Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 1, 43-46

Effects of encapsulated *Peperomia pellucida* extract and *Lactobacillus plantarum* on intestinal bacteria, fat and cholesterol contents of meat in broiler chicken

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ARTICLE INFO

Received: 04 November 2025

Accepted: 30 November 2025

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Keywords:

Broiler chickens, Carcass, Encapsulation, *Lactobacillus plantarum*, *Peperomia pellucida*

ABSTRACT

The study looked at how adding *Peperomia pellucida* extract and encapsulated *Lactobacillus plantarum* (PPELPE) affects the fat content, chemical makeup, and meat yield of broiler chickens. Researchers used 200 Ross strain broilers, 8 days old, weighing about 177.09 grams each. They used a randomized design with five treatments, each repeated four times, with 10 birds per group. The treatments were: T0 = basic feed (BF) without PPELPE, T1 = BF + 0.1% PPELPE, T2 = BF + 0.2% PPELPE, T3 = BF + 0.3% PPELPE, and T4 = BF + 0.4% PPELPE. They measured total bacteria (lactic acid bacteria (LAB) and coliforms in the ileum), ileum pH, fat digestibility, relative abdominal fat weight, and meat chemistry (fat and cholesterol) in the breast, femur, and tibia. Results showed that adding PPELPE significantly affected ($p<0.05$) total LAB, coliforms, ileum pH, fat digestibility, relative abdominal fat weight, tibia meat fat content, and cholesterol levels in breast and femur meat. However, it did not significantly change the fat content of breast and femur meat or the cholesterol content of the tibia. The study concluded that adding 0.4% PPELPE to the diet can increase total LAB and meat production while reducing total coliforms, ileum pH, fatty meat, tibia meat fat content, and cholesterol levels in breast and femur meat.

Introduction

Broiler chickens are genetically selected broilers with fast growth and high feed conversion efficiencies. Broiler meat is a known source of animal protein because of its high protein content and relatively low fat content, making it a top choice for consumers. The superior growth and feed efficiency of broilers makes them a key commodity in the modern poultry industry. In Indonesia, broiler production has increased significantly, in line with the increasing demand for animal proteins. Broiler production in 2023 reached approximately 3.8 million tons and is projected to increase to 4.2 million tons in 2024, with a production surplus of 195.84 thousand tons, in 2025, it is estimated to increase to 3.98 million tons, with a growth of 5.61% compared to the previous year, and is predicted to reach 4.48 million tons in 2029, with an average annual growth rate of 2.73%.

The success of broiler production is not only influenced by genetic factors but also by rearing management, especially in terms feed, environment, and health. Therefore, efforts to increase productivity and carcass quality shoul be supported by feed management strategies. Proper feed management has been shown to improve feed efficiency, animal health, and broiler performance (Aftab *et al*., 2018). A well-designed feed and supplementation strategy can improve broiler growth and carcass quality.

Approaches to improve production efficiency and meat quality natural feed additives. *Peperomia pellucida* is a tropical herbal plant that contains bioactive compounds such as flavonoids, polyphenols, alkaloids, saponins, and tannins, which have antioxidant, antibacterial, and immunomodulatory activities. These compounds help to reduce oxidative stress, improve intestinal mucosal health, and strengthen the broiler immune system (Li *et al*., 2024). In addition, *Lactobacillus plantarum*, a type of lactic acid bacteria, functions as a probiotic that improves gut microbial balance, produces lactic acid and short-chain fatty acids (SCFA), and supports immune capacity and nutrient absorption (Kopec and Slizewska, 2020).

The combination of *Peperomia pellucida* extract and *Lactobacillus plantarum* had a synergistic effect on the growth performance and digestive health of broiler chickens. However, the efficacy of bioactive compounds and probiotics may decrease during processing and digestion. To overcome this, encapsulation technology has been used to improve the stability, bioavailability, and controlled release of the active compounds. Maltodextrin is often used as a coating agent for encapsulation because it is compatible with hydrophilic compounds and can protect active compounds during storage (Zabot *et al*., 2022). The novelty of this study is that the combination of encapsulated *Peperomia pellucida* and *Lactobacillus plantarum* extracts can act synergistically as immunodulators and inhibitors of the amount of fat and cholesterol in the body. Therefore, this study aimed to evaluate the effect of the combination of encapsulated *Peperomia pellucida* extract and *Lactobacillus plantarum* on total small intestinal bacteria, meat fatty acids, meat chemical content, and broile chicken carcass production.

Materials and methods

The study took place from May to June 2025 in digestion cages at the Animal Nutrition Laboratory, part of the Faculty of Animal and Agricultural Sciences at Universitas Diponegoro in Semarang.

Animals and requirements

A group of 200 Ross broilers, each 8 days old and unsexed, with an average weight of 177.09±1.8 g, were utilized in the study. The natural feed additive comprised *Peperomia pellucida* extract serving as a phytobiotic and *Lactobacillus plantarum* functioning as a probiotic. The equipment employed included a digital thermo-hygrometer and precision digital scales. Materials for extraction and encapsulation consisted of 96% ethanol, maltodextrin, distilled water, and various tools such as sonicators, filter paper, and beakers. The composition and nutrient content of the experimental diets are detailed in Table 1.

Table 1. Composition and calculated chemical analysis of the experimental diets for broiler chicken.

Feed stuff	Composition	
	Starter (8 – 21 d)	*Finisher* (22 – 35 d)
Yellow Corn	50.41	55.41
Rice Bran	14.74	14.74
Soybean Meal	24	19
Meat Bone Meal	10	10
Limestone	0.3	0.3
Premix	0.25	0.25
L-Lisin***	1.2	1.1
DL-Metionin***	0.58	0.47
Total	100	100
Nutrien Content		
Metabolizable Energy **(kkal/kg)	3,062.85	3,073.69
Crude Protein*(%)	21.63	19.68
Crude Fat*(%)	4.37	4.45
Crude Fiber*(%)	4.39	4.33
Calcium*(%)	1.44	1.42
Phosphor*(%)	0.75	0.72

Source: * Feed Tested for Proximate and Minerals in the Laboratory of Animal Nutrition, Faculty of Anima and Agricultural Sciences, Universitas Diponegoro (2025). ** Metabolic Energy Calculated Using Bolton Formula (1967). *** Analysis results at the Feed Quality and Certification Center (2025).

Experimental design

A completely randomized design was implemented, featuring five different treatments and four repetitions, with each replicate comprising 10 birds. The treatments included: T0: Basal feed
T1: Basal feed + 0.1% PPELPE
T2: Basal feed + 0.2% PPELPE
T3: Basal feed + 0.3% PPELPE
T4: Basal feed + 0.4% PPELPE

Preparation of extract and encapsulation

Peperomia pellucida plants underwent a drying and grinding process before being extracted using a modified version of the method by Gouda *et al.* (2021), employing 96% ethanol in a 1:10 (b/v) ratio. The solution was subjected to sonication at 37°C for 60 minutes and then left to macerate for a day. Following filtration and evaporation at 45°C, a dense extract was produced. The encapsulation was carried out using a modified approach from Agusetyaningsih *et al.* (2020), with a 1:1 ratio of extract to *L. plantarum*, combined with skim milk and maltodextrin. This mixture was homogenized and freeze-dried until it formed a powder.

Chicken rearing

Over a period of 35 days, 200 chicks were raised, beginning from their first day. During the initial week, the chicks were fed commercial feed in the form of fine crumble. From days 8 to 21, they received research feed in crumble form, and this continued from days 22 to 35. The treatments were determined by the amount of feed consumed, and water was always made available.

Data collection

Parameters measured included: Lactic Acid Bacteria (LAB) and coliform populations: The total plate count method was used with MRS agar media for LAB and MacConkey agar for coliforms. Ileal pH was measured using a digital pH meter. Fat Digestibility: Determined through the total collection method and fat extraction using the Soxhlet method. Abdominal Fat: Calculated as a percentage of total body weight. Meat Fat: Measured in the breast, upper thigh, and lower thigh using the Soxhlet method. Meat Cholesterol: Analyzed using the Liebermann-Burchard method and spectrophotometer at 340 nm wavelength.

Statistical analysis

Variance analysis was conducted at a 5% significance level. When the treatment showed a significant impact, Duncan's multiple range test was applied at the same significance level. The statistical analysis utilized SPSS version 25.

Results

Intestinal microflora

The effects of Chinese betel extract and *Lactobacillus plantarum* (ESCLP) on the intestinal bacterial population and ileal pH of broiler chickens are presented in Table 2. Analysis of variance showed that the treatment had a significant effect ($p<0.05$) on the number of lactic acid bacteria (LAB), coliform bacteria, and ileal pH. Duncan's test showed that the LAB population in the T3 and T4 treatments increased significantly compared to T0 and T1, but not significantly different from T2. Coliform populations in treatments T3 and T4 were significantly lower than those in treatments T0, T1, and T2. The ileal pH value at T0 was not significantly different from that at T1, but was significantly different from that at T2, T3, and T4.

Fat digestibility and body fat deposition in broiler chickens

The administration of encapsulated *Peperomia pellucida* extract and *Lactobacillus plantarum* (PPELP) had a significant effect ($P<0.05$) on fat digestibility, abdominal fat, and tibial fat, but did not significantly affect breast fat or femoral fat (Table 3). Fat digestibility and the relative weight of abdominal fat in T1-T4 were significantly ($p<0.05$) lower than those in T0. The fat content of tibia meat in T4 was significantly ($p<0.05$) lower than that in other treatments.

Meat cholesterol in broiler chicken

Supplementation with encapsulated *Peperomia pellucida* extract and *Lactobacillus plantarum* (PPELP) had a significant effect on cholesterol

Table 2. Intestinal Bacteria in Broiler Chickens Fed with *Peperomia pellucida* Extract and *Lactobacillus plantarum*.

Parameter	Treatment				
	T0	T1	T2	T3	T4
BAL (log cfu/g)	$9.27±0.2^{b}$	$9.32±0.24^{b}$	$9.45±0.15^{ab}$	$9.58±0.12^{a}$	$9.63±0.15^{a}$
Coliform (log cfu/g)	$3.04±0.11^{a}$	$2.90±0.11^{ab}$	$2.80±0.14^{b}$	$2.73±0.09^{b}$	$2.72±0.1^{b}$
pH Ileum	$6.96±0.12^{a}$	$6.88±0.11^{ab}$	$6.72±0.11^{bc}$	$6.64±0.11^{cd}$	$6.51±0.1^{d}$

Different superscripts within the same row indicate significant differences ($P<0.05$) T0 = Basal diet (control) T1–T4 = Basal diet + ESCLP at 0.1%, 0.2%, 0.3%, and 0.4%, respectively LAB = Lactic acid bacteria

Table 3. Body Fat Deposition in Broiler Chickens Fed with *Peperomia pellucida* Extract and *Lactobacillus plantarum*.

Parameter	Treatment				
	T0	T1	T2	T3	T4
Fat digestibility (%)	78.44±0.18[a]	75.22±0.12[b]	74.50±0.13[b]	74.40±0.15[b]	74.02±0.12[b]
Abdominal fat (%)	1.42±0.04[a]	1.13±0.06[b]	1.01±0.07[b]	1.00±0.05[b]	1.00±0.02[b]
Breast fat (%)	5.75±0.09	5.62±0.11	5.55±0.08	5.55±0.14	5.52±0.09
Femur fat (%)	6.69±0.05	6.64±0.07	6.57±0.05	6.39±0.08	6.39±0.048
Tibia fat (%)	9.47±0.01[a]	8.39±0.08[b]	8.34±0.09[b]	8.32±0.12[b]	7.92±0.09[c]

Different superscripts within the same row indicate significant differences (P<0.05): T0 = Basal diet (control); T1–T4 = Basal diet + ESCLP at 0.1%, 0.2%, 0.3%, and 0.4%.

Table 4. Meat Cholesterol Levels in Broiler Chickens Fed with *Peperomia pellucida* Extract and *Lactobacillus plantarum*.

Parameter	Treatment				
	T0	T1	T2	T3	T4
Cholesterol breast (%)	106.02±0.2[a]	102.50±0.17[a]	100.18±0.2[a]	97.92±0.17[a]	85.50±0.19[b]
Femur Cholesterol (%)	118.70±0.16[a]	110.64±0.2[ab]	106.50±0.14[b]	104.70±0.17[b]	105.70±0.18[b]
Tibia Cholesterol (%) [ns]	145.24±0.22	144.14±0.26	140.65±0.23	140.61±0.2	139.40±0.21

Different superscripts within the same row indicate significant differences (P<0.05): T0 = Basal diet (control); T1–T4 = Basal diet + ESCLP at 0.1%, 0.2%, 0.3%, and 0.4%.

levels in the breast and femoral meat of broiler chickens, but no significant effect was observed on tibial meat cholesterol levels (Table 4). The cholesterol levels of breast meat in T4 were significantly (p<0.05) lower than those in other treatments. Cholesterol levels in femur meat at T2, T3, and T4 were not significantly different (p>0.05) from those at T1, but were significantly (p<0.05) lower than those at T0.

Discussion

The increase in LAB populations and reduction in coliforms in the treatment groups indicate that PPELP exerts a synergistic effect as a synbiotic. Flavonoids in *Peperomia pellucida* extract act as prebiotics, providing substrates for fermentation by *Lactobacillus plantarum*, thereby enhancing the production of organic acids, such as short-chain fatty acids (SCFAs), which contribute to the creation of an acidic intestinal environment (Chau and Tran, 2024). SCFAs, such as acetate, propionate, and butyrate, function to lower the intestinal pH and inhibit the growth of pathogenic microbes, including coliforms (El-Hack *et al*., 2020). This mechanism is further explained by Louis and Flint (2017), who reported that the reduction in gut pH due to SCFA production promotes microbial competition and increases selectivity in favor of beneficial microorganisms such as LAB.

Furthermore, low pH in the ileum prevents the colonization of pathogenic bacteria, maintains intestinal mucosal integrity, and enhances nutrient absorption (Rinttilä and Apajalahti, 2013). Therefore, the combination of prebiotics from *Peperomia pellucida* extract and the probiotic *Lactobacillus plantarum* not only improves microflora balance, but also creates an optimal physiological condition for broiler chicken growth.

The control group (T0) exhibited the highest fat digestibility, which was notably greater than that of all groups supplemented with PPELP. This outcome is likely attributed to the action of bile salt hydrolase (BSH) enzymes produced by *Lactobacillus plantarum*, which deconjugate bile salts and consequently diminish their capacity to emulsify dietary fats (Wang *et al*., 2024). This reduction in emulsification hinders micelle formation and impairs lipid absorption in the small intestine. Furthermore, bioactive compounds in *Peperomia pellucida* extract, such as flavonoids and phenolics, serve as prebiotics that enhance lactic acid bacteria (LAB) fermentation. This fermentation process produces short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which lower intestinal pH and further reduce the efficiency of fat absorption (Jiang *et al*., 2023).

The decrease in abdominal fat observed in all PPELP treatment groups compared to the control group further confirms the beneficial impact of the probiotic-phytobiotic combination. SCFAs generated during LAB fermentation not only reduce gut pH but also boost hepatic fatty acid oxidation and decrease lipogenesis (Deng *et al*., 2023; Dev *et al*., 2021). The reduced abdominal fat in PPELP-treated birds suggests that the combination effectively inhibited systemic fat accumulation. While no significant differences were found in breast and femoral fat, a decreasing trend was noted in both areas with higher PPELP levels. This trend was also linked to BSH enzyme activity and increased SCFA production by LAB. BSH enzymes transform bile salts into their deconjugated forms, which diminishes lipid emulsification and ultimately reduces fat absorption (Begley *et al*., 2006). Propionate, in particular, contributes to the inhibition of hepatic lipogenesis, thereby preventing the buildup of intramuscular fat (Dev *et al*., 2021).

Tibial fat content was significantly reduced in T4 compared to all other treatments, suggesting that the highest PPELP inclusion level had the most pronounced effect on reducing fat in hard tissues. This finding is closely related to the increased LAB population stimulated by phytobiotics in *P. pellucida*, which supports fermentative activity, while enhancing mucosal immunity and intestinal integrity (Uchechukwu *et al*., 2024). Reduction in tibial fat also contributes to the production of poultry meat with improved nutritional quality. Therefore, PPELP supplementation has proven effective in reducing fat deposition parameters by decreasing fat digestibility and enhancing LAB fermentative activity, facilitated by the bioactive compounds in *P. pellucida* extract.

The significant reduction in cholesterol levels in broiler breast and femoral meat observed in the T4 treatment group can be attributed to the polyphenol content of the *Peperomia pellucida* extract. Polyphenols are known to inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the endogenous cholesterol biosynthesis pathway in the liver (Theriault *et al*., 2000). Inhibition of this enzyme reduces the production of mevalonate, a critical precursor of cholesterol formation, thereby physiologically lowering tissue cholesterol levels. In addition, this enzyme facilitates the breakdown of conjugated bile salts into less soluble free forms, reducing intestinal reabsorption, and increasing cholesterol excretion via feces. This enhanced excretion compels the body to utilize endogenous cholesterol to synthesize new bile acids, resulting in decreased tissue cholesterol concentration (Zhu *et al*., 2022; Feng *et al*., 2022).

Lactobacillus plantarum in the PPELP formulation also contributed to cholesterol reduction through two primary mechanisms: cholesterol assimilation and bile salt deconjugation. In the assimilation mechanism, lactic acid bacteria (LAB) bind to cholesterol within the intestinal lumen and incorporate it into their cell membranes (Pan *et al*., 2011). In the deconjugation mechanism, bile salt hydrolase (BSH) enzymes produced by LAB hydrolyze conjugated bile acids into free, less soluble forms that cannot be reabsorbed or excreted via feces. This process increases cholesterol excretion and compels the liver to utilize circulating cholesterol for the synthesis of new bile acids (Begley *et al*., 2006; Hernández-Gómez *et al*., 2021).

Although no statistically significant differences were found in tibial meat cholesterol levels, the downward trend observed in the ES-CLP-treated groups suggested a systemic effect on lipid metabolism. This reduction is likely due to the activity of the BSH enzymes produced by *L. plantarum*, which deconjugate bile acids, thereby reducing intestinal cholesterol absorption and increasing its excretion. Wang *et al*. (2021) reported that different strains of *L. plantarum* exhibit varying degrees of cholesterol-lowering ability via BSH activity depending on environmental conditions and strain specificity. Furthermore, Zhao *et al*. (2023) demonstrated that supplementation of hyper scholesterolemic rats with Lactiplantibacillus plantarum WLPL21reduced serum and hepatic cholesterol levels by enhancing cholesterol and bile acid excretion. These effects were supported by BSH activity, regulation of cholesterol metabolism-related genes, and modulation of gut microbiota, confirming the systemic cholesterol-lowering role of probiotics, even in tissues where statistical significance was not observed.

Therefore, the combination of antioxidants from *P. pellucida* extract and the probiotic properties of *L. plantarum* in PPELP represents a promising natural strategy for reducing cholesterol content in broiler meat, thereby enhancing the nutritional quality and health value of poultry products.

Conclusion

Supplementation with 0.4% encapsulated *Peperomia pellucida* extract and *Lactobacillus plantarum* enhanced the gut microflora, improved fat digestibility, and reduced meat cholesterol levels in broiler chickens. This supplementation is recommended as a natural feed additive for improving broiler performance. Further research is needed to evaluate its long-term effects and economic feasibility.

Acknowledgements

The authors express their gratitude for the financial support provided by the Institute of Research and Community Service, Universitas Diponegoro, Semarang, Indonesia, grant number 222-285/UN7.D2/PP/IV/2025).

Conflict of interest

This study does not conflict with any other organization. These findings are distinct from those of previous studies. The authors declare no financial conflict of interest associated with this study.

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