Research Article

Fermented Ginger Turmeric Teak Improves Broiler Growth Performance and Intestinal Morphology

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Abstract | Fermentation with *Lactobacillus plantarum* was employed to enhance the bioactive metabolite profile of a combined ginger, turmeric, and teak leaf extract for use as a phytobiotic feed additive. The optimal fermentation duration was determined by evaluating total phenolic content, total flavonoid content, and antioxidant activity. A 72-hour fermentation period was selected for *in vivo* testing as it yielded the highest flavonoid content, the most potent antioxidant capacity (indicated by the lowest IC_{50}), and substantially elevated phenolic levels. In a 35-day broiler trial, this optimized fermented extract was incorporated into a basal diet at inclusion levels of 0, 0.2, 0.4, 0.6, and 0.8%. Supplementation significantly ($p < 0.05$) improved final body weight from 2.186 kg (control) to 2.433 kg (0.8% group) and enhanced feed conversion ratio from 1.593 to 1.365. These performance gains were concomitant with profound improvements in intestinal morphology. Birds fed the 0.8% diet exhibited significant ($p < 0.05$) increases in villus number (63.25 vs. 50.50), villus height (819.25 µm vs. 575.50 µm), villus width, total absorptive surface area, and crypt depth compared to the control. The study concludes that 72-hour fermentation optimally enriches the extract's bioactivity, and its dietary inclusion enhances broiler growth performance by fundamentally improving gut health, as evidenced by the expansion of the absorptive surface and support of active mucosal renewal.

Keywords | Antibiotics alternative, Fermentation, Fermented phytobiotics, Sustainable agriculture, Production performance

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INTRODUCTION

Broiler chickens have been intensively selected for rapid growth and efficient feed conversion, making their performance highly sensitive to dietary interventions. For many years, antibiotic growth promoters were routinely used to enhance growth rate, feed efficiency, and resistance to disease in intensive poultry systems (Fajri *et al.*, 2025; Natsir *et al.*, 2025). Mounting concerns about antibiotic residues in poultry products and the global spread of antimicrobial resistance have prompted strict regulations and voluntary reductions in antibiotic growth promoter use (Kiskó *et al.*,

2025). These constraints have stimulated interest in natural alternatives such as probiotics, phytobiotics, and organic acidifiers that can maintain productivity and health while avoiding the adverse consequences associated with antibiotic use (Natsir *et al*., 2024).

Phytobiotics, defined as plant-derived bioactive substances incorporated into feed, are particularly attractive because they may exert antimicrobial, antioxidant, anti-inflammatory, and digestion-enhancing effects (Prabakar *et al*., 2016; Natsir *et al*., 2024). Herbs and spices such as ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and teak leaves (*Tectona grandis*) provide essential oils, phenolic acids, and flavonoids that can modulate nutrient utilization and gut health (Hermanto *et al*., 2024). Curcumin from turmeric, beta-sesquiphellandrene from ginger, and quercetin from teak leaves have been implicated in improving oxidative balance, shaping the intestinal microbiota, and supporting epithelial barrier integrity, which jointly contribute to better growth performance and resilience to stress (Ardiansyah *et al*., 2024; Hermanto *et al*., 2024; Pan *et al*., 2024; Katrolia *et al*., 2025). However, conventional herbal powders often show variable phytochemical content, limited storage stability, and low bioavailability in the gastrointestinal tract, and their high moisture and nutrient content can favor spoilage, thereby compromising safety and efficacy (Lestari *et al*., 2023).

Fermentation has been proposed as an effective strategy to overcome these limitations. Lactic acid bacteria such as *Lactobacillus plantarum* can stabilize herbal substrates, biotransform complex compounds into more active or more readily absorbed forms, and enhance antioxidant capacity (Jiang *et al*., 2025; Ma *et al*., 2025). Fermentation has been shown to increase total phenolic and flavonoid content and to improve radical scavenging activity in plant-based preparations, thereby increasing their functional value as natural growth promoters (Predescu *et al*., 2024). Nevertheless, most work has examined single herbs or simple mixtures in non-fermented forms and has focused mainly on performance traits, often omitting a detailed evaluation of intestinal histomorphometry (Edi *et al*., 2018; Nuningtyas and Widodo, 2018; Fajri *et al*., 2025). Because villus number, height, and surface area, together with crypt depth, are critical determinants of absorptive capacity and mucosal renewal, comprehensive studies that integrate performance, economic indices, and intestinal morphology in response to multi-herb fermented phytobiotics remain limited.

Ginger, turmeric, and teak leaves constitute a coherent phytobiotic formulation because ginger and turmeric supply essential oils and curcuminoids with notable antimicrobial and antioxidant properties (Dusabumuremyi *et al*., 2022), whereas teak leaves contribute flavonoids such as quercetin that may enhance gut integrity and modulate immune functions (Uyanga *et al*., 2021; Hermanto *et al*., 2024). Previous work has indicated that combined phytogenic preparations can improve gastrointestinal health, regulate immune activity, enhance carcass quality, and support growth performance in broiler chickens (Ardiansyah *et al*., 2024; Hermanto *et al*., 2024; Suwito *et al*., 2025a, b). Fermentation of these mixed extracts with *L. plantarum* is expected to elevate total phenolic and flavonoid contents and increase overall antioxidant capacity. The present study therefore investigated the influence of dietary inclusion of fermented ginger, turmeric, and teak leaf extracts on broiler performance and intestinal morphology by applying graded supplementation levels and assessing feed intake, body weight, feed conversion ratio, performance index, income over feed cost, and detailed morphometric parameters of the small intestine. It was hypothesized that this fermented multi-herb preparation would enhance growth efficiency and economic returns and that these improvements would be associated with beneficial modifications in villus architecture and crypt depth, supporting its potential application as a natural growth promoter in antibiotic-free broiler production systems.

MATERIALS AND METHODS

Preparation of extract

Extract was prepared according to the previous protocol (Ardiansyah *et al*., 2024; Suwito *et al*., 2025a). Dried powders of turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and teak leaves (*Tectona grandis*) were prepared and combined in equal proportions (w/w). The mixture was subjected to solvent extraction using analytical-grade ethanol at a herb-to-solvent ratio of 1:5 (w/v). The suspension was sealed and maintained under maceration for twenty-four hours to facilitate diffusion of ethanol-soluble phytochemicals.

Following maceration, the mixture was transferred to a microwave-assisted extraction system to enhance extraction efficiency in accordance with the procedure described by Suwito *et al*. (2025b). Extraction was performed at moderate microwave power while maintaining a controlled temperature range of 50 to 60 °C for ten minutes. The extract was subsequently cooled to ambient temperature and filtered through qualitative filter paper to remove residual plant particulates. Ethanol was removed from the filtrate using controlled thermal evaporation at 50 to 60 °C until a liquid concentrated crude extract was obtained.

Fermentation of extract

The concentrated herbal extract obtained from the initial extraction process was subjected to fermentation using *Lactobacillus plantarum*. The bacterial strain was maintained

in De Man, Rogosa, and Sharpe (MRS) broth and subcultured twice prior to use to ensure optimal viability. For each fermentation batch, the extract was inoculated with *L. plantarum* at a final concentration of 10^6 CFU per milliliter of extract. The inoculated extract was incubated at 37 °C under constant agitation at 150 rpm to maintain sufficient oxygen transfer and homogeneous bacterial distribution.

To determine the optimal fermentation duration, independent fermentation sets were prepared and incubated for 24, 48, 72, 96, and 120 hours. At the end of each incubation period, fermentation period was terminated by passing the fermented extract through a sterile 0.22 µm syringe filter. This filtration step effectively removed viable bacterial cells and prevented any further metabolic activity that could alter the chemical profile of the extract. The fermented extract then was stored in 4°C until further use.

To minimize variability in the fermentation process, all plant materials were obtained from the same supplier or region within a single procurement period and processed using standardized washing, slicing, and drying procedures. The fermented product was stored in airtight containers and used within a defined storage period to prevent quality deterioration.

Determination of phytochemical contents and antioxidant activity

The fermented extracts obtained at each time point were evaluated for their total phenolic content, total flavonoid content, and antioxidant activity to determine the optimal duration of fermentation. All analyses were performed under minimal-light conditions to prevent photodegradation of phenolic and flavonoid compounds. The protocols were adopted from the previous method with modification (Purwanti *et al.*, 2023).

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu colorimetric method. An aliquot of each fermented extract (100 µL) was mixed with 1 mL of ten-fold diluted Folin–Ciocalteu reagent. The mixture was incubated for five minutes at room temperature before the addition of 1 mL of 7.5 percent sodium carbonate solution. The reaction mixture was then incubated for ninety minutes in the dark, after which absorbance was measured at 725 nm using a UV–visible spectrophotometer. A standard calibration curve was prepared using gallic acid, and total phenolic concentration was expressed as mg/L gallic acid equivalent (GAE). Sample concentrations were calculated using the linear regression equation obtained from the standard curve.

Total flavonoid content (TFC) was determined following an aluminum chloride colorimetric assay using quercetin as the reference standard. A total 500 µL of sample was combined with 100 µL of 10 percent aluminum chloride, followed by 1.5 mL of ethanol and 100 µL of 1 M sodium acetate. All mixtures were incubated for forty minutes at room temperature in the dark. Absorbance was recorded at 415 nm, and flavonoid concentrations were interpolated from a quercetin standard curve. Results were expressed as mg/L quercetin equivalents (QE).

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay. Fermented extracts were diluted ten-fold prior to analysis to ensure measurable absorbance within the linear detection range. A 0.4 mM DPPH solution was prepared in methanol and stored in a sealed amber container. For each sample, 1 mL of the diluted extract was mixed with 1 mL of the DPPH solution in test tubes. A negative control consisted of 1 mL of DPPH solution mixed with 1 mL of methanol, while ascorbic acid (100 ppm stock with serial dilutions) served as the positive control. Reaction mixtures were incubated for thirty minutes at room temperature in reduced-light conditions, and absorbance was measured at 517 nm. Antioxidant activity was calculated as percentage radical inhibition based on the reduction in absorbance relative to the negative control. A linear regression curve was generated using the percent inhibition values of the extract dilutions, and the IC_{50} value was determined by interpolating the concentration corresponding to 50 percent inhibition. This method was adapted from the previous described protocol (Purwanti *et al.*, 2023).

The combined results of TPC, TFC, and DPPH-based antioxidant capacity were used to identify the fermentation duration that yielded the highest enrichment of bioactive compounds and the strongest free-radical scavenging activity. The optimal duration was defined as the time point at which all three parameters reached their maximum or plateaued without further improvement.

In vivo experimental design

The *in vivo* trial was conducted using two hundreds day-old chicks (DOC) of the Lohmann MB 202 strain. The birds were reared in an open-house system, and the facility consisted of twenty floor pens, each measuring 1 × 1 × 0.7 meters. Each pen accommodated ten chicks, resulting in a total of twenty experimental units.

The birds were assigned to five dietary treatments following a randomized block design with four replications per treatment and ten birds per replication. The experimental treatments included: A control group receiving a standard commercial diet (P0), a diet supplemented with 0.2 percent fermented extract combined with probiotic (P1), a diet supplemented with 0.4 percent fermented extract combined

with probiotic (P2), a diet supplemented with 0.6 percent fermented extract combined with probiotic (P3), and a diet supplemented with 0.8 percent fermented extract combined with probiotic (P4). The probiotics used in this feed formulation was a commercial liquid probiotic preparation containing Lactic Acid Bacteria (LAB) and *Bacillus* spp., with a concentration of 2.58×10^9 CFU/mL, as described from the earlier protocol (Nuningtyas *et al*., 2023).

The feeding trial was carried out for a duration of 35 days. Feed mixing procedures were standardized to ensure uniform inclusion of the fermented product across diets. Feed and drinking water were offered *ad libitum* throughout the experiment. This research was carried out in compliance with the institution's ethical guidelines for animal experimentation (Approval No. 91/EC/KEPK/04/2025). The ethical clearance certificate was issued by the Research Ethics Committee of Universitas Brawijaya, and all procedures were performed according to established animal welfare standards to minimize stress and discomfort.

Performance data measurement

Growth performance variables, including feed intake, body weight, feed conversion ratio, and performance index, were evaluated based on replicate-level measurements. Feed intake was determined by subtracting the remaining feed from the total feed provided to each replicate throughout the study period. Final body weight was recorded individually at day 35, and the mean value for each replicate was used for performance calculations. Feed conversion ratio (FCR) was obtained by dividing the total feed consumed by the corresponding body weight gain. The performance index was calculated using a formula that integrates livability, final body weight, FCR, and production age, providing a comprehensive indicator of productivity (Sartono *et al*., 2025).

Intestinal histomorphometry

Intestinal histomorphometry was performed according to the previous described method (Maghfiroh *et al*., 2025). Briefly, intestinal samples were processed for paraffin histology using standard dehydration, clearing, and paraffin infiltration steps, followed by embedding and microtomy. Sections of approximately 3 µm thickness were mounted on glass slides, deparaffinized, rehydrated, and stained with Mayer's Hematoxylin and Eosin to visualize mucosal architecture. Stained tissues were examined under a light microscope at 100× and 400× magnifications, and digital images were obtained for quantitative analysis. Histomorphometric measurements were conducted using ImageJ software. For each specimen, three sections were evaluated, and ten well-oriented villi per section were selected for analysis. Villus height was measured from the villus apex to the villus–crypt junction, villus width from the basal or mid-villus region, and crypt depth from the base of the crypt to the villus–crypt interface. Villus number was determined by counting vertically oriented villi within standardized microscopic fields. The absorptive surface area of each villus was estimated by modelling the villus as a cylinder and applying height and width measurements accordingly. Mean values for each parameter were calculated from all measurements obtained across the three sections and subsequently used for statistical analysis.

Data analysis

The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences among fermentation durations. All data were expressed as mean ± standard deviation for each treatment group. Differences between means were considered statistically significant at $p < 0.05$. The analysis was conducted using R statistical software with the agricolae package, and the results were presented with Duncan grouping letters to indicate homogeneous subsets where means sharing the same letter are not significantly different from each other.

RESULT AND DISCUSSION

Effect of fermentation period on total phenol, flavonoid, and radical scavenging activity

The fermentation duration significantly influenced the production of bioactive compounds, as illustrated in Figure 1. Phenolic content demonstrated a substantial increase throughout the fermentation period, with the highest accumulation observed at 96 hours, followed by a moderate decline at 120 hours (Figure 1A). Similarly, flavonoid content exhibited a pronounced enhancement, reaching its peak at 72 hours before gradually decreasing at longer fermentation times (Figure 1B). The statistical analysis revealed distinct grouping patterns, where different alphabetical notations indicate significant differences ($p < 0.05$) among treatment means, confirming that fermentation time exerts a profound effect on bioactive compound synthesis.

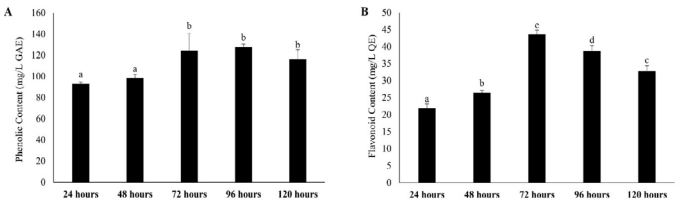

Figure 1: Changes in bioactive compound profiles during fermentation. (A) Total phenolic content expressed as mg/L gallic acid equivalents (GAE). (B) Total flavonoid content expressed as mg/L quercetin equivalents (QE). Values represent mean ± standard deviation (n=3). Different lowercase letters above bars indicate significant differences among fermentation durations according to Duncan's multiple range test ($p < 0.05$).

The observed trends in bioactive compound production can be attributed to the dynamic metabolic activities during fermentation. The initial increase in phenolic and flavonoid contents likely results from the enzymatic hydrolysis of complex polyphenols into simpler, more extractable forms, facilitated by microbial enzymes (Zhang *et al*., 2025). The subsequent decline after peak periods may be explained by the possible degradation of these compounds or their conversion into other metabolites by microbial activity (Vollmer *et al*., 2018). These findings align with previous studies demonstrating that optimal fermentation duration is crucial for maximizing bioactive compound yield, as prolonged fermentation may lead to compound instability or further metabolic transformations (Andika *et al*., 2025). The significant ($p < 0.05$) variations among different time points, as denoted by the Duncan grouping letters, underscore the importance of precise timing control in fermentation processes to achieve optimal bioactive compound production.

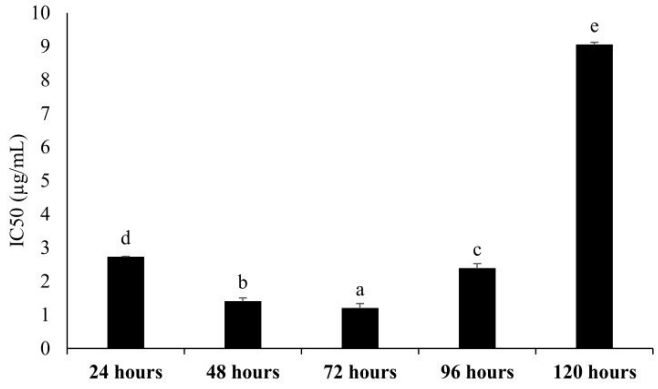

Figure 2: Antioxidant activity determined by DPPH radical scavenging assay. Values represent IC50 (half maximal inhibitory concentration) in µg/mL, with lower values indicating higher antioxidant capacity. Data shown as mean ± standard deviation (n=3). Different lowercase letters above bars denote significant differences among fermentation durations based on Duncan's multiple range test ($p < 0.05$).

The DPPH radical scavenging assay revealed a significant ($p < 0.05$) effect of fermentation duration on antioxidant activity, as measured by IC50 values (Figure 2). The antioxidant capacity showed a notable improvement with the lowest IC50 value, indicating the strongest activity, observed at 72 hours of fermentation. This was preceded by a gradual enhancement from 24 to 72 hours and followed by a substantial decrease in activity at 96 and 120 hours. This pattern of antioxidant activity closely aligns with the temporal profile of flavonoid content, which similarly peaked at 72 hours, suggesting a strong contribution of flavonoid compounds to the observed radical scavenging capacity. The correlation between the high flavonoid levels and enhanced antioxidant activity at 72 hours is consistent with the established role of flavonoids as potent hydrogen donors and free radical terminators (Stepanić *et al*., 2013). Although phenolic content reached its maximum later at 96 hours, the superior antioxidant potential at 72 hours underscores that the quality and specific composition of bioactive compounds, particularly flavonoids, may be more critical for antioxidant efficacy than the total phenolic content alone (Huang *et al*., 2014). Therefore, based on the integration of all parameters the peak flavonoid content, optimal antioxidant activity (lowest IC50), and substantially high phenolic content the 72-hour fermentation period is determined to be the most effective for maximizing the production of bioactive compounds with potent antioxidant properties.

Effect of fermented ginger turmeric teak extract on the performance of broiler

Since the 72 hours of fermentation yielded in greater phenolic and flavonoid contents with strong antioxidant activity, that fermentation period was selected for in vivo performance experiment.

The inclusion of fermented ginger, turmeric, and teak leaf extract in broiler diets produced clear effects on growth and efficiency, while exerting only a limited influence on feed intake. Cumulative feed intake over the 35 days rearing period showed a slight but consistent numerical decline from the control diet (P0) to the highest inclusion level (0.8%; P4), despite no statistical difference was observed. Birds in the control group consumed 3487.25 ± 27.66 g per bird, whereas those receiving 0.8% fermented phytobiotics consumed 3324.63 ± 36.34 g per bird. Intermediate treatments (0.2, 0.4, and 0.6%; P1–P3) exhibited intake levels between these extremes, and statistical analysis indicated that the differences among treatments were not significant ($p > 0.05$, Table 1). These findings suggest that the presence of fermented phytobiotic did not impair palatability or induce feed refusal. Rather, the modest reduction in intake at higher inclusion levels likely reflects improved efficiency of nutrient utilization rather than an adverse effect on appetite.

In contrast to the relatively stable feed intake, final body weight and FCR were markedly influenced by the dietary treatments. Final body weight increased significantly ($p < 0.05$) with increasing levels of fermented herbal. Birds in the control group reached a final weight of 2.186 ± 0.04 kg per bird at 35 days, whereas those in P4 attained 2.433 ± 0.03 kg per bird. Treatments P1, P2, and P3 yielded intermediate weights of 2.258 ± 0.02, 2.360 ± 0.03, and 2.383 ± 0.03 kg per bird, respectively, indicating a dose responsive pattern in which higher inclusion levels of fermented phytobiotics were associated with greater weight gain.

Table 1: Production performance of broiler chickens fed diets containing fermented ginger, turmeric, and teak leaf extract.

Treatment	FI (g/bird/week)	BW (kg)	FCR	IOFC (IDR)	Performance index (PI)
P0	3,487.25±27.66	2.186±0.04^{d}	1.592±0.035^{a}	4,438.56±866.25^{c}	382.137±165.03^{c}
P1	3,453.50±98.78	2.258±0.02^{c}	1.530±0.041^{ab}	5,885.57±576.02^{bc}	422.323±122.10^{b}
P2	3,411.87±63.51	2.360±0.03^{b}	1.445±0.025^{b}	6,124.71±663.03^{b}	443.188±106.63^{b}
P3	3,383.75±148.63	2.383±0.03^{b}	1.417±0.076^{c}	8,270.76±1862.97^{a}	444.211±266.03^{b}
P4	3,324.62±36.34	2.433±0.03^{a}	1.365±0.012^{c}	9,080.31±389.75^{a}	483.401±98.91^{a}

Body weight (BW), feed intake (FI), feed conversion ratio (FCR), performance index, and income over feed cost (IOFC) of broiler chickens fed a basal diet supplemented with 0, 0.2, 0.4, 0.6, or 0.8% fermented ginger, turmeric, and teak leaf extract (denoted as P0 to P4, respectively) for 35 days. Values are presented as mean ± standard deviation (n = 4 pens per treatment, 10 birds per pen). Means within a same column with different superscript letters differ significantly ($p < 0.05$).

Table 2: Intestinal morphometry of broiler chickens fed diets containing fermented ginger, turmeric, and teak leaf extract.

Treatment	Intestinal characteristics				
	Number of Villi	Villi Height (µm)	Villi Width (µm)	Total Area of Villi (mm²)	Cryptic Depths (µm)
P0	50.50 ± 3.70^{b}	575.50 ± 24.96^{b}	196.75 ± 17.99^{d}	815.00 ± 48.96^{c}	136.00 ± 15.03^{b}
P1	51.25 ± 6.99^{b}	579.00 ± 24.60^{b}	215.50 ± 10.25^{c}	818.75 ± 106.44^{b}	141.00 ± 20.51^{b}
P2	53.00 ± 6.05^{b}	592.75 ±136.56^{b}	230.00 ± 9.05^{c}	853.25 ± 74.29^{b}	146.00 ± 6.98^{b}
P3	55.00 ± 3.65^{b}	618.50 ± 35.63^{b}	257.75 ± 14.86^{b}	910.00 ± 33.09^{b}	149.75 ± 16.78^{b}
P4	63.25 ± 4.19^{a}	819.25 ± 118.85^{a}	289.50 ± 4.79^{a}	1,154.75 ± 107.73^{a}	183.50 ± 15.26^{a}

Note: Number of villi, villus height, villus width, villus surface area, and crypt depth in the small intestine of broiler chickens fed a basal diet supplemented with 0, 0.2, 0.4, 0.6, or 0.8% fermented ginger, turmeric, and teak leaf extract (denoted as P0 to P4, respectively) for 35 days. Values are presented as mean ± standard deviation (n = 4 birds per treatment). Means within a same column with different superscript letters differ significantly ($p < 0.05$).

This improvement in body weight agrees with previous findings that phytobiotics from teak-ginger-turmeric and probiotics can act as natural growth promoters in broilers. Phytogenic compounds such as essential oils, phenolic acids, and flavonoids have been reported to enhance nutrient digestion (Suwito *et al*., 2025b), stabilize the intestinal microflora (Sartono *et al*., 2025), and improving gut health (Hermanto *et al*., 2024), thereby increasing the proportion of ingested nutrients that are converted into body tissue. The current study extends these observations by demonstrating that a combined and fermented preparation of ginger, turmeric, and teak leaves can provide measurable benefits in broiler growth performance.

The FCR showed a pattern that paralleled body weight. As presented in Table 1, FCR decreased significantly from 1.5925 ± 0.035 in the control group to 1.365 ± 0.012 in P4 ($p < 0.05$). Values for P1, P2, and P3 were 1.530 ± 0.041, 1.445 ± 0.025, and 1.417 ± 0.076, respectively. Since FCR expresses the amount of feed required to produce a unit of body weight gain, lower values indicate improved efficiency. The combination of slightly reduced feed intake and substantially increased body weight gain in the higher inclusion groups therefore translated into markedly better feed efficiency.

Several mechanisms may account for the improved FCR observed in the supplemented treatments. Fermentation with *L. plantarum* likely increased the stability and bioavailability of phytochemical constituents (Jiang *et al*., 2025; Ma *et al*., 2025), while simultaneously introducing balanced gut microbiota and immunity along with the probiotic (Idowu *et al*., 2025). Probiotic bacteria can produce digestive enzymes, organic acids, and bacteriocins that enhance mucosal integrity, suppress potential pathogens, and improve nutrient absorption (Idowu *et al*., 2025). In addition, ginger and turmeric are known to stimulate pancreatic secretions and activities of enzymes such as amylase, lipase, and protease (Chowdhury *et al*., 2021), thereby promoting more complete digestion of dietary carbohydrates, lipids, and proteins. The combined action of these processes can explain the more efficient conversion of feed into body mass.

Economic indicators reinforce the biological benefits of the supplementation strategy. Performance index and income over feed cost (IOFC) both increased significantly ($p < 0.05$) with higher inclusion levels of fermented herbal (Table 1). The performance index, which integrates survival rate, body weight, FCR, and age at slaughter, rose from 382.14 ± 165.03 in the control group to 483.40 ± 98.91 in P4. Improvements in this index are primarily driven by

higher final weights and lower FCR values, suggesting a more efficient production process (Zalfa *et al*., 2026). The IOFC, calculated as the difference between revenue from live bird sales and feed costs, increased from 4438.56 ± 866.25 IDR per bird in P0 to 9080.31 ± 389.75 IDR per bird in P4. Despite some variability in economic returns between replicates, a clear and significant positive trend in IOFC was observed with increasing supplementation levels. Improvements in FCR and final body weight are central determinants of IOFC in broiler operations, and the present results are consistent with this conclusion (Ibrahim *et al*., 2025).

Previous studies have shown that appropriate use of herbal and probiotic additives can enhance profitability in broiler production by increasing growth rate, improving feed efficiency, and reducing mortality or morbidity (Nurhayati *et al*., 2015; Astuti and Suripta, 2021). The present data suggest that the additional cost associated with 0.8% inclusion of fermented ginger, turmeric, and teak leaf is offset by the gains in productivity and economic return. Overall, the production performance findings support the potential of this combined fermented phytobiotics preparation as a viable natural alternative to conventional antibiotic growth promoters in broiler diets.

Intestinal characteristics of broiler fed with fermented ginger turmeric teak extract

The dietary inclusion of fermented ginger–turmeric–teak extract significantly ($p < 0.05$) influenced the intestinal morphometry of broiler chickens, as detailed in Table 2. The number of villi increased progressively with higher supplementation levels, rising from 50.50 ± 3.70 in the control group (P0) to 63.25 ± 4.19 in the 0.8% inclusion group (P4). Similarly, villus height exhibited a significant ($p < 0.05$) enhancement, increasing from 575.50 ± 24.96 µm in P0 to 819.25 ± 118.85 µm in P4. Villus width and the calculated total villus surface area also showed significant ($p < 0.05$) dose-dependent increases, expanding from 196.75 ± 17.99 µm to 289.50 ± 4.79 µm and from 815.00 ± 48.96 mm^2 to 1154.75 ± 107.73 mm^2, respectively. Furthermore, crypt depth was significantly ($p < 0.05$) greater in the P4 group (183.50 ± 15.26 µm) compared to the control (136.00 ± 15.03 µm).

The morphometric alterations indicate a substantial expansion of the intestinal absorptive surface in response to the fermented phytobiotic supplementation. The increases in villus number, height, and width collectively contribute to a larger surface area available for nutrient absorption. The concurrent deepening of the crypts, which house epithelial progenitor cells, suggests an active state of mucosal renewal. This combination of morphological changes taller and more numerous villi alongside deeper crypts is indicative of a healthy and dynamically renewing intestinal epithelium, which is conducive to enhanced digestive and absorptive functions.

The observed improvements in villus architecture are consistent with the known benefits of phytobiotics and probiotics on gastrointestinal health. Probiotics incorporated with the phytobiotics can modulate the intestinal microbiota, reduce pathogen load, and stimulate epithelial cell proliferation (Ardiansyah *et al*., 2024; Idowu *et al*., 2025). The phytochemical compounds from ginger, turmeric, and teak leaves may further contribute by providing antioxidant and anti-inflammatory support (An *et al*., 2019; Hermanto *et al*., 2024), thereby creating a more favorable gut environment for mucosal development (Apalowo *et al*., 2024; Ardiansyah *et al*., 2024; Hermanto *et al*., 2024; Li *et al*., 2025). The synergistic action of the fermented multi-herb preparation likely promoted a balanced mucosal turnover, facilitating the development of more numerous, taller, and wider villi.

The significant enhancement in intestinal morphology provides a plausible mechanistic explanation for the improved growth performance and feed efficiency reported in this study. A greater villus surface area permits more efficient absorption of nutrients, while adequately deep crypts ensure a sustained supply of healthy enterocytes to maintain the integrity of the expanded absorptive surface (Almet *et al*., 2020; Maghfiroh *et al*., 2025). The correlation between superior gut structure and enhanced production parameters underscores the role of optimal intestinal health as a foundation for broiler productivity. These findings align with the concept that natural growth promoters can exert their positive effects, at least in part, by optimizing gut structure and function.

Collectively, the intestinal characteristics observed in this study support the conclusion that fermented ginger, turmeric, and teak leaf extract, when included at 0.8% in the diet, can enhance gut morphology in a manner that is coherent with improved biological performance and economic outcomes. The positive effects on villus architecture and crypt renewal, alongside the gains in body weight, feed efficiency, and IOFC, indicate that this combined phytobiotic and probiotic approach represents a promising natural strategy to support broiler production in systems that aim to reduce or eliminate antibiotic growth promoters.

This study has several limitations that should be considered when interpreting the findings. First, the fermentation process and the broiler trial can be influenced by multiple factors that may either support or hinder experimental outcomes. Variability in the phytochemical composition of ginger, turmeric, and teak leaves due to differences in plant

maturity, seasonality, geographic region, and post-harvest handling may affect fermentation kinetics and the resulting bioactive profile. In addition, fluctuations in substrate moisture, ambient temperature, and hygiene during processing may alter microbial dynamics and increase the risk of contamination, which in turn can influence product consistency and biological responses. Although these sources of variability were minimized through standardized raw-material handling and pre-processing, routine monitoring of fermentation indicators, sanitary handling and sealed fermentation conditions, controlled storage, and uniform animal management with random allocation and consistent feeding procedures, residual variability may still remain. Second, the present work evaluated only one fixed formulation using equal proportions of the three botanical materials. Therefore, the individual contribution of each component and the optimal mixing ratio cannot be concluded from the current data. Because altering the ratio is expected to change the phytochemical balance and potentially shift the trade-off between gut-modulating benefits and possible anti-nutritional effects (particularly from higher leaf-derived polyphenols/tannins), future studies should apply mixture-design optimization coupled with batch-to-batch phytochemical profiling, fermentation quality metrics, and expanded biological endpoints to define the most effective and safest ratio for broiler application.

CONCLUSION

Dietary supplementation with fermented ginger, turmeric, and teak leaf extract improved broiler growth performance and intestinal morphology without increasing feed intake. Birds receiving the highest inclusion level showed greater body weight, better feed conversion ratio, higher performance index, and higher income over feed cost, indicating clear biological and economic benefits. These gains were associated with more numerous, taller, and wider villi and deeper crypts, suggesting enhanced absorptive capacity and balanced mucosal renewal. The study contributes to the evidence that fermented multiherb can serve as promising natural growth promoters in antibiotic-free broiler systems. Further work should explore underlying microbiota and immune responses.

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NOVELTY STATEMENT

This study is novel because it integrates fermentation optimization and in vivo validation of a multi-herb phytobiotic made from ginger, turmeric, and teak leaves for broiler production. Unlike previous reports on single herbs or non-fermented preparations, it first selects the optimal fermentation time using phenolic content, flavonoid content, and DPPH antioxidant activity, then tests the optimized product in broilers. The study further links growth performance, feed efficiency, and economic returns with detailed intestinal histomorphometry, providing mechanistic evidence that improved villus architecture and crypt renewal underlie the benefits of fermented phytobiotics in antibiotic-free production systems using a practical graded inclusion design.

AUTHOR'S CONTRIBUTION

FEH contributed to the methodology, conducted data analysis, and prepared the original draft. DTA performed the experimental work and assisted with data analysis, while YFN carried out the experiments and managed project administration. FM was responsible for data analysis, visualization, and data curation. OS provided conceptualization and supervision, and MHN contributed to conceptualization, supervision, and secured funding for the study. MHR assisted with data analysis and participated in reviewing and editing the manuscript, whereas NH contributed to the methodological design and the review and editing processes.

Generative AI and AI-assisted technology statement

The authors used ChatGPT (OpenAI) solely to polish the language of the manuscript to improve readability, clarity, and conciseness. The tool was not used to generate scientific content, design the study, analyze data, interpret results, or draw conclusions. All manuscript contents, including data presentation, scientific interpretation, and conclusions, were reviewed and approved by all authors. The authors take full responsibility for the accuracy, integrity, and originality of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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