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Optimal level of *Bacillus* spp. fermented soybean meal utilization in broiler diets under commercial conditions: effect on gut morphology and microbiota

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ABSTRACT
Fermented soybean meal (FSM) using Bacillus spp. has the potential to be used as a functional feed ingredient to improve the intestinal health of broiler chickens, but the optimal level of use in feed in the commercial broiler industry has not yet been determined. A total of 20.000 cobb 500 broilers were allotted to four dietary treatments: control (T0) and rations containing 1.5% (T1), 2.5% (T2), and 5.0% (T3) FSBM, each with five replications. The chickens were reared under commercial industry conditions. Over a 28-day period, parameters measured included Growth performance indicators, small intestine histomorphometry, microbial populations, catalase and motility activity, and lactic acid bacteria counts. FSBM supplementation had no significant ($P>0.05$) effect on performance (feed intake, body weight gain, feed change ratio). However, T2 significantly increased basal and apical pH in the jejunum ($P<0.05$), along with elevated LAB counts and reduced intestinal pH, compared to the control. In conclusion, fermentation with Bacillus spp. represents an effective strategy to reduce anti-nutritional factors and enhance feed quality. The improved intestinal morphology and lactic acid bacteria populations in broilers observed in this study suggest that 2.5% FSBM. Therefore, Bacillus spp. fermented soybean meal has potential as a functional feed ingredient for commercial broiler production.
Keywords: *Bacillus* spp., broiler, fermented soybean meal, intestinal morphology

1. Introduction

Broiler production is one of the fastest-growing livestock sectors worldwide due to the increasing demand for animal protein. Feed represents approximately 60–70% of total production costs, and soybean meal (SBM) remains the major protein source in broiler diets because of its high protein content and balanced amino acid profile. However, the nutritional utilization of SBM is limited by anti-nutritional factors such as trypsin inhibitors, oligosaccharides, lectins, and allergenic proteins, which can impair nutrient digestibility and intestinal health. Consequently, improving the nutritional quality and biological utilization of SBM has become an important objective in poultry nutrition research.

Fermented soybean meal (FSBM) has been increasingly used as a functional feed ingredient because fermentation can reduce anti-nutritional compounds and improve nutrient availability [1], [2]. Previous studies demonstrated that fermentation increases protein digestibility, enhances amino acid and mineral

bioavailability, and produces beneficial metabolites that support digestive function [3], [4]. Among various microorganisms, *Bacillus* spp. are widely utilized due to their ability to produce protease, amylase, and other extracellular enzymes that degrade complex substrates and improve feed quality. In addition to improving nutrient composition, *Bacillus* spp. function as probiotics by modulating intestinal microbiota, improving intestinal morphology, enhancing intestinal barrier function, and stimulating immune responses in broiler chickens [5]. Recent studies further reported that FSBM fermented with *Bacillus* species improved villus development, antioxidant activity, gut integrity, and microbial balance in broilers [4].

Although several studies have shown the benefits of FSBM on poultry productivity, the most effective level of FSBM use in feed still varies. Husnain *et al.* [6] reported that using 2–2.5% FSBM in feed resulted in better performance. Meanwhile, Abdel-Raheem *et al.* [7] stated that supplementation above 2.5% showed improvements in nutrient digestibility, growth performance, and immune function. The use of *Bacillus* spp. in the fermentation process has been consistently reported by several studies to improve nutrient quality [3], [4], [8], [9]. Therefore, this study was conducted to determine the optimal level of *Bacillus spp*. FSBM use in feed on growth performance, intestinal morphology, and microbiota in commercial broiler conditions. In addition, information regarding the efficacy of different FSBM inclusion levels under commercial broiler production conditions is scarce, despite commercial environments often presenting different challenges compared with controlled experimental settings.

2. Materials and Methods

2.1. Animals, Diet Treatment and Management

A total of 20.000 DOC Cobb500 broilers were reared in a semi-closed house under commercial industry conditions. The treatment used was the provision of FSBM mixed into the ration as follows: T0 (control), T1 (1.5% FSBM in ration), T2 (2.5% FSBM in ration), and T3 (5.0% FSBM in ration), with five replications per group (1000 chickens per replicate). The research protocol was approved by the Animal Research Ethics Committee (No.0031/KEPH-FMIPA/2025) at the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia. FSBM was prepared by fermenting soybean meal with *Bacillus* spp. as follows: 1 kg of SBM was moistened with distilled water at a 1:1 ratio (w/v), inoculated with 10 mL of *Bacillus* spp. culture (10^8 CFU/ml), mixed thoroughly, and incubated aerobically at 37°C for 72 hours in a sterile environment. After fermentation, the product was dried at 50°C to reach <10% moisture content and ground to uniform particle size before mixing into the diets. The chemical composition of feed ingredients and experimental diets was analyzed at the PT. New Hope Indonesia Laboratory (2024). Dry matter, crude protein, ether extract, crude fiber, and ash contents were determined according to the procedures of the AOAC International [10]. Chickens were fed the experimental diets from day 1 to day 28. Diet composition and nutrient content are presented in Table 1.

Table 1. Ingredients and chemical composition of the diets

Ingredients (%)	Pre-starter Diets (1-14 d)				Finisher Diets (15-28 d)			
	T0	**T1**	**T2**	**T3**	**T0**	**T1**	**T2**	**T3**
Corn	31	31	31	31	33	33	33	33
Wheat Flour	15	15	15	15	15	15	15	15
Meat Bone Meal	5	5	5	5	5	5	5	5
Soybean Meal	27	25.5	24.5	22	28	26.5	25.5	23
FSBM	0	1.5	2.5	5	0	1.5	2.5	5
Corn Germ	2	2	2	2	3	3	3	3
Rice Bran Flour	4	4	4	4	8	8	8	8
Copra Meal	5	5	5	5	3	3	3	3
Noodle Flour	5	5	5	5	-	-	-	-
Fish Meal	2	2	2	2	-	-	-	-
Crude Palm Oil	1.6	1.6	1.6	1.6	2.5	2.5	2.5	2.5
Additive and Mineral	2.4	2.4	2.4	2.40	2.5	2.5	2.5	2.5

Table 1. Continued

Ingredients (%)	Pre-starter Diets (1-14 d)				Finisher Diets (15-28 d)			
	T0	T1	T2	T3	T0	T1	T2	T3
Chemical composition (%)								
Metabolizable Energy (Mcal/kg)	3.00	3.00	3.00	3.00	3.02	3.02	3.02	3.02
Crude Protein	23.33	23.55	23.55	23.59	22.22	22.47	22.66	22.78
Calcium	1.09	0.96	1.07	0.98	0.82	0.86	0.83	0.93
Phoporus	0.77	0.76	0.75	0.74	0.81	0.79	0.74	0.86
Fat	6.84	7.13	7.12	6.49	7.22	7.36	7.52	7.49
Ash	5.65	5.69	5.49	5.73	5.70	5.60	5.43	5.68
Crude Fiber	3.01	2.68	2.61	3.08	3.02	222.94	2.86	2.82
Lysine	1.41	1.41	1.41	1.41	1.30	1.30	1.30	1.30
Metionine + Cystine	1.00	1.00	1.00	1.00	0.92	0.92	0.92	0.92
Threonine	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85

Source: Primer Data (Laboratory of PT. New Hope Indonesia, 2024)

2.2. Performance Measurement

The performance parameters calculated included feed intake (FI), average body weight, average daily gain, feed conversion ratio (FCR), performance index, and depletion. FI was calculated to determine the ratio of the number of chickens to the amount of feed consumed. The average daily gain is calculated as the difference between the final and initial body weight of chickens weighed every week. The feed conversion ratio is a conversion based on the amount of feed consumed and the weight of meat produced. The FCR is a comparative number representing the total ratio required to obtain one kilogram of meat. A lower FCR value indicates that a lower ratio is needed to produce one kilogram of meat. Meanwhile, Performance Index (PI) was used as an indicator of overall broiler production efficiency by integrating growth performance, feed utilization, livability, and age at marketing into a single value. Higher PI values indicate better productive performance and management efficiency. Depletion was used to represent the proportion of birds lost during the rearing period due to mortality or culling. Lower depletion values indicate better flock health, welfare, and management conditions.

Small Intestine Profile: The evaluated intestinal parameters included intestinal pH, small intestine length, intestinal wall thickness, small intestine weight, and intestinal histomorphometry. Small intestinal samples were collected from 28-day-old broiler chickens, representing 0.5% of the total birds in each replicate, for both histological and microbial analyses. Histomorphometric examination was conducted by measuring villus height, basal villus width, and apical villus width using an Olympus CX31 light microscope equipped with a calibrated eyepiece micrometer [11].

Gut Microbial Profile: The gut microbial profile was analyzed by enumerating total bacterial colonies using the pour plate method on MRS agar from a 10^{-9} dilution, incubated for 24 hours. Dominant colonies were subsequently isolated and identified through morphological assessment and Gram staining. Total lactic acid bacteria (LAB) were quantified using MRS broth for dilution and MRS agar for culture, with serial dilutions up to 10^{-6}, incubated anaerobically for 24 hours at 37°C, and counted with a colony counter. Isolated colonies were subjected to Gram staining to determine cell wall type, followed by a catalase test using 3% H_2O_2 to observe gas bubble formation [12] . Motility was assessed by inoculating isolates vertically into semi-solid NA media and incubating for 24 hours at 37°C; motility was determined based on the distribution pattern of bacterial growth around the puncture line. Catalase activity test using a scoring system as follows: 0 = catalase negative (no bubble formation), 1 = catalase positive (few bubbles formed), 2 = catalase positive (many bubbles formed), and 3 = catalase positive (many large bubbles formed). Motility test using scoring system as follows: 0 = non-motile (no growth spreading from the stab line) and 1 = motile (growth spreading from the stab line).

2.3. Statistical Analysis

The experimental data were analyzed using a Completely Randomized Design (CRD) according to the following statistical model: $Yij = \mu + Ti + \varepsilon ij$, where Yij is the observed value of the dependent variable, μ is the overall mean, Ti is the effect of the i-th dietary treatment (i = 1, 2, 3, 4), and εij is the random error associated with each experimental unit, assumed to be independently and normally distributed. Data were subjected to one-way analysis of variance (ANOVA) using SPSS software. When a significant treatment effect was detected ($P < 0.05$), differences among treatment means were further compared using Duncan's Multiple Range Test.

3. Results and Discussion

3.1. Performances

Dietary supplementation with fermented soybean meal (FSBM) did not significantly affect ($P>0.05$) on FI, ABW, ADG, depletion, FCR, and IP, indicating that FSBM neither enhanced nor impaired the overall growth performance of broiler chickens under normal rearing conditions. Furthermore, the mortality rate remained within the normal range, suggesting that FSBM can be safely incorporated into broiler diets without causing adverse physiological effects. These findings are consistent with previous reports [2][13], which demonstrated that although FSBM may improve intestinal function and gut health, such improvements do not necessarily translate into enhanced growth performance. In contrast, several studies have reported significant improvements in broiler productivity following FSBM supplementation [14]–[16]. These inconsistencies may be explained by differences in dietary inclusion levels, fermentation procedures, microbial strains, and environmental or physiological conditions experienced by the birds. The beneficial effects of FSBM appear to be more evident under challenging conditions, such as pathogen exposure or environmental stress, where its probiotic microorganisms and fermentation-derived organic acids can enhance intestinal microbial balance, inhibit pathogenic bacterial proliferation, and strengthen host immune responses. Supporting this hypothesis, Jazi *et al*. [17] demonstrated that broilers challenged with *Salmonella typhimurium* and fed an FSBM-containing diet exhibited superior growth performance compared with untreated birds, suggesting that the growth-promoting effects of FSBM are largely mediated through improvements in gut health and disease resistance rather than direct stimulation of growth under normal production conditions.

Table 2. The effect of fermented soybean meal (FSBM) using *Bacillus spp*. on chicken performance

Parameter	Treatment			
	T0	T1	T2	T3
Feed Intake (g/head) ns	2480±57.01	2495±44.72	2545±92.53	2550±79.06
Final body weight (kg/head) ns	1.87±0.09	1.82±0.11	1.82±0.10	1.84±0.08
Depletion (%) ns	5.90±0,02	5.40±0,02	5.30±0,02	4.50±0,02
Feed Conversion Ratio (g/g) ns	1.41±0.03	1.46±0.07	1.48±0.02	1.45±0.06
Average Daily Gain (g/day/head) ns	63.90±2.56	61.94±3.31	61.72±2.38	62.69±2.31
Performances Indeks (IP) ns	427±23.75	403.60±38.68	393.60±15.99	413.80±35.56

Description: ns = non significance ($P>0.05$)

3.2. Small Intestine Profile

In the present study, no significant differences ($P>0.05$) were observed in jejunal pH, intestinal thickness, length, or relative jejunal weight among the experimental groups, suggesting that dietary supplementation with fermented soybean meal (FSBM) did not adversely affect the overall structural integrity or physiological condition of the jejunum. These findings indicate that intestinal morphology remained relatively stable following FSBM inclusion, which is consistent with the report of Feng et al [18], who suggested that dietary interventions do not necessarily induce measurable changes in gross intestinal morphology, such as intestinal length or wall thickness. In contrast, significant differences ($P<0.05$) were detected in the relative weights of the duodenum and ileum (Table 3). Broilers receiving T1 and T2 diets exhibited a higher relative duodenal weight, whereas increased ileal weight was observed in the T1 and T3 groups. These responses may reflect region-specific physiological adaptations within the small intestine following FSBM supplementation. The increased duodenal weight likely indicates enhanced digestive and absorptive activity in the proximal small intestine, while the greater ileal weight may be associated with improved protein digestion and increased processing of residual nutrients reaching the distal intestinal segment. Collectively, these findings suggest that

FSBM supplementation supports intestinal function and may contribute to improved gut health in broiler chickens. This beneficial effect is likely related to the probiotic properties of *Bacillus* spp. present during the fermentation process, which have been reported to inhibit the proliferation of pathogenic bacteria and promote a healthier intestinal environment [19].

Table 3. The effect of fermented soybean meal (FSBM) using *Bacillus spp*. on small intestine profile

Research Variables	Treatment			
	T0	T1	T2	T3
Intestine Thickness(mm) ^{ns}	1.00±0.29	0.91±0.33	0.90±0.35	0.97±0.35
Small Intestine Length (cm) ^{ns}	185.84±17.92	184.84±20.84	181.96±17.14	188.88±18.86
% Duodenum Weight	0.85±0.19^{a}	0.98±0.18^{c}	0.97±0.18^{bc}	0.90±0.18^{ab}
% Jejunum Weight ^{ns}	1.57±0.25	1.58±0.23	1.51±0.22	1.57±0.25
% Ileum Weight	1.10±0.21^{ab}	1.16±0.20^{b}	1.08±0.16^{a}	1.18±0.21^{b}

Description:numbers in rows followed by different letters indicate significant differences (P<0.05); ns = non-significance (P>0.05)

3.3. Histomorphometric

The results showed a significant effect (P<0.05) on small intestine histomorphometrics (Figure 1). The appearance of small intestine histomorphometrics in broiler intestines can be seen in Table 4. T1 and T3 exhibited higher villi height compared to T2. Nevertheless, when compared with the control group (T0), T1 generally produced the most favorable intestinal histomorphometric profile, characterized by increased villus height and adequate villus width, indicating enhanced absorptive capacity. Although T2 and T3 improved certain structural parameters, excessive supplementation tended to reduce villus height or narrow villus dimensions in some intestinal segments. FSBM supplementation improved several aspects of intestinal morphology, particularly villus height in the jejunum and ileum. These findings suggest that the beneficial effects of FSBM are concentration-dependent and that moderate inclusion levels may optimize intestinal development more effectively than higher levels.

Table 4. The effect of fermented soybean meal (FSBM) using *Bacillus spp*. on small intestine histomorphometry (μm)

Parameter	Treatment			
	T0	T1	T2	T3
Villi Height				
Duodenum	1476±109.68^{a}	1682±206.69^{a}	1336±205.11^{a}	1195.99±692.69^{a}
Jejunum	779.19±63.41^{a}	1144±103.34^{b}	831.91±50.82^{a}	1256±86.78^{c}
Ileum	615.18±74.34^{ab}	743±33.31^{b}	653.36±171.17^{ab}	556.60±141.26^{a}
Basal Width				
Duodenum	114.05±25.37^{ab}	159.01±36.36^{b}	158.41±51.45^{b}	82.06±26.25^{a}
Jejunum	110.71±26.92^{a}	105.86±37.06^{a}	200.29±51.88^{b}	69±24.18^{a}
Ileum	82.93±11.00^{a}	74.70±26.75^{a}	120.24±13.13^{a}	165.54±56.49^{b}
Apical Width				
Duodenum	122.46±82.31^{b}	47.33±24.04^{a}	97.12±32.77^{ab}	34.99±12.47^{a}
Jejunum	54.96±12.83^{a}	35.24±8.95^{a}	152.43±129.40^{b}	17.79±4.71^{a}
Ileum	56.96±27.54^{ab}	43.35±22.16^{a}	67.80±13.70^{ab}	84.60±26.94^{b}

Description: numbers in rows followed by different letters indicate significant differences (P<0.05)

Consistent with the findings of the study by Zhang *et al.* [20], the addition of FSBM in a ratio increased the height of the duodenal villi, thereby expanding the surface area for the absorption of feed nutrients. In another study, Jazi *et al.* [17] reported that FSBM reduced the growth performance and villus height in the duodenum and jejunum in broilers aged 1-24 days infected with *Salmonella typhimurium*. Broiler feed with the addition of FSBM fermented with one type of *Bacillus* can still decrease intestinal villus height. This condition is generally followed by narrowing of the villi's basal and apical widths. The decrease in basal width observed in T3 indicates that the optimal effect of FSBM in broiler feed can be achieved at a certain concentration. Meanwhile, the decrease in apical width in the intestines of broilers fed with FSBM supplementation is thought

to occur due to changes in the intestinal microflora composition. *Bacillus* spp. can produce excess amounts of organic acid metabolites, potentially slowing enterocyte regeneration in the villi's apical portion [17]. Despite the decrease in apical villus width, the villi surface area remains increased, thus maintaining feed absorption efficiency and supporting lactic acid bacteria proliferation [15].

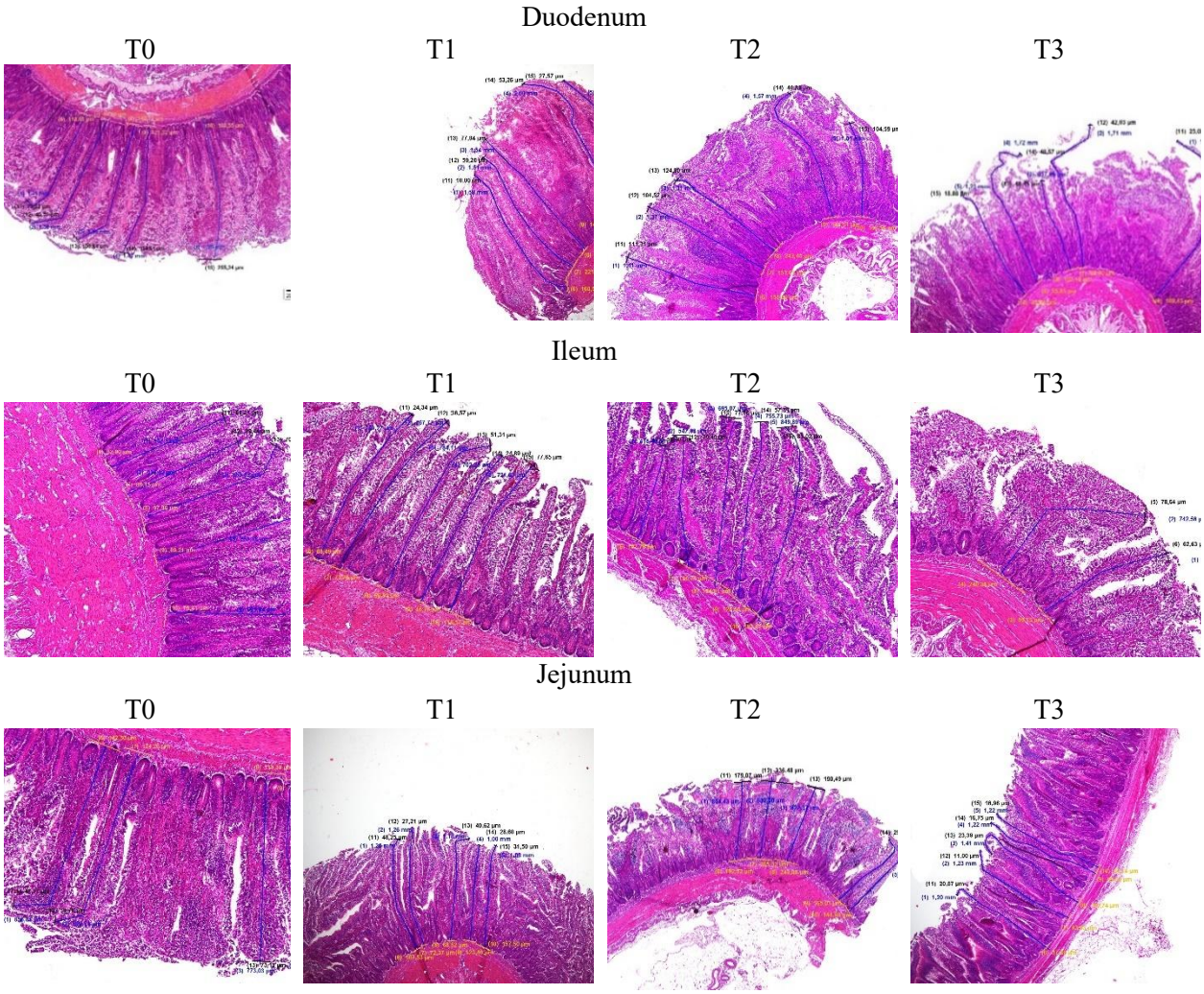

Figure 1. Histological analysis of villus height, basal width, and apical width of chicken intestine chiken

3.4. Total Bacterial Colonies and Lactic Acid Bacteria

The results showed significant effects ($P<0.05$) of dietary FSBM supplementation on intestinal pH, total bacterial colonies, and lactic acid bacteria (LAB) populations (Table 5). Broilers fed the T3 diet generally exhibited lower pH values throughout the small intestine compared with the control group, indicating a more acidic intestinal environment. This effect may be attributed to the organic acids, particularly lactic acid and acetic acid, produced during the soybean meal fermentation process. Previous studies have reported that FSBM supplementation decreases intestinal pH and promotes a more favorable microbial environment in broilers [21]. The lower intestinal pH observed in the present study may have contributed to changes in microbial populations by suppressing acid-sensitive pathogenic bacteria while supporting the growth of beneficial microorganisms. Consistent with this observation, total bacterial colonies were higher in T3, particularly in the duodenum and ileum, indicating increased microbial activity and colonization within the intestinal tract.

FSBM supplementation also significantly affected LAB populations, suggesting that it modified the composition of the intestinal microbiota rather than simply increasing total bacterial abundance. The highest LAB population was observed in T2, whereas LAB counts were lower in T3 despite its lower intestinal pH. This finding suggests that moderate FSBM supplementation may provide more favorable conditions for LAB proliferation than higher inclusion levels. FSBM contains bioactive compounds and fermentation metabolites that can serve as substrates for beneficial bacteria and enhance microbial balance. LAB contribute to intestinal health through the production of organic acids and antimicrobial compounds that inhibit pathogenic bacteria such as Escherichia coli and Salmonella spp. [14], [22]. Therefore, the higher LAB population observed in T2

may indicate a more balanced intestinal microbial ecosystem, while the increase in total bacterial colonies in T3 suggests that higher FSBM inclusion levels promote the growth of a broader microbial community. Overall, these results demonstrate that FSBM modulates the intestinal microbiota in a dose-dependent manner, with moderate supplementation appearing more effective in promoting beneficial bacterial populations.

Table 5. The effect of fermented soybean meal (FSBM) using *Bacillus spp*. on total bacterial colonies and *lactic acid bacteria* (log CFU/g)

Parameter	Treatment			
	T0	T1	T2	T3
Duodenum				
pH	5.40±0.89^{ab}	4.80±0.45^{ab}	5.20±0.45^{a}	4.00±0.00^{b}
Number of Colonies	11.41±10.73^{ab}	11.38±10.91^{ab}	11.23±10.88^{a}	11.45±10.65^{b}
Jejunum				
pH	5.20±0.84^{ab}	5.40±0.89^{b}	5.20±0.84^{ab}	4.20±0.45^{a}
Number of Colonies	11.46±10.34^{b}	11.09±10.79^{a}	11.11±11.11^{a}	11.40±10.69^{b}
Ileum				
pH	5.00±0.71^{ab}	5.60±0.55^{b}	5.20±0.84^{ab}	4.40±0.55^{a}
Number of Colonies	11.46±10.34^{b}	11.09±10.79^{a}	11.11±11.11^{a}	11.40±10.69^{b}
Number of LAB	8.42±7.77^{b}	8.42±7.96^{b}	8.46±7.54^{b}	8.25±8.09^{a}

Description: The results of the analysis of variance showed a significant difference (P<0.05)

3.5. Catalase, Motility and Gram Test

The results showed that the addition of FSBM had no effect (P>0.05) on catalase and motility tests (Figure 2). The average catalase test value in this study was 0.00-1.40, indicating that the probiotic species used were capable of producing catalase. The motility test results showed relatively similar motility values, namely 0.4-0.8. Decreased motility was observed in the ileum in treatments T2 and T3. This condition is considered an adaptive response of the intestine to increase nutrient absorption efficiency, which can improve feed conversion. Although the catalase and motility tests did not show significant differences, the results of the Gram stain test in broiler intestines provide an overview of the balance of Gram-positive and Gram-negative bacteria. Figure 2 shows that T1 produced fewer Gram-positive bacteria than T1, T2 and T3. The higher dominance of Gram-positive bacteria reflects the probiotic effect of FSBM, which can create a healthier intestinal microflora environment, thereby reducing the risk of infection and improving broiler growth performance [15].

Table 6. The effect of fermented soybean meal (FSBM) using *Bacillus spp*. on catalase and motility test results in broiler chicken digestion

Parameter	Treatment			
	T0	T1	T2	T3
Catalase ^{ns}				
Duodenum	0.00±0.00	1.40±0.89	0.60±1.34	1.20±0.60
Jejunum	0.20±0.45	1.20±0.84	1.00±1.41	0.60±1.34
Ileum	0.00±0.00	0.80±0.84	0.00±0.00	1.20±1.64
Motility ^{ns}				
Duodenum	0.80±0.45	0.60±0.55	0.60±0.55	0.80±0.45
Jejunum	0.80±0.45	0.80±0.45	0.80±0.45	0.80±0.45
Ileum	0.80±0.45	0.80±0.45	0.40±0.55	0.40±0.55

Description: The results of the analysis of variance showed no significant difference (P>0.05). Catalase activity test using a scoring system as follows: 0 = catalase negative (no bubble formation), 1 = catalase positive (few bubbles formed), 2 = catalase positive (many bubbles formed), and 3 = catalase positive (many large bubbles formed). Motility test using scoring system as follows: 0 = non-motile (no growth spreading from the stab line) and 1 = motile (growth spreading from the stab line).

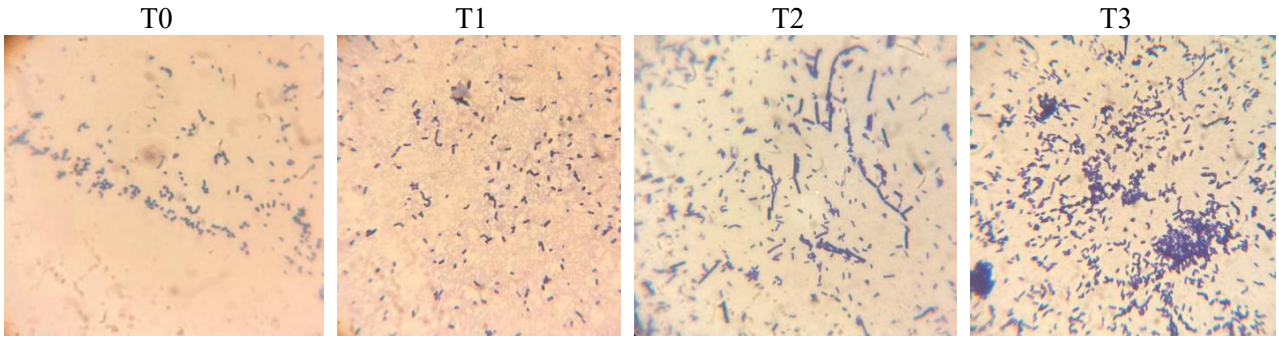

Figure 2. Results of gram testing on broiler chicken small intestine

4. Conclusion

In conclusion, fermentation with *Bacillus* spp. represents an effective strategy to reduce anti nutritional and enhance feed quality. The improved intestinal morphology and lactic acid bacteria populations in broilers observed in this study suggest that 2.5% FSBM. Therefore, *Bacillus* spp. fermented soybean meal has potential as a functional and sustainable feed ingredient for commercial broiler production.

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6. Conflict of Interest

The authors state no conflict of interest.

References

[1] B. Dastar, A. Ashayerizadeh, F. Sharifi, and V. Jazi, "Replacement of soybean meal with fermented rapeseed meal in broiler diets: impacts on growth performance, gut health, and nutrient digestibility," *Poult. Sci.*, p. 105616, Jul. 2025, doi: 10.1016/j.psj.2025.105616.

[2] M. T. Lambo *et al.*, "Mechanism of action, benefits, and research gap in fermented soybean meal utilization as a high-quality protein source for livestock and poultry," *Anim. Nutr.*, vol. 16, pp. 130–146, Mar. 2024, doi: 10.1016/J.ANINU.2023.10.003.

[3] G. Deveci, E. Çelik, D. Ağagündüz, E. Bartkiene, J. M. F. Rocha, and F. Özogul, "Certain Fermented Foods and Their Possible Health Effects with a Focus on Bioactive Compounds and Microorganisms," *Fermentation*, vol. 9, no. 11, Nov. 2023, doi: 10.3390/FERMENTATION9110923.

[4] N. Miraalami, S. N. Mousavi, M. R. Abedini, and G. A. Jafarabadi, "The effect of heat and fermentation treatments of soybean meal on performance, immune system, microbial population, intestinal morphology, and PepT1 gene expression in broiler chickens," *J. Anim. Sci.*, vol. 103, 2025, doi: 10.1093/JAS/SKAF040.

[5] T. Y. Lee, Y. S. Lee, R. H. Yeh, K. H. Chen, and K. L. Chen, "Bacillus amyloliquefaciens CU33 fermented feather meal-soybean meal product improves the intestinal morphology to promote the growth performance of broilers," *Poult. Sci.*, vol. 101, no. 9, p. 102027, 2022, doi: 10.1016/j.psj.2022.102027.

[6] A. Husnain *et al.*, "Effects of replacement of soybean meal with fermented soybean meal on growth performance, nutrient digestibility and carcass characteristics in broiler," *Livest. Sci.*, vol. 299, p. 105779, Sep. 2025, doi: 10.1016/J.LIVSCI.2025.105779.

[7] S. M. Abdel-Raheem *et al.*, "Double-Fermented Soybean Meal Totally Replaces Soybean Meal in Broiler Rations with Favorable Impact on Performance, Digestibility, Amino Acids Transporters and Meat Nutritional Value," *Anim. 2023, Vol. 13, Page 1030*, vol. 13, no. 6, p. 1030, Mar. 2023, doi: 10.3390/ANI13061030.

[8] W. Liu, W. Wang, J. Li, H. Li, T. Gao, and B. Zhu, "Anaerobic fermentation of soybean meal by Bacillus subtilis ED-3-7 and its effect on the intestinal microbial community of chicken," *Poult. Sci.*, vol. 104, no. 1, p. 104564, Jan. 2024, doi: 10.1016/J.PSJ.2024.104564.

[9] M. Zhao *et al.*, "The investigation into the physicochemical properties of fermented soybean meal and its effects as a substitute for soybean meal on the growth performance and intestinal development of broiler chickens," *Poult. Sci.*, p. 105670, Aug. 2025, doi: 10.1016/J.PSJ.2025.105670.

[10] A. International, *Official Methods of Analysis of AOAC International*, 21st Editi. Gaithersburg, MD,

USA: AOAC International, 2019.
[11] P. A. Iji, A. A. Saki, and D. R. Tivey, "Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide," *J. Sci. Food Agric.*, vol. 81, no. 12, pp. 1186–1192, Sep. 2001, doi: 10.1002/JSFA.925.
[12] Hadioetomo, "Mikrobiologi Dasar Dalam Praktek," *Jakarta: Gramedia*. 1990.
[13] S. Guo *et al.*, "Partial Substitution of Fermented Soybean Meal for Soybean Meal Influences the Carcass Traits and Meat Quality of Broiler Chickens," *Anim. an Open Access J. from MDPI*, vol. 10, no. 2, p. 225, Feb. 2020, doi: 10.3390/ANI10020225.
[14] A. Irawan *et al.*, "Effect of feeding fermented soybean meal on broiler chickens' performance: a meta-analysis," *Anim. Biosci.*, vol. 35, no. 12, p. 1881, Dec. 2022, doi: 10.5713/AB.21.0546.
[15] Y. Li *et al.*, "Effects of Fermented Soybean Meal Supplementation on the Growth Performance and Cecal Microbiota Community of Broiler Chickens," *Anim. 2020, Vol. 10, Page 1098*, vol. 10, no. 6, p. 1098, Jun. 2020, doi: 10.3390/ANI10061098.
[16] R. Pérez–Velasco, B. Gómez-Gil, E. Martínez-Montaño, A. F. González-Córdova, and C. Hernández, "Nutritional attributes and microbial metagenomic profile during solid-state fermentation of soybean meal inoculated with Lactobacillus acidophilus under non-sterile conditions," *J. Sci. Food Agric.*, vol. 104, no. 13, Oct. 2024, doi: 10.1002/JSFA.13657.
[17] V. Jazi, H. Mohebodini, A. Ashayerizadeh, A. Shabani, and R. Barekatain, "Fermented soybean meal ameliorates Salmonella Typhimurium infection in young broiler chickens," *Poult. Sci.*, vol. 98, no. 11, pp. 5648–5660, Nov. 2019, doi: 10.3382/PS/PEZ338.
[18] J. Feng, X. Liu, Z. R. Xu, Y. Z. Wang, and J. X. Liu, "Effects of Fermented Soybean Meal on Digestive Enzyme Activities and Intestinal Morphology in Broilers," *Poult. Sci.*, vol. 86, no. 6, pp. 1149–1154, Jun. 2007, doi: 10.1093/PS/86.6.1149.
[19] A. Y. L. Teo and H. M. Tan, "Effect of Bacillus subtilis PB6 (CloSTAT) on Broilers Infected with a Pathogenic Strain of Escherichia coli," *J. Appl. Poult. Res.*, vol. 15, no. 2, pp. 229–235, Jul. 2006, doi: 10.1093/JAPR/15.2.229.
[20] S. T. Zhang, Y. Shi, S. L. Zhang, W. Shang, X. Q. Gao, and H. K. Wang, "Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation," *Food Control*, vol. 41, no. 1, pp. 1–6, Jul. 2014, doi: 10.1016/J.FOODCONT.2013.12.026.
[21] Y. H. Cheng, F. S. H. Hsiao, C. M. Wen, C. Y. Wu, A. Dybus, and Y. H. Yu, "Mixed fermentation of soybean meal by protease and probiotics and its effects on the growth performance and immune response in broilers," *J. Appl. Anim. Res.*, vol. 47, no. 1, pp. 339–348, Jan. 2019, doi: 10.1080/09712119.2019.1637344.
[22] S. Sugiharto and S. Ranjitkar, "Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review," *Anim. Nutr.*, vol. 5, no. 1, pp. 1–10, Mar. 2019, doi: 10.1016/J.ANINU.2018.11.001.