talenta PUBLISHER

InJAR
Indonesian Journal of Agricultural Research
Journal homepage: https://injar.usu.ac.id

Modulation of broiler carcass cholesterol levels through *Lactobacillus plantarum* intervention

Mursalim[1], Hafsan[1*], Fatmawati Nur[1], Fatin Khudaer[2]
[1]*Department of Biology, Universitas Islam Negeri Alauddin, Gowa, South Sulawesi, Indonesia*
[2]*Department of Medical Science, Al-Manara College for Medical Sciences Amarah, Iraq*
[*]Corresponding author: hafsan.bio@uin-alauddin.ac.id

ARTICLE INFO

Article history:
Received 25-03-2025
Revised 16-05-2025
Accepted 23-08-2025
Available online 12-12-2025

E-ISSN: 2615-5842
P-ISSN: 2622-7681

How to cite:
Mursalim, Hafsan, Fatmawati Nur, and F. Khudaer, "Modulation of broiler carcass cholesterol levels through Lactobacillus plantarum intervention", *Indonesian J. Agric. Res*, vol. 8, no. 3, pp. 161–171, Nov. 2025

https://doi.org/10.32734/injar.v8i3.20284

ABSTRACT

High cholesterol in poultry meat poses a serious public health concern, driving research on healthier animal products. Probiotics such as *Lactobacillus plantarum* have been investigated for their potential to reduce meat cholesterol through gut microbiota modulation. This study evaluated the effectiveness of *L. plantarum* derived from Dangke in lowering carcass cholesterol of broiler chickens. The intervention was conducted orally before feeding to ensure precise dosage intake. Four treatment groups were applied: P0 (control), P1 (10^6 CFU/ml), P2 (10^8 CFU/ml), and P3 (10^{10} CFU/ml). Results showed that P0 had the highest cholesterol level (0.069 mg/dL), while P2 recorded the lowest (0.061 mg/dL), indicating the most significant reduction. This effect is attributed to enhanced colonization of *L. plantarum* in the cecum, which modulates lipid metabolism and increases bile acid excretion. Beyond cholesterol reduction, broiler performance parameters such as feed conversion ratio (FCR) and body weight gain also improved in probiotic groups, especially at 10^8 CFU/ml, supporting previous evidence on probiotic efficacy in poultry production. The findings highlight *L. plantarum* as a promising functional probiotic for reducing cholesterol while enhancing growth efficiency in broilers. From a practical standpoint, incorporation into drinking water may represent a more feasible application strategy, ensuring uniform distribution and easier adoption at the farm level. Future research should address optimal intervention strategies, the sustainability of effects on meat quality, and potential synergistic applications with other probiotics or prebiotics to further enhance poultry health and product safety.

Keywords: broiler carcass, cholesterol reduction, Lactobacillus plantarum, poultry meat quality, probiotic intervention

1. Introduction

The increase in broiler chicken meat consumption globally demands production that is not only oriented towards rapid growth but also towards consumer health aspects, including the cholesterol content in the carcasses consumed. High cholesterol levels in meat are one of the risk factors for cardiovascular disease in humans [1], [2]. Therefore, strategies to reduce chicken meat cholesterol levels are becoming a topic that is getting more and more attention in the field of livestock nutrition and biotechnology. One of the approaches that has begun to be studied is the use of lactic acid bacteria (LAB) as a probiotic [3]-[5], in particular *Lactobacillus plantarum*, which is known to have the potential to lower cholesterol levels through various biological mechanisms [6]-[9].

Several previous studies have shown that BAL can lower cholesterol levels by mechanisms such as bile salt deconjugation by bile salt hydrolase (BSH) enzymes, increased excretion of cholesterol through faeces, as well as competition with intestinal microorganisms that play a role in lipid metabolism [10]. Several other studies have also reported that *L. plantarum* supplementation may reduce cholesterol levels in broiler chicken serum and liver, but its effect on carcass cholesterol is still poorly studied. In addition, most previous studies have mixed *L. plantarum* into feed, which can lead to variability in consumption and degradation of probiotic viability before it reaches the gastrointestinal tract [11], [12], [7]. In this study, we used a strain of *L. plantarum* isolated from Dangke, a traditional Indonesian fermented dairy product from South Sulawesi, prepared by

coagulating buffalo milk using papaya latex, which supports the growth of diverse lactic acid bacteria with potential probiotic properties [13].

This study aims to evaluate the effectiveness of giving *L. plantarum* orally directly to the mouth of broiler chickens in reducing carcass cholesterol levels. This approach was chosen to ensure each chicken received an appropriately measured dose of probiotic before feeding so that its biological effects could be more clearly observed. In addition, this study also considers the possibility of measuring the colonization of *L. plantarum* in the digestive tract to understand the extent to which this bacterium can survive and interact with the chicken intestinal microbiota by microbial culture methods in selective media [14]. This study not only focuses on the effects of *L. plantarum* intervention on the cholesterol levels of broiler chicken carcasses but also provides insights into more effective probiotic administration approaches and the potential of probiotics in modulating the gut microbial ecosystem to support overall chicken health and performance.

2. Methods

2.1. Research design

This study used a complete randomized design (RAL) with four doses of *Lactobacillus plantarum* administered orally directly into the mouth of broiler chickens every morning before feeding. This approach aims to ensure that each chicken receives an appropriately measured dose of probiotics, thus allowing for a more accurate evaluation of the biological effects on carcass cholesterol levels [11]. The treatment provided consisted of: P0 (control, without *L. plantarum* intervention), P1 (10^6 CFU/ml), P2 (10^8 CFU/ml), and P3 (10^{10} CFU/ml). All chickens were fed a standard basal diet formulated according to NRC recommendations (specify composition if possible), and water was provided ad libitum. Environmental parameters were maintained at 32 °C (week 1) and gradually decreased to 24 °C (week 5), with a 23L:1D lighting program. Chickens were raised in identical cages to minimize environmental variability.

A total of 100 Cobb strain broiler chickens were used in the study, which was randomly divided into four treatment groups, each with 25 chickens. The study was conducted during a 35-day maintenance period, with an adaptation period of 7 days before probiotic treatment began on day 8 until harvest on day 35. All chickens were fed a standard basal diet formulated according to NRC recommendations (specify composition if possible), and water was provided ad libitum. Environmental parameters were maintained at 32°C (week 1) and gradually decreased to 24°C (week 5), with a 23L:1D lighting program. Chickens were raised in identical cages to minimize environmental variability. All chickens are raised under controlled environmental conditions, with temperature, humidity, and lighting that conform to commercial broiler chicken rearing standards [12].

2.2. Isolation and preparation of Lactobacillus plantarum

The *L. plantarum* strain used in this study was isolated from Dangke, a typical South Sulawesi dairy food that is rich in BAL [14]. Isolation was carried out by the selective culture method using de Man, Rogosa, and Sharpe (MRS) media enriched with calcium carbonate ($CaCO_3$) to facilitate the identification of BAL colonies based on the clear zones around the colony [10]. Colonies showing typical BAL morphology were confirmed by Gram staining and catalase assays [13]. After isolation, *L. plantarum* is cultured in MRS broth at 37 °C for 24 hours under anaerobic conditions to obtain sufficient bacterial biomass. Next, the bacterial suspension was standardized with a UV-Vis spectrophotometer at a wavelength of 600 nm to obtain concentrations of 10^6 CFU/ml, 10^8 CFU/ml, and 10^{10} CFU/ml, which were then administered orally to the chickens according to the prescribed treatment [6].

2.3. Intervention of Lactobacillus plantarum

Probiotics are administered every morning before feeding, with a volume of 1 ml per head using an automatic pipette or needleless syringe to ensure that each chicken receives the right dose. This method was chosen to avoid bacterial degradation in the feed and ensure high viability when reaching the digestive tract [15]. In addition, the consumption of chicken feed and drinking water is monitored daily to ensure there are no significant changes in consumption patterns due to probiotic interventions. Observations of chicken health were also carried out, including aspects of mortality rate, stress symptoms, and faecal consistency as indicators of gastrointestinal tolerance and health status [12].

2.4. Evaluation of broiler growth performance

To assess the effect of *L. plantarum* intervention on the growth and production efficiency of broiler chickens, several growth performance parameters were measured periodically. The observed parameters included Final Body Weight, Average Daily Gain, feed consumption, feed conversion ratio, mortality (%), and Production Efficiency Factor (PEF) [16].

Chicken body weight was recorded weekly using a digital scale with an accuracy of 0.01 g to obtain data on daily body weight gain and final body weight on day 35. Feed consumption is calculated based on the amount of feed given minus the rest of the feed each day. Feed conversion ratio is calculated by the formula (1):

$$\text{FCR} = \frac{\text{Total feed consumption (g)}}{\text{Total weight gain (g)}} \quad (1)$$

Mortality is observed daily, and every dead chicken is recorded for further analysis. The Production Efficiency Index (PEF) is calculated using the formula (2):

$$\text{PEF} = \frac{\text{[Average body weight (kg) x Average percentage of life]}}{\text{FCR x Length of maintenance (hari)}} \text{x } 100 \quad (2)$$

All performance data were analyzed to evaluate whether *L. plantarum* intervention affected growth efficiency without negatively impacting broiler health [17].

2.5. Measurement of broiler carcass cholesterol levels

At the end of the study (day 35), the whole chicken was harvested and slaughtered for analysis of carcass cholesterol levels. Meat samples were taken from the chest and thigh and then dried by the freeze-drying method before being extracted using chloroform-methanol solvent (2:1 v/v) [10]. Cholesterol levels were analyzed using the Enzymatic Colorimetric Assay method using a total cholesterol kit based on the enzyme cholesterol oxidase-peroxidase (CHOD-PAP). Absorbance readings were performed at a wavelength of 500 nm using a spectrophotometer [13].

2.6. Observation of L. plantarum colonization in the broiler gastrointestinal tract

To evaluate the colonization of *L. plantarum* in the digestive tract of chickens, small intestinal and cecum samples were collected after slaughter. These samples were weighed and homogenized in a saline buffer phosphate solution (BPS), then serial dilution and inoculation were carried out on MRS to selectively contain vancomycin to suppress the growth of other bacteria [6]. After incubation at 37°C for 48 hours under anaerobic conditions, colonies were counted using the total plate count (TPC) method [15].

2.7. Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA), with treatment as the main factor. If a significant difference is found ($P < 0.05$), the analysis is continued with Tukey's Honest Significant Difference (HSD) test to compare between treatments [15]. Statistical analysis was performed using SPSS software version 25.0, and the data were presented in the form of mean±standard deviation (SD) [6].

3. Results and Discussion

3.1. Broiler growth performance

Broiler growth performance is one of the main parameters in this study because it determines the efficiency of poultry production in the poultry industry. *Lactobacillus plantarum* intervention as a probiotic has been reported to improve feed efficiency, gut health, and chicken growth performance through the mechanism of modulating the intestinal microbiota and increasing nutrient absorption [18]. However, the effects of probiotic supplementation can vary depending on the species, dosage, and method of administration used [5], [11], [17] [19].

In this study, *L. plantarum* was administered orally directly into the chicken's mouth before feeding, aiming to ensure each individual received a uniform dose and reduce the likelihood of bacterial degradation during mixing with feed or water. Therefore, evaluation of final body weight, daily body weight gain, feed consumption, feed conversion ratio (FCR), mortality, and production efficiency index is important to understand the impact of these interventions on broiler production performance. The results of the measurement of broiler growth performance parameters are presented in Table 1.

Table 1. Broiler growth performance with *Lactobacillus plantarum* intervention

Parameter	P0 (Control)	P1 (10^6 CFU/ml)	P2 (10^8 CFU/ml)	P3 (10^{10} CFU/ml)	p-Value
Final Body Weight (kg)	2.35±0.12	2.38±0.10	2.42±0.08	2.36±0.11	0.07^{ns}
Daily Weight Gain (g)	58.70±2.30	59.40±2.10	60.10±1.90	58.90±2.50	0.09^{ns}
Feed Consumption (g/day)	97.50±3.50	96.90±3.20	95.80±2.90	97.10± 3.60	0.11^{ns}
Feed Conversion (FCR)	1.66±0.07	1.63±0.050	1.59±0.04	1.65±0.06	0.05^{ns}
Mortality (%)	4.50±1.20	4.20±1.00	3.9±0.80	4.40±1.10	0.08^{ns}
Production Efficiency Factor Index (PEF)	280.00±15.00	285.00±12.00	292.00±10.00	281.00±14.00	0.06^{ns}

Note: Data presented in Mean±SD (n = 20 per group); bold letter indicates the best value in each parameter; *ns* = nonsignificant (P > 0.05).

The results showed that *L. plantarum* intervention did not cause significant differences in final body weight, daily weight gain, and feed consumption between treatments (P > 0.05). The final body weight of the broiler ranges from 2.35 to 2.42 kg, which is still in line with the growth standards of commercial broilers at 35 days of age [20].

However, there was a trend of improving feed efficiency (lower FCR) in the P2 treatment (10^8 CFU/ml), although statistically insignificant (P = 0.05). The lower FCR suggests that chickens in this group can convert feed into meat more efficiently, which is supported by the hypothesis that *L. plantarum* can improve nutrient absorption through the modulation of gut microbiota and digestive enzymes [5].

Previous studies have also reported that administration *of L. plantarum* can lower FCR and improve feed efficiency in broilers, primarily through increased expression of nutrient transporters in the small intestine and reduction of inflammation of the intestinal mucosa [10], [21], [22]. In addition, the effects of probiotics in improving the balance of the gut microbiota can reduce populations of pathogenic bacteria such as *Escherichia coli*, *Clostridium,* and *Campylobacter*, which are often associated with indigestion and production inefficiency [23], [24].

Compared to previous studies, these results show a pattern that is in line with some previous studies that indicated that probiotic supplementation has a positive effect on poultry production efficiency, although its effect on growth still varies. For example, a study found that *L. plantarum* supplementation was able to improve feed efficiency (lower FCR), but did not make a significant difference to the broiler's final body weight [6]. Similar results were also reported to show that LAB supplementation with an optimal dose of 10^8–10^9 CFU/g in feed was able to improve nutrient retention and feed efficiency, without causing indigestion or physiological stress in chickens [25], [3]. In addition, other research revealed that the combination of several species of Lactobacillus can lower intestinal inflammation as well as increase the absorption of essential amino acids, which ultimately contributes to improved feed efficiency and gut health [15], [26].

Based on these findings, it can be concluded that *L. plantarum* intervention in this study provides potential benefits for broiler production efficiency, especially in terms of feed conversion (FCR) and gut microbiota balance. Although the difference in final body weight was not significant, the trend of increasing feed efficiency still indicated the role of *L. plantarum* in increasing nutrient utilization. Therefore, this probiotic supplementation can be considered as a potential strategy in supporting more efficient and sustainable poultry production.

3.2. Effect of Lactobacillus plantarum on broiler carcass cholesterol levels

The results showed that *Lactobacillus plantarum* intervention contributed to a decrease in broiler carcass cholesterol levels, with effects that varied depending on the dose administered. The cholesterol in the carcass is an important parameter because it is directly related to the quality of the meat and the health implications for the consumer. A decrease in cholesterol levels due to LAB (Lactic Acid Bacteria) supplementation has been widely reported in various previous studies, especially those highlighting the mechanism of bile acid

deconjugation and regulation of lipid metabolism by LAB [4], [27], [28]. To understand the effectiveness of this intervention, broiler carcass cholesterol level data at each treatment are presented in the following Figure 2.

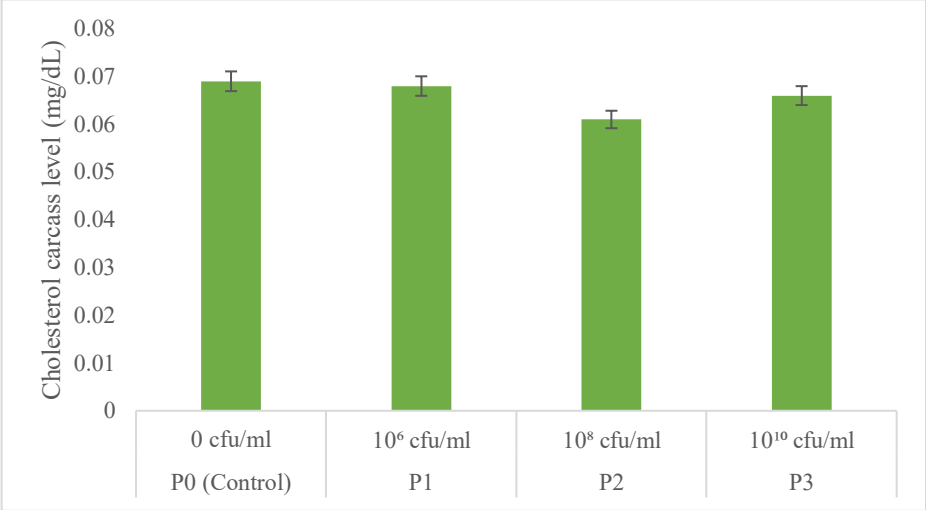

Figure 1. Broiler carcass cholesterol level at various doses of *L. plantarum* intervention; different letter (a, b) show significant differences ($P < 0.05$)

Based on these findings, P2 treatment (10^8 cfu/ml) resulted in the lowest carcass cholesterol levels, which differed significantly compared to controls (P0) and other treatments ($P < 0.05$). Meanwhile, the P1 (10^6 cfu/ml) and P3 (10^{10} cfu/ml) treatments showed a slight decrease compared to controls, but the difference was not significant. This trend indicates that the optimal dose to lower carcass cholesterol levels in this study was 10^8 cfu/ml, with a statistically significant effect. This dosage is likely optimal because it provides a sufficient bacterial load to achieve effective colonization and exert metabolic activity in the gut, particularly in the cecum, without exceeding the threshold that may trigger intraspecific competition or lead to excessive bacterial clearance through feces, phenomena commonly observed at higher probiotic dosages.

The results of these measurements are consistent with studies reporting that *L. plantarum* supplementation in poultry contributes to a decrease in plasma and meat cholesterol levels [8], [29], [30], through the mechanism of bile salt hydrolysis and increased bile acid excretion, which indirectly inhibits cholesterol synthesis in the liver [21], [28]. Another study showed that administration of *L. plantarum* at a dose of 10^8–10^9 cfu/ml was able to lower meat cholesterol levels, with a similar mechanism through inhibition of the enzyme HMG-CoA reductase, which is a key enzyme in cholesterol biosynthesis [27], [28] [31].

Several molecular mechanisms have been used to explain the cholesterol-lowering effects of *Lactobacillus plantarum*. One of the central pathways involves the regulation of hepatic cholesterol biosynthesis through inhibition of the 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) enzyme, a rate-limiting enzyme in the mevalonate pathway responsible for endogenous cholesterol synthesis. Experimental studies in mammalian models have demonstrated that *L. plantarum* administration can downregulate the expression of the HMGCR gene, leading to reduced de novo cholesterol production in the liver [27], [28].

L. plantarum has been reported to influence cholesterol absorption in the intestine by modulating the expression of Niemann-Pick C1-Like 1 (NPC1L1), a key transporter located in the brush border membrane of enterocytes. NPC1L1 facilitates the uptake of luminal cholesterol into intestinal cells. Suppression of NPC1L1 expression has been associated with reduced intestinal absorption of dietary cholesterol, leading to lower systemic cholesterol levels. Certain strains of *L. plantarum* are hypothesized to exert this effect through the production of short-chain fatty acids (SCFAs), particularly acetate and propionate, which act as signaling molecules to alter gene expression in intestinal epithelial cells [27], [28], [31].

Furthermore, bile salt hydrolase (BSH) activity, which is characteristic of many *L. plantarum* strains, contributes to increased deconjugation and subsequent excretion of bile acids. This mechanism forces the host to synthesize new bile acids from hepatic cholesterol pools, thereby reducing overall cholesterol levels. It is

also suggested that this microbial activity may alter the expression of genes involved in bile acid metabolism, such as CYP7A1, which catalyzes the rate-limiting step in bile acid synthesis.

These insights suggest that the hypocholesterolemic effect observed in this study may not solely result from microbial colonization, but also involve complex host-microbe interactions at the molecular level. Further studies involving qRT-PCR or transcriptomic profiling of intestinal and hepatic tissues are recommended to validate the regulation of these genes in broiler chickens receiving *L. plantarum* supplementation.

On the other hand, it was also found that BAL combined with a low-fat diet can increase the expression of genes that play a role in lipid metabolism, thereby increasing cholesterol degradation in muscle tissue [4], [24], [32]. The results obtained in this study support the idea that *L. plantarum* may play a role as a natural hypocholesterolemic agent, especially in the poultry production system.

From a human health perspective, the observed reduction of approximately 11.6% in carcass cholesterol levels can be considered modest but potentially relevant, particularly for consumers who are concerned about dietary cholesterol intake from poultry products. Although poultry meat contributes only a fraction of total cholesterol intake in the general diet, even small reductions at the production level may cumulatively impact public health when integrated into broader dietary interventions. Moreover, it is important to note that the cholesterol reduction achieved in this study did not appear to compromise growth performance parameters, as no significant differences were found in body weight gain, feed intake, or feed conversion ratio across the treatment groups. This suggests that *L. plantarum* intervention at the tested doses may provide health-related benefits without negatively affecting broiler production efficiency. Nevertheless, further research is warranted to comprehensively assess potential interaction effects between cholesterol metabolism modulation and broiler growth physiology, including long-term implications for meat quality and nutritional value.

3.3. Colonization of Lactobacillus plantarum in the digestive tract

To understand the effectiveness of *L. plantarum* intervention, it is important to evaluate the rate of colonization of these bacteria in the broiler digestive tract. Successful colonization suggests that *L. plantarum* can survive in the intestinal environment, compete with endogenous microbiota, and provide expected physiological benefits, such as modulation of lipid metabolism and competition with pathogenic bacteria.

The colonization of *L. plantarum* in the broiler gastrointestinal tract was evaluated by microbial culture method on de Man, Rogosa, and Sharpe (MRS) selective media in order to enrich with certain antibiotics for selectivity against *Lactobacillus* spp. Samples were taken from the contents of the digestive tract (duodenum, ileum, and cecum) at the end of the study period, and then diluted in a saline phosphate buffer solution (PBS) before planting. The number of colonies that grow is calculated in Colony-Forming units per gram (CFU/g) of intestinal content and compared between treatments. The results of the calculation are presented in the following Table 2.

Table 2. Colonization of *Lactobacillus plantarum* in the broiler digestive tract

Treatment	Dose Treatments (*L. plantarum*)	Duodenum (CFU/g) ± STDV	Ileum (CFU/g) ± STDV	Cecum (CFU/g) ± STDV
P0 (Control)	0 cfu/ml	$4.2\times10^5\pm0.3^a$	$5.1\times10^5\pm0.5^a$	$7.8\times10^5\pm0.4^a$
P1	10^6 cfu/ml	$5.1\times10^5\pm0.4^a$	$6.0\times10^5\pm0.3^a$	$8.5\times10^5\pm0.5^a$
P2	10^8 cfu/ml	$7.8\times10^5\pm0.3^b$	$8.3\times10^5\pm0.6^b$	$1.2\times10^5\pm0.4^b$
P3	10^{10} cfu/ml	$6.5\times10^5\pm0.5^b$	$7.2\times10^5\pm0.4^b$	$9.9\times10^5\pm0.6^b$

Note: Different letters (a, b) show significant differences (P>0.05) based on ANOVA and post-hoc Tukey statistical tests.

The results showed that the colonization of *L. plantarum* in the broiler gastrointestinal tract was significantly increased in the treatment group compared to the control group (P<0.05). The highest increase in the number of Lactobacillus was found in the P2 group (10^8 cfu/ml), especially in the cecum segment (1.2×10^6 CFU/g), which is the main site of microbial fermentation in the digestive tract of poultry.

These results are in line with previous studies that showed that Lactobacillus intervention in poultry diets can increase the colonization of such bacteria in the digestive tract, especially in the ileum and cecum. Other studies have also shown that *L. plantarum* supplementation increases the dominance of probiotic microbiota in the broiler small intestine, which contributes to the modulation of gut microbiota homeostasis as well as decreased colonization of pathogenic bacteria [19], [22], [25], [33], [34].

The increased amount of *L. plantarum* in the P2 group compared to P3 suggests that too high a dose (10^{10} CFU/ml) does not necessarily result in better colonization, likely due to the mechanism of intraspecific competition between *L. plantarum* itself, which limits the number of bacteria that can survive in the intestine [16], [33]. In addition, the excess amount of uncolonized probiotics may be eliminated through faeces, as described in a study that observed the dynamics of probiotic populations in the broiler gastrointestinal tract [5], [12], [17].

In addition to increasing *L. plantarum* populations, some studies have shown that the successful colonization of probiotics correlates with increased production of bioactive metabolites, such as organic acids, biosurfactant bacteria, and enzymes that modulate lipid metabolism [18], [26], [35]. It is further explained that increased colonization of Lactobacillus in the intestines of chickens can inhibit the absorption of cholesterol in the digestive tract by increasing the production of lactic acid and acetic acid, which inhibit the activity of the lipase enzyme in the small intestine [27], [28], [29], [36], [37].

These results indicate that a dose of 10^8 CFU/ml is the optimal dose to increase the colonization of *L. plantarum* in the broiler digestive tract, especially in the ileum and cecum. The success of this colonization is an important indicator of the effectiveness of probiotics in improving gut health, feed efficiency, and lipid metabolism regulation. As a practical implication, the application of probiotics in the poultry farming industry should consider the appropriate dosage and optimal administration method.

Although administering probiotics through drinking water offers a more feasible and scalable option compared to direct oral gavage, it is important to acknowledge that the effectiveness of this delivery route might be influenced by individual variations in water consumption, environmental factors affecting probiotic viability in drinking systems, and the lack of control over precise dosage intake per bird. In contrast, the oral gavage approach used in this study ensured a uniform and accurate probiotic dose for each chicken, likely contributing to the consistent colonization outcomes observed. Therefore, while drinking water administration is more practical in commercial settings, strategies such as dose adjustment, microencapsulation, or stabilization techniques might be necessary to optimize its efficacy and ensure comparable colonization levels to those achieved by direct oral administration.

3.4. Correlation between colonization of Lactobacillus plantarum and decreased carcass cholesterol levels

It is important to understand the extent to which colonization of *Lactobacillus plantarum* in the gastrointestinal tract contributes to a decrease in carcass cholesterol levels. Based on the results of the study, there was a correlation between the colonized population of *L. plantarum* in the intestine and the effectiveness of reducing cholesterol levels in broiler carcasses. To quantify this relationship, statistical analysis was carried out using the Pearson correlation test. The results of the analysis, as shown in Table 4, show that there is a significant negative correlation ($r = -0.79$, $P<0.01$) between the amount of L. plantarum colonized in the cecum and the cholesterol level of broiler carcasses. This indicates that the higher the colonization rate of *L. plantarum*, the lower the carcass cholesterol level produced.

Table 3. Correlation between *L. Plantarum* colonization and carcass cholesterol levels

Parameter	r (Pearson Correlation Coefficients)	p-Value
Colonization in the duodenum vs. Carcass cholesterol	-0.61	<0.05
Colonization in the ileum vs. Carcass cholesterol	-0.74	<0.01
Colonization in the cecum vs. Carcass cholesterol	-0.79	<0.01

The present study revealed a significant negative correlation between the colonization *of L. plantarum* in the gastrointestinal tract and broiler carcass cholesterol levels (Table 3). Among the evaluated intestinal segments, the cecum exhibited the strongest correlation (-0.79, $P < 0.01$), followed by the ileum (-0.74, $P < 0.01$), while

the duodenum displayed a comparatively weaker correlation (-0.61, $P < 0.05$). These findings suggest that the cecum is the primary site where *L. plantarum* exerts its most profound effects on cholesterol metabolism modulation.

This observation aligns with the established role of the cecum as a central site of microbial fermentation and lipid metabolism in poultry, providing an anaerobic environment with extended retention time that facilitates the proliferation of probiotic bacteria and their metabolic interactions with the host [18]. One of the key mechanisms implicated is the activity of *L. plantarum* in deconjugating bile acids through bile salt hydrolase (BSH) enzymes, thereby promoting bile acid excretion and reducing cholesterol reabsorption in the intestine [28], [38]. Additionally, the production of organic acids, such as lactic and acetic acid, by *L. plantarum* in the cecum may inhibit intestinal lipase activity, further contributing to the reduction of dietary cholesterol absorption [39], [8], [9], [29].

The stronger correlation in the ileum compared to the duodenum can also be attributed to anatomical and physiological differences along the intestinal tract. The ileum offers a relatively stable environment with slower transit time and more developed microbial communities, enabling *L. plantarum* to colonize effectively and maintain metabolic activities critical for lipid metabolism modulation. In contrast, the duodenum represents a harsh environment due to high concentrations of bile acids and pancreatic enzymes, rapid content movement, and relatively unfavorable pH, which may limit the survival, colonization, and functional expression of probiotic bacteria [16], [33]. Consequently, although some colonization occurs, the capacity of *L. plantarum* to exert significant lipid-modulating effects in the duodenum appears limited, as reflected in the weaker correlation coefficient.

These results emphasize the importance of targeting the distal parts of the intestine, particularly the ileum and cecum, for maximizing the probiotic effects of *L. plantarum* on cholesterol metabolism in poultry. From a practical perspective, while the current study employed direct oral gavage to ensure precise dosing and maximize colonization efficiency, such an approach may not be practical for large-scale poultry farming. The suggestion to deliver probiotics through drinking water systems represents a more feasible alternative for industrial applications. However, it should be noted that compared to oral gavage, administration via drinking water may result in lower colonization efficiency due to inter-individual variability in water intake, environmental exposure that may affect probiotic viability, and imprecise dosing. Therefore, additional strategies, such as microencapsulation, dose optimization, or incorporation of prebiotics, may be necessary to enhance the efficacy of *L. plantarum* delivered via drinking water and achieve colonization outcomes comparable to those obtained through controlled oral administration.

Furthermore, higher colonization of *L. plantarum* in the intestine may also inhibit the growth of pathogenic microorganisms that play a role in increasing the synthesis of endogenous cholesterol, such as Clostridium spp. [40]-[43]. Some studies have also shown that the presence of probiotics may affect the expression of genes involved in lipid transport in enterocytes, such as decreased expression of the Niemann-Pick transporter C1-Like 1 (NPC1L1), which is responsible for the absorption of cholesterol in the small intestine [44].

The findings in this study are in line with previous studies that showed *that L. plantarum* intervention can lower carcass cholesterol levels through a mechanism involving modulation of the gut microbiota and lipid excretion [21], [45]. It has been reported that *L. plantarum* supplementation lowers serum and chicken cholesterol levels by increasing the excretion of bile acids [7], [28], [38]. Similarly, it has been reported that the use of Lactobacillus in poultry feed contributes to the decrease in lipids in meat through improved gut microbiota balance and increased production of organic acid metabolites [46], [35]. Meanwhile, it has also been reported that the effectiveness of probiotics in lowering cholesterol levels depends on the dose administered, where *L. plantarum* intervention at a concentration of 10^8 CFU/ml shows a more significant reduction in cholesterol compared to higher doses such as 10^{10} CFU/ml, possibly due to colonization saturation that limits the effects of probiotics on the digestive tract [8], [9], [29], [30].

The results of this study confirm that the successful colonization of *L. plantarum* in the broiler intestine plays an important role in lowering carcass cholesterol levels, with cecum as the main location of probiotic activity. The implications of these findings suggest that in industrial applications, probiotic formulation strategies that support optimal colonization, such as administration through potable water with microencapsulation

techniques to improve bacterial viability, may be a more effective approach in sustainably lowering chicken meat cholesterol levels.

4. Conclusion

This study confirms that oral administration of *Lactobacillus plantarum* from Dangke at a dose of 10^8 CFU/ml effectively reduced broiler carcass cholesterol level by approximately 11.6%. This reduction was closely linked to enhanced colonization of *L. plantarum* in the cecum, evidenced by a strong negative correlation ($r = -0.79$, $P<0.01$). The intervention also showed a favorable trend in improving feed efficiency without adversely affecting broiler growth performance, indicating dual benefits for poultry production and meat quality.

Considering industrial applications, administering *L. plantarum* via drinking water is a more practical approach than direct oral administration, although challenges such as variability in intake and probiotic stability must be addressed through formulation improvements. Regulatory measures should focus on probiotic strain certification and dosage standardization to ensure safety, efficacy, and consistency. The promotion of reduced-cholesterol poultry meat as a functional food can provide added consumer value and contribute to healthier dietary options.

References

[1] P. J. Nestel and T. A. Mori, "Dietary patterns, dietary nutrients and cardiovascular disease," *Rev. Cardiovasc. Med.*, vol. 23, no. 1, p. 17, 2022, doi: 10.31083/j.rcm2301017.

[2] D. Milićević *et al.*, "The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors," *Lipids Health Dis.*, vol. 13, no. 1, p. 42, 2014, doi: 10.1186/1476-511X-13-42.

[3] E. H. Chandra, W. P. Lokapirnasari, S. Hidanah, M. A. Al-Arif, W. M. Yuniarti, and E. M. Luqman, "Probiotic Potential of lactic acid bacteria on feed efficiency, weight, and carcass percentage in ducks," *J. Med. Vet.*, vol. 5, no. 1, pp. 69–73, 2022, doi: 10.20473/jmv.vol5.iss1.2022.69-73.

[4] X. Song, Y. Liu, X. Zhang, P. Weng, R. Zhang, and Z. Wu, "Role of intestinal probiotics in the modulation of lipid metabolism: implications for treatments," *Food Sci. Hum. Wellness*, vol. 12, no. 5, pp. 1439–1449, 2023, doi: 10.1016/j.fshw.2023.02.005.

[5] J. Patterson and K. Burkholder, "Application of prebiotics and probiotics in poultry production," *Poult. Sci.*, vol. 82, no. 4, pp. 627–631, 2003, doi: 10.1093/ps/82.4.627.

[6] R. Widajati, A. Aryati, and H. Notopuro, "Pengaruh perbedaan konsentrasi *Lactobacillus plantarum* terhadap perubahan kadar kolesterol total pada tikus putih," *J. Manaj. Kesehat. Yayasan RSDr Soetomo*, vol. 6, no. 1, p. 53, 2020, doi: 10.29241/jmk.v6i1.286.

[7] L. Guo *et al.*, "Effect of bile salt hydrolase-active *Lactobacillus plantarum* KLDS 1.0344 on cholesterol metabolism in rats fed a high-cholesterol diet," *J. Funct. Foods*, vol. 61, p. 103497, 2019, doi: 10.1016/j.jff.2019.103497.

[8] C. Li *et al.*, "Cholesterol-lowering effect of *Lactobacillus plantarum* NCU116 in a hyperlipidaemic rat model," *J. Funct. Foods*, vol. 8, pp. 340–347, 2014, doi: 10.1016/j.jff.2014.03.031.

[9] Y. Huang *et al.*, "*Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol-lowering activity," *J. Dairy Sci.*, vol. 96, no. 5, pp. 2746–2753, 2013, doi: 10.3168/jds.2012-6123.

[10] H. S. Lye, G. R. Rahmat-Ali, and M. T. Liong, "Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract," *Int. Dairy J.*, vol. 20, no. 3, pp. 169–175, 2010, doi: 10.1016/j.idairyj.2009.10.003.

[11] Y. Wang and Q. Gu, "Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers," *Res. Vet. Sci.*, vol. 89, no. 2, pp. 163–167, Oct. 2010, doi: 10.1016/j.rvsc.2010.03.009.

[12] H. S. Al-Khalaifah, "Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry," *Poult. Sci.*, vol. 100, no. 2, p. 1330, Feb. 2021, doi: 10.1016/j.psj.2020.12.055.

[13] Y. Wang, J. Wang, and W. Dai, "Use of GFP to trace the colonization of Lactococcus lactis WH-C1 in the gastrointestinal tract of mice," *J. Microbiol. Methods*, vol. 86, no. 3, pp. 390–392, 2011, doi: 10.1016/j.mimet.2011.06.009.

[14] F. Nur, H. Hafsan, A. Wahdiniar, "Isolasi bakteri asam laktat berpotensi probiotik pada dangke, makanan tradisional dari susu kerbau di Curio Kabupaten Enrekang," *Biog. J. Ilm. Biol.*, vol. 3, no. 1, pp. 60–65, 2015, doi: 10.24252/bio.v3i1.568.

[15] A. D. Andriani *et al.*, "Efektifitas probiotik *Lactobacillus casei* dan *Lactobacillus rhamnosus* sebagai pengganti antibiotic growth promoter terhadap total kolesterol, low density lipoprotein dan high density

lipoprotein ayam broiler," *J. Med. Vet.*, vol. 3, no. 1, p. 114, 2020, doi: 10.20473/jmv.vol3.iss1.2020.114-122.
[16] F. Zaefarian, M. R. Abdollahi, and V. Ravindran, "Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health," *World's Poult. Sci. J.*, vol. 72, no. 2, pp. 277–290, 2016, doi: 10.1017/s0043933916000222.
[17] P. Shokryazdan, M. Faseleh Jahromi, J. B. Liang, and Y. W. Ho, "Probiotics: From Isolation to Application," *J. Am. Coll. Nutr.*, vol. 36, no. 8, pp. 666–676, 2017, doi: 10.1080/07315724.2017.1337529.
[18] T. Rinttilä and J. Apajalahti, "Intestinal microbiota and metabolites—Implications for broiler chicken health and performance," *J. Appl. Poult. Res.*, vol. 22, no. 3, pp. 647–658, 2013, doi: 10.3382/japr.2013-00742.
[19] GBIF Secretariat, *Lactobacillus plantarum* (Orla-Jensen, 1919) Bergey *et al.*, 1923, in *GBIF Backbone Taxonomy*, Checklist dataset. 2023. [Online]. Available: https://doi.org/10.15468/39omei.
[20] Y. Su, G. Chang, J. Liu, P. Huang, and J. Zeng, "Dietary sanguinarine supplementation improves the growth performance and intestinal immunity of broilers," *Anim. Nutr.*, vol. 19, pp. 76–89, 2024, doi: 10.1016/j.aninu.2024.05.009.
[21] W. Wang, K. Zhang, K. Zhang, R. Wu, Y. Tang, and Y. Li, "Gut microbiota promotes cholesterol gallstone formation through the gut-metabolism-gene axis," *Microb. Pathog.*, p. 107446, 2025, doi: 10.1016/j.micpath.2025.107446.
[22] K. Leal, L. Truong, E. Maga, and A. King, "Lactobacillus (*L. plantarum* & *L. rhamnosus*) and Saccharomyces (*S. cerevisiae*): Effects on performance, biochemical parameters, ammonium ion in manure, and digestibility of broiler chickens," *Poult. Sci.*, vol. 102, no. 4, p. 102525, 2023, doi: 10.1016/j.psj.2023.102525.
[23] I. H. Choi, W. Y. Park, and Y. J. Kim, "Effects of dietary garlic powder and α-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken," *Poult. Sci.*, vol. 89, no. 8, pp. 1724–1731, 2010, doi: 10.3382/ps.2009-00052.
[24] E. Zanardi, E. Novelli, G. P. Ghiretti, and R. Chizzolini, "Oxidative stability of lipids and cholesterol in salame Milano, coppa and Parma ham: dietary supplementation with vitamin E and oleic acid," *Meat Sci.*, vol. 55, no. 2, pp. 169–175, 2000, doi: 10.1016/s0309-1740(99)00140-0.
[25] B. Wang *et al.*, "Probiotic *Paenibacillus polymyxa* 10 and *Lactobacillus plantarum* 16 enhance growth performance of broilers by improving the intestinal health," *Anim. Nutr.*, vol. 7, no. 3, pp. 829–840, 2021, doi: 10.1016/j.aninu.2021.03.008.
[26] S. Haase, N. Wilck, A. Haghikia, R. Gold, D. N. Mueller, and R. A. Linker, "The role of the gut microbiota and microbial metabolites in neuroinflammation," *Eur. J. Immunol.*, vol. 50, no. 12, pp. 1863–1870, 2020, doi: 10.1002/eji.201847807.
[27] A. Wahlstrom, "Crosstalk between bile acids and the gut microbiota - influence on host metabolism," *Endocr. Abstr.*, 2018, doi: 10.1530/endoabs.56.s3.1.
[28] G. Hou *et al.*, "*Lactobacillus delbrueckii* Interfere with bile acid enterohepatic circulation to regulate cholesterol metabolism of growing–finishing pigs via its bile salt hydrolase activity," *Front. Nutr.*, vol. 7, 2020, doi: 10.3389/fnut.2020.617676.
[29] H. Julendra, A. E. Suryani, L. Istiqomah, E. Damayanti, M. Anwar, and N. Fitriani, "Isolation of lactic acid bacteria with cholesterol-lowering activity from digestive tracts of indonesian native chickens," *Media Peternak.*, vol. 40, no. 1, pp. 35–41, 2017, doi: 10.5398/medpet.2017.40.1.35.
[30] A. Hameed *et al.*, "Isolation and characterization of a cholesterol-lowering bacteria from *Bubalus bubalis* raw milk," *Fermentation*, vol. 8, no. 4, p. 163, 2022, doi: 10.3390/fermentation8040163.
[31] R. Rinto, R. Dewanti, S. Yasni, and M. T. Suhartono, "Isolasi dan identifikasi bakteri asam laktat penghasil inhibitor enzim hmg-koa reduktase dari bekasam sebagai agen pereduksi kolesterol," *J. Agritech*, vol. 35, no. 03, p. 309, 2015, doi: 10.22146/agritech.9342.
[32] J. K. Drackley, "Lipid metabolism.," in *Farm Animal Metabolism and Nutrition.*, EU: CABI, pp. 97–119. doi: 10.1079/9780851993782.0097.
[33] S. Shini and W. L. Bryden, "Probiotics and gut health: linking gut homeostasis and poultry productivity," *Anim. Prod. Sci.*, vol. 62, no. 12, pp. 1090–1112, 2021, doi: 10.1071/an20701.
[34] P. S. Lim, C. F. Loke, Y. W. Ho, and H. Y. Tan, "Cholesterol homeostasis associated with probiotic supplementation *in vivo*," *J. Appl. Microbiol.*, vol. 129, no. 5, pp. 1374–1388, 2020, doi: 10.1111/jam.14678.
[35] X. Li, Y. Liu, X. Guo, Y. Ma, H. Zhang, and H. Liang, "Effect of *Lactobacillus casei* on lipid metabolism and intestinal microflora in patients with alcoholic liver injury," *Eur. J. Clin. Nutr.*, vol. 75, no. 8, pp. 1227–1236, 2021, doi: 10.1038/s41430-020-00852-8.

[36] A. R. A. Al-Fataftah, S. M. Herzallah, K. Alshawabkeh, and S. A. Ibrahim, "Administration of lactic acid bacteria to enhance the synthesis of vitamin B12 and B6 and lower cholesterol levels in poultry meat," *J. Food Agric. Environ.*, no. 11, no. 2, pp. 604-609, 2013.
[37] L. Wang *et al.*, "Effects of lactic acid bacteria isolated from Tibetan chickens on the growth performance and gut microbiota of broiler," *Front. Microbiol.*, vol. 14, p. 1171074, 2023, doi: 10.3389/fmicb.2023.1171074.
[38] M. Begley, C. Hill, and C. G. M. Gahan, "Bile salt hydrolase activity in probiotics," *Appl. Environ. Microbiol.*, vol. 72, no. 3, pp. 1729–1738, 2006, doi: 10.1128/aem.72.3.1729-1738.2006.
[39] A. I. Klimko, T. A. Cherdyntseva, A. L. Brioukhanov, and A. I. Netrusov, "In vitro evaluation of probiotic potential of selected lactic acid bacteria strains," *Probiotics Antimicrob. Proteins*, vol. 12, no. 3, pp. 1139–1148, 2019, doi: 10.1007/s12602-019-09599-6.
[40] R. A. Bailey, "Intestinal microbiota and the pathogenesis of dysbacteriosis in broiler chickens," PhD thesis, Uni. East Anglia, Norwich, England, 2010.
[41] C. M. C. Chapman, G. R. Gibson, and I. Rowland, "In vitro evaluation of single- and multi-strain probiotics: Inter-species inhibition between probiotic strains, and inhibition of pathogens," *Anaerobe*, vol. 18, no. 4, pp. 405–413, 2012, doi: 10.1016/j.anaerobe.2012.05.004.
[42] J. P. Mills, K. Rao, and V. B. Young, "Probiotics for prevention of *Clostridium difficile* infection," *Curr. Opin. Gastroenterol.*, vol. 34, no. 1, pp. 3–10, 2018, doi: 10.1097/MOG.0000000000000410.
[43] A. Schoster, B. Kokotovic, A. Permin, P. D. Pedersen, F. D. Bello, and L. Guardabassi, "In vitro inhibition of *Clostridium difficile* and *Clostridium perfringens* by commercial probiotic strains," *Anaerobe*, vol. 20, pp. 36–41, 2013, doi: 10.1016/j.anaerobe.2013.02.006.
[44] R. Zhang *et al.*, "Niemann-Pick C1-Like 1 inhibitors for reducing cholesterol absorption," *Eur. J. Med. Chem.*, vol. 230, p. 114111, 2022, doi: 10.1016/j.ejmech.2022.114111.
[45] K. Grond, B. K. Sandercock, A. Jumpponen, and L. H. Zeglin, "The avian gut microbiota: community, physiology and function in wild birds," *J. Avian Biol.*, vol. 49, no. 11, p. e01788, 2018, doi: 10.1111/jav.01788.
[46] H. Li *et al.*, "Probiotic mixture of *Lactobacillus plantarum* Strains improves lipid metabolism and gut microbiota structure in high fat diet-fed mice," *Front. Microbiol.*, vol. 11, p. 512, 2020, doi: 10.3389/fmicb.2020.00512.