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Effect of fermented gambir (*Uncaria gambir*) leaves on broiler performance and blood lipids

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Abstract. *Widjastuti T, Faadhilah A, Adriani L. 2025. Effect of fermented gambir (*Uncaria gambir*) leaves on broiler performance and blood lipids. Asian J Agric 9: 615-622.* Increasing broiler chicken productivity must be supported by proper management during rearing. Efforts to maintain production efficiency with potential alternatives to replace antibiotic growth promoters (AGP) include gambir leaves (*Uncaria gambir*). Fermented gambir leaves aim to improve performance and reduce blood lipid levels in broiler chickens, serving as an alternative ingredient to replace antibiotic growth promoters. The leaves are fermented with *Lactobacillus plantarum* at 1.5% and *Rhizopus oligosporus* at 1%. The study used a completely randomized design (CRD) with five treatments and four replicates. R0: 100% basal diet; R1: 100% basal diet + 0.5% fermented gambir leaf product; R2: 100% basal diet + 1% gambir leaf fermentation product; R3: 100% basal diet + 1.5% fermented gambir leaf product; R4: 100% basal diet + 2% gambir leaf fermentation product. Parameters measured included performance, blood, and meat lipid levels. Results showed significant differences (P<0.05) among treatments R1-R4 compared to R0, indicating increased body weight gain (R1=2393 g) and decreased cholesterol (R1=115 mg/dL), triglyceride (R1 = 49 mg/dL), and fat levels in blood and meat. Based on these findings, adding 1% fermented gambir leaves to the basal diet can improve broiler performance while reducing lipid content in blood plasma and meat. Using 0.5% to 1% gambir leaf fermentation in feed is recommended as a feed additive to replace AGP.

Keywords: Blood lipids, fermentation, gambir leaves, meat lipids, performance

INTRODUCTION

Broiler chickens are a type of chicken raised explicitly for meat production. They provide a significant source of animal protein and enjoy widespread popularity globally. In Indonesia, broiler chicken production has reached 3,997,652.70 tons. Renowned for their rapid growth and efficient feed-to-meat conversion, broiler chickens greatly influence the country's meat consumption. Chicken remains the top meat source in Indonesia, with annual consumption increasing; by 2023, it is projected to be 10.62 kg per person (Mushawwir et al. 2025a).

Enhancing broiler chicken productivity depends on proper management during rearing to ensure high-quality meat production. As consumers become more aware of their food's nutritional content, particularly low-fat options (Mir et al. 2017; Fajar et al. 2021; Dudi et al. 2023), the demand for healthier foods is rising. Elevated fat levels, including cholesterol and triglycerides, can negatively impact broiler productivity. Therefore, efforts to improve production efficiency and develop safe, natural feed additives for livestock are crucial. Recently, AGP in poultry feed have been prohibited because they can leave residues on meat or eggs, presenting health risks to consumers.

Research on natural extracts in poultry is extensive. Ginger powder supplementation has shown benefits in reducing fat content and boosting immunity (Mushawwir et al. 2023), but it did not significantly increase body weight gain in broilers (Irwani et al. 2021). Other studies found that chitosan from shrimp shells can increase body weight in local chickens (Irwani et al. 2021; Rahmania et al. 2022; Aritonang et al. 2024). *Parinarium glaberrimum* seed flour can be used as green additive to antibiotic growth promoters in broiler diets, enhancing gut microflora (Sangadji et al. 2024). Additionally, mangosteen fruit extract and turmeric exhibit lipolipidemic effects, enhance immunity (Purwanti et al. 2024), and improve egg production. Gambir has been researched, though its use in fermentation technology remains unexplored (Rosmiati et al. 2017), and it has not shown significant effects on poultry performance. Gambir leaves (*Uncaria gambir* Roxb.) present potential alternative to antibiotics as a feed additive.

Gambir leaves contain polyphenolic compounds, specifically catechins and tannins. The catechin content functions as an antioxidant and antibacterial agent that inhibits fatty acid synthesis, reducing gambir's toxic effects on the kidneys, liver, and heart, while also decreasing the absorption of blood lipids such as triglycerides, cholesterol, and other lipophilic components in the intestine. Tannins act as antioxidants by binding free radicals, preventing cell damage, and reducing the risk of various diseases. However, high tannins can impair nutrient digestibility, so it's crucial to process them through fermentation with specific microbiota like *Rhizopus oligosporus* and *Lactobacillus plantarum*. This practice ensures the mitigation of tannin effects and the extraction of their beneficial properties (Setiarto and Widhyastuti 2016).

Fermentation of feed ingredients with *R. oligosporus* produces enzymes like proteases that break down proteins into peptides and amino acids, improving digestibility for poultry. This process enhances nutrient availability and feed efficiency. Additionally, *R. oligosporus* generates antimicrobial compounds that inhibit pathogenic bacteria such as coliforms in the digestive tract, lowering infection risks (Endrawati and Kusumaningtyas 2017). *L. plantarum* can reduce harmful bacteria like *Escherichia coli* in the poultry gut by producing lactic acid and antimicrobial substances, including bacteriocins, which create an acidic environment that suppresses pathogens and supports gut health (Haqqi et al. 2021). Fermentation generally increases the bioaccessibility and bioavailability of bioactive compounds, such as polyphenols, through enzymatic breakdown of complex molecules and microbial synthesis of new compounds, like short-chain fatty acids and vitamins (Annunziata et al. 2020; Verardo et al. 2020). These changes can boost antioxidant, antimicrobial, and anti-inflammatory properties by altering existing phytochemicals and producing new ones (Luo et al. 2024; Chouhan et al. 2019). Specifically, fermentation converts bound metabolites into free, active forms, maximizing their therapeutic effects (Kaur and Purewal 2023).

R. oligosporus and *L. plantarum* produce protease enzymes that break down gambir protein into simple polypeptides and amino acids. *L. plantarum* may contain 1.29% crude fiber because it can ferment into SCFA (Short-Chain Fatty Acid) compounds. Short-Chain Fatty Acids (SCFAs), such as acetate, propionate, and butyrate, are bioactive compounds generated by the fermentation of crude fiber and other substrates by gut microbes. SCFAs can activate GPR43/GPR41 receptors on immune cells, reduce the production of pro-inflammatory cytokines (e.g., TNF-α), and increase regulatory T cells (Tregs), thereby suppressing inflammatory responses (Tan et al. 2014; Kim 2023). Fermented gambir leaves enhance broiler chicken quality by boosting performance and lowering blood lipid levels, serving as an alternative to antibiotic growth promoters (AGPs).

MATERIALS AND METHODS

Animal sample

The animal sample consisted of a DOC of 100 broiler chickens. They were housed in a 1×1×1 m pen, with five birds per cage. Each cage had a bottom litter system, a chicken feeder, a drinking area, and a 60-watt bulb. The chickens received commercial 511 feed from day-old until seven days, then switched to the commercial feed BR1 from day 7 to 35. These broiler chickens, with no sex separation, were raised from day-old (DOC) until 35 days old. The study involved five treatments, each repeated four times, with each experimental unit including five chickens. Each chicken was marked with a wing tag on its right wing to facilitate observation and data collection, ensuring a comprehensive study. During the study, the temperature and humidity of the cage and the environment were recorded in real time. The temperature and humidity of the cage during the study were 26°C and 76%, respectively, while those around the animal housing ranged from 27-29°C and 80-85%.

Broiler chickens were maintained for 35 days. Their body weight is recorded at the beginning of the maintenance period. During the first week, they are fed commercial rations, then switched to treatment rations from the second week. The chickens' remaining ration and body weight are measured weekly for each cage. Rations are supplied twice daily, and water is available freely. The composition of these rations, including the specific nutrients and their proportions, is detailed in Table 1, providing a comprehensive understanding of the broiler chickens' nutritional intake.

Preparing fermented gambir leaf

Fermentation of gambir leaves using *R. oligosporus* and *L. plantarum* starters was conducted under anaerobic conditions. The process involved liquid fermentation. The chopped gambir leaves were placed in a container, then sterile distilled water was added, inoculated with *L. plantarum* (1.5%) and *R. oligosporus* (1%), and soaked about 5 cm below the water surface. Incubation was done at room temperature for 24 hours. The fermented gambir leaves were dried in an oven at 50°C until completely dry. Afterwards, the leaves were ground with a grinding machine and filtered through an 80-mesh sieve to produce fermented gambir leaf powder.

Parameters and measurement methods

Performance

Consumption ratios: Feed consumption is the total feed consumed by livestock daily, calculated by subtracting the remaining amount from the total.

Feed consumption (g/head) = Feed given (g) – remaining feed (g)

Body weight gain: Body weight gain (BWG) is the final and initial body weights.

Body Weight Gain (g/head) = final body weight (g) – initial body weight (g)

Conversion ratios: Feed conversion is a comparison between feed consumption and body weight gain.

$$\text{Conversion} = \frac{\textit{Feed Comsumption}}{\textit{Body weight gain}}$$

Table 1. Requirements and content nutrient rations for broiler chickens

Nutrient components and energy metabolism	Broiler chicken requirements (%)	Commercial rating content
Metabolic energy (Kcal/kg)	Min. 3000	-
Crude protein (%)	Min. 20.0	21-23
Crude fat (%)	Max. 5.0	Max. 5.0
Crude fiber (%)	Mask. 5.0	Max. 5.0
Calcium (%)	0.8-1.10	0.8-1.1
Phosphor (%)	Min. 0.5	Min. 0.5
Lysine (%)	Min. 1,2	Min. 1,2
Methionine (%)	Min. 0.45	Min. 0.45

Table 2. Lipid analysis procedure based on Biolabo KIT

Pipette into well-identified test tubes:	Reagent Blank	Specimen blank	Standard	Assay
Saline solution		1 mL		
Reagent R1*	1 mL		1 mL	1 mL
Standard			20 µL	
Specimen		20 µL		20 µL
Demineralized water	20 µL			

Note: Mix well. Let stand for 10 minutes at room temperature—record absorbances against the reagent blank. Read the specimen blank against the saline solution. *based on the parameters, respectively

Blood lipid profile levels

The complete lipid analysis was examined based on the protocol in the Biolabo kit using the following procedure (Table 2).

Blood cholesterol: Blood cholesterol analysis uses the CHOD-POD Enzymatic Colorimetric method. Three test tubes, one blank, one standard, and one assay, were filled with 1 mL of reagent. To the blank, 10 µL of demineralized water was added. The reaction was incubated for 5 minutes at 37°C or 10 minutes at room temperature. Absorbance was read at a wavelength of 500 nm.

HDL (High-Density Lipoprotein): The HDL content in the blood is measured using the Enzymatic Photometric Test method, which utilizes a spectrophotometer. This method is based on the reaction principle of forming Quinoneimine from cholesteryl, and the absorbance is measured with a spectrophotometer at a wavelength of 600 nm. The HDL results of the object are recorded, and the results are calculated using the following formula:

$$\text{HDL} = \frac{Abs\ (assay)}{Abs\ (calibrator)} \times \text{Calibrator concentration}$$

LDL (Low-Density Lipoprotein): This results from a calculation conversion by comparing the values of blood cholesterol, blood HDL, and blood triglycerides. Using the Aritonang et al. (2024) formula as follows:

$$\text{LDL} = \text{Cholesterol Total} - \text{HDL} - \left(\frac{Trigliserida}{5}\right)$$

Triglycerides: The GPO-POD Enzymatic Colorimetric method is used for triglyceride analysis. Three test tubes, one blank, one standard, and one assay, were filled with 1 mL of reagent. To the blank, 10 µL of demineralized water was added. The reaction was incubated for 5 minutes at 37°C or 10 minutes at room temperature. Absorbance was read at a wavelength of 500 nm.

Meat lipid levels

Cholesterol meat: The determination rate of cholesterol in meat can be performed using the CHOD-PAP (Cholesterol Oxidase Phenylperoxidase Amino Phenazone) method. This method directly oxidizes cholesterol in plasma, and the cholesterol ester reaction is hydrolyzed. The 3-OH group is then hydrolyzed, resulting in a response (Kharazi et al. 2022) similar to the analysis rate of cholesterol in meat.

$$\text{Cholesterol Levels of Meat } (\mu g/mg) = \frac{A\ Sample}{A\ Standard} \text{x } 10$$

Where:
A Sample: Absorbance sample
A Standard: Absorbance standard
10: Concentration standard

Meat fat: The fat content of meat is analyzed from samples of breasts and thighs. This is done using Soxhlet's extraction method, which involves extracting fat with a fat solvent (chloroform). After the solvent is evaporated, the fat can be weighed and the percentage calculated.

$$\text{Fat content } (\%) = \frac{(B-C)}{A} \times 100\%$$

Where:
A: Weight sample beginning
B: Weigh the sample dry before extracting
C: Weight sample after extraction and drying

Meat triglycerides: The materials for the triglyceride test are PA alcohol, PA acetone, and a triglyceride kit. Triglycerides in chicken meat were extracted using a mixture of ethanol and acetone. One gram of the sample was placed in a test tube, and then 10 mL of a 1:1 (v/v) ethanol: acetone mixture was added. Triglyceride levels were measured using a spectrophotometer at a wavelength of 546 nm.

Experimental design and statistical analysis

The experiment used a completely randomized design, involving five treatments and four repetitions, analyzed with ANOVA. Significant treatment effects were further tested with Duncan's Multiple Range Test. All data were analyzed via ANOVA and Duncan's test in SPSS version 22.0, with a significance level set at $P<0.05$.

The treatments in this study were as follows:
R0 = 100% Basal ratio
R1 = 100% Basal ration + 0.5% gambir leaf fermentation product
R2 = 100% Basal ration + 1% gambir leaf fermentation product
R3 = 100% Basal ration + 1.5% gambir leaf fermentation product
R4 = 100% Basal ration + 2% gambir leaf fermentation product

RESULTS AND DISCUSSION

Performance

Based on the results of this study, the performance of broilers treated with gambir leaves is shown in Table 3. Consumption results show leaf gambir fermentation influences real P (<0.05). The best results are achieved in PBB and FCR, with an R2 value of 2578 and an FCR value of 1.314. Based on the study results, adding flour leaf gambir fermentation in feed has different influences on feed consumption ($P<0.05$).

Blood lipid profile levels

Based on the results of this study, Table 4 shows the lipid profile of experimental chickens treated with gambir leaves. In addition, total lipids in blood plasma (including lipoprotein transport) are shown in Figure 1. The analysis of adding ferrofoliate leaf flour to the feed showed a significantly different effect (P<0.05) on blood cholesterol. Total lipids in blood plasma, including cholesterol, triglycerides, and lipoproteins, seem lower with R2 treatment.

Meat lipid content

Based on the results of this study, Table 5 shows the lipid content of chicken meat treated with gambir leaves. The study on adding fermented gambir leaf flour to feed revealed a significantly different effect (P<0.05) on cholesterol levels in broiler chicken meat.

Discussion

Performance

The results indicate that adding fermented gambir leaf flour to feed influences feed consumption differently (P<0.05). According to Rosmiati et al. (2017), adding 0.5% gambir flour had no significant effect compared to other studies. This addition of gambir leaf flour has already been fermented using *R. oligosporus* and *L. plantarum* to achieve substantial results. The fermentation process with gambir leaf flour not only enhances feed palatability but also introduces antioxidants. This aspect, as highlighted by Haqqi et al. (2021), is crucial in influencing feed intake. The energy chickens consume is used for growth, production, and activity (Andriyanto et al. 2015). The bioactive compounds in gambir leaves, such as tannins, catechins, flavonoids, and polyphenols, contribute to this process. Fermentation of gambir leaves with *R. oligosporus* and *L. plantarum* was carried out to decrease the harmful effects of tannins, enhance digestibility, reduce bitterness, and boost catechin levels. Catechin functions as an antioxidant, preventing oxidation caused by free radicals. Higher catechin levels are linked with increased antioxidant activity because catechins can inhibit oxidative stress (Sari et al. 2018). Reducing oxidative stress is a significant health benefit for the chickens, stimulating their appetite. The role of fermented gambir leaf flour in improving feed palatability is intriguing for poultry farmers.

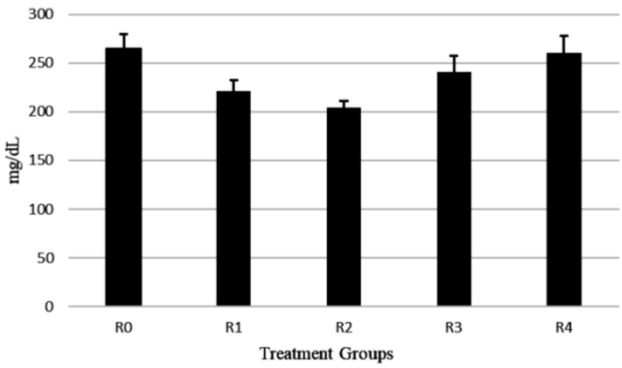

Figure 1. Total lipid profile in blood plasma (including lipoprotein transport)

Table 3. Performance results of broiler chickens

Parameter	Treatment					p-value
	R0	R1	R2	R3	R4	
Consumption ration (g)	3290.38[a]	3409.89[a]	3380.28[ab]	3400.32[ab]	3520.17[b]	0.007
Weight gain (g)	2202.00[a]	2393.00[b]	2578.00[c]	2371.00[a]	2370.00[a]	0.003
Feed conversion	1.49[a]	1.41[a]	1.31[b]	1.45[a]	1.49[a]	0.044

Note: [a,b]: Different superscript letter in the same column indicates a significant effect (P<0.05). R0: 100% Basal ration; R1: 100% Basal ration + 0.5% fermented gambir leaf product; R2: 100% Basal ration + 1% fermented gambir leaf product; R3: 100% Basal ration + 1.5% fermented gambir leaf product; R4: 100% Basal ration + 2% fermented gambir leaf product

Table 4. Results of blood lipid profile levels

Parameter	Treatment					p-value
	R0	R1	R2	R3	R4	
Cholesterol (mg/dL)	134.0[a]	115.0[b]	124.0[b]	134.5[a]	134.0[a]	0.041
HDL (mg/dL)	25.0[a]	29.5[b]	27.7[b]	29.0[b]	28.7[b]	0.035
LDL (mg/dL)	91.5[a]	71.7[b]	77.2[b]	84.7[c]	82.5[c]	0.001
Triglycerides (mg/dL)	83.5[a]	49.0[b]	67.7[c]	60.5[d]	60.5[d]	0.002

Note: [a,b]: Different superscript letter in the same column indicates a significant effect (P<0.05). R0: 100% Basal ration; R1: 100% Basal ration + 0.5% fermented gambir leaf product; R2: 100% Basal ration + 1% fermented gambir leaf product; R3: 100% Basal ration + 1.5% fermented gambir leaf product; R4: 100% Basal ration + 2% fermented gambir leaf product

Table 5. Results of meat lipid content

Parameter	Treatment					p-value
	R0	R1	R2	R3	R4	
Cholesterol (mg/dL)	65.60[a]	54.60[b]	54.93[b]	55.92[b]	58.83[c]	0.011
Fat (mg/dL)	8.83[a]	5.57[a]	6.05[a]	7.71[a]	5.59[a]	0.055
Triglyceride (mg/dL)	184.08[a]	165.85[a]	149.09[a]	174.15[a]	181.35[a]	0.062

Note: [a,b]: Different superscript letter in the same column indicates a significant effect (P<0.05). R0: 100% Basal ration; R1: 100% Basal ration + 0.5% fermented gambir leaf product; R2: 100% Basal ration + 1% fermented gambir leaf product; R3: 100% Basal ration + 1.5% fermented gambir leaf product; R4: 100% Basal ration + 2% fermented gambir leaf product

Research results show that adding gambir leaf flour fermentation to feed influences various absolute values, leading to increased body weight (R2, P<0.05) compared to R0, as shown in Table 3. This study's optimal body weight gain is achieved with 1% (R2) at 2578 g. The significant differences are due to adding natural feed additives from gambir leaves, which can boost feed efficiency and result in higher weight gain compared to R0. This finding aligns with Djaelani et al. (2022), who state that herbal feed additives serve as growth stimulants and improve feed efficiency in broiler chickens. Nilawati et al. (2024) conducted a study using unfermented gambir leaf flour and reported lower results than this study, indicating that fermentation with *R. oligosporus* and *L. plantarum* improves broiler performance by increasing digestibility, enhancing enzyme production, and reducing anti-nutritional substances like tannins. Generally, gambir leaves contain significant tannin levels, which can make the feed bitter and hinder digestion. According to Djunu et al. (2022), tannins are anti-nutrients that inhibit poultry growth.

Fermentation of gambir leaves is performed to increase the polyphenol compound catechin. Catechins can promote body weight gain in broiler chickens through various mechanisms, including antioxidant, antibacterial, and anti-inflammatory effects, by improving gut health, reducing oxidative stress, and enhancing palatability. These effects improve nutrient absorption and support more optimal growth, leading to increased body weight. A study by Manullang et al. (2018) found that gambir extract effectively inhibits the growth of *E. coli* bacteria associated with colibacillosis, resulting in increased body weight.

The study's results on adding fermented gambir leaves to feed showed a significant difference in ration conversion (P<0.05), as seen in Table 3. The lowest conversion ratio was in R2 at 1.314. According to Rosmiati et al. (2017), adding gambir flour at 0.5% and 1% improved broiler feed conversion, but their results averaged 1.67, higher than this study's average of 1.43. This difference may be due to increased nutritional value from fermentation. Feed conversion factors include feed type and quality (Irwani et al. 2021; Muhammad et al. 2023). Fermentation of gambir leaves with *R. oligosporus* and *L. plantarum* increases protein content, as these microorganisms produce protease enzymes that break down proteins into peptides and amino acids. Because protein influences livestock growth, proper digestion promotes body weight gain and improves feed conversion (Hendalia et al. 2021). Additionally, fermentation enhances polyphenol compounds like catechins, which help boost chickens' immune response (Mushawwir et al. 2023; Adriani et al. 2024), making them more disease-resistant. Healthier chickens exhibit better feed conversion efficiency because they do not need to allocate energy to fighting disease, allowing more energy for growth and weight gain.

Blood lipid profile levels

The analysis of adding fermented gambir leaf flour to the feed showed a significantly different effect (P<0.05) on blood cholesterol levels (Table 4). The lowest cholesterol level in this study, R1, was 115 mg/dL. Normal blood cholesterol levels in broiler chickens range from 125 to 200 mg/dL (Rakhmawati and Sulistyoningsih 2020). R1 in this study had a blood cholesterol value below the normal range. This is because of the addition of fermented gambir leaf flour, which contains many active substances, including flavonoids and polyphenols such as catechin and tannin.

Gambir leaves fermented with *R. oligosporus* and *L. plantarum* can boost their pharmacological activity, such as increasing flavonoids, to help lower blood cholesterol levels. The flavonoid content may protect damaged arteries and remove cholesterol buildup from the surfaces of blood vessel walls (Nurazizah et al. 2020; Kharazi et al. 2022; Rahmania et al. 2022). Other active compounds in gambir leaves include catechins and tannins. According to Dahlia et al. (2017), polyphenols like catechins have antihypercholesterolemic effects, which can reduce cholesterol levels. This is because catechin compounds inhibit the activity of the HMG-CoA reductase enzyme by binding directly to its active site, causing allosteric changes, increasing enzyme breakdown, and regulating HMG-CoA enzyme gene expression. These actions block cholesterol biosynthesis and decrease blood cholesterol. A study by Yunarto et al. (2021) showed that catechins in gambir leaves effectively inhibit cholesterol absorption in mice's blood plasma. Besides catechins, tannins can also aid in lowering cholesterol levels. According to Waznah et al. (2019), several tannin derivatives from traditional herbs are known to inhibit HMG-CoA reductase, which is linked to hyperlipidemia.

The study on adding fermented gambir leaf flour to feed showed a significantly different effect (P<0.05) on HDL (High-Density Lipoprotein) and LDL (Low-Density Lipoprotein). The highest increase in blood HDL levels was at R1 of 29.5 mg/dL, while the lowest decrease in LDL levels was at R1 of 71.7 mg/dL.

The increase in HDL is attributed to the rise in levels of fermented catechins in gambir leaves with *R. oligosporus* and *L. plantarum* inoculum. Catechins possess potent antioxidant properties that prevent lipid oxidation in the body. The mechanism behind catechins' increasing HDL levels involves an increase in the activity of the LCAT enzyme (Lecithin-Cholesterol Acyltransferase) and greater expression of the ABCA1 gene (ATP-Binding Cassette transporter A1), making the transport of cholesterol from cells to HDL more efficient and thereby raising HDL levels. According to Millar et al. (2017), catechins can boost LCAT enzyme activity, which helps sterolize free cholesterol in the blood and convert it into cholesterol esters, which HDL then absorbs. ABCA1 mediates unidirectional cholesterol flux, resulting in a net removal of cholesterol from cells (Zanotti et al. 2015; Tanuwiria et al. 2022, 2023; Manin et al. 2024; Mushawwir et al 2025a). The combined effect of these mechanisms facilitates cholesterol transport into HDL, leading to increased HDL levels in the blood and supporting cardiovascular health.

Similar to HDL, decreased LDL levels in the blood occur due to increased levels of catechins during gambir leaf fermentation. Catechins reduce LDL by boosting the activity of LDL receptors in the liver, which move LDL from the bloodstream back to the liver for breakdown and removal. According to Ahmad et al. (2020), catechins block cholesterol production by boosting the activity of LDL receptors. The more bonds formed, the more stable the catechins are when binding to the HMG-CoA reductase enzyme, leading to a more substantial pharmacological effect. According to Yunarto et al. (2021), the presence of hydrophobic bonds in the interaction between catechins and the HMG-CoA reductase enzyme loosens the hydrogen bonds around it, thus increasing the needed bond energy. Catechins are compounds that can potentially manage LDL levels and maintain health.

The results of the study on adding fermented gambir leaf flour to feed showed a significantly different effect ($P<0.05$) on triglycerides (TG). This study observed a decrease in TG levels, with the lowest results, specifically R1, at 49 mg/dL. Broiler chickens typically have TG levels around 70-90 mg/dL. This is because gambir leaves contain bioactive compounds that can reduce blood TG, such as catechins, tannins, flavonoids, alkaloids, phenolic acids, saponins, and terpenoids, which contribute to the mechanism of inhibiting fat absorption, anti-inflammatory effects (Mushawwir et al. 2025b), antioxidant activity, cholesterol binding, and modulation of lipid enzymes, including HMG-CoA.

Catechins can inhibit enzymes involved in lipogenesis in the liver and increase lipoprotein lipase activity. According to Binugraheni and Nastiti (2015), catechins and flavonoids can boost lipoprotein lipase activity and decrease MTP (Microsomal Triglyceride-Transfer Protein) activity. The lipoprotein lipase enzyme causes about 90% of triglycerides in chylomicrons to be removed from circulation, leaving behind chylomicron remnants rich in cholesterol and cholesterol esters. Besides catechins and flavonoids, saponins can also promote cholesterol excretion through feces, which helps lower triglyceride levels in the blood. According to Waznah et al. (2019), saponins lower cholesterol by forming an insoluble complex with β-hydroxysteroids, reducing intestinal cholesterol absorption, and increasing fecal sterol excretion. Combining these compounds makes gambir a potential agent for managing triglyceride levels and improving overall lipid health. However, in this experiment, it appears that adding gambir at concentrations >1.5% did not stimulate weight gain. This suggests that excessive concentrations of bioactive compounds do not stimulate overall anabolism.

The effectiveness of fermentation in boosting the role of active compounds in current research is consistent with previous findings. Rodríguez-Durán et al. (2021) and Aharwar and Parihar (2021) reported that fermentation can increase the production of microbial enzymes, such as tannase, which are essential for breaking down complex tannins into simpler phenolic compounds. This process affects their subsequent metabolism and bioavailability. Such enzymatic activity can reduce the anti-nutritional effects often linked to high tannin intake, potentially enhancing the bioavailability of beneficial phenolic metabolites (Ojo 2022; Zuo et al. 2022). Additionally, this enzymatic modification can influence the overall metabolic profile of the gut microbiome, promoting pathways that produce short-chain fatty acids and other beneficial metabolites from tannin precursors (Sharma et al. 2020).

Meet lipid content

The study's results of adding fermented gambir leaf flour to feed showed a significantly different effect ($P<0.05$) on cholesterol in broiler chicken meat (Table 5). In this study, meat cholesterol decreased with the lowest result, R1, at 54.60 mg/dL. This study showed lower cholesterol levels compared to the survey by Waskita et al. (2023), which indicated that fermentation using butterfly pea flower kombucha can reduce cholesterol levels in broiler chicken meat. The decrease in cholesterol occurs due to fermentation using *R. oligosporus* and *L. plantarum*, which is carried out on gambir leaves, leading to an increase in active substances such as polyphenols and flavonoids. According to Yansen (2021) and Rosiyanti et al. (2025), using feed ingredients that have undergone fermentation can suppress cholesterol formation through the activity of the HMG-CoA reductase enzyme. Antioxidants like polyphenols and flavonoids can reduce the synthesis of the HMG-CoA reductase enzyme, resulting in lower cholesterol production. They also inhibit the HMG-CoA reductase enzyme and increase LDL receptor activity (Yunarto et al. 2021).

Catechin, tannin, saponin, and alkaloid compounds are present in gambir leaves. Catechin is a type of polyphenol that can help prevent high blood pressure, reduce cholesterol buildup in the blood, promote cholesterol excretion through feces, and neutralize free radicals (Tana and Djaelani 2021; Gurning et al. 2024; Mushawwir et al. 2025a, b). Catechin can lower the risk of cardiovascular disease. Tannins and saponins decrease during fermentation but remain in gambir leaves, reducing cholesterol. According to Manafe (2022), tannins and saponins block cholesterol absorption and promote its excretion through feces. These findings align with research by Auclair et al. (2019), which shows that oral administration of catechins over six weeks can decrease the average size of atherosclerotic lesions in the aorta.

The study's results on adding fermented gambir leaf flour to feed showed a significantly different effect ($P<0.05$) on broiler chicken meat fat. In this study, meat cholesterol decreased, with the lowest result, R1, at 5.57%. Similar to cholesterol, fat reduction occurs due to the active substances in fermented gambir leaves. Besides these active substances, crude fiber in gambir leaves also reduces fat by eliminating bile, which is excreted along with feces. The liver secretes bile salts into the bloodstream, which then passes through the liver. The increase in bile salt levels in plasma during digestion causes the liver to produce more, which inhibits fat absorption and leads to its excretion through feces (Prabowo et al. 2023; Purwanti et al. 2024; Mushawwir et al. 2024). The higher crude fiber content results in lower metabolic energy, causing stored fat in adipose tissue to be broken down to meet the body's energy needs. These novel findings are sure to pique the interest of researchers in the field of animal feed and meat quality.

Adding fermented gambir leaf flour to broiler chicken feed significantly affected meat fat content ($P<0.05$). Specifically, meat cholesterol levels decreased, reaching a 54.60 mg/dL low. This reduction is linked to fermented gambir leaves produced with *R. oligosporus* and *L. plantarum*. As noted by Kinasih and Sopandi (2017), incorporating sauerkraut into drinking water can lower triglyceride levels in chicken meat by 69.4-79.2%. Fermentation with these inoculants releases extra lipase enzymes that facilitate fat breakdown in the feed before digestion and can also diminish antinutrients that inhibit fat absorption, thereby enhancing lipase activity.

Lipase enzymes are crucial in breaking down fats, enhancing fat digestion, optimizing metabolism, supporting gut health, and improving overall digestion. Adriani et al. (2015) and Yansen (2021) explain that lipase production reduces triglyceride levels by breaking large fats into smaller, more digestible molecules. Besides lipase, fermentation generates Short Chain Fatty Acids (SCFA), which inhibit cholesterol synthesis in the liver, thus decreasing triglyceride formation. Nilawati et al. (2024) and Aritonang et al. (2025) identified propionate as an SCFA that can block endogenous cholesterol production. Together, these mechanisms from gambir leaf fermentation help lower triglyceride levels in broiler meat, resulting in leaner, healthier meat.

Based on current research, administering fermented gambir can stimulate weight gain of up to 2393 g (R1) and reduce cholesterol (R1=115 mg/dL), triglycerides (R1=49 mg/dL), and fat levels in the blood and meat. These findings suggest that adding 1% fermented gambir leaves to the basic diet can enhance broiler chicken performance while lowering lipid content in blood plasma and meat. Using 0.5% to 1% fermented gambir leaves as a feed supplement is recommended to replace AGP. Future research is highly encouraged to develop coating technology to improve the absorption of active compounds in gambir, ensuring they reach target cells more effectively.

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