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The Effects of Synbiotic Supplementation with Galoba (*Hornstedtia alliacea*) Fruit Extract and *Lactobacillus plantarum* on Broiler Chicken Performance and Intestinal Health

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ABSTRACT

Restrictions on antibiotic use in poultry have encouraged the search for natural alternatives to maintain gut health and performance. This study assesses the effects of supplementing broiler chickens with *Lactobacillus plantarum* and galoba fruit (*Hornstedtia alliacea*) extract on their intestinal health and growth performance. Two hundred and fifty broiler chickens raised together from day 0 to day 7 participated in an in vivo investigation. Beginning on day 8, treatments GL1, GL2, GL3, and GL4 received dosages of 0.5, 1.0, 1.5, and 2.0mL/kg feed of synbiotics, while the control group (CONT) did not receive any synbiotic supplementation. Galoba extract and *L. plantarum* supplementation significantly improved growth performance, increased the length of villi in the duodenum, jejunum, and ileum, decreased the pH in the ileum and cecum, increased the population of lactic acid bacteria, and decreased the coliform counts in the ileum and cecum ($P<0.05$), according to the results. To sum up, using synbiotics made of *L. plantarum* and galoba fruit extract has a lot of promise as a practical nutraceutical approach to enhance the intestinal morphology, microbial balance, and performance of broiler chickens. These findings provide a practical basis for developing sustainable, locally sourced feed additives in antibiotic-free poultry production systems.

Keywords: Sinbiotik, *Hornstedtia alliacea*, *Lactobacillus plantarum*, Broiler performance, Intestinal health.

INTRODUCTION

In Indonesia's poultry farming industry, broiler chickens are an important commodity that is relied upon to meet the domestic demand for animal protein (Fadilah et al. 2025). Formerly, farmers typically used Antibiotic Growth Promoters (AGPs) as feed additives to enhance growth performance and production efficiency (Wickramasuriya et al. 2024). However, since January 2018, the Indonesian government has banned their use due to the risks of antimicrobial resistance and food residues. This has encouraged the exploration of natural supplements using probiotics, prebiotics and synbiotics as healthier alternatives (Amenyogbe et al. 2020; Ramaiyulis et al. 2023; Dharmayanti et al. 2025).

Galoba fruit (*Hornstedtia alliacea*) has been recognized in several studies as containing antioxidants, flavonoids, tannins, saponins, and other bioactive compounds, signifying its potential use as a prebiotic that promotes intestinal health (Elim 2021; Julianto et al. 2025). Gustaman et al. (2020), for example, have measured its antioxidant activity using the DPPH method and found that the ethyl acetate extract of Galoba fruit has an IC50 value of 23.43ppm, which is categorized as strong. In addition, Galoba extract has been reported to exhibit antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in in vitro studies (Sule et al. 2023).

Meanwhile, *L. plantarum* is one of the most widely explored probiotics in studies on broiler chickens (Yilmaz et al. 2022; Yin et al. 2023; Sapsuha et al. 2025). Numerous studies have revealed that supplementation with *L. plantarum* can enhance growth performance and feed efficiency, reduce pathological infections, improve gut health, and boost immunity (Yang et al. 2024; Mirsalami and Mirsalami 2024). Previous studies have also reported that *L. plantarum* supplementation can increase feed

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intake ratio, body weight gain, cecal microbiota composition, and immune response (Chen et al. 2023; Liu et al. 2025), raise the levels of organic acids (short-chain fatty acids, SCFAs), enhance antioxidant activity and reduce pathogenic load in the intestine (Xiao et al. 2024; Jiao et al. 2025; Du et al. 2025).

Synbiotics are a combination of prebiotics, indigestible substrates that support the growth of beneficial microbes and probiotics, live microorganisms that bring considerable health benefits to the host. This combination is considered more effective than their individual use in improving gut microbiota balance, enhancing nutrient metabolism, and boosting immunity. Previous studies have shown that synbiotics can increase feed intake, body weight gain, and feed conversion in broilers (Beski and Al-Sardary 2015; Mohammed et al. 2018; Sapsuha et al. 2023; Rahmi et al. 2025). The usage of synbiotics made from *L. plantarum* and galoba fruit extract, however, has not been reported in the relevant literature. Thus, the purpose of this study was to examine the effects of supplementing broiler chickens with galoba fruit extract and *L. plantarum* as a synbiotic on their intestinal health and growth performance, as well as the microbial composition and intestinal morphology of these birds. It was hypothesized that synbiotic supplementation would improve intestinal morphology, microbiota balance, and performance in broilers.

MATERIALS AND METHODS

Preparation of synbiotic from galoba fruit extract and *L. plantarum*

Ripe galoba fruits from the forests of Halmahera Island in Indonesia's North Maluku Province were used in this investigation. With minor adjustments, the Sule et al. (2023) method was used to prepare the galoba fruit extract. One kilogram of galoba fruit powder was extracted using the maceration procedure by soaking it in four liters of 96% ethanol solution for three by twenty-four hours. During the maceration process, stirring was carried out twice a day, in the morning and in the afternoon. The soaking result in the form of filtrate was then filtered and evaporated using a rotary evaporator to obtain a thick extract of galoba fruit.

With a few minor adjustments, the synbiotic preparation was made using the methodology described by Sapsuha et al. (2023). First, 100mL of distilled water was combined with 25mL of galoba fruit extract. The solution was then supplemented with 10mL of *L. plantarum* suspension (bacterial concentration 1×10^9CFU/mL). The mixture was then plated on MRSA using the pour plate method, incubated at 37°C for 24 hours, and then incubated at 37°C for 48 hours. Before being used, the resultant synbiotic culture was kept in a refrigerator.

In vivo experiment

From day 0 to day 7, the total of 250 broiler chicks were raised collectively. On day 8, they (average body weight 153.07 ± 2.14g) were randomly allocated into five treatment groups: CONT (basal diet without synbiotic supplementation with galoba fruit extract and *L. plantarum*), GL1 (basal diet + synbiotic consisting of galoba fruit extract and *L. plantarum* at 0.5g/kg feed), GL2 (basal diet + synbiotic supplementation at 1.0g/kg feed), GL3 (basal diet + synbiotic supplementation at 1.5g/kg feed), and GL4 (basal diet + synbiotic supplementation at 2.0g/kg feed). Each treatment was replicated five times.

The broilers were fed mash diets formulated (Table 1) as starter feed (days 1–21) and finisher feed (days 22–35). They were housed in 1 × 1m² broiler pens bedded with wood shavings, at a stocking density of 10 broilers per pen. The environmental temperature was controlled at 32 ± 1°C at the beginning of the rearing period and gradually reduced to approximately 27 ± 1°C after day 21.

Table 1: Broiler feed formulations as as starters (days 1-21) and finishers (days 22-35).

Items	%, Unless specified otherwise	
	Starter (days 1-21)	Finisher (days 22-35)
Yellow corn	5.61	65.87
Soybean meal	33.47	26.21
Palm oil	2.42	2.42
DL-methionine	0.46	0.46
Bentonite	1.18	1.18
Limestone	1.98	1.98
Monocalcium phosphate	1.24	1.24
Premix[1]	0.28	0.28
Chlorine chlorite	0.07	0.07
NaCl	0.29	0.29
Nutrient contents:		
Metabolizable energy (kcal/kg)[2]	2942	3116
Crude protein (%)	21.87	19.32
Crude fiber (%)	3.23	3.12
Crude fat (%)	4.22	4.57

Broiler performance

Throughout the rearing stage, body weight, feed intake, and feed conversion ratio (FCR) were recorded in order to gather data on broiler performance. Every week, each broiler's body weight was determined using a digital scale with an accuracy of 0.01g, and feed intake was computed by subtracting the weekly feed residual from the amount of feed that was fed.

Intestinal microbial population

By taking digesta samples from the ileum and cecum and keeping them in sterile containers, the intestinal microbial population was identified. Prior to inoculation onto selective media, the samples were serially diluted with physiological saline solution (0.9% NaCl). MacConkey agar (Merck KGaA, Darmstadt, Germany) was used to count coliform bacteria, which were then cultured aerobically for 24 hours at 37–38°C. Lactic acid bacteria (LAB) were counted on de Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA) and then incubated anaerobically for 48hours at the same temperature. Bacterial counts were represented as log10 colony-forming units per gram (cfu/g) of digesta, and colonies growing on the media were counted using a colony counter.

Intestinal morphology

Tissue samples from the ileum, jejunum, and duodenum were taken in order to evaluate intestinal morphology. About 2cm long tissue segments were fixed in 10% buffered formalin solution after being washed with physiological saline solution (0.9% NaCl). The samples were then cleaned, dehydrated, and paraffin-embedded. A microtome was used to cut histological slices that were 5µm thick, and the hematoxylin-eosin (HE) procedure was used to stain them. A light microscope with a 40× magnification was used to quantify intestinal wall thickness, villus height, crypt depth, villus-to-crypt ratio, and other morphological parameters on the produced slides. For every sample, five randomly chosen fields were measured, and the findings were presented as mean values.

Statistical Analysis

The collected data were analyzed statistically using ANOVA with SPSS software version 25.0. When the treatments showed a significant influence on the measured variables (P<0.05), Duncan's multiple range test was applied to identify differences among the treatment means.

RESULTS

Broiler performance

The broiler performance data is shown in Table 2. The findings demonstrated that broiler performance was significantly (P<0.05) impacted by supplementing with synbiotics including *L. plantarum* and galoba fruit extract. In comparison to CONT, GL1, and GL2, the average daily weight growth of broilers in GL4 was considerably higher (P<0.05). But there was no discernible difference between CONT and GL1 or between GL4 and GL3. Likewise, GL4's daily feed consumption was considerably higher (P<0.05) than CONT, GL1, GL2, and GL3. Additionally, feed efficiency did not differ significantly from that in GL2, but it was significantly lower (P<0.05) in GL1 when compared to CONT, GL3, and GL4.

Intestinal microbial population

Table 3 shows the average pH and intestinal microbial population levels in broilers fed synbiotics made from *L. plantarum* and galoba fruit extract. The findings showed that while synbiotic supplementation had no significant effect (P>0.05) on the pH of the duodenum and jejunum, it significantly (P<0.05) lowered the pH of the ileum and cecum. Additionally, supplementation with *L. plantarum* synbiotics and galoba fruit extract significantly (P<0.05) decreased the coliform population in the ileum and cecum while increasing the lactic acid bacterial population.

Intestinal morphology

Table 4 shows the intestinal morphology data of broilers fed with *L. plantarum* and galoba fruit extract synbiotics. The study found that the villus height in the duodenum, jejunum, and ileum, as well as the villus height-to-crypt depth ratio in the jejunum and ileum, were considerably (P<0.05) increased by synbiotic supplementation. However, neither the villus height-to-crypt depth ratio in the duodenum nor the crypt depth in any region of the small intestine were significantly affected (P>0.05) by the treatment.

DISCUSSION

Synbiotics from galoba fruit extract and *L. plantarum* have been shown to improve broiler growth performance. This can be explained through the synergistic interaction between the prebiotic (galoba fruit extract) and the probiotic (*L. plantarum*). The bioactive compounds in galoba fruit, particularly phenolics and flavonoids, act as

Table 2: Performance of chicken broilers

Items	Treatment groups					SE	P-value
	CONT	GL1	GL2	GL3	GL4		
Initial BW (g)	153.68	152.98	153.14	152.62	152.94	12.25	0.18
Final BW (g)	1567.24^{c}	1555.68^{a}	1615.74^{b}	1698.62^{c}	1712.43^{c}	153.55	<0.01
Weight gain (g)	1413.56^{c}	1402.70^{a}	1462.60^{b}	1546.00^{c}	1559.49^{c}	151.61	<0.01
Accumulative FI (g)	2783.42^{c}	2788.72^{c}	2772.98^{ab}	2766.05^{a}	2770.78^{a}	0.18	0.03
FCR	1.96^{c}	1.98^{c}	1.89^{c}	1.78^{a}	1.77^{a}	1.73	<0.01

^{a,b,c}On the same row, different superscripts indicated a significant variation (P<0.05). CONT (basal diet without synbiotic supplementation with galoba fruit extract and *L. plantarum*), GL1 (basal diet + synbiotic consisting of galoba fruit extract and *L. plantarum* at 0.5g/kg feed), GL2 (basal diet + synbiotic supplementation at 1.0g/kg feed), GL3 (basal diet + synbiotic supplementation at 1.5g/kg feed), and GL4 (basal diet + synbiotic supplementation at 2.0g/kg feed). SE: standard error. FI: feed intake. BW: body weight. FCR: feed conversion ratio.

Table 3: Treatment diet-fed broiler chickens' pH and bacterial population

Items	Treatment groups					SE	p value
	CONT	GL1	GL2	GL3	GL4		
Ph							
Duodenum	6.34	6.55	6.38	6.42	6.51	0.36	0.64
Jejunum	5.34	5.35	5.32	5.20	5.22	0.28	0.38
Ileum	6.14^{b}	6.11^{b}	5.53^{a}	5.23^{b}	5.22^{b}	0.54	0.04
Cecum	7.84^{c}	7.73^{c}	6.79^{ab}	6.48^{a}	6.41^{a}	0.42	<0.01
Lactic Acid Bacteria (log cfu/g)							
Ileum	7.55^{a}	8.52^{b}	9,31^{bc}	9.30^{bc}	9.41^{c}	0.56	p<0.01
Cecum	8.04^{a}	9.02^{b}	9.61^{c}	9.63^{c}	9.66^{c}	0.62	p<0.01
Coliform (log cfu/g)							
Ileum	6.39^{b}	5.89^{b}	5.26^{a}	5.21^{a}	5.18^{a}	1.18	<0.01
Cecum	7.39^{b}	6.41^{b}	6.24^{b}	6.89^{ab}	5.24^{a}	1.54	0.02

^{a,b,c} Means on the same row with different superscripts differ at P<0.05). CONT (basal diet without synbiotic supplementation with galoba fruit extract and *L. plantarum*), GL1 (basal diet + synbiotic consisting of galoba fruit extract and *L. plantarum* at 0.5g/kg feed), GL2 (basal diet + synbiotic supplementation at 1.0g/kg feed), GL3 (basal diet + synbiotic supplementation at 1.5g/kg feed), and GL4 (basal diet + synbiotic supplementation at 2.0g/kg feed), SE: standard error.

Table 4: Treatment diet-fed broiler chickens' intestinal morphology

Items	Treatment groups					SE	P-value
	CONT	GL1	GL2	GL3	GL4		
Duodenum							
Villi height (µm)	1058.42[a]	1069.32[a]	1089.32[b]	1106.42[c]	1120.34[c]	98.34	<0.01
Crypt depth (µm)	101.84	105.16	111.23	112.85	113.20	11.54	0.28
VH/CD	10.39	10.16	9.79	9.08	9.89	1.80	0.32
Jejunum							
Villi height (µm)	1154.28[a]	1167.21[a]	1189.24[b]	1198.78[b]	1193.24[b]	87.56	<0.01
Crypt depth (µm)	82.64	83.52	84.56	83.32	82.89	3.16	0.87
VH/CD	13.96[a]	13.97[a]	14.06[ab]	14.38[b]	14.39[b]	1.39	0.03
Ileum							
Villi height (µm)	737.16[a]	732.54[a]	752.98[a]	888.31[b]	889.98[b]	138.90	<0.01
Crypt depth (µm)	70.29	71.43	70.84	70.68	70.42	2.84	0.43
VH/CD	10.48[a]	10.25[a]	10.62[a]	12.56[b]	12.63[b]	1.54	0.02

[a,b]On the same row, different superscripts indicated a significant variation (P<0.05). CONT (basal diet without synbiotic supplementation with galoba fruit extract and *L. plantarum*), GL1 (basal diet + synbiotic consisting of galoba fruit extract and *L. plantarum* at 0.5g/kg feed), GL2 (basal diet + synbiotic supplementation at 1.0g/kg feed), GL3 (basal diet + synbiotic supplementation at 1.5g/kg feed), and GL4 (basal diet + synbiotic supplementation at 2.0g/kg feed). SE: standard error.

prebiotic substrates that stimulate the growth of beneficial bacteria (Sule et al. 2023; Salamena et al. 2025). Meanwhile, *L. plantarum* enhances the colonization of lactic acid bacteria in the gastrointestinal tract, decreases the population of pathogenic bacteria, and promotes a more balanced gut microbiota. Such colonization results in reduced digesta pH, improved intestinal mucosal integrity, and enhanced nutrient absorption efficiency (Vernocchi et al. 2020). Furthermore, the phenolic compounds found in galoba fruit have antimicrobial and antioxidant properties that contribute to strengthening the immune system and reducing oxidative stress (Surai et al. 2019; Sultana et al. 2025). The combination of these effects has been demonstrated to increase feed intake, body weight gain, and feed conversion efficiency in broiler chickens.

Supplementation with synbiotics containing galoba fruit extract and *L. plantarum* positively affects the performance of broiler chickens. Khosravi et al. (2025) reported in their study that combining *Enterococcus faecium* and galactooligosaccharides can significantly improve broiler performance. Acharya et al. (2024) also demonstrated that synbiotic supplementation at levels higher than the recommended dose enhances body weightgain and feed conversion efficiency, as well as reduces intestinal damage caused by necrotic enteritis. Similarly, the addition of synbiotics to the feed has been shown to improve feed conversion efficiency and immune response (White et al. 2024). Furthermore, Zhao et al. (2024) confirmed that synbiotic supplementation combined with enzymes improves growth performance, intestinal morphology, and immune organs development.

Supplementation with galoba fruit extract and *L. plantarum* synbiotics can improve the composition and function of the intestinal microbial ecosystem through dual mechanisms: firstly, the prebiotic–polyphenol effect of the galoba fruit, which modulates the microbial community and its metabolites; and secondly the probiotic activity of *L. plantarum*, which enhances the colonization of beneficial bacteria, strengthens barrier integrity, and reduces mucosal inflammation. Galoba fruit is rich in phenolic compounds (tannins, flavonoids), saponins, and steroids, with demonstrated in vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Sule et al. 2023), signifying its potential as a bioactive source for microbiota modulation.

The mechanism by which galoba fruit extract and *L. plantarum* synbiotics improve gut microbiota involves both prebiotic and probiotic actions. The polyphenols present in galoba fruit act as prebiotics as they are largely not absorbed in the small intestine, thus being able to reach the colon where they are metabolized by microbes into simple phenolic compounds that can promote the growth of commensal bacteria such as *L.* and *Bifidobacterium*. Recent studies have highlighted the central role of polyphenols in modulating the gut ecosystem and their importance as key components of plant-based synbiotics (Rodríguez-Daza et al. 2021). *L. plantarum*, on the other hand, acts as a probiotic; this lactic acid bacterium is capable of producing lactic acid and acetic acid, lowering intestinal pH, producing bacteriocins, and competing for epithelial adhesion receptors, thereby inhibiting pathogen colonization. This confirms the finding of a prior study that *L. plantarum* increases microbial diversity, improves metabolite profiles, and strengthens mucosal immune function (Xiao et al. 2024; Setiani et al. 2025).

According to the study's findings, broiler chickens' villus height-to-crypt depth ratio in the jejunum and ileum as well as their villus height in the duodenum, jejunum, and ileum increase when supplemented with galoba fruit extract and *L. plantarum* synbiotics. Such improvement of intestinal morphology is encouraged by the enhanced production of fermentation metabolites, including short-chain fatty acids (SCFAs)—such as acetate, propionate, and butyrate—generated from the fermentation of prebiotics by probiotic bacteria. SCFAs serve as the main energy source for enterocytes, stimulate epithelial cell proliferation, and enhance the mucosal tissue's differentiation and integrity. These changes lead to increased villus height and deeper crypts, which histologically reflect an improved absorptive capacity of the intestine. In addition, the probiotics contained in synbiotics reduce intestinal pH (Table 3), produce bacteriocins, and fight pathogenic bacteria, thereby creating a favorable environment for mucosal recovery. This study's findings regarding this mechanism support those of several previous studies. For instance, synbiotic supplementation with *L. plantarum* has been shown to increase villus height and villus-to-crypt ratio in animal models with intestinal damage due to stress or infection (Dong et al. 2025). Similarly, another study has reported that combining probiotics with polyphenol or soluble fiber substrates enhances mucosal thickness and reduces the degree of histological damage caused by inflammation (Xiao et al. 2024). Nonetheless, this research has a number of limitations that need to be recognized. The duration of the experiment was fairly brief (35 days), which might not adequately reflect the long-term impacts of synbiotic supplementation on gut health and performance. Furthermore, molecular techniques like 16S rRNA sequencing were not conducted to identify specific changes in gut microbiota composition, hindering the comprehension of microbial mechanisms behind the observed physiological responses. Additionally, immune-related biomarkers, including cytokine profiles and intestinal immunoglobulin levels, were not assessed, potentially offering greater understanding of the immunomodulatory impacts of the synbiotic therapy. Future studies should hence incorporate extended feeding trials alongside thorough microbiome and immune evaluations to clarify the involved mechanistic pathways.

Conclusion

Synbiotic supplementation combining *Lactobacillus plantarum* and galoba (*Hornstedtia alliacea*) fruit extract improved broiler growth performance, intestinal morphology, and microbial balance. The synergistic effects enhanced villus structure, reduced intestinal pH, and increased beneficial bacteria. This led to better nutrient absorption and feed efficiency. This synbiotic shows strong potential as a natural, sustainable alternative to antibiotic growth promoters in tropical poultry production. Future research should examine its long-term effects, immune and microbiome responses, and practical applications in commercial feeding systems.

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Author's Contribution: Yusri Sapsuha arranged the experimental design and wrote the original manuscript. Sundari Sundari conducted the experiment in the laboratory. Amran Nur finalized the draft. Sri Utami data collection. hasriani Ishak analyzed the data. The final version of the manuscript was revised and approved by all authors.

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