



Effects of *Durio zibethinus* Waste Fermented with *Lentinus Edodes* as a Functional Feed Ingredient in Broiler Chickens

Nuraini Nuraini ^{1*}, Ade Trisna ², Rahmad Fani Ramadhan ³, Jamila Mustabi ⁴ and Mirzah Mirzah ¹

¹Department of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Andalas, Padang

²Animal Science Department, Faculty of Agriculture, Universitas Sumatera Utara, Indonesia

³Department of Animal Nutrition and Feed Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Indonesia

⁴Department of Nutrition and Feed Technology, Faculty of Animal Science, Universitas Hasanuddin 90245, Indonesia

*Corresponding author: nuraini@ansci.unand.ac.id

ABSTRACT

Durian (*Durio zibethinus*) processing generates substantial agro-industrial waste that remains underutilized despite its potential as a functional feed ingredient. Fermenting *Lentinus edodes* can enhance the nutritional value and bioavailability of durian waste by improving fiber degradability and releasing bioactive compounds; however, information on the safe inclusion level and physiological effects in broiler diets is limited. This study evaluated the effects of graded dietary levels of *L. edodes*-fermented durian waste (DzWF) on growth performance, gut health, immune response, lipid metabolism, and meat fatty acid composition in broiler chickens. A total of 200 Lohmann MB 202 broilers were assigned to five dietary treatments containing 0, 5, 10, 15, or 20% DzWF with four replicates of 10 birds each, and fed isoenergetic and isonitrogenous starter and finisher diets. Dietary DzWF up to 20% did not affect growth performance, feed efficiency, carcass yield, or abdominal fat deposition. Thymus index increased linearly with increasing DzWF levels, while bursa and spleen indices were unaffected. DzWF supplementation significantly improved intestinal morphology by increasing villus height and villus height-to-crypt depth ratio and reducing crypt depth, accompanied by decreased ileal *Escherichia coli* counts and increased lactic acid bacteria populations. Blood lipid profiles were favorably modulated, with increased high-density lipoprotein levels and reduced meat cholesterol content. Omega-3 fatty acids increased at all inclusion levels, and omega-6 fatty acids increased notably at 20% DzWF. These findings indicate that DzWF can be safely included in broiler diets up to 20% and functions as a sustainable feed ingredient that enhances gut health, immune development, and meat lipid quality.

Keywords: Broiler chickens, Fermented durian waste, *Lentinus edodes*, Intestinal morphology, Blood lipid profile.

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INTRODUCTION

The poultry industry continues to experience rapid global expansion, driving increased demand for high-quality feed ingredients that support optimal broiler growth and production efficiency. However, competition for conventional feed resources such as maize and soybean meal has intensified, leading to higher feed costs and prompting the search for alternative, sustainable, and locally available feedstuffs (Wang & Wei, 2021; Lundberg & Abman, 2022). Agro-industrial by-products represent a

promising solution due to their abundance, low cost, and potential nutritive value when properly processed (Hadidi et al., 2024). In tropical regions, *Durio zibethinus* (durian) is widely cultivated, producing substantial amounts of waste, including peel and seeds that are typically discarded. These materials contain structural carbohydrates, polysaccharides, and phytochemicals that could be used as feed, although their high fiber content and the presence of anti-nutritional factors limit direct inclusion in monogastric diets (Sha minutes-Shazwana et al., 2022; Yusof et al., 2024).

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Fermentation has emerged as an effective biotechnological approach for improving the nutritional quality of agricultural residues. Through microbial fermentation, crude fiber can be reduced, crude protein increased, a minuteso acid profiles enhanced, and bioactive compounds produced with antioxidant, antimicrobial and immunomodulatory activities (Jiang et al., 2021; Zhao et al., 2021). Filamentous fungi and edible mushrooms, such as *Lentinus edodes*, are particularly effective fermenters due to their ability to degrade lignocellulosic materials via extracellular enzymes, while simultaneously producing β -glucans such as lentinan—known for their immunostimulatory properties (Roszczyk et al., 2022; Hsiao et al., 2022). Previous research has shown that fungal fermentation of plant-based substrates can enhance their digestibility and functional value in poultry diets, thereby improving growth, intestinal health and immune function (Sun et al., 2022; Goliomytis et al., 2025).

In broiler production, gut health is recognized as a critical deter minutesant of nutrient absorption, feed efficiency, and disease resistance. Improved intestinal morphology characterized by increased villus height, reduced crypt depth and higher villus height-to-crypt depth (VH/CD) ratios has been strongly associated with enhanced digestive and absorptive capacity (Wang et al., 2025). Likewise, modulation of the intestinal microbial community, particularly an increase in beneficial lactic acid bacteria (LAB) and reduction of pathogenic bacteria such as *Escherichia coli*, is essential for maintaining immune homeostasis and preventing enteric disturbances (Li et al., 2024). Fermented feed ingredients rich in prebiotic fibers, polysaccharides, and phenolic compounds have been shown to promote the proliferation of beneficial microbes while suppressing harmful bacteria (Borrego-Ruiz et al., 2024).

In addition to gut-related benefits, functional feed ingredients may influence systemic physiological responses, including lipid metabolism. Bioactive compounds derived from fermentation, such as β -glucans and flavonoids, have been reported to reduce serum cholesterol, increase high-density lipoprotein (HDL) and modulate fatty acid deposition in muscle tissues (Ahn et al., 2020). Such improvements elevate the nutritional quality of broiler meat, contributing to healthier lipid profiles with reduced cholesterol and increased omega-3 fatty acid content, which is increasingly valued by consumers (Ghobashy et al., 2023).

Despite the growing interest in agro-industrial by-products as alternative feed resources, scientific evidence on the use of durian waste fermented specifically with *Lentinus edodes* in broiler diets remains limited. Existing studies have largely focused on chemical composition, antioxidant activity, or fermentation characteristics, with relatively few *in vivo* evaluations in poultry (Sknepnek et al., 2021; Abuajamieh et al., 2025). Moreover, available feeding trials generally assess isolated outcomes (e.g., growth performance or blood lipids) and rarely integrate intestinal morphology, gut microbial populations, immune organ development, and meat lipid quality within a single experimental framework. As a result, the safe inclusion

threshold, as well as the functional links between gut health, immune responses, and lipid metabolism, remain poorly defined for DzWF in broiler production systems.

Unlike prior studies that typically evaluate fermented plant by-products in a limited set of outcomes, this work tests *Lentinus edodes*–fermented durian waste flour (DzWF) as a functional ingredient in broiler diets across graded inclusion levels up to 20% while simultaneously assessing an integrated response profile—growth performance, carcass traits, immune organ indices, small-intestinal morphology, ileal microbial populations, systemic lipid profile, and meat cholesterol/fatty acid composition. This combined design allows us to link gut structural and microbial changes with lipid-related outcomes, providing a more comprehensive functional evaluation of DzWF as a sustainable feed ingredient.

Therefore, the present study was conducted to evaluate the effects of dietary *Durio zibethinus* waste fermented with *Lentinus edodes* on growth performance, carcass traits, immune organ indices, intestinal morphology, microbial populations, blood lipid profile, and meat cholesterol and fatty acid composition in broilers. It was hypothesized that DzWF, through its improved nutrient profile and bioactive properties, would enhance gastrointestinal structure and function, modulate lipid metabolism, and positively influence overall health without impairing growth performance.

MATERIALS & METHODS

Study Design and Ethics

This study was conducted from June to December 2025 at the Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia. The experiment was conducted using a Completely Randomized Design (CRD) with five dietary treatments and four replications. Each replicate consisted of 10 broilers housed in an individual pen, for a total of 200 two-day-old, unsexed Lohmann MB 202 broiler chickens (5 treatments \times 4 replicates \times 10 broilers). The dietary treatments were formulated by supplementing *Durio zibethinus* waste fermented by *Lentinus edodes* (DzWF) at different inclusion levels in the diet: 0% (DZWF0), 5% (DZWF5), 10% (DZWF10), 15% (DZWF15), and 20% (DZWF20) of the total ration. The trial was carried out in an open-sided broiler house equipped with separate floor pens (150 \times 150 \times 100cm) for each replicate. Each pen was equipped with a hanging feeder, a drinker, and a 60-watt incandescent bulb that served as both a heat source and a light. Before placement, the broiler house and equipment were cleaned, disinfected, and preheated to achieve a suitable brooding temperature. During the first week, broilers were reared under continuous light, and from day 8 onward, a lighting schedule of 23 h light and 1 h darkness per day was implemented using fluorescent bulbs. Feed and water were provided *ad libitum* throughout the 35-day experimental period, which was divided into a starter phase (1–21 days) and a finisher phase (22–35 days). Broilers were vaccinated against Newcastle disease and infectious bronchitis with commercial live vaccines by spray on days 2 and 14 of age, following the manufacturer's

recommendations. Health status, behavior, and mortality of broilers were monitored daily, and any abnormal signs were recorded. All procedures involving animals complied with the institutional guidelines for the care and use of experimental animals. The experimental protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No. 362/UN.16.2/KEPFK/2025).

Fermentation of *Durio zibethinus* Waste with *Lentinus Edodes*

Durio zibethinus waste consisted of a mixture of 40% durian fruit peel and 40% durian seeds, combined with 20% fresh tofu pulp as a nitrogen- and energy-rich co-substrate. The fresh substrate mixture was adjusted to approximately 65% moisture and placed into heat-resistant plastic containers. The mixture was sterilized in an autoclave at 121°C for 15 minutes and cooled to room temperature. The sterilized substrate was inoculated with 8% *Lentinus edodes* inoculum (w/w), thoroughly mixed to ensure homogeneous distribution, and incubated for 10 days under solid-state fermentation conditions. At the end of fermentation, the product was dried in a forced-air oven at 50°C until constant weight, then ground to pass through a fine-mesh sieve to obtain fermented durian waste flour (DzWF). Previous analyses showed that fermentation increased crude protein, reduced crude fiber, and improved a minuteso acid profiles compared with the unfermented substrate (Zhao et al., 2021).

Dietary Treatments

The 35-day experimental period was chosen to encompass both the starter (1–21 days) and finisher (22–35 days) phases of broiler production, during which

intestinal development, immune organ maturation, and lipid deposition are most dynamic. Evaluating responses at market age enables assessment of the practical relevance of DzWF supplementation on growth performance, gut health, and meat quality traits under standard broiler production conditions.

For each phase, five diets were prepared with DzWF inclusion levels of 0% (DzWF0), 5% (DzWF5), 10% (DzWF10), 15% (DzWF15), and 20% (DzWF20). The graded inclusion levels of DzWF were selected to evaluate both nutritional safety and functional responses across a wide range of dietary levels. Lower inclusion levels (5–10%) reflect typical substitution rates of alternative feed ingredients in broiler diets, whereas higher levels (15–20%) were used to determine the upper practical utilization threshold without compromising growth performance. The stepwise design allows assessment of potential dose-dependent effects on gut morphology, microbial populations, and lipid metabolism, as reported for other fermented plant-based feed ingredients in poultry nutrition. Maize and soybean meal (SBM) were the main conventional ingredients and were progressively replaced by DzWF in the experimental diets.

Tables 1 and 2 present the ingredient composition and calculated nutrient contents of the starter and finisher diets. All diets were formulated to be isoenergetic and isonitrogenous, in accordance with Lohmann MB 202 recommendations and PT Japfa Comfeed Indonesia standards. The nutrient profile included crude protein, metabolizable energy, crude fat, crude fiber, calcium, available phosphorus, and key amino acids (lysine, methionine, glutamate), as well as functional compounds such as lentinan, linoleic acid, flavonoids, and phenolic compounds contributed by DzWF.

Table 1: Ingredient composition of the experimental diets containing different levels of DzWF

Feed Ingredient	Treatment (%)									
	Starter Period					Finisher Period				
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20
Maize	62.00	57.85	53.35	44.85	44.25	65.00	60.60	54.90	49.58	43.75
SBM	18.90	18.00	17.00	16.00	15.00	14.50	13.50	12.50	11.90	11.20
Fish Meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
F. Product	0.00	5.00	10.00	15.00	20.00	0.00	5.00	10.00	15.00	20.00
Tapioca M	1.00	1.00	1.00	1.00	1.00	1.50	1.50	2.65	3.00	4.00
Bone Meal	1.00	0.70	0.70	0.80	0.80	0.80	0.80	0.90	0.90	1.00
Coconut Oil	1.40	1.75	2.25	2.75	3.25	2.50	2.90	3.15	3.90	4.30
PreMix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.22	0.25
Total	100	100	100	100	100	100	100	100	100	100

Table 2: Nutrient contents of the experimental diets containing different levels of DzWF

Nutrient Content	Treatment									
	Starter Period					Finisher Period				
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20
Protein (%)	22.06	22.11	22.08	22.04	22.00	20.07	20.05	20.02	20.02	20.04
Fat (%)	3.96	4.34	4.86	5.38	5.90	5.03	5.46	5.71	6.47	6.87
Crude Fiber (%)	3.61	4.09	4.57	5.04	5.51	3.51	3.99	4.43	4.88	5.32
Calcium (%)	1.11	1.02	1.01	1.02	1.02	1.04	1.04	1.04	1.04	1.03
A.Phosphor (%)	0.61	0.57	0.57	0.59	0.55	0.57	0.57	0.59	0.59	0.60
Lisine (%)	1.37	1.34	1.30	1.27	1.34	1.26	1.22	1.19	1.17	1.14
Methionine (%)	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14
Glutamate (%)	4.04	3.93	3.79	3.66	3.93	3.75	3.62	3.48	3.34	3.21
Lentinan (%)	0.33	0.34	0.34	0.35	0.35	0.33	0.34	0.34	0.35	0.35
Linoleate (%)	2.79	2.81	2.83	2.84	2.71	2.84	2.86	2.84	2.85	2.83
ME (kcal/kg)	3006	3009	3011	3010	3012	3118	3115	3101	3114	3105

Growth Performance and Carcass Traits

Body weight (BW) and feed intake (FI) were recorded at the beginning of the trial and at 21 and 35 days of age for each replicate pen. Feed intake was calculated as the difference between the amount offered and the amount refusals. Average Daily Feed Intake (ADFI), Average Daily Weight Gain (ADWG), and Feed Conversion Ratio (FCR) were calculated for the starter (1–21d), finisher (22–35d), and entire experimental period (1–35d). At 35 days of age, after a 12-hour fasting period with free access to water, all broilers in each replicate were individually weighed to determine final BW. Four broilers per replicate, with BW close to the pen mean, were selected for carcass evaluation. Broilers were humanely slaughtered, bled, defeathered, and eviscerated. Carcass weight was recorded, and carcass yield was calculated as carcass weight divided by live BW \times 100%. Abdominal fat and selected non-carcass organs (heart and gizzard) were removed and weighed. Carcass percentage, abdominal fat percentage, and non-carcass organ percentage were expressed relative to live BW (Danladi et al., 2022).

Immune Organ Indices

Immune organs, including the bursa of Fabricius, spleen, and thymus, were carefully excised from the selected broilers, blotted dry, and weighed individually. Immune organ indices were calculated as organ weight divided by live BW \times 100%. These indices were used as indirect indicators of immune organ development and immune status in broilers, rather than direct measures of immune function (Mauludin et al., 2025).

Intestinal Morphology

On day 35, tissue samples from the duodenum, jejunum, and ileum were collected from one representative broiler per replicate. Approximately 2–3cm segments from each intestinal region were gently flushed with physiological saline to remove digesta and then fixed in 10% neutral-buffered formalin. Fixed tissues were dehydrated, embedded in paraffin and sectioned at 2 μ m. Sections were mounted on glass slides and stained with hematoxylin and eosin. Histological measurements were performed using a light microscope equipped with an image analysis system

(Fig. 1). Villus height (VH) was measured from the tip of the villus to the villus–crypt junction, while crypt depth (CD) was measured from the villus–crypt junction to the base of the crypt. The villus height-to-crypt depth ratio (VH/CD) was calculated for each intestinal segment. For each broiler and intestinal segment, several well-oriented villi and crypts were measured and averaged to obtain representative values (Li et al., 2022).

Intestinal Microbial Population

For microbial enumeration, intestinal digesta from the same intestinal segments (with emphasis on the ileum) were collected into sterile containers on day 35. Samples were transported on ice to the microbiology laboratory and processed immediately. Serial dilutions of digesta were prepared in sterile saline. *Escherichia coli* was enumerated by plating on MacConkey agar and incubating aerobically at 37–38°C for 2h, whereas lactic acid bacteria (LAB) were enumerated on de Man, Rogosa, and Sharpe (MRS) agar under anaerobic conditions at 37–38°C for 48h. Colony-forming units (CFU) were counted and expressed as log₁₀ CFU per gram of digesta (Hwang et al., 2024).

Blood Lipid Profile

At 35 days of age, blood samples were collected from the carotid artery or jugular vein of fasted broilers (four broilers per replicate). Approximately 2–3mL of blood was drawn into tubes without anticoagulant and allowed to clot at room temperature for about 30 minutes. The samples were then centrifuged at 4,000 rpm for 20 minutes to separate serum. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were determined using commercial DiaSys kits in a photometric system. Kit catalogue for serum total cholesterol (REF 1 1300 30 83 030), for HDL (REF 1 33521 30 83 030), for LDL (REF 1 4131 30 83 030) and for triglycerides (REF 1 4131 30 83 030), all manufactured by Diasys Diagnostic Systems GmbH, Holzheim (Germany). Cholesterol was measured using the CHOD-PAP method and triglycerides using the GPO-PAP method, following the manufacturer's instructions. Absorbance was read at 546nm using a MicroLab 300 analyzer (Deba et al., 2024; Liu et al., 2024).

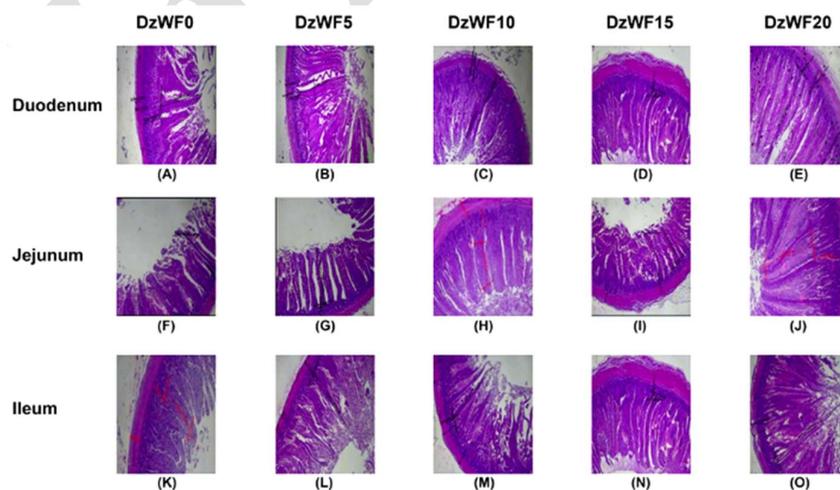


Fig. 1: Representative histological sections of the small intestine (H&E staining). Duodenum (A–E), jejunum (F–J), and ileum (K–O) from animals treated with DzWF at doses of 0, 5, 10, 15, and 20. Images were captured at the same magnification to illustrate overall intestinal architecture and villus morphology.

Meat Cholesterol, Fat Content, and Fatty Acid Composition

Thigh meat samples were collected from slaughtered broilers at 35 days of age. Samples were trimmed of visible fat and skin, then dried and ground to a fine powder. Meat cholesterol content was determined using a SpectraLab 752 Pro according to established procedures. Meat fat content was analyzed using the Soxhlet extraction method. For fatty acid analysis, lipids were converted to fatty acid methyl esters (FAMES) using boron trifluoride-methanol reagent. FAMES were analyzed by gas chromatography with a flame-ionization detector (GC-FID) (Zhou et al., 2023; Ng et al., 2024). Individual fatty acids, including omega-3, omega-6, and omega-9, were identified and quantified based on retention times and comparison with known standards. Results were expressed as a percentage of total fatty acids in thigh meat.

Statistical Analyses

The sample size and experimental design (five dietary treatments with four replicates of ten birds each) were based on previous broiler feeding trials with similar designs evaluating alternative and fermented feed ingredients. This replication level has been shown to provide sufficient sensitivity to detect biologically relevant differences in growth performance, intestinal morphology, and metabolic parameters under controlled experimental conditions.

All collected data were analyzed using analysis of variance (ANOVA) appropriate for a Completely Randomized Design (CRD), with dietary DzWF level as the main effect. When significant treatment effects were detected ($P < 0.05$), mean differences among treatments were separated using suitable post-hoc tests (e.g., Tukey's honestly significant difference). Statistical analyses were performed using IBM SPSS Statistics (version 22.0; IBM Corp., Armonk, NY, USA). Results are presented as means \pm SD, and significance was declared at $P < 0.05$.

RESULTS

Growth Performance

Dietary inclusion of DzWF at levels up to 20% did not significantly influence growth performance during the starter, finisher, or overall experimental periods ($P > 0.05$; Table 3). Throughout the 1–35-day period, average daily feed intake was comparable across treatments, ranging from 86.22 to 87.44g/day, while average daily weight gain

was 55.89–56.75g/day. Feed conversion ratio also remained stable (1.52–1.56), indicating that partial replacement of maize and soybean meal with DzWF maintained feed utilization efficiency under the conditions of this study.

Carcass Traits

Dietary DzWF supplementation did not significantly affect carcass yield, abdominal fat deposition, or relative weights of physiological organs ($P > 0.05$; Table 4). Carcass percentage ranged from 70.80% to 73.98%, and abdominal fat remained low across treatments (1.11–1.31% of live body weight). Similarly, the relative weights of the gizzard (1.24–1.31%), heart (0.43–0.45%) and liver (1.91–2.09%) were comparable across dietary groups, suggesting that inclusion of up to 20% DzWF did not alter carcass composition or organ development.

Immune Organ Indices

Immune organ indices of broilers fed diets containing *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) are presented in Fig. 2. Bursa Fabricius percentage was not significantly affected by dietary treatments ($P = 0.201$), with mean values of 0.31 ± 0.03 , 0.41 ± 0.03 , 0.39 ± 0.09 , 0.43 ± 0.12 , and 0.41 ± 0.04 for DzWF0, DzWF5, DzWF10, DzWF15, and DzWF20, respectively. Spleen index also did not differ among treatments ($P = 0.169$), ranging from 0.135 ± 0.006 (DzWF5) to 0.178 ± 0.015 (DzWF20), with intermediate values of 0.143 ± 0.019 (DzWF0), 0.150 ± 0.034 (DzWF10), and 0.150 ± 0.032 (DzWF15). In contrast, thymus percentage was significantly influenced by DzWF inclusion level ($P < 0.001$). Thymus indices increased from 0.073 ± 0.003 in DzWF0 to 0.102 ± 0.013 , 0.117 ± 0.002 , 0.130 ± 0.007 , and 0.148 ± 0.030 in DzWF5, DzWF10, DzWF15, and DzWF20, respectively. Tukey's post hoc test showed that DzWF0 had significantly lower thymus values than DzWF10, DzWF15, and DzWF20, while DzWF5 was intermediate and did not differ from either the control or the higher inclusion levels. Overall, dietary DzWF supplementation at up to 20% selectively increased the thymus index without altering the Bursa Fabricius or spleen indices in broiler chickens.

Intestinal Morphology

The effects of dietary DzWF supplementation on intestinal morphology are presented in Tables 5–7. In the duodenum, increasing levels of DzWF significantly improved villus height and the villus height – to – crypt

Table 3: Growth performance of broilers fed diets containing DzWF

Parameter	Treatment					P-Value
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	
Starter						
ADFI (g/day)	59.07 \pm 1.50	59.69 \pm 1.93	59.10 \pm 1.59	59.98 \pm 1.22	60.02 \pm 1.65	0.638
ADWG (g/day)	41.61 \pm 1.02	41.36 \pm 1.49	41.63 \pm 1.34	41.13 \pm 1.81	41.17 \pm 1.38	0.363
FCR	1.42 \pm 0.02	1.44 \pm 0.01	1.42 \pm 0.02	1.46 \pm 0.03	1.46 \pm 0.08	0.453
Finisher						
ADFI (g/day)	113.52 \pm 1.65	113.13 \pm 1.35	114.50 \pm 1.67	114.89 \pm 1.25	114.14 \pm 1.13	0.703
ADWG (g/day)	70.48 \pm 2.65	70.10 \pm 2.38	70.14 \pm 1.34	71.21 \pm 2.29	71.16 \pm 2.35	0.890
FCR	1.61 \pm 0.01	1.61 \pm 0.02	1.63 \pm 0.05	1.61 \pm 0.02	1.60 \pm 0.07	0.591
Total						
ADFI (g/day)	86.22 \pm 1.25	86.41 \pm 1.23	86.81 \pm 1.60	87.44 \pm 1.04	86.78 \pm 1.24	0.692
ADWG (g/day)	56.05 \pm 1.62	56.75 \pm 1.45	55.89 \pm 1.55	55.92 \pm 1.67	56.16 \pm 1.56	0.457
FCR	1.54 \pm 0.01	1.52 \pm 0.05	1.55 \pm 0.03	1.56 \pm 0.01	1.55 \pm 0.02	0.567

Note: Values are expressed as mean \pm SD (n=4 replicates per treatment). ADFI=average daily feed intake; ADWG=average daily weight gain; FCR=feed conversion ratio. Differences were considered significant at $P < 0.05$.

Table 4: Carcass traits and physiological organ weights of broilers fed diets containing DzWF

Parameter	Treatment					P-Value
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	
Carcass (%)	73.09 ± 2.91	70.80 ± 1.77	71.51 ± 2.55	72.12 ± 2.98	73.98 ± 2.08	0.432
Abdominal fat (%)	1.28 ± 0.12	1.11 ± 0.20	1.23 ± 0.36	1.31 ± 0.15	1.31 ± 0.15	0.648
Gizzard (%)	1.24 ± 0.07	1.30 ± 0.09	1.29 ± 0.07	1.31 ± 0.09	1.29 ± 0.06	0.707
Heart (%)	0.43 ± 0.03	0.45 ± 0.00	0.44 ± 0.01	0.45 ± 0.02	0.45 ± 0.01	0.232
Liver (%)	2.09 ± 0.20	1.99 ± 0.08	2.05 ± 0.30	1.94 ± 0.08	1.91 ± 0.09	0.593

Table 5: Duodenal morphology of broilers fed diets containing DzWF

Parameter	Treatment					P-value
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	
Villus height (µm)	930.71 ± 40.08 ^a	1154.09 ± 109.68 ^b	1400.93 ± 52.15 ^c	1511.05 ± 31.03 ^c	1526.59 ± 23.79 ^c	0.001
Crypt depth (µm)	335.77 ± 9.86 ^c	269.36 ± 47.08 ^b	188.67 ± 6.88 ^a	154.65 ± 3.81 ^a	141.89 ± 6.00 ^a	0.001
VH/CD ratio	2.77 ± 0.14 ^a	4.38 ± 0.88 ^b	7.43 ± 0.43 ^c	9.77 ± 0.24 ^d	10.77 ± 0.30 ^d	0.001

Note: Duodenal morphology of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) at 35 days of age. Villus height (VH) and crypt depth (CD) are expressed in micrometers (µm), and the villus height-to-crypt depth ratio (VH/CD) was calculated as an indicator of intestinal absorptive capacity. Values are presented as mean ± SD (n=4 replicates per treatment). Different superscript letters within a row indicate significant differences (P<0.05).

Table 6: Jejunal morphology of broilers fed diets containing DzWF

Parameter	Treatment					P-value
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	
Villus height (µm)	663.94 ± 35.98 ^a	897.23 ± 70.71 ^b	928.01 ± 47.04 ^b	1095.87 ± 11.49 ^c	1100.52 ± 15.27 ^c	0.001
Crypt depth (µm)	160.75 ± 4.90 ^d	131.11 ± 3.93 ^c	123.17 ± 3.39 ^b	120.11 ± 4.16 ^b	108.16 ± 1.98 ^a	0.001
VH/CD ratio	4.13 ± 0.26 ^a	6.85 ± 0.59 ^b	7.54 ± 0.50 ^b	9.13 ± 0.30 ^c	10.18 ± 0.25 ^d	0.001

Jejunal morphology of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) at 35 days of age. Villus height (VH) and crypt depth (CD) are expressed in micrometers (µm), and the villus height-to-crypt depth ratio (VH/CD) was calculated as an indicator of intestinal absorptive capacity. Values are presented as mean ± SD (n=4 replicates per treatment). Different superscript letters within a row indicate significant differences (P<0.05).

Table 7: Ileal morphology of broilers fed diets containing DzWF

Parameter	Treatment					P-value
	DzWF0	DzWF5	DzWF10	DzWF15	DzWF20	
Villus height (µm)	678.12 ± 7.40 ^a	899.21 ± 7.40 ^b	973.72 ± 43.81 ^c	1057.90 ± 26.51 ^d	1088.41 ± 47.81 ^d	0.001
Crypt depth (µm)	173.86 ± 6.58 ^c	157.81 ± 7.60 ^b	151.02 ± 9.68 ^b	140.92 ± 2.83 ^a	129.11 ± 5.12 ^a	0.001
VH/CD ratio	3.91 ± 0.17 ^a	5.71 ± 0.27 ^b	6.46 ± 0.41 ^c	7.51 ± 0.33 ^d	8.44 ± 0.43 ^d	0.001

Ileal morphology of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) at 35 days of age. Villus height (VH) and crypt depth (CD) are expressed in micrometers (µm), and the villus height-to-crypt depth ratio (VH/CD) was calculated as an indicator of intestinal absorptive capacity. Values are presented as mean ± SD (n=4 replicates per treatment). Different superscript letters within a row indicate significant differences (P<0.05).

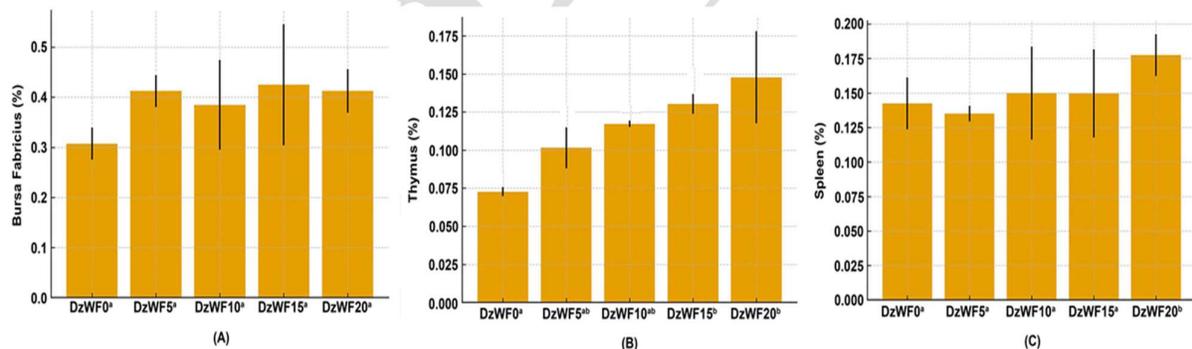


Fig. 2: Immune organ indices of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) for 35 days. (A) Bursa of Fabricius, (B) thymus, and (C) spleen, expressed as a percentage of live body weight. Values are presented as mean ± SD (n=4 replicates per treatment). Different superscript letters indicate significant differences among treatments (P<0.05).

depth (VH/CD) ratio, while crypt depth progressively decreased across treatments (P<0.001). All DzWF-supplemented groups (DzWF5–DzWF20) exhibited markedly better duodenal morphology compared with the control (DzWF0), with the greatest improvements observed at higher inclusion levels.

A similar response was observed in the jejunum. Villus height and VH/CD ratio increased significantly with higher DzWF inclusion, whereas crypt depth decreased consistently among treatments (P<0.001). Broilers receiving DzWF displayed a more developed jejunal mucosa than the control

group, with morphological enhancement becoming more pronounced at DzWF15 and DzWF20. In the ileum, DzWF supplementation also resulted in significant improvements (P<0.001), characterized by increased villus height and VH/CD ratio along with reduced crypt depth. The pattern mirrored that of the duodenum and jejunum, indicating a consistent positive effect of DzWF on the entire small intestinal mucosa. Overall, supplementation with DzWF up to 20% consistently enhanced intestinal morphology in broilers across all segments evaluated, as indicated by taller villi, shallower crypts, and greater VH/CD ratios.

Intestinal Microbial Population

The effects of dietary DzWF supplementation on intestinal microbial populations are summarized in Fig. 3. A significant reduction in *Escherichia coli* counts was observed across treatments ($P < 0.001$). Broilers in DzWF0, DzWF5, and DzWF10 showed similar *E. coli* populations, with mean values of 7.44 ± 0.02 , 7.34 ± 0.02 , and $7.33 \pm 0.02 \log_{10}$ CFU/g, respectively, whereas a marked decline occurred at higher DzWF inclusion levels, dropping to $7.10 \pm 0.55 \log_{10}$ CFU/g in DzWF15 and $6.23 \pm 0.06 \log_{10}$ CFU/g in DzWF20. This pattern indicates that DzWF inhibits coliform proliferation at higher inclusion levels. Conversely, lactic acid bacteria (LAB) populations increased significantly with the inclusion of DzWF in the diet ($P < 0.001$). LAB counts rose from $6.18 \pm 0.06 \log_{10}$ CFU/g in DzWF0 to $6.88 \pm 0.51 \log_{10}$ CFU/g in DzWF5 and $7.17 \pm 0.09 \log_{10}$ CFU/g in DzWF10, and further increased to $7.37 \pm 0.06 \log_{10}$ CFU/g in DzWF15 and $8.17 \pm 0.06 \log_{10}$ CFU/g in DzWF20. This progressive rise demonstrates a strong positive response of LAB to DzWF supplementation. Overall, DzWF supplementation clearly modulated the intestinal microbiota, decreasing *E. coli* abundance while promoting LAB proliferation, particularly at inclusion levels of 15% and 20%, thereby shifting the intestinal environment toward a more favorable microbial profile.

Blood Lipid Profile

Dietary supplementation with DzWF exerted a measurable effect on the blood lipid profile of broilers (Fig. 4). The HDL-to-total cholesterol ratio increased significantly across treatments ($P < 0.05$). Broilers in the control group (DzWF0) showed an HDL percentage of $34.54 \pm 4.25\%$, while HDL% gradually increased in DzWF5 ($38.67 \pm 3.67\%$) and DzWF10 ($39.40 \pm 1.13\%$), and reached markedly higher values in DzWF15 ($44.11 \pm 2.67\%$) and

DzWF20 ($51.61 \pm 5.22\%$). These results indicate a clear dose-responsive enhancement in HDL% with increasing DzWF inclusion.

In contrast, LDL percentage did not differ significantly among treatments ($P > 0.05$). However, numerical reductions were evident with increasing DzWF inclusion, with LDL% decreasing from $73.52 \pm 13.91\%$ in DzWF0 to $65.61 \pm 8.18\%$ in DzWF5, $61.21 \pm 9.42\%$ in DzWF10, $63.95 \pm 9.62\%$ in DzWF15, and $60.83 \pm 11.43\%$ in DzWF20. Although not statistically significant, this pattern suggests a biologically relevant downward trend in LDL%. Triglyceride percentage showed a similar decline ($P = 0.052$). The DzWF0 and DzWF5 groups showed comparable TG% values ($23.34 \pm 1.32\%$ and $23.40 \pm 4.49\%$, respectively), while DzWF10 and DzWF15 demonstrated moderately lower percentages ($21.89 \pm 3.81\%$ and $21.31 \pm 3.47\%$, respectively). The lowest TG% was observed in DzWF20 ($16.60 \pm 1.72\%$), suggesting a potential hypolipidemic response at higher DzWF inclusion levels. Overall, DzWF supplementation shifted the broiler lipid profile toward a more favorable composition, characterized by a significant increase in HDL% and a concurrent numerical decrease in LDL% and triglycerides, with the most pronounced improvements at the 20% inclusion level.

Meat Cholesterol and Fatty Acid Composition

Dietary supplementation with DzWF affected several parameters of meat lipid composition (Table 8). Meat cholesterol concentration differed significantly among treatments ($P = 0.020$). The highest cholesterol level was observed in broilers fed the control diet (DzWF0), whereas dietary inclusion of DzWF resulted in progressively lower values, reaching the lowest level in DzWF20. Although intermediate inclusion levels (DzWF5–DzWF15) did not differ significantly from either the control or the highest inclusion level, the overall pattern reflected a decreasing

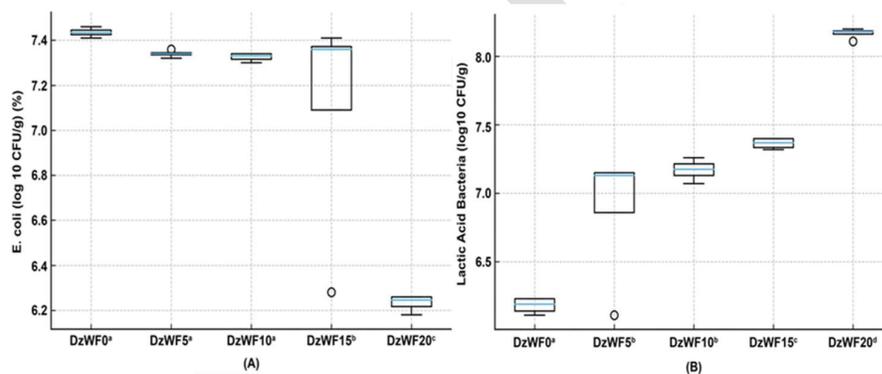


Fig. 3: Ileal microbial populations of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) at 35 days of age. A) *Escherichia coli* and B) lactic acid bacteria (LAB) counts, expressed as \log_{10} CFU/g of digesta. Values are presented as mean \pm SD ($n = 4$ replicates per treatment). Different superscript letters indicate significant differences among treatments ($P < 0.05$).

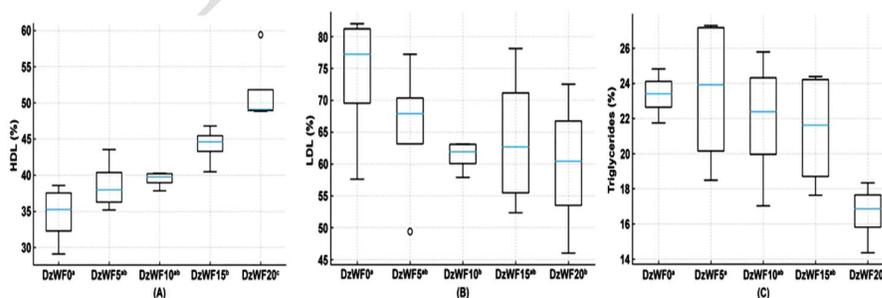


Fig. 4: Blood lipid profile of broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) for 35 days. A) High-density lipoprotein (HDL), B) low-density lipoprotein (LDL), and C) triglycerides, expressed as a percentage of total cholesterol. Values are presented as mean \pm SD ($n = 4$ replicates per treatment). Different superscript letters indicate significant differences among treatments ($P < 0.05$).

Table 8: Meat cholesterol, intramuscular fat, and fatty acid composition of broiler thigh meat

Treatment	Parameter				
	Meat Cholesterol (mg/100 g)	Meat Fat (%)	Omega-3 (%)	Omega-6 (%)	Omega-9 (%)
DzWF0	101.73 ± 8.67 ^a	6.94 ± 0.58 ^a	0.722 ± 0.009 ^a	16.18 ± 0.74 ^a	39.56 ± 0.81 ^a
DzWF5	99.68 ± 3.95 ^{ab}	7.82 ± 0.81 ^{ab}	0.733 ± 0.009 ^{ab}	16.35 ± 0.32 ^a	39.75 ± 0.53 ^{ab}
DzWF10	93.70 ± 1.75 ^{ab}	8.20 ± 0.48 ^b	0.743 ± 0.012 ^b	16.39 ± 0.41 ^a	40.04 ± 0.63 ^{ab}
DzWF15	90.45 ± 4.99 ^{ab}	7.64 ± 0.18 ^{ab}	0.748 ± 0.010 ^b	16.35 ± 0.42 ^a	40.18 ± 0.60 ^{ab}
DzWF20	90.10 ± 4.58 ^a	8.12 ± 1.23 ^b	0.813 ± 0.010 ^c	17.65 ± 1.02 ^b	40.64 ± 0.82 ^b
P-value	0.020	0.173	5.24 × 10 ⁻⁸	0.031	0.264

Meat cholesterol content, intramuscular fat, and fatty acid composition of thigh meat from broiler chickens fed diets containing graded levels of *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) at 35 days of age. Fatty acid values are expressed as percentage of total identified fatty acids. Values are presented as mean ± SD (n=4 replicates per treatment). Different superscript letters within a column indicate significant differences (P<0.05).

trend in meat cholesterol as DzWF supplementation increased. Intramuscular fat content did not differ significantly among treatments (P=0.173). Meat fat percentages remained relatively comparable across all groups, indicating that DzWF inclusion did not adversely affect the deposition of intramuscular fat in broiler thigh meat.

In contrast, dietary DzWF exerted a pronounced effect on omega-3 fatty acid deposition (P<0.001). Omega-3 levels increased steadily from DzWF0 through DzWF20, with DzWF20 exhibiting the highest concentration among all treatments. This enhancement in omega-3 enrichment demonstrates a dose-dependent response to DzWF supplementation. Omega-6 fatty acid concentration also differed significantly among treatments (P=0.031), with DzWF20 showing noticeably higher values than the other groups. Treatments DzWF0–DzWF15 remained statistically similar, indicating that a marked increase occurred only at the highest DzWF inclusion level. Omega-9 fatty acid concentration did not differ significantly across treatments (P=0.264), although a slight numerical increase was observed from DzWF0 to DzWF20. Taken together, these results show that DzWF supplementation predominantly affected cholesterol content and fatty acid composition by lowering meat cholesterol while enhancing omega-3 and, to a lesser extent, omega-6 enrichment in broiler meat.

DISCUSSION

The present study demonstrates that *Durio zibethinus* waste fermented with *Lentinus edodes* can be incorporated into broiler diets at inclusion levels up to 20% without compromising growth performance or carcass yield. The absence of significant differences in ADFI, ADWG, FCR, carcass yield, and organ weights indicates that DzWF is nutritionally adequate when diets are formulated to be isoenergetic and isonitrogenous. These findings are consistent with previous reports showing that fermented plant-based by-products can partially replace conventional feed ingredients without adverse effects on performance (Li et al., 2022; Konkol et al., 2023; Tretola et al., 2024). From a practical perspective, maintaining growth and carcass traits at high inclusion levels underscores DzWF as a sustainable and economically viable feed alternative that contributes to the valorization of agro-industrial waste.

The absence of significant differences in ADFI, ADWG, and FCR across all phases and treatments (Table 3) indicates that DzWF is nutritionally adequate and does not exert antinutritional or depressant effects on feed intake and growth. All diets were formulated to be isoenergetic and

isonitrogenous, which explains the comparable performance across treatments and suggests that replacing maize and soybean meal with DzWF did not impair the supply of metabolizable energy or essential amino acids. Similar results have been reported when fermented plant by-products were incorporated into broiler diets at moderate to high inclusion levels, where growth performance was maintained due to improved digestibility and reduced crude fiber content after fermentation (Li et al., 2022; Konkol et al., 2023).

Carcass yield and relative weights of abdominal fat, gizzard, heart, and liver were likewise unaffected by DzWF supplementation (Table 4), indicating that nutrient partitioning and organ development were not adversely influenced. The lack of change in abdominal fat percentage is consistent with the absence of major differences in energy intake among treatments and suggests that DzWF did not lead to excessive fat deposition or energy deficit (Kolnes et al., 2021). Comparable carcass responses have been observed in broilers fed diets containing fermented fruit or seed by-products, where carcass traits were largely preserved despite partial replacement of conventional ingredients (Tretola et al., 2024). From a practical standpoint, the maintenance of performance and carcass yield at DzWF levels up to 20% indicates that this ingredient can be used as a sustainable and economical feed alternative, contributing to the valorization of durian waste and the reduction of environmental burden associated with its disposal (Peng et al., 2022; Sha minutes-Shazwana et al., 2022).

A key finding of this study is the selective effect of DzWF on immune organ indices. While the bursa of Fabricius and spleen indices remained unchanged, the thymus index increased significantly and dose-dependently with DzWF inclusion, particularly at 15–20% of the diet. The thymus is the primary site of T-lymphocyte maturation; therefore, an increase in thymus relative weight is often interpreted as an indirect indicator of enhanced T-cell-related immune development, rather than direct evidence of immune function (Asadova, 2021).

The observed thymic response is likely linked to the presence of bioactive components in DzWF, notably lentinan (a β-glucan from *L. edodes*) and other fungal polysaccharides enriched during fermentation. β-glucans from mushrooms are well documented as immunomodulators that stimulate T-cell proliferation, macrophage activity and cytokine production (Kumar et al., 2024; Rutckeviski et al., 2024). In addition, phenolic compounds and flavonoids detected in DzWF may exert

antioxidant and immunoprotective effects by reducing oxidative stress in lymphoid tissues (Bar-Dagan et al., 2023; Khaksar et al., 2024). The lack of effect on bursa and spleen indices suggests that DzWF acts more strongly on the cellular arm of the immune system rather than on humoral immunity, at least under the conditions of this experiment. However, the enhanced thymus index, in combination with improved intestinal health and microbiota, may indicate a more favorable immune-related condition, particularly in relation to thymus development and gut health.

The most pronounced effects of DzWF were observed on intestinal morphology. Across the duodenum, jejunum, and ileum, DzWF supplementation linearly increased villus height and VH/CD ratio while decreasing crypt depth (Table 5–7). These changes are indicative of improved intestinal integrity and absorptive capacity. Taller villi increase the surface area for nutrient absorption, whereas shallower crypts are associated with lower epithelial turnover and reduced energy expenditure for mucosal renewal (Sundaram & Borthakur, 2021; Zhu et al., 2023).

These morphological improvements are likely associated with enhanced nutrient availability and the presence of prebiotic polysaccharides and antioxidant compounds generated during fermentation, which collectively support enterocyte proliferation and intestinal integrity (Jiang et al., 2021; Gois et al., 2023). The consistency of the morphological response along all three segments of the small intestine indicates a systemic effect rather than a localized change, suggesting that DzWF exerts a global trophic effect on intestinal mucosa. Such improvements in gut structure are commonly associated with increased absorptive capacity and may support more efficient nutrient utilization, even when performance parameters do not differ statistically, and may provide a physiological buffer under environmental or pathogenic challenges.

DzWF supplementation markedly improved the microbial balance in the gut, as evidenced by reduced *E. coli* counts and increased LAB populations, particularly at 15–20% inclusion levels (Fig. 3). A reduction in coliforms, including *E. coli*, coupled with an increase in LAB, is recognized as a shift toward a more favorable microbiota that is generally considered indicative of a more favorable gut microbial balance, which may support intestinal health (Li et al., 2024). This microbial modulation may be attributed to the combined effects of fermentable substrates, prebiotic polysaccharides, antimicrobial phytochemicals, and improved intestinal morphology that collectively create a more stable gut environment (Liang et al., 2024; Song et al., 2023; do Nascimento & da Costa, 2025). The combination of prebiotic effects, antimicrobial phytochemicals, and improved mucosal health likely explains the strong dose-dependent enhancement of LAB and the reduction of *E. coli* counts in this study.

DzWF supplementation was associated with favorable changes in lipid-related indicators, including increased HDL proportion and numerical reductions in LDL and triglycerides (Fig. 4). An elevated HDL fraction is generally associated with enhanced reverse cholesterol transport and reduced cardiovascular risk in humans and is considered a favorable biomarker of lipid metabolism in animals (Pownall

et al., 2021). Several mechanisms may underlie these changes. Mushroom-derived β -glucans and polysaccharides have been shown to bind bile acids and increase their excretion, thereby stimulating hepatic conversion of cholesterol into bile acids and reducing circulating cholesterol levels (Belobrajdic et al., 2024). Additionally, phenolic compounds and flavonoids may modulate key enzymes involved in lipid synthesis and oxidation, such as HMG-CoA reductase and lipoprotein lipase, thereby lowering LDL and triglyceride concentrations (Balasubramanian & Maideen, 2021). The improved gut microbiota, with higher LAB populations, may also contribute to cholesterol reduction by deconjugating bile salts and co-precipitating cholesterol in the intestinal lumen. Although the reductions in LDL% and triglycerides did not reach statistical significance, the biological trend, particularly at 20% DzWF, suggests that higher inclusion levels can exert clinically relevant hypolipidemic effects. This interpretation is supported by the parallel reduction observed in meat cholesterol.

In addition to its systemic effects, DzWF improved the lipid profile of broiler thigh meat. Meat cholesterol decreased progressively with increasing DzWF inclusion, with the lowest value observed at 20% DzWF (Table 8). This reduction is consistent with the observed increase in HDL% and the numerical decrease in LDL% and triglycerides, suggesting a systemic effect of DzWF on lipid mobilization and deposition. Lower meat cholesterol content is desirable from a consumer health perspective and may enhance the functional value of broiler meat in human diets (Barthwal et al., 2025). Omega-3 fatty acid content in thigh meat increased significantly and dose-dependently, while omega-6 content increased significantly at the highest DzWF level. The enrichment of omega-3 fatty acids may result from multiple factors, including improved protection of unsaturated fatty acids from oxidative degradation due to antioxidant phytochemicals, modulation of hepatic fatty acid synthesis and desaturation, and altered incorporation of fatty acids into muscle tissues. The increase in omega-6 content at 20% DzWF suggests that DzWF may provide precursors or improve the metabolic conversion and deposition of polyunsaturated fatty acids. Interestingly, intramuscular fat content remained statistically unchanged, indicating that DzWF altered the qualitative rather than the quantitative aspects of meat lipids. This is advantageous, as it allows the production of meat with an improved fatty acid profile and reduced cholesterol without compromising juiciness and the sensory characteristics associated with intramuscular fat (Predescu et al., 2024; Derewicka et al., 2025). Taken together, the present findings show that DzWF is a promising functional feed ingredient for broiler chickens. At inclusion levels up to 20%, DzWF maintained growth performance and carcass yield while improving intestinal morphology, enhancing beneficial microbiota, stimulating thymus development, and favorably modulating both blood and meat lipid profiles.

The most pronounced functional benefits, improved VH/CD ratio, enhanced LAB populations, elevated HDL%, and reduced meat cholesterol with higher omega-3 enrichment, were generally observed at inclusion levels of

15–20%, suggesting that these levels may be close to the optimal range for functional outcomes under the conditions of this study. From a sustainability perspective, the use of fermented durian peel and seeds, combined with tofu pulp, represents a circular-economy approach that transforms low-value agro-industrial waste into a high-value, protein- and bioactive-rich feed ingredient (Sha minutes-Shazwana et al., 2022; Wicaksono et al., 2025). Future studies should investigate the effects of DzWF under commercial rearing conditions, its interactions with dietary fat sources, and its potential to replace antibiotic growth promoters through its combined prebiotic, immunomodulatory, and hypolipidemic properties.

Despite the comprehensive scope of the present study, several limitations should be acknowledged. Immune organ indices and intestinal morphology were used as indirect indicators of immune status and gut health, and no direct immunological measurements (such as antibody titers, cytokine expression, or immune challenge tests) were performed. Intestinal microbial populations were assessed using culture-based methods, which provide limited taxonomic resolution compared with molecular approaches such as 16S rRNA sequencing. In addition, the experiment was conducted under controlled experimental conditions, and responses under commercial production systems may differ. Therefore, further studies incorporating molecular immune markers, advanced microbiome analyses, and commercial-scale trials are warranted to confirm and extend the present findings.

Conclusion

In conclusion, *Durio zibethinus* waste fermented with *Lentinus edodes* (DzWF) can be incorporated into broiler diets at up to 20% under controlled experimental conditions without adversely affecting growth performance, feed efficiency, carcass yield, or physiological organ weights. Although productive performance was unchanged, DzWF supplementation induced biologically meaningful functional responses, including improvements in small-intestinal morphology, favorable modulation of ileal microbial populations, selective enhancement of thymus development, and beneficial alterations in blood and meat lipid profiles, particularly increased HDL proportion, reduced meat cholesterol, and enrichment of omega-3 fatty acids. These findings indicate that, under the controlled conditions of this study, DzWF was associated with gut-related and immunometabolic responses rather than direct growth stimulation, supporting its potential use as a functional feed ingredient in broiler nutrition. However, further studies under commercial production conditions and with additional functional endpoints are required to confirm the consistency and broader applicability of these effects.

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Conflict of Interest: The authors declared that there is no conflict of interest.

Data Availability: All data supporting this study's findings are available within the manuscript.

Ethics Statement: All experimental procedures involving animals were conducted in accordance with institutional guidelines for the care and use of experimental animals. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No. 362/UN.16.2/KEPFK/2025). All efforts were made to minimize animal discomfort and to ensure humane handling throughout the experimental period.

Author's Contribution: All authors equally contributed and approved the manuscript.

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