan *et al.* (2021), who reported that a lower FCR is indicative of improved efficiency in feed use and enhanced growth performance in broilers.

In contrast, the higher FCR observed in treatment A1 (without probiotic supplementation) was primarily associated with excessive crude fiber content exceeding the tolerance level in broiler diets. High dietary fiber impairs digestive processes and nutrient absorption, resulting in suboptimal utilization of feed nutrients. Higher dietary fiber levels increase gut fill and satiety, resulting in reduced feed intake and lower energy and protein consumption. At the same time, fiber is poorly digested by broilers and interferes with nutrient availability, thereby limiting nutrient allocation for growth. Consequently, the imbalance between feed intake and BWG leads to an increased FCR. Ginindza *et al.* (2022) similarly reported that higher crude fiber levels in broiler diets reduce nutrient digestibility and increase FCR due to decreased efficiency of feed utilization for growth.

Notably, comparable low FCR values observed in treatments B2 and B3 alongside increasing probiotic doses indicate that higher inclusion levels of PKM did not compromise feed efficiency when supported by probiotic supplementation. This suggests that the presence of beneficial microbes plays a critical role in maintaining feed efficiency under high-fiber dietary conditions by improving nutrient digestion and utilization.

In the present study, treatment A3B3 resulted in the most favorable FCR value of 1.66, achieved with a diet containing 30% PKM and a probiotic dose of 1.21×10^{14} CFU/mL. This value was lower than that reported by Mirnawati *et al.* (2025a), who utilized fermented PKM diets supplemented with a similar combination of *L. fermentum* and *B. subtilis* and obtained an FCR of 1.75. These results demonstrate that high-dose probiotic supplementation via drinking water can effectively improve feed efficiency in broilers fed high-PKM diets, even without prior feed fermentation.

Effect of treatment on carcass quality

Effect of treatments on carcass percentage

According to the data presented in Table 4, the highest ($P < 0.01$) carcass yield was observed in treatment A3B3, which combined a probiotic dose of 1.21×10^{14} CFU/mL with a dietary inclusion of 30% PKM. The improvement in carcass percentage indicates enhanced efficiency of nutrient utilization, which is likely associated with the metabolic activity of *L. fermentum* and *B. subtilis* as producers of cellulase and mannanase enzymes. Cellulase contributes to the breakdown of structural fiber components, while mannanase specifically hydrolyzes β-mannan, a predominant non-starch polysaccharide in PKM. The enzymatic degradation of these fiber fractions improves the availability of energy and nutrients, allowing broilers to more effectively utilize high-PKM diets and redirect absorbed nutrients toward carcass tissue deposition, thereby resulting in a higher carcass percentage.

Table 4: Effect of treatments on carcass quality.

Parameters	Factor A (Probiotic dose)	Factor B (PKM percentage)			SEM	SE
		B1 (0%)	B2 (25%)	B3 (30%)		
Carcass percentage (%)	A1 (without probiotic)	72.21 ^{aB}	71.18 ^{bC}	70.72 ^{bC}	0.44	0.27
	A2 (1.21x 10^{12} CFU/ml)	72.73 ^{bB}	73.17 ^{bB}	74.34 ^{aB}	0.48	
	A3 (1.21x 10^{14} CFU/ml)	74.64 ^{cA}	76.02 ^{bA}	77.28 ^{aA}	0.76	
Abdominal fat (%)	A1 (without probiotic)	1.41 ^{aA}	1.34 ^{bA}	1.30 ^{cA}	0.03	0.01
	A2 (1.21x 10^{12} CFU/ml)	1.25 ^{aB}	1.12 ^{bB}	1.07 ^{cB}	0.05	
	A3 (1,21x 10^{14} CFU/ml)	0.99 ^{aC}	0.86 ^{bC}	0.74 ^{cC}	0.07	
Thigh meat cholesterol (mg/100g)	A1 (without probiotic)	126.67 ^{aA}	120.93 ^{bA}	118.43 ^{cA}	2.44	0.75
	A2 (1.21x 10^{12} CFU/ml)	115.73 ^{aB}	110.83 ^{bB}	103.27 ^{cB}	3.63	
	A3 (1.21x 10^{14} CFU/ml)	101.17 ^{aC}	96.00 ^{bC}	93.70 ^{cC}	2.21	

Description: Different lowercase letters in rows ($P<0.01$) and different uppercase letters in columns ($P<0.01$) indicate a very significant difference.

In contrast, the lowest carcass yield recorded in treatment A1B3 suggests that the inclusion of PKM at a high level (30%) requires adequate probiotic support to ensure optimal nutrient utilization. The reduced carcass percentage in this treatment may be attributed to the absence of probiotic supplementation capable of producing cellulase and mannanase, leading to suboptimal degradation of complex fiber fractions, particularly β-mannan, which dominates PKM fiber composition. Consequently, a substantial portion of dietary components remained poorly digested, limiting the availability of metabolizable energy and essential nutrients for growth.

This inefficiency in nutrient utilization subsequently impaired growth performance and muscle tissue accretion. Diets high in fiber content without enzymatic support may also reduce palatability and increase gut fill, accelerating satiety and ultimately suppressing feed intake (Jha and Mishra, 2021). These conditions result in suboptimal final body weight, which directly influences carcass yield, given that carcass percentage is closely related to relative body weight and the proportion of muscle tissue formed.

Effect of treatments on abdominal fat

Based on the data presented in Table 4, the lowest ($P < 0.01$) abdominal fat percentage was observed in treatment A3B3, which combined a probiotic dose of 1.21×10^{14}CFU/mL with a dietary inclusion of 30% PKM. This response may be attributed to improvements in intestinal morphology induced by probiotic supplementation, which enhanced nutrient absorption efficiency. Structural improvements in the intestinal mucosa increase the absorptive surface area, allowing more effective uptake of essential nutrients, including energy and micronutrients (Walton *et al.*, 2018). Enhanced nutrient absorption and improved energy availability can elevate metabolic activity, directing energy utilization toward muscle growth and other lean tissue accretion rather than lipid storage. Consequently, improved intestinal morphology contributes to reduced abdominal fat deposition in broilers fed high-PKM diets supplemented with probiotics.

In contrast, the higher abdominal fat percentage observed in treatment A1B1 may be associated with a lower population of beneficial bacteria in the gastrointestinal tract due to the absence of adequate probiotic supplementation which can lead to intestinal dysbiosis, facilitating the overgrowth of pathogenic bacteria that produce toxins and trigger inflammatory responses in the intestinal epithelium. Inflammation compromises mucosal integrity, disrupts barrier function, and slows epithelial regeneration (Obianwuna *et al.*, 2023). These conditions negatively affect digestive and absorptive processes, including lipid metabolism, ultimately promoting excessive lipid accumulation in the abdominal region.

According to Hidayat (2015), normal abdominal fat levels in broilers range from 0.73% to 3.78% of live body weight, with values exceeding 3% considered excessive. In the present study, abdominal fat percentages remained within the normal range and were lower than those reported by Tang *et al.* (2021), who observed an abdominal fat percentage of 1.52% in Arbor Acres broilers supplemented with *B. subtilis*. Similarly, Wang et al. (2017) reported an abdominal fat percentage of 1.17% in Cobb 500 broilers fed *Lactobacillus johnsonii* BS15 at 1.0×10^{6}CFU/g of diet.

Effect of treatments on thigh meat cholesterol

Based on the data presented in Table 4, the lowest ($P < 0.01$) thigh meat cholesterol content was observed in treatment A3B3, which combined a probiotic dose of 1.21×10^{14}CFU/mL with a dietary inclusion of 30% PKM. This reduction in cholesterol level is likely associated with the higher probiotic dosage administered. Probiotic bacteria are known to modulate cholesterol metabolism

by converting cholesterol into less harmful compounds, thereby reducing its accumulation in animal tissues. This mechanism is primarily mediated through the activity of bile salt hydrolase (BSH), an enzyme that deconjugates bile salts into forms that are less efficiently reabsorbed in the intestine. As bile salt reabsorption decreases, the host compensates by utilizing more endogenous cholesterol for the synthesis of new bile acids, ultimately lowering cholesterol concentrations in the bloodstream and peripheral tissues, including muscle. This finding is consistent with Mursalim *et al.* (2025), who reported that probiotics can reduce cholesterol absorption through bile acid metabolism and cholesterol transformation.

In contrast, the highest thigh meat cholesterol content was recorded in treatment A1B1. The increase in cholesterol levels under this treatment may result from impaired nutrient absorption and reduced metabolic efficiency due to insufficient probiotic supplementation. A low probiotic dose limits the ability of beneficial microbes to lower intestinal pH and suppress pathogenic bacteria, resulting in a less stable and suboptimal gut environment. Such conditions negatively affect nutrient utilization and metabolic processes, particularly lipid metabolism, thereby promoting cholesterol accumulation in body tissues, including muscle. This observation aligns with the findings of Getachew (2016), who reported that an unhealthy intestinal environment contributes to increased cholesterol levels by disrupting overall lipid metabolism.

The lowest cholesterol concentration observed in the present study was 93.70 mg/100 g of thigh meat, which is substantially lower than values reported in previous studies. Lokapirnasari *et al.* (2025) documented a cholesterol concentration of 135.88 mg/100 g in broiler meat following probiotic supplementation, while Hasanah and Hartoyo (2023) reported an even higher value of 265.76 mg/100 g in broilers receiving probiotic-supplemented diets. These comparisons suggest that the probiotic strategy applied in the present study was more effective in reducing meat cholesterol content.

Effect of Treatment on Intestinal Morphology

Effect of Treatments on Villi Height

Based on the data presented in Figure 1, a significant interaction between factor A (probiotic dosage) and factor B (PKM inclusion level) was observed on the villus height of the broiler small intestine. Increasing the probiotic dose up to 1.21×10^{14}CFU/mL in drinking water in combination with PKM inclusion levels up to 30% in the diet resulted in a marked increase ($P < 0.05$) in villus height. This interaction effectively enhanced villus development in the duodenum, jejunum, and ileum. These findings indicate that higher probiotic supplementation alongside increased PKM utilization promotes optimal intestinal villus growth, thereby enlarging the absorptive surface area and improving nutrient absorption capacity and overall gut health in broilers.

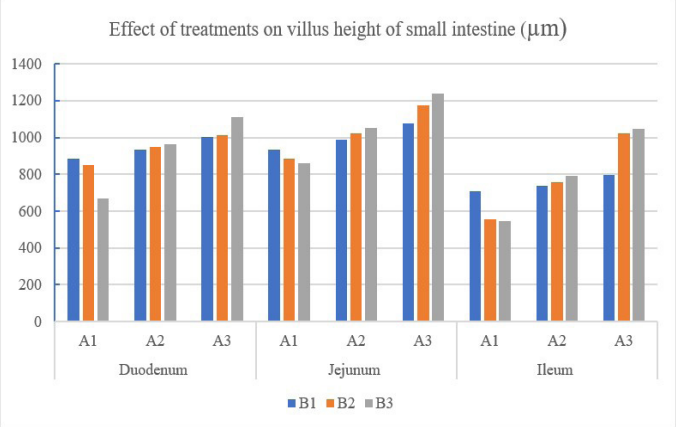

Figure 1: Effect of treatments on villus height of small intestine (µm).

The increased ($P < 0.05$) villus height in the duodenum, jejunum, and ileum observed in treatments A2 and A3 can be attributed to the supplementation of *L. fermentum* and *B. subtilis* (1.21×10^{12} and 1.21×10^{14}CFU/mL, respectively) in drinking water combined with dietary PKM. Probiotics contribute to intestinal health by modulating gut microbiota through competitive exclusion, whereby beneficial microbes compete with pathogenic bacteria for nutrients and adhesion sites on the intestinal mucosa. In addition, probiotics produce antimicrobial substances, including lactic acid bacteria metabolites and bacteriocins, which lower intestinal pH and suppress acid-sensitive pathogens. The reduction in pathogenic populations decreases toxin production and intestinal inflammation, thereby minimizing villus damage and supporting optimal crypt cell proliferation and epithelial regeneration (Li *et al.*, 2021). Improved intestinal integrity under these conditions facilitates efficient nutrient absorption and contributes positively to broiler performance.

In contrast, treatment A1 (without probiotic supplementation) combined with higher PKM inclusion levels (25% and 30%) resulted in reduced ($P < 0.05$) villus height across all intestinal segments. This response is associated with the elevated crude fiber content of the diet, which exceeds the tolerance threshold of broilers (approximately 7–9%) (Zhang *et al.*, 2023). In the absence of probiotics, dietary fiber is poorly degraded, leading to accelerated digesta passage and reduced nutrient absorption efficiency. Limited nutrient availability impairs epithelial cell proliferation and crypt regeneration, resulting in villus shortening or atrophy. Moreover, the lack of probiotic support may favor the proliferation of pathogenic bacteria that produce toxins and induce intestinal inflammation,

further exacerbating epithelial damage and villus cell loss (Antonissen *et al.*, 2016). When the rate of villus cell damage exceeds that of new cell formation, villus length cannot be maintained, leading to a reduced absorptive surface area and compromised intestinal function.

In the present study, treatment A3B3 (probiotic supplementation of *L. fermentum* and *B. subtilis* at 1.21×10^{14}CFU/mL combined with 30% PKM inclusion) produced the most favorable results, with villus heights of 1111.48 µm in the duodenum, 1237.98 µm in the jejunum, and 1048.42 µm in the ileum. These values were higher than those reported by Lisnahan and Nahak (2020), who observed villus heights of 867.50 µm, 989.50 µm, and 766.00 µm in the duodenum, jejunum, and ileum, respectively, under control conditions. Similarly, Roa *et al.* (2018) reported lower villus heights in broilers supplemented with *B. subtilis*, namely 1022.4 µm in the duodenum, 1144.1 µm in the jejunum, and 825.9 µm in the ileum. These comparisons highlight the synergistic effect of high-dose probiotic supplementation and elevated PKM inclusion in enhancing intestinal morphology.

Effect of treatments on villi width

Based on the data presented in Figure 2, a significant interaction between factor A (probiotic dosage) and factor B (PKM inclusion level) was observed on the villus width of the broiler small intestine. Supplementation of probiotics up to 1.21×10^{14} CFU/mL via drinking water, combined with increasing dietary PKM levels up to 30%, significantly ($P < 0.05$) enhanced villus width in the duodenum, jejunum, and ileum. This finding indicates a synergistic interaction between probiotic supplementation and PKM inclusion in supporting intestinal villus growth and development. Concurrent increases in probiotic dose and PKM level were consistently associated with broader intestinal villi in broilers.

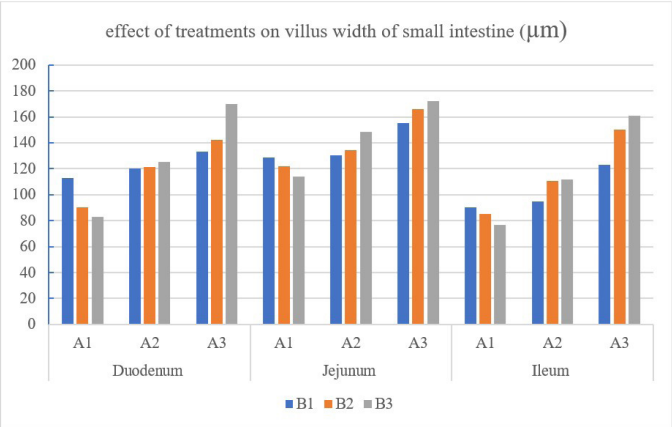

Figure 2: Effect of treatments on villus width of small intestine (µm).

The increased ($P < 0.05$) villus width observed in treatments A2 and A3 can be attributed to supplementation with *L. fermentum* and *B. subtilis* (1.21×10^{12} and 1.21×10^{14} CFU/mL, respectively) administered through drinking water alongside dietary PKM. As beneficial live microorganisms, probiotics colonize the intestinal lumen and exert their effects by competing with pathogenic bacteria for nutrients and adhesion sites, while simultaneously creating an acidic environment unfavorable for pathogen survival. A reduction in pathogenic populations alleviates chronic inflammatory responses in the intestinal mucosa that otherwise lead to epithelial damage and villus atrophy. With diminished mucosal injury, epithelial cells originating from the crypts can proliferate and regenerate more efficiently, resulting in improved villus architecture characterized by increased villus width and a higher villus-to-crypt ratio (Awad *et al.*, 2018).

In contrast, treatment A1 (without probiotic supplementation) combined with higher PKM inclusion levels (25% and 30%) resulted in reduced ($P < 0.05$) villus width due to the elevated crude fiber content of the diet in the absence of probiotic support. Dietary fiber exhibits bulky properties that promote early satiety in broilers. Moreover, high fiber content accelerates digesta passage through the gastrointestinal tract, thereby shortening the contact time between nutrients and digestive enzymes. This condition reduces the efficiency of nutrient degradation and absorption required to support epithelial cell proliferation and hypertrophy, leading to narrower and less developed villi. Additionally, the absence of probiotics facilitates increased colonization of pathogenic bacteria along the intestinal epithelium. These pathogens can damage epithelial cells, increase intestinal permeability, and release virulence factors that trigger inflammatory responses (Tomal *et al.*, 2023). Sustained inflammation accelerates mucosal damage, resulting in villus atrophy manifested by villus narrowing and a reduced absorptive surface area, ultimately impairing nutrient uptake. Rinttilä and Apajalahti (2013) further reported that the lack of probiotic supplementation disrupts gut microbial balance, reduces the availability of epithelial growth-stimulating metabolites, and contributes to villus narrowing in the small intestine.

In the present study, treatment A3B3 (probiotic supplementation with *L. fermentum* and *B. subtilis* at 1.21×10^{14} CFU/mL combined with 30% PKM inclusion) yielded the most favorable villus width measurements, reaching 169.92 µm in the duodenum, 172.32 µm in the jejunum, and 160.98 µm in the ileum. These values exceeded those reported by Khatun *et al.* (2022), who documented villus widths of 79.08 µm, 168.63 µm, and 47.37 µm in the duodenum, jejunum, and ileum, respectively, under control conditions. Similarly, Iryos *et al.* (2025) reported narrower villi following supplementation with *L. fermentum*

CMUL-54 at 1.42 × 10^{12} CFU/mL combined with 25% PKM, with villus widths of 160.58 µm in the duodenum, 166.02 µm in the jejunum, and 133.80 µm in the ileum. These comparisons further emphasize the superior effectiveness of high-dose probiotic supplementation in conjunction with elevated PKM inclusion in enhancing intestinal morphology.

Effect of treatments on crypt depth

Data presented in Figure 3 indicate a significant interaction between the dosage of *L. fermentum* and *B. subtilis* probiotics and the dietary inclusion level of palm kernel meal (PKM) on reducing crypt depth in the small intestine of broilers. Increasing the probiotic dose up to 1.21×10^{14}CFU/mL via drinking water, together with PKM inclusion levels up to 30% in the diet, exerted a beneficial effect on crypt depth in the duodenum, jejunum, and ileum. This interaction demonstrates the combined contribution of probiotic supplementation and PKM utilization in supporting intestinal health, as evidenced by a progressive reduction in crypt depth with increasing probiotic dose and PKM level.

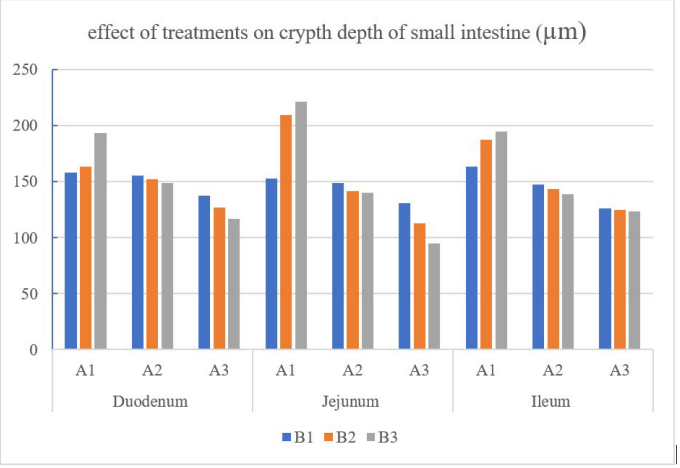

Figure 3: Effect of treatments on crypth depth of small intestine (µm).

The reduced (P < 0.05) crypt depth observed in treatments A2 and A3 can be attributed to supplementation with *L. fermentum* and *B. subtilis* at 1.21×10^{12} and 1.21×10^{14} CFU/mL, respectively, administered through drinking water in combination with dietary PKM. These findings suggest that appropriate probiotic supplementation can effectively modulate crypt morphology. Shallower crypts reflect improved intestinal homeostasis, as probiotics enhance gut microbial balance and reduce mucosal stress, thereby supporting efficient villus regeneration and digestive function (Huang *et al*., 2024). A decrease in crypt depth is commonly associated with reduced epithelial turnover demands and improved intestinal integrity.

In contrast, treatment A1 (without probiotic supplementation) combined with higher PKM inclusion levels (25% and 30%) resulted in increased (P < 0.05) crypt depth across all intestinal segments. This response is likely due to the elevated crude fiber content of the diet in the absence of probiotic support. High dietary fiber negatively affects intestinal histomorphology by reducing nutrient absorption efficiency and increasing the functional workload of the intestinal epithelium, thereby stimulating excessive crypt cell proliferation. This hyperproliferative response may occur as a compensatory mechanism to repair mucosal damage and inflammation induced by increased pathogenic bacterial populations. Increased crypt depth is indicative of intestinal stress and is associated with a reduced absorptive surface area and compromised nutrient utilization. Consistent with this observation, Kumar *et al.* (2016) reported that inadequate probiotic function can decrease the villus height to crypt depth ratio, ultimately impairing gastrointestinal health.

In the present study, treatment A3B3 (probiotic supplementation with *L. fermentum* and *B. subtilis* at 1.21 × 10^{14} CFU/mL combined with 30% PKM inclusion) produced the most favorable crypt depth values, measuring 116.63 µm in the duodenum, 94.82 µm in the jejunum, and 123.28 µm in the ileum. These values were notably lower, particularly in the jejunum and ileum, than those reported in control treatments by Bogusławska (2021), who documented crypt depths of 180.6 µm, 150.5 µm, and 149.3 µm in the duodenum, jejunum, and ileum, respectively. Similarly, de Souza *et al.* (2024) reported greater crypt depths following *Lactobacillus* spp. supplementation, with values of 164.57 µm in the duodenum, 122.39 µm in the jejunum, and 137.01 µm in the ileum. These comparisons further emphasize the effectiveness of high-dose probiotic supplementation combined with elevated PKM inclusion in improving intestinal morphology.

CONCLUSION

In conclusion, palm kernel meal can be used up to 30% in the ration followed by supplementation with the consortium probiotic (*L. fermentum* and *B. subtilis)* at a dose of 1.21 x 10^{14} CFU/mL which can improve broiler performance, carcass quality and intestinal morphology. Furthermore, this probiotic consortium can serve as a potential alternative to antibiotic growth promoters (AGPs) in broilers.

ACKNOWLEDGMENTS

The authors would like to thank National Research and Innovation Agency (BRIN) of Indonesia and the Indonesia Endowment Fund for Education Agency (LPDP) for financially supporting this research through the Riset dan Inovasi untuk Indonesia Maju (RIIM) batch 4 (Main

contract no. 188/IV/KS/11/2023 and sub contract no. 3/UN16.19/PT.01.03/RIIM/2025).

NOVELTY STATEMENT

This study demonstrated that a probiotic consortium consisting of *Lactobacillus fermentum* and *Bacillus subtilis*, possessing cellulolytic, mannanolytic, and proteolytic activities, effectively enhanced the utilization of palm kernel meal in broiler diets. Supplementation at 1.21 × 10^14 CFU/mL enabled the inclusion of up to 30% palm kernel meal while improving broiler performance and health. These findings highlight the strong potential of this probiotic consortium as a sustainable and cost-effective strategy to optimize alternative feed utilization and reduce broiler production costs.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Antonissen G, Eeckhaut V, Van Driessche K, Onrust L, Haesebrouck F, Ducatelle R, Moore RJ, Van Immerseel F (2016). Microbial shifts associated with necrotic enteritis. Avian Pathol., 45(3): 308–312. https://doi.org/10.1080/03079457.2016.1152625

Awad EA, Idrus Z, Soleimani Farjam A, Bello A, Jahromi MF (2018). Growth performance, duodenal morphology and caecal microbial population in female broiler chickens fed glycine-fortified low protein diets under heat stress conditions. Br. Poult. Sci., 59(3): 340–348. https://doi.org/10.1080/00071668.2018.1440377

Bogusławska-Tryk M, Ziółkowska E, Sławińska A, Siwek M, Bogucka J (2021). Modulation of intestinal histology by probiotics, prebiotics and synbiotics delivered *in ovo* in distinct chicken genotypes. Animals, 11(11): 3293. https://doi.org/10.3390/ani11113293

Burke RW, Diamondstone BI, Velapoldi RA, Menis O (1974). Mechanisms of the Liebermann–Burchard and Zak color reactions for cholesterol. Clin. Chem., 20(7): 794–801. https://doi.org/10.1093/clinchem/20.7.794

Ciptaan G, Mirnawati, Djulardi, A (2021). Utilization of fermented soy-milk waste with *Aspergillus ficuum* in broiler ration. IOP Conf. Ser. Earth Environ. Sci., 709: 012044. https://doi.org/10.1088/1755-1315/709/1/012044

De Souza M, Baptista AAS, Menck-Costa MF, Justino L, da Glória EM, Shimizu GD (2024). Modulation of broiler intestinal changes induced by *Clostridium perfringens* and deoxynivalenol through probiotic, paraprobiotic, and postbiotic supplementation. Toxins, 16(1): 46. https://doi.org/10.3390/toxins16010046

Djulardi A, Mirnawati, Ciptaan G, Kurnia R, Srifani A, Adriani L, Makmur M (2023). Improving the quality and nutritional value of a mixture of sago pith and Indigofera leaves fermented with *Rhizopus oligosporus*. World's Vet. J., 13(4): 580–586. https://doi.org/10.54203/scil.2023.wvj62

Getachew T (2016). A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. J. World Poult. Res., 6(1): 31–36.

Ginindza MM, Mbatha KR, Ng'ambi JW (2022). Dietary crude fiber levels for optimal productivity of male Ross 308 broiler and Venda chickens aged 1 to 42 days. Animals, 12(10): 1333. https://doi.org/10.3390/ani12101333

Gomez-Osorio LM, Nielsen JU, Martens HJ, Wimmer, R (2022). Upgrading the nutritional value of PKC using a *Bacillus subtilis*-derived monocomponent β-mannanase. Molecules, 27(2): 563. https://doi.org/10.3390/molecules27020563

Harimurti S, Endang S (2009). Morfologi usus ayam broiler yang disuplementasi dengan probiotik strain tunggal dan campuran. Agritech., 29(3): 179–183.

Hasanah A, Hartoyo, B (2023). Effects of encapsulated Fermeherbafit in feed towards blood and meat cholesterol levels of broiler chicken. J. Ilmiah Ilmu-Ilmu Peternakan, 26(1): 36–45. https://doi.org/10.22437/jiiip.v26i1.24547

Hidayat C (2015). Reducing abdominal fat deposition in broiler through feeding management. Indones. Bull. Anim. Vet. Sci., 25(3): 125–134. https://doi.org/10.14334/wartazoa.v25i3.1157

Huang CW, Liu SY, Bhattarai BP, Lee TY, Chang HT, Lin HC (2024). Live multi-strain probiotics enhance growth performance by regulating intestinal morphology and microbiome population in weaning piglets. Microorganisms, 12(11): 2334. https://doi.org/10.3390/microorganisms12112334

Idowu PA, Mpofu TJ, Magoro AM, Modiba MC, Nephawe KA, Mtileni B (2025). Impact of probiotics on chicken gut microbiota, immunity, behavior, and productive performance. A systematic review. Front. Anim. Sci., 6: 1562527. https://doi.org/10.3389/fanim.2025.1562527

Iryos AR, Mirnawati, Harnentis H, Srifani A and Yanti G (2025). Effectiveness of *Lactobacillus fermentum* CMUL-54 and *Lactobacillus fermentum* B978 as probiotic candidates producing mannanase, cellulase and protease activities for poultry. Online J. Anim. Feed Res., 15(2). https://doi.org/10.51227/ojafr.2025.14

Jha R, Mishra P (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and the environment: A review. J. Anim. Sci. Biotechnol., 12(1): 1–16. https://doi.org/10.1186/s40104-021-00576-0

Khatun A, Chowdhury S, Roy B, Gani S, Ray B, Ahmed T (2022). Effects of feeding multi-strain probiotics and multi-enzymes to broilers on growth performance, intestinal morphology and cost effectiveness of production. Adv. Anim. Vet. Sci., 10(2): 389–396. https://doi.org/10.17582/journal.aavs/2022/10.2.389.396

Kumar A, Anbazhagan AN, Coffing H, Chatterjee I, Priyamvada S, Gujral T (2016). *Lactobacillus acidophilus* counteracts inhibition of NHE3 and DRA expression and alleviates diarrheal phenotype in mice infected with *Citrobacter rodentium*. Am. J. Physiol. Gastrointest. Liver

Physiol., 311(5): G817–G826. https://doi.org/10.1152/ajpgi.00173.2016

Li HY, Zhou DD, Gan RY, Huang SY, Zhao CN, Shang AO (2021). Effects and mechanisms of probiotics, prebiotics, synbiotics, and postbiotics on metabolic diseases targeting gut microbiota: A narrative review. Nutrients, 13(9): 3211. https://doi.org/10.3390/nu13093211

Lisnahan CV, Nahak OR (2020). Growth performance and small intestinal morphology of native chickens after feed supplementation with tryptophan and threonine during the starter phase. Vet. World, 13(12): 2765. https://doi.org/10.14202/vetworld.2020.2765-2771

Lokapirnasari WP, Anggun R, Hana E (2025). Potensi penambahan bakteri asam laktat *Lactobacillus casei* dan *Lactobacillus rhamnosus* terhadap konsumsi ransum dan konversi pakan ayam pedaging. Agro Vet., 5(1): 43–49.

Melia U, Nafiu LO, Badaruddin R (2022). Performans produksi ayam broiler yang diberi probiotik berbeda. J. Ilmiah Petern. Halu Oleo, 4(1): 57–60. https://doi.org/10.56625/jipho.v4i1.23547

Mirnawati M, Akhadiarto S, Harnentis H, Ciptaan G, Zurmiati, Yanti G, Srifani, A (2025b). Synergistic probiotic consortium of *Bacillus subtilis* and *Lactobacillus fermentum* enhances palm kernel meal utilization and functional feed potential in poultry. Vet. World, 18(11): 3447–3463. https://doi.org/10.14202/vetworld.2025.3447-3463

Mirnawati, Ciptaan G, Akhadiarto S, Adriani L, Devi PC, Azzahra H, Srifani A (2025a). Utilization of fermented palm kernel meal with a combination of *Bacillus subtilis* and *Lactobacillus fermentum* in broiler rations. J. Anim. Health Prod., 13(3): 711–720. https://doi.org/10.17582/journal.jahp/2025/13.3.711.720

Mirnawati, Ciptaan G, Martaguri I, Ferawati, Srifani, A (2023). Improving quality and nutrient content of palm kernel meal with *Lactobacillus fermentum*. Int. J. Vet. Sci., 12(4): 615–622. https://doi.org/10.47278/journal.ijvs/2023.013

Mursalim, Hafsan, Fatmawati N, Khudaer F (2025). Modulation of broiler carcass cholesterol levels through *Lactobacillus plantarum* intervention. Indones. J. Agric. Res., 8(3): 161–171. https://doi.org/10.32734/injar.v8i3.20284

Obianwuna U, Wang J, Zhang H, Qi G, Qiu K, Wu S (2023). Recent trends on mitigative effect of probiotics on oxidative-stress-induced gut dysfunction in broilers under necrotic enteritis challenge: A review. Antioxidants, 12(4): 911. https://doi.org/10.3390/antiox12040911

Rinttilä T, Apajalahti J (2013). Intestinal microbiota and metabolites implications for broiler chicken health and performance. J. Appl. Poult. Res., 22(3): 647–658. https://doi.org/10.3382/japr.2013-00742

Roa ML, Guzmán Y.E, Navarro CA (2018). Efecto del uso de probióticos en la morfometría intestinal de pollos de engorde. Arch. Zootec., 67(260): 486–492. https://doi.org/10.21071/az.v0i0.3878

Sabo SdS, Mendes MA, Araújo ES, Muradian LBA, Makiyama EN, LeBlanc JG (2020). Bioprospecting of probiotics with antimicrobial activities against *Salmonella heidelberg* and that produce B-complex vitamins as potential supplements in poultry nutrition. Sci. Rep., 10: 7235. https://doi.org/10.1038/s41598-020-64038-9

Srifani A, Mirnawati, Marlida Y, Rizal Y, Nurmiati N (2024). Isolation and characterization of cellulolytic lactic acid bacteria from soymilk waste as probiotic candidates for broiler. Int. J. Vet. Sci., 13(1): 108–114.

Srifani A, Mirnawati, Marlida Y, Rizal Y, Nurmiati N, Lee KW (2024). Identification of novel probiotic lactic acid bacteria from soymilk waste using the 16S rRNA gene for potential use in poultry. Vet. World, 17(5): 1001–1011. https://doi.org/10.14202/vetworld.2024.1001-1011

Steel RGD, Torrie JH (1997). Principles and procedures of statistics: A biometrical approach. 3rd ed. McGraw-Hill, New York, pp. 352–358.

Tang X, Liu X, Liu H (2021). Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. Front. Vet. Sci., 8: 767802. https://doi.org/10.3389/fvets.2021.767802

Tejeda OJ, Kim WK (2021). Role of dietary fiber in poultry nutrition. Animals, 11(2): 461. https://doi.org/10.3390/ani11020461

Tomal F, Sadrin G, Gaboriaud P, Guitton É, Sedano L, Lallier N (2023). The caecal microbiota promotes the acute inflammatory response and the loss of intestinal barrier integrity during severe *Eimeria tenella* infection. Front. Cell. Infect. Microbiol., 13: 1250080. https://doi.org/10.3389/fcimb.2023.1250080

Walton KD, Mishkind D, Riddle MR, Tabin CJ, Gumucio DL (2018). Blueprint for an intestinal villus: species-specific assembly required. Wiley Interdis. Rev. Dev. Biol., 7(4): e317. https://doi.org/10.1002/wdev.317

Wang H, Ni X, Liu L (2017). Controlling growth performance, lipid deposits and fatty acid composition of chicken meat through a probiotic, *Lactobacillus johnsonii*, during subclinical *Clostridium perfringens* infection. Lipids Health Dis., 16: 38 https://doi.org/10.1186/s12944-017-0408-7.

Zhang C, Hao E, Huang CX, Liu G, Chen H, Wang DH (2023). Dietary fiber level improve growth performance, nutrient digestibility, immune and intestinal morphology of broilers from day 22 to 42. Animals, 13(7): 1227. https://doi.org/10.3390/ani13071227

Zhang L, Zhang R, Jia H, Zhu Z, Li H, Ma Y (2021). Supplementation of probiotics in water beneficial growth performance, carcass traits, immune function, and antioxidant capacity in broiler chickens. Cent. Eur. J. Biol., 16(1): 311–322. https://doi.org/10.1515/biol-2021-0031