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Fermented Prebiotic-Assisted Enriched Acidifiers on the Growth Performance and Physiological Traits of Broiler Chickens

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Abstract

Banana peel is a readily available agro-industrial material that is a probiotic with potential properties. Although banana peel has prebiotic potential, its high fibre content limits its direct use in poultry diets. Additionally, it is an easily accessible supplementary dietary supplement. In this study, the effects of fermented banana peels produced using *Averrhoa bilimbi* filtrate as a natural organic acid source were evaluated. A total of 280 broiler chickens were randomly assigned to the groups, which included a control diet and diets supplemented with graded amounts of fermented peel powder. All the subjects were then evaluated for their health. Fermentation increased the organic acidity of the material and promoted lactic acid bacteria. The growth performance parameters, intestinal pH level, carcass features, blood composition, and other parameters were measured using standard protocols. Fermented banana peel powder as a dietary supplement significantly improved body weight gain and the feed conversion ratio at optimal inclusion levels ($p < 0.05$). A number of performance traits showed quadratic responses. Improved hematological values within physiological ranges and reduced intestinal pH were observed in birds receiving moderate supplementation compared with those in the control group. Carcass traits were not adversely affected. In summary, when *Averrhoa bilimbi* filtrate was used as an acidifier for the fermentation process, it was demonstrated that fermented banana peel could increase the growth and physiological parameters of broiler chickens without affecting their carcass characteristics.

Keywords: *Averrhoa bilimbi*; acidifier; banana peel; broiler chickens

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1. Introduction

Poultry production faces increasing challenges related to livestock health management and the demand for safe growth-promoting strategies. As one of the fastest-growing livestock sectors, poultry farming plays a vital role in supplying affordable, high-quality animal protein worldwide [1]. In recent decades, the productivity of broiler chickens has improved markedly because of genetic selection and advancements in feed formulation [2]. Among feed-based strategies, phytobiotics have been widely adopted as natural growth promoters to increase nutrient utilization and feed efficiency, particularly following restrictions on antibiotic growth promoters (AGPs) in many countries [3,4]. With the increasing use of antibiotic growth promoters in poultry production, interest in discovering natural feed additives that can enhance growth and support gut health is growing. Prebiotics, probiotics, and organic acids are being increasingly recognized for their essential roles in improving intestinal health, immune function, and growth performance in broiler chickens. Prebiotics are

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indigestible compounds that selectively stimulate beneficial microorganisms in the gastrointestinal tract, thereby promoting microbial balance and optimizing digestive processes [5,6].

Among the fruits grown in tropical areas, banana peel is a common agricultural product that contains both dietary fibre and bioactive compounds that may possess prebiotic qualities. The presence of antinutritional factors and high crude fibre content in poultry diets hinder their direct consumption. Agricultural byproducts such as banana peels—rich in fibre, pectin, polyphenols, and other phytochemicals—present a promising, sustainable source of natural prebiotics for poultry feed [7]. Fruit peels generally contain diverse bioactive compounds, including antioxidants and antifungal and antimicrobial agents [8,9], and several studies have demonstrated that banana peel supplementation enhances beneficial gut bacteria and supports digestive health in poultry and other livestock species [10,11]. Nutritionally, banana peel is reported to contain approximately 9.4–11.7% crude protein [12] and approximately 2,930 kcal/kg metabolizable energy [13]. For instance, [14] reported that supplementation with fermented banana peel flour up to 15% did not negatively affect broiler health but did not significantly enhance growth performance. These variations highlight the need for improved processing methods to maximize its functional value. However, the effects of banana peel flour in poultry diets have been inconsistent. Its high crude fibre content and the presence of antinutritional factors such as tannins, oxalates, and phytates may impair nutrient digestibility and growth performance if not properly processed [12,15]. Fermentation has been suggested to increase its nutritive value by decreasing fibre fractions, improving nutrient bioavailability, and increasing beneficial microbial populations. The use of prebiotic substrates can promote the growth of beneficial microbial populations through fermentable fibres, whereas organic acids may hinder the proliferation of pathogenic bacteria by reducing their gastrointestinal pH. However, studies combining both methods in a single fermented product are still limited.

Moreover, the filtrate of *Averrhoa bilimbi (A. bilimbi)* fruit, known for its high organic acid content, particularly citric acid, has been identified as a potential natural acidifier source [16,17]. These organic acids can lower intestinal pH and inhibit pathogenic microorganisms, thereby improving gut health and digestive efficiency in broiler chickens [18].

Fermentation is a well-established method to increase the nutritional value of feed, reduce antinutritional compounds, and produce beneficial bioactive metabolites. Fermenting banana peel flour with *A. bilimbi* fruit filtrate is expected to generate a synergistic combination of prebiotics from banana peel [7,14], probiotics from naturally occurring lactic acid bacteria, and organic acids from *A. bilimbi* [17], creating a functional additive capable of improving digestion, growth performance, immune response, and overall gut health in broiler chickens [19]. Fermenting natural feed ingredients can increase nutrient digestibility, improve feed quality, and modulate the intestinal microbiota of poultry [10,20].

Therefore, by integrating the prebiotic and phytochemical properties of banana peel with the naturally occurring probiotic activity and organic acids present in *Averrhoa bilimbi* filtrate through a controlled fermentation process, this study aims to evaluate the efficacy of fermented banana peel powder as a functional feed additive for broiler chickens. Despite the promising attributes of banana peels fermented with natural acidifiers, information on the dose–response effects on growth performance, hematological parameters, and the intestinal environment in broiler chickens is limited. These findings are expected to contribute to the development of safe, sustainable, and locally sourced natural additives capable of enhancing broiler chicken growth performance, physiological responses, and intestinal health.

2. Materials and Methods

2.1. Preparation of the Fermented Banana Peels

Fresh banana peels were collected from a local banana market in Semarang. The banana variety used in this study was the *Musa paradisiaca* "Tanduk" cultivar, which is commonly used for fried bananas. The peels were washed under running water, sun-dried until they reached a constant

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weight, and subsequently ground using a hammer mill to obtain banana peel flour. The *Averrhoa bilimbi L.* fruit filtrate was prepared from ripe fruits collected from home gardens around Universitas Diponegoro. Fruits were washed thoroughly under running water, the calyx ends were removed, and the fruit was blended to produce a juice. The juice was then filtered using a chiffon-mesh filter to obtain a clear fruit filtrate.

Fermentation was performed by mixing banana peel flour with bilimbi filtrate at a ratio of 1:4 (g:mL). The mixture was placed in airtight anaerobic jars and incubated anaerobically at 38 °C for 48 hours following the methods of [17]. After fermentation, the product was sun-dried until fully dry and then ground using an electric grinder to produce powder. The fermented banana peel additive was analysed prior to feed formulation. The lactic acid bacteria (LAB) population was 3.46×10^8 CFU/g, and the antioxidant activity measured using the DPPH method was 13.39 mg/L.

2.2. Birds, Diets, and Experimental Design

Experimental procedures were approved by the Animal Ethics Committee of the Faulty of Animal and Agricultural Sciences, Universitas Diponegoro (Ethical approval No. 61-12/A-32/KEP-FPP). A total of 280-day-old Cobb broiler chicks with an initial average body weight of 39.26±0.18 g were randomly allocated into four dietary treatments, each consisting of seven replicates with 10 birds per replicate (pen). The birds were reared in an open-house broiler facility using colony cages measuring 1 m^2 per pen. Broiler chickens had *ad libitum* access to clean water and were vaccinated according to commercial broiler vaccination standards for Newcastle disease (ND-IB) through eye drops on day 4, infectious bursal disease (IBD) by drinking water on day 12, and a Newcastle disease booster by drinking water on day 18. Broiler chickens were provided continuous 24-hour lighting. The incandescent light bulbs were used both as a heat source and to regulate the house temperature. The brooding temperature was maintained at 32–34 °C for days 1–4 and then gradually decreased each week to 25–29 °C for the remainder of the experiment.

Broiler chickens were reared for five weeks and received antibiotic growth promoters (AGPs) in accordance with the guidelines imposed by PERMENTAN/14/16/2017 for AGPs in Indonesia. The feed formulated for the broiler chickens was grain-based and did not include any animal-based protein sources, such as meat-bone-meal, fishmeal, or bone meal. The feed formulation included yellow maize, soya bean meal, palm oil, DL-methionine, bentonite, limestone, monocalcium phosphate, and premix, as shown in Table 1. During days 1–7, the birds received commercial prestarter feed containing 22% crude protein, 5% crude fat, 4% crude fibre, and 8% ash. From days 8 to 35, the experimental diets were offered according to the formulations shown in Table 1. The dietary treatments were as follows: T0, basal diet without additive; T1, basal diet + 0.25% fermented banana peel additive; T2, basal diet + 0.5% fermented banana peel additive; and T3, basal diet + 1% fermented banana peel additive. The broiler chicken experiment was conducted using a completely randomized design (CRD).

Table 1. Diet and analysed chemical compositions.

Raw Materials (%)	Starter (d 8-21)	Finisher (d 22-35)
Yellow maize	50.25	57.95
Soya bean meal	43.00	34.8
Palm oil	2.90	3.5
DL-methionine, 099 g	0.19	0.19
Bentonite	0.75	0.75
Limestone	1.00	1.00
Monocalcium phosphate	1.10	1.00
Premix[1]	0.34	0.34
Salt (fine)	0.40	0.40
Chlorine chloride	0.07	0.07
Calculated composition		

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Dry matter (%)	84.39	83.53
Crude Protein (%)	23.62	20.71
Fat (%)	5.81	6.56
Crude Fibre (%)	3.16	2.91
Ash (%)	5.11	4.49
Metabolizable energy (kcal/kg)	2,670.94	2,728.51
Chemicals composition		
Crude Protein(%)	26.85	22.04
Metabolizable energy (kcal/kg)	3,489.66	3419.47
Crude Fibre (%)	2.27	2.49
Crude Fat (%)	5.59	4.90
Ash (%)	7.59	6.20

[1] The premix supplied (per kg of diet): vitamin A, 7750 IU; vitamin D_3, 1550 IU; vitamin E, 1.88 mg; vitamin B_1, 1.25 mg; vitamin B_2, 3.13 mg; vitamin B_6, 1.88 mg; vitamin B_{12}, 0.01 mg; vitamin C, 25 mg; folic acid, 1.50 mg; Ca-D-pantothenate, 7.5 mg; niacin, 1.88 mg; biotin, 0.13 mg; Co, 0.20 mg; Cu, 4.35 mg; Fe, 54 mg; I, 0.45 mg; Mn, 130 mg; Zn, 86.5 mg; Se, 0.25 mg; L-lysine, 80 mg; choline chloride, 500 mg; DL-methionine, 900 mg; $CaCO_3$, 641.5 mg; and dicalcium phosphate, 1500 mg.[2]Metabolizable energy values were estimated using the equation proposed by Bolton (1967): 40.81 × {0.87 × (crude protein + 2.25 × crude fat + nitrogen-free extract) + 2.5}.d—days; g—gram; kcal—kilocalorie; kg—kilogram.

2.3. Data Collection and Analysis

2.3.1. Growth Performance, Carcass Quality, and Relative Organ Weight

The initial body weight was recorded on a pen basis, with the pens divided similarly to ensure the uniformity of the replicates and treatments on the first day of the experiment. Additionally, final body weight (FBW), feed intake (FI), body weight gain (BWG), and the feed conversion ratio (FCR) were recorded on a weekly basis (1, 7, 14, 21, 28 and 35 days of age) following [21]. Feed intake was calculated as the difference between the feed offered and the feed refused. The number of mortalities was recorded on a daily basis for any broiler chicken in unusual conditions, such as lame, ruffled feathers, lethargy, broken wings, diarrhea or any injury, reported as the percentage of birds culled. The feed conversion ratio was adjusted for the body weight of the broiler chickens that died during the experiments. On day 35, the same birds were selected for recording of the relative organ weight in comparison between body weight and various intestinal compartments. The live body weight of the broiler chickens was subsequently determined by direct cutting of the jugular vein, trachea, and carotid artery. Additionally, the abdominal cavity was opened, and all the visceral organs were carefully excised to obtain the eviscerated carcass. Individual organs, such as the bursa of Fabricius, gizzard, liver, proventriculus, thymus, caecum, spleen, ileum, duodenum, jejunum, caecum, and abdominal fat, were weighed using an analytical balance. Major commercial carcass components, including the breast, wings, thighs, drumsticks, and back, were separated and weighed to determine their proportional yields.

2.3.2. Hematological and Biochemical Indices

At the end of the experiment (day 35), male birds whose body weights were representative of their respective pens were chosen to minimize the physiological variations associated with sex and body weight. Blood samples (approximately 1 ml) from each broiler chicken from each pen were collected from the wing via the *vena branchialis*. The remaining blood was transferred into tubes without anticoagulants for serum preparation. The samples were allowed to clot at room temperature for 2 hours and then centrifuged at 5,000 rpm for 10 minutes. The resulting serum was collected and stored at −10 °C until analysis. The parameters observed included erythrocytes, haematocrit, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width–coefficient of variation (RDW–CV) and red cell distribution width–standard deviation (RDW–SD). Moreover, the leukocyte,

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lymphocyte, heterophil, and heterophil-to-lymphocyte ratios (H/L ratios) were determined. Moreover, thrombocytes, mean platelet volume (MPV), and platelet distribution width (PDW) were measured.

Serum biochemical parameters, including total cholesterol, total triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, and creatinine, were determined using enzyme-based colorimetric methods. Biochemical measurements, including total protein, albumin, serum gluatamic oxaloacetic transaminase (SGOT) and serum gluatamic oxaloacetic transaminase (SGPT), were measured spectrophotometrically according to the manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany). The globulin concentration was calculated as the difference between total protein and albumin. Blood samples were subsequently collected into anticoagulant agent ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis. Red blood cell count, hemoglobin concentration, and white blood cell count were determined using an automated hematology analyser (Prima Fully Auto Hematology Analyser, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia), following the procedures of [21].

2.3.3. Intestinal Total Bacteria and pH

Digesta samples from the ileum and cecum were aseptically collected immediately after slaughter for microbiological analysis. Approximately 1–2 g of digesta from each segmen was transferred into sterile tubes, placed in an ice box, transported to the laboratory, and then stored at a temperature below 4 °C to maintain bacterial viability in a dormant state prior to analysis. Before enumeration, the samples were allowed to equilibrate at room temperature for approximately 2 hours, after which they were homogenized and serially diluted with sterile physiological saline solution following previously described methods [20]. LAB were quantified on de Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany) after anaerobic incubation using AnaeroCult systems at 38 °C for 48 h, with colonies identified as creamy-white or yellowish-white and expressed as log CFU/g of digesta. Coliform and lactose-negative *enterobacteria* (LNE) were enumerated on MacConkey agar (Merck KGaA, Darmstadt, Germany) following aerobic incubation at 38 °C for 24 hours, and red colonies represented coliform and colorless LNE, with the results expressed as log CFU/g of digesta. For intestinal pH measurement, digesta samples were collected from the duodenum, jejunum, ileum, and caecum, and the pH values were measured immediately using a calibrated portable pH meter (OHAUS ST300, OHAUS Corporation, Parsippany, NJ, USA). The instrument was standardized using buffer solutions of pH 4.0 and 7.0 prior to measurement, and the electrode was rinsed with distilled water between samples to prevent cross-contamination.

2.3.4. Villus Height and Crypt Depth

Small intestinal segments (approximately 2 cm) from the duodenum, jejunum, and ileum were collected immediately after slaughter. The intestinal contents were gently removed using physiological saline (0.9% NaCl) to preserve the integrity of the villi. The samples were fixed in 10% neutral buffered formalin for at least 24 hours to prevent tissue autolysis. Histological preparations were carried out following standard procedures. Briefly, the fixed samples were dehydrated through a graded series of ethanol solutions (70%, 80%, 90%, 95%, and absolute), followed by clearing in xylol. The tissues were then embedded in paraffin blocks and sectioned at a thickness of 5 µm using a microtome (Leica HistoCore BIOCUT, Leica Biosystems, Wetzlar, Germany). The sections were mounted on glass slides and stained with hematoxylin and eosin (H&E). Morphometric analysis was performed using a light microscope equipped with a digital imaging system (Olympus CX23; Olympus Corporation, Tokyo, Japan). Villus height was measured from the tip of the villus to the villus–crypt junction, whereas crypt depth was measured from the base of the villus to the base of the crypt. For each sample, 5 well-oriented villi and crypts were selected and measured to obtain representative mean values. The villus height to crypt depth ratio (V/C ratio) was also calculated.

2.3.5. Statistical Analysis

All the data were analysed using analysis of variance (ANOVA) with SPSS software (Version 27.0; IBM Corp., Armonk, NY, USA). When a significant treatment effect was detected ($p < 0.05$), mean comparisons among treatments were performed using Duncan's multiple range test.

To further evaluate the dose–response relationship associated with increasing levels of fermented banana peel supplementation, orthogonal polynomial contrasts were applied to determine linear, quadratic, and cubic trends in the measured variables. The polynomial regression model is expressed as follows:

$$Y_i = \beta_0 + \beta_i X_i + \varepsilon_i \quad (1)$$

$where: Y_i\ represents\ observed\ variable$

$\beta_i\ represents\ the\ linear\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$X_i\ is\ level\ of\ fermented\ banana\ peel\ powder\ in\ the\ diet\ (\%)$

$\varepsilon_i\ is\ the\ random\ error$

$\beta_i > 0\ if\ the\ responses\ increase\ with\ supplementation$

$\beta_i < 0\ if\ the\ responses\ increase\ with\ supplementation$

$$Y_i = \beta_0 + \beta_i X_i + \beta_2 X_i^2 + \varepsilon_i \quad (2)$$

$where: Y_i\ represents\ observed\ variable$

$\beta_i\ represents\ the\ linear\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$\beta_2\ represents\ the\ quadratic\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$X_i\ is\ level\ of\ fermented\ banana\ peel\ powder\ in\ the\ diet\ (\%)$

$\varepsilon_i\ is\ the\ random\ error$

$$Y_i = \beta_0 + \beta_i X_i + \beta_2 X_i^2 + \beta_3 X_i^3 + \varepsilon_i \quad (3)$$

$where: Y_i\ represents\ observed\ variable$

$\beta_i\ represents\ the\ linear\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$\beta_2\ represents\ the\ quadratic\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$\beta_3\ represents\ the\ cubic\ effect\ of\ fermented\ banana\ peel\ inclusion\ levels$

$X_i\ is\ level\ of\ fermented\ banana\ peel\ powder\ in\ the\ diet\ (\%)$

$\varepsilon_i\ is\ the\ random\ error$

Additional statistical analyses and graphical visualizations were conducted using Python (version 3.1.0), employing the Pandas, SciPy, Matplotlib, and Seaborn following [21] methods for data visualization. Statistical significance was defined as $p < 0.05$, whereas $0.05 \leq p < 0.10$ was considered to indicate statistical significance.

3. Results

3.1. Growth Performance

The growth performance of broilers fed diets supplemented with fermented banana peel powder produced using Averrhoa bilimbi L. filtrate is summarized in Table 2, while the distribution and relationships among the performance variables are illustrated in Figures 1–6. On day 21, no significant differences ($P > 0.05$) in body weight, body weight gain (BWG), feed intake, or the feed conversion ratio (FCR) were detected among the treatments, as presented in Table 2. The distribution patterns of these variables across treatments are further illustrated by the boxplots in Figures 2–5, which show relatively similar medians and overlapping interquartile ranges among treatments. The relationships among performance parameters at this stage are visualized in the pair plot (Figure 1), indicating a positive association between body weight and BWG, whereas the FCR tended to decrease as growth increased.

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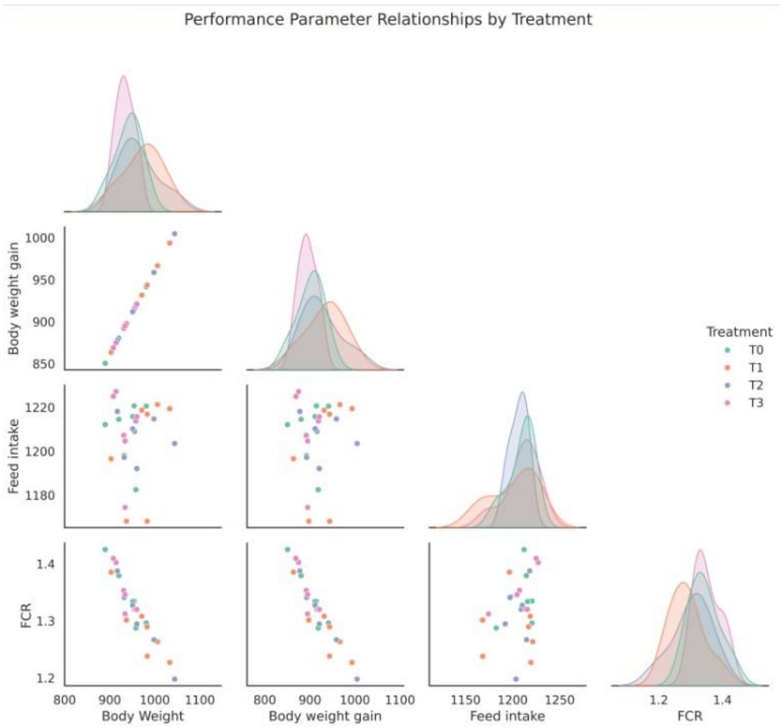

Figure 1. Pair plot of fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on broiler chickens growth performance at 21 days.

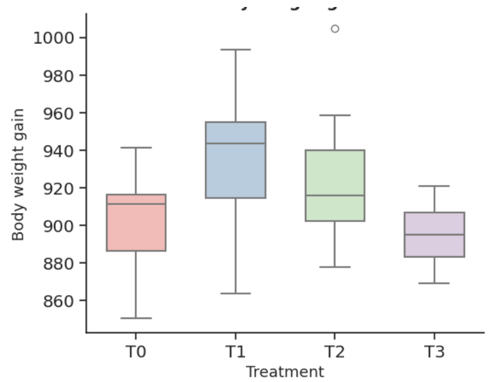

Figure 2. Boxplot of body weight gain (g) of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

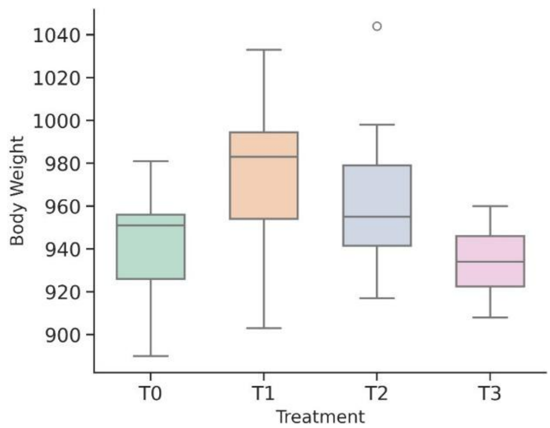

Figure 3. Boxplot of body weight (g) at 21 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

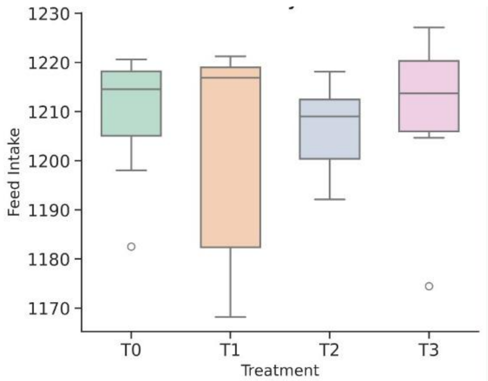

Figure 4. Boxplot of feed intake (g) at 21 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

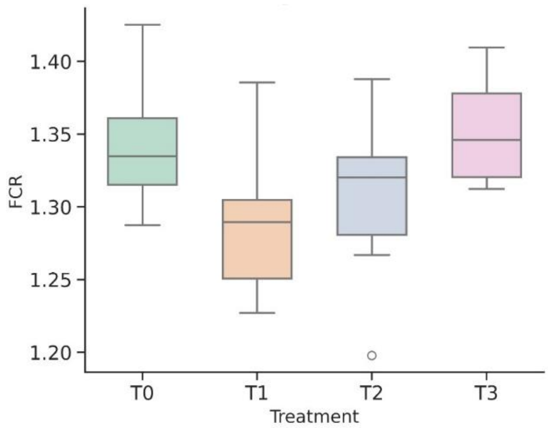

Figure 5. Boxplot of FCR at 21 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

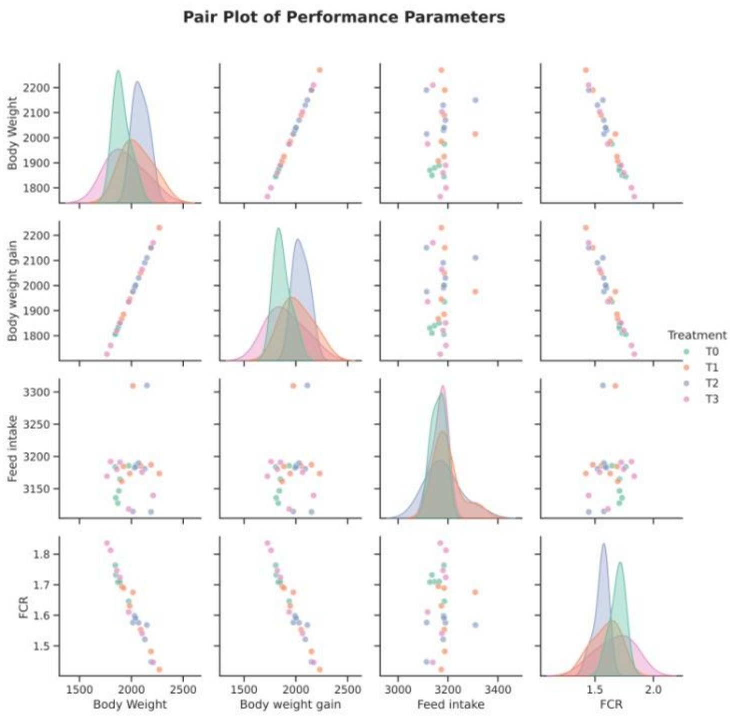

Figure 6. Pair plot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens growth performance at 35 days.

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On day 35, dietary supplementation significantly influenced body weight (Figure 7), BWG (Figure 8), and FCR (P < 0.01), as shown in Table 2. Broiler chickens receiving diets containing 0.25% and 0.5% fermented banana peel powder presented higher final body weight and BWG, together with improved FCR, than birds fed the control diet and those receiving the highest inclusion level did. The food intake (Figure 9) remained unaffected by the dietary treatments (P > 0.05). The pair plot presented in Figure 6 further illustrates the relationships among performance variables on day 35. A strong positive relationship between body weight and BWG was observed, whereas FCR (Figure 10) was negatively correlated with both body weight and BWG, indicating improved feed efficiency with increased growth. The association of feed intake with the other variables was weaker, supporting the finding that feed intake was not significantly affected by dietary supplementation. Quadratic responses (P < 0.01) were detected for body weight, BWG, and FCR, suggesting that moderate inclusion levels of fermented banana peel powder resulted in optimal growth performance.

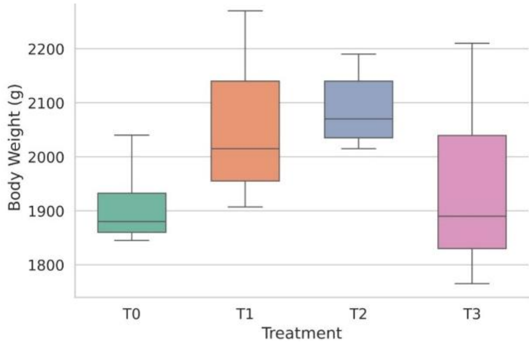

Figure 7. Boxplot of body weight (g) at 35 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

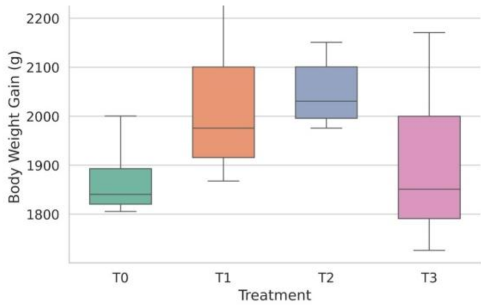

Figure 8. Boxplot of body weight gain (g) at 35 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

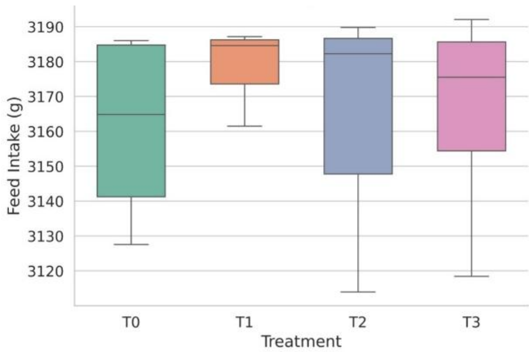

Figure 9. Boxplot of feed intake (g) at 35 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

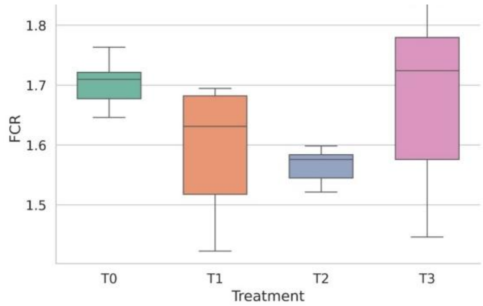

Figure 10. Boxplot of FCR at 35 days of broiler chickens fed diets supplemented with different levels of fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

Table 2. Effects of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler performance from day 21 to day 35 of age.

Parameters	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
d 21								
Body weight (g)	940.86	973.71	965.28	934.14	7.03	0.13	0.66	0.06
BWG (g/bird)	901.43	934.36	926.08	895.14	7.02	0.14	0.67	0.06
Feed intake (g)	1209.17	1201.30	1206.41	1209.70	3.12	0.78	0.81	0.66
FCR	1.34	1.2876	1.30	1.35	0.01	0.08	0.63	0.04
d 35								
Body weight (g)	1907.14^{a}	2070.00^{b}	2103.57^{b}	1823.57^{a}	27.84	<0.01	0.39	<0.01
BWG (g/bird)	1867.64^{a}	2030.65^{b}	2064.37^{b}	1784.57^{a}	27.83	<0.01	0.39	<0.01
Feed intake (g)	1209.17	1201.30	1206.41	1209.70	3.12	0.78	0.81	0.66
FCR	1.69^{b}	1.57^{a}	1.54^{a}	1.78^{c}	0.02	<0.01	0.29	<0.01

a–c Values within the same row bearing different superscripts indicate significant differences among treatment means ($P < 0.05$).T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A: analysis of variance; L: linear regression effect; Q: quadratic regression effect. BWG: body weight gain; FCR: feed conversion ratio.

3.2. Carcass Traits

The carcass characteristics of the broiler chickens are presented in Table 3. Dietary supplementation with fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate did not significantly affect the carcass yield or the thigh, breast, or back proportions ($P > 0.05$). However, the wing proportion significantly increased linearly and quadratically with increasing levels of fermented banana peel supplementation ($P < 0.05$). Overall, the carcass composition remained relatively stable across the treatments.

Table 3. Effects of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on the carcass traits of broiler chickens.

Parameters (% live BW)	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Carcass	69.88	71.41	71.58	71.64	0.37	0.29	0.10	0.16
Thigh	10.20	10.02	10.28	10.06	0.01	0.91	0.89	0.98
Breast	27.85	27.29	28.17	28.67	0.33	0.5S3	0.27	0.39
Back	14.11	15.73	14.94	14.57	0.22	0.05	0.78	0.07
Wings	7.01	7.58	7.71	7.95	0.13	0.08	0.01	0.03

T1: basal diet without additives; T2: basal diet supplemented with 0.25% fermented banana peel powder; T3: basal diet supplemented with 0.5% fermented banana peel powder; T4: basal diet supplemented with 1%

fermented banana peel powder. SEM: standard error of the mean; A: analysis of variance; L: linear regression effect; Q: quadratic regression effect.

3.3. Relative Internal Organ Weights

The effects of dietary fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on the relative weights of internal organs are presented in Table 4. No significant differences in the relative weights of the bursa of Fabricius, duodenum, gizzard, liver, ileum, jejunum, abdominal fat, spleen, proventriculus, or caecum were detected among the treatments ($P > 0.05$). In contrast, thymus weight was significantly affected by the dietary treatments, with both linear and quadratic responses to increasing levels of fermented banana peel supplementation ($P < 0.05$). The relative weights of digestive, intestinal, and immune organs in broiler chickens fed fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate are presented in Figures 11–24. Overall, the treatments showed only modest variation among the groups, indicating that dietary inclusion of the fermented product did not markedly alter organ development. The pair plot in Figure 11 illustrates the relationships among liver, proventriculus, gizzard, and abdominal fat weights across treatments (T0–T3). The distributions appear largely overlapping, suggesting comparable organ development among groups. Scatter patterns also indicate weak associations between most digestive organs.

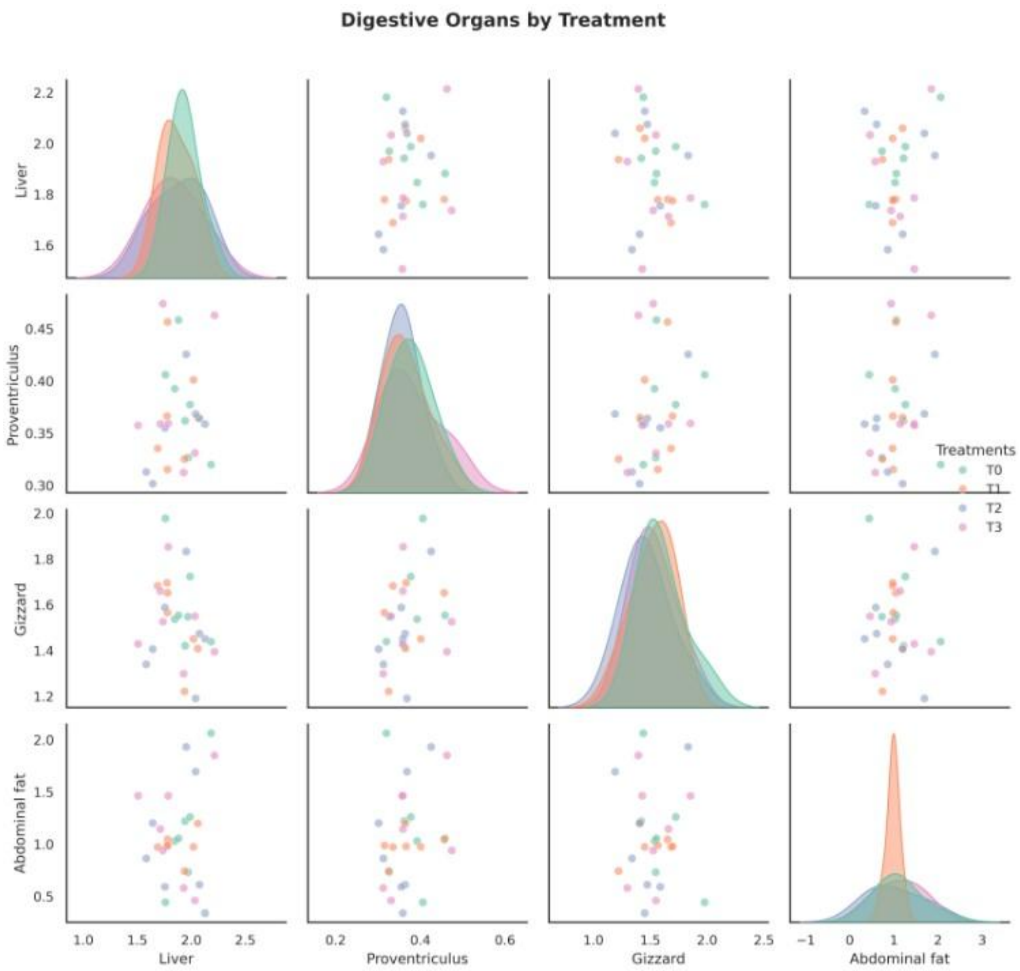

Figure 11. Pair plot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens organ weight.

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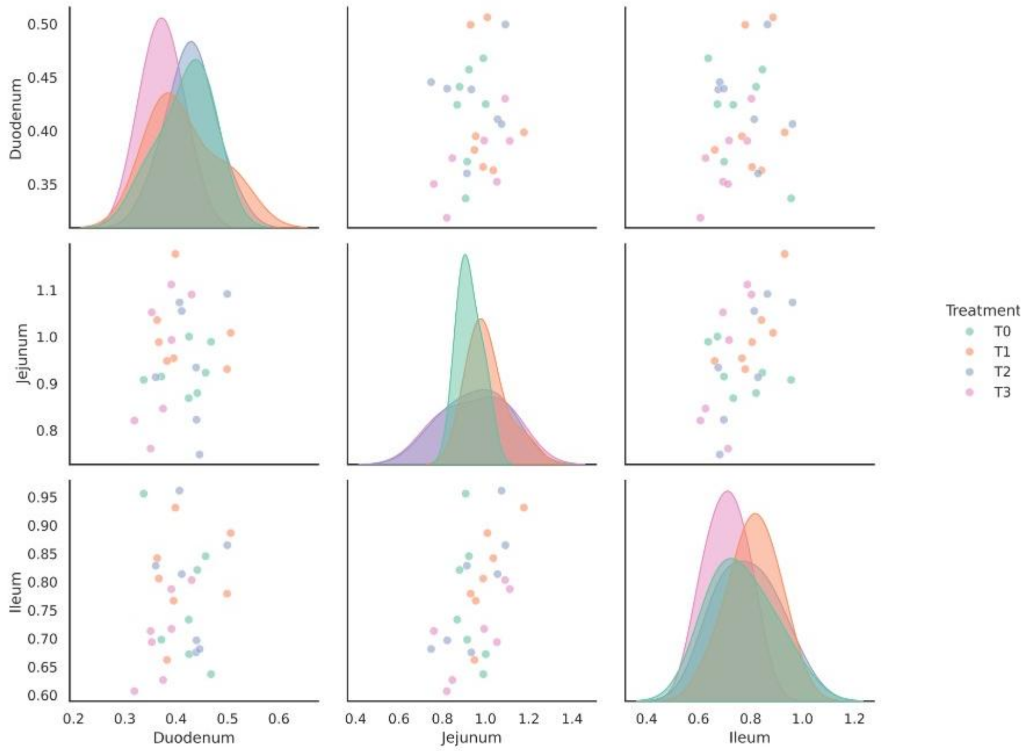

Figure 12. Pair plot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens small intestine segment.

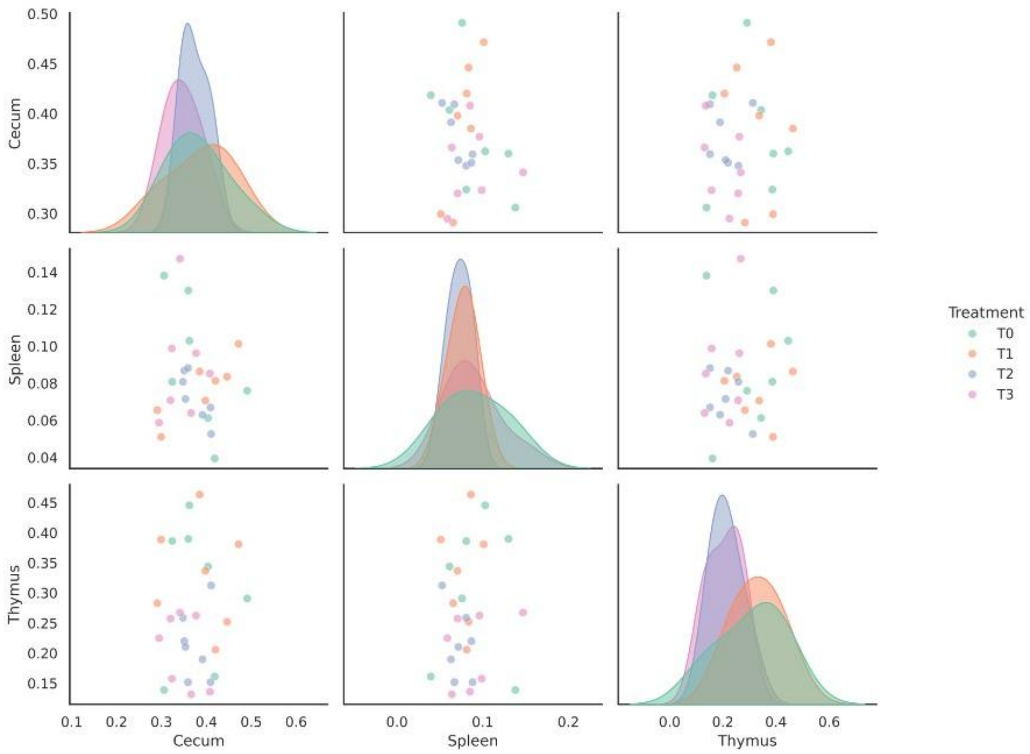

Figure 13. Pair plot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens cecum and immune organs.

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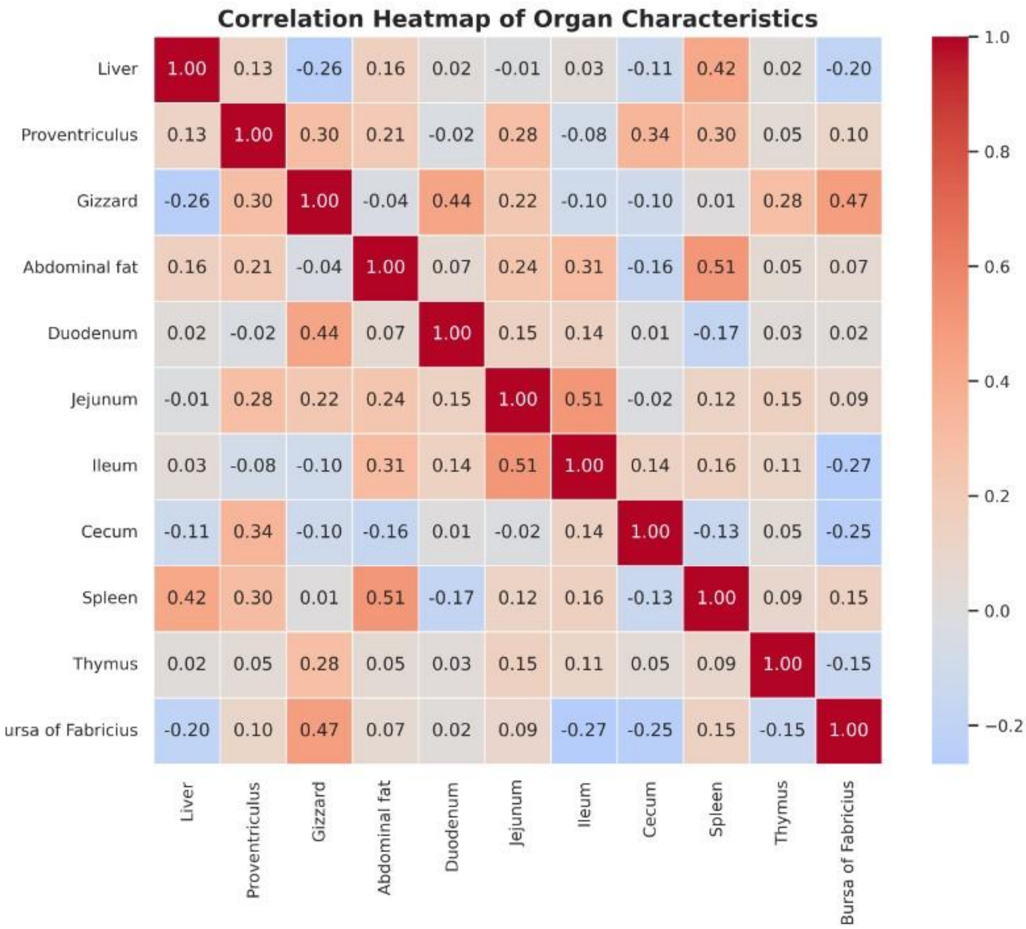

Figure 14. Heatmap of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens organ weight.

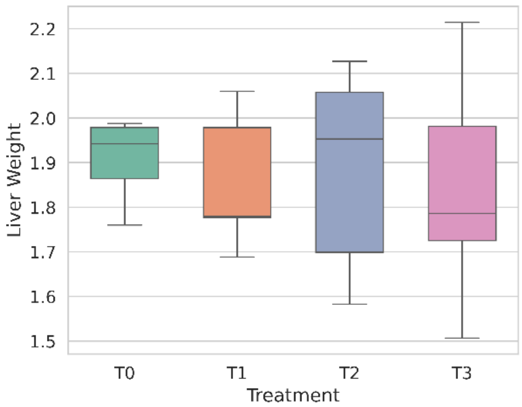

Figure 15. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens liver weight.

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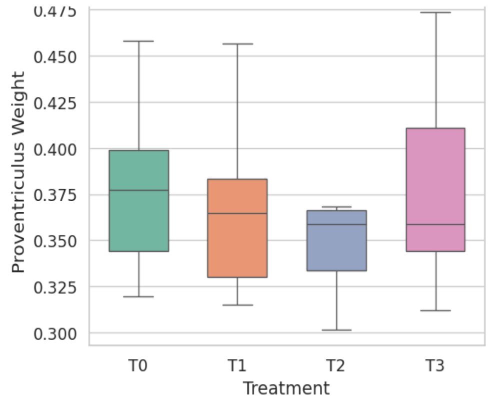

Figure 16. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens liver weight.

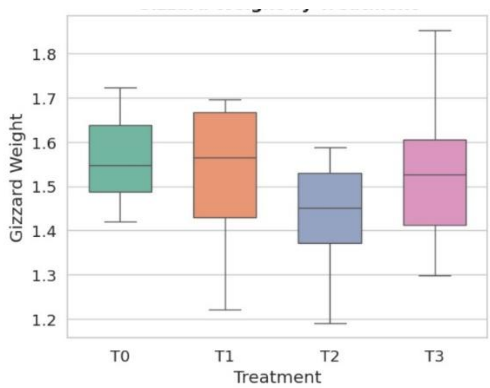

Figure 17. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens gizzard weight.

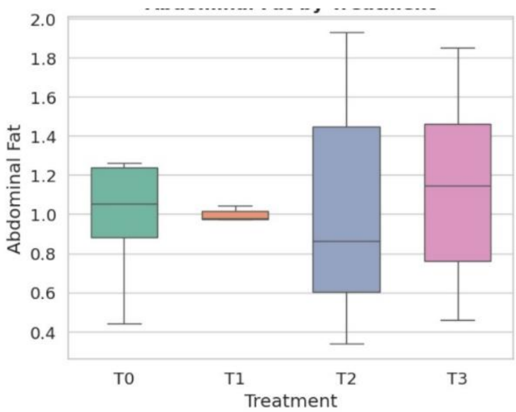

Figure 18. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens gizzard weight.

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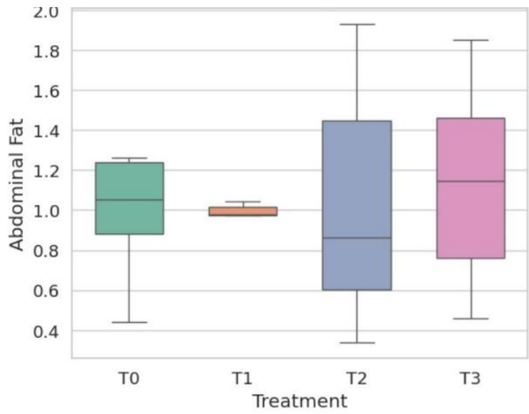

Figure 19. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens abdominal fat.

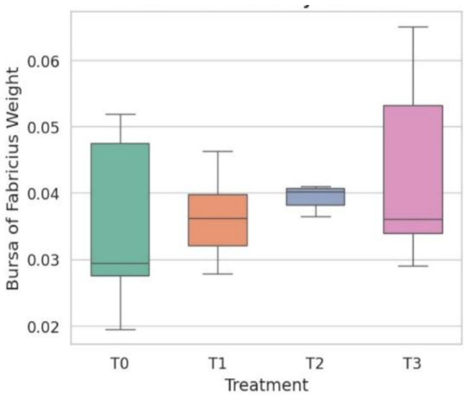

Figure 20. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens bursa of fabricius.

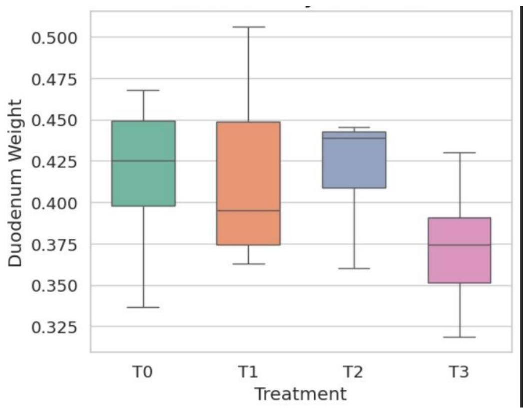

Figure 21. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens duodenum.

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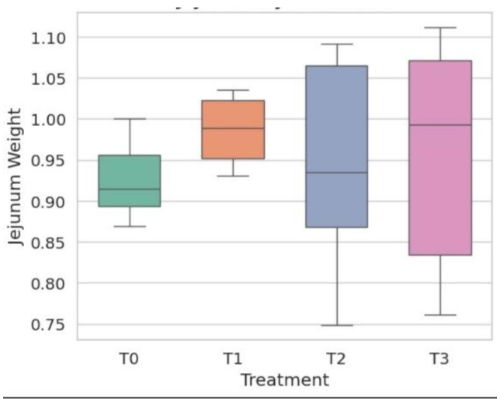


Figure 22. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens jejunum.

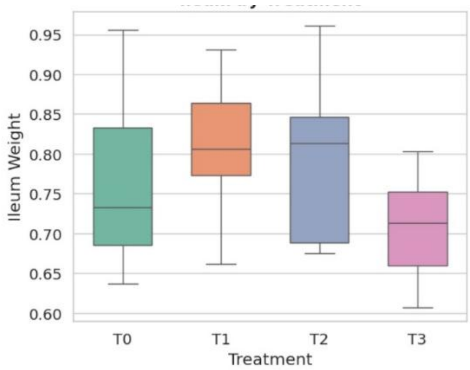


Figure 23. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens ileum.

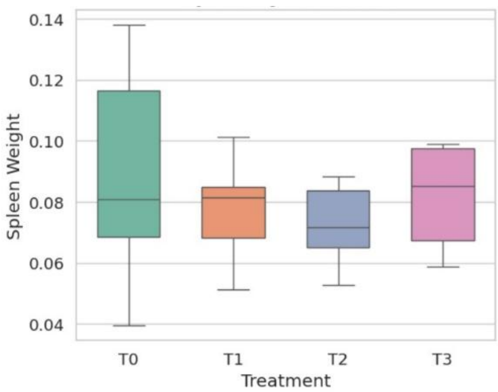
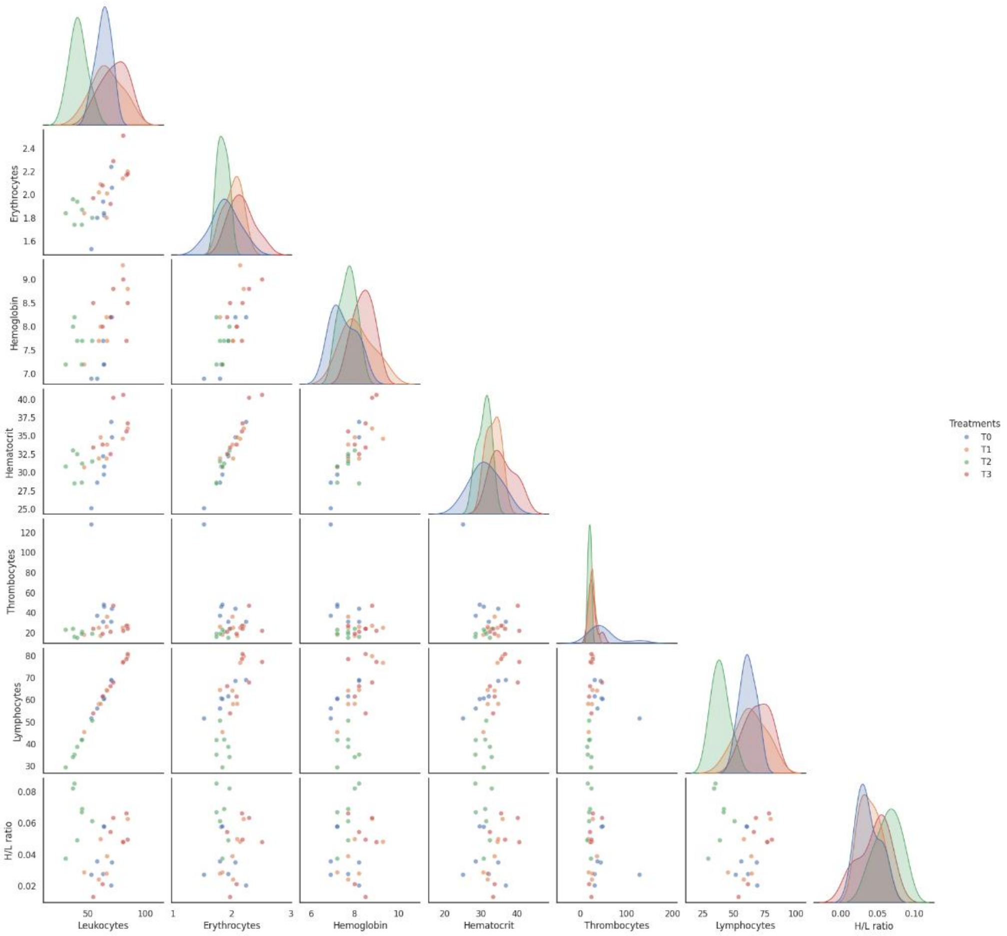


Figure 24. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens ileum.


Erythrocytes
Hemoglobin
Hematocrit
Thrombocytes
Lymphocytes
H/L ratio
Leukocytes
Treatments
T0
T1
T2
T3

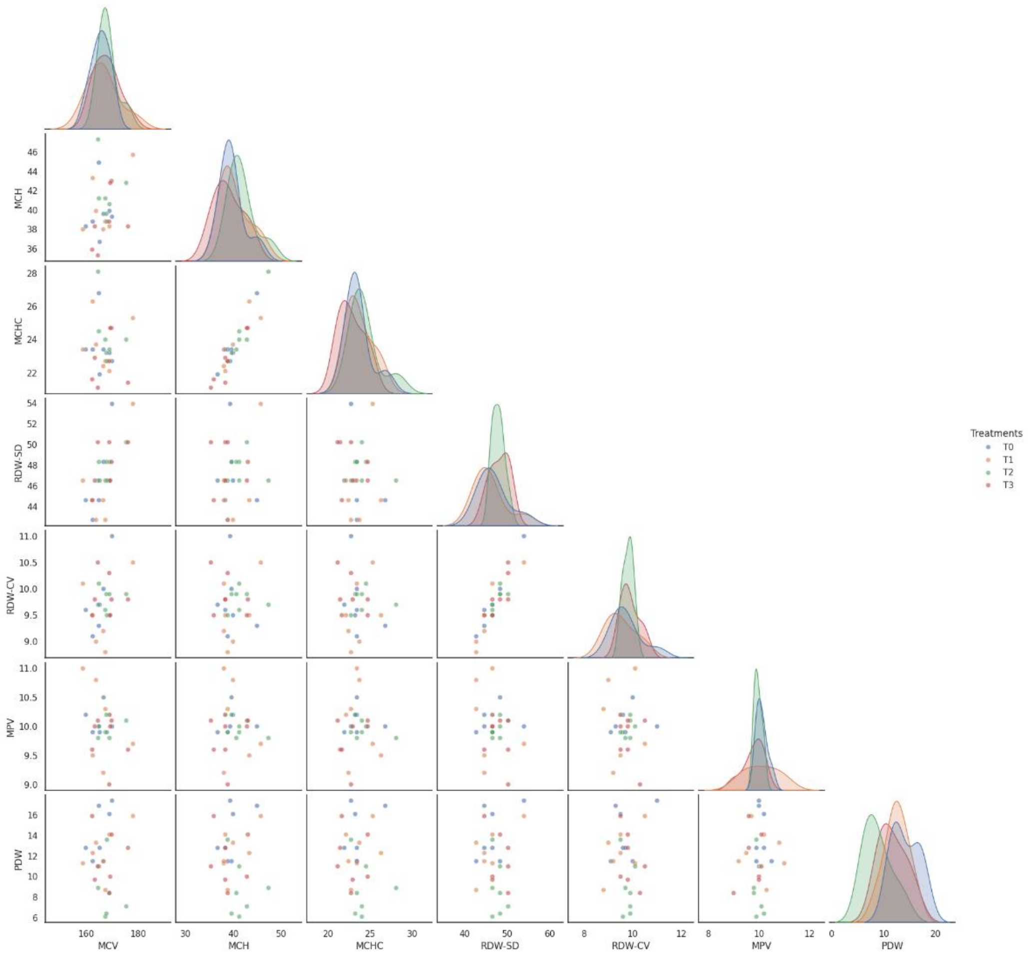

MCH
MCHC
RDW-SD
RDW-CV
MPV
PDW
MCV
Treatments
T0
T1
T2
T3

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Figure 25. Pair plot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens hematological indices.

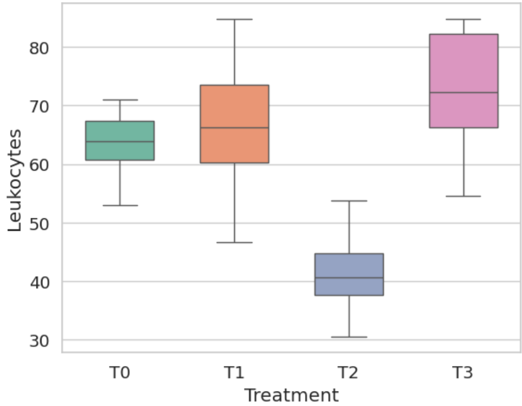

Figure 26. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens leukocytes.

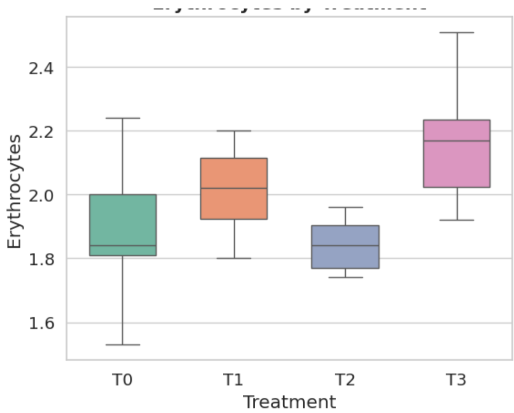

Figure 27. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens erythrocytes.

The boxplot analyses further support these observations. As shown in Figure 15, the relative liver weight varied slightly among treatments, with T2 showing a marginally higher median value than the other groups did. However, the interquartile ranges overlapped substantially, suggesting no pronounced treatment effect. Similarly, Figure 16 shows relatively consistent proventriculus weights across treatments, with only minor variation in the median and spread. The relative weight of the gizzard (Figure 17) showed moderate variability, particularly in T2 and T3, although the overall distributions remained comparable to those of the control group. In contrast, abdominal fat (Figure 18) exhibited a wider distribution, especially in T2, indicating greater variability in fat deposition among the birds in this group. The pair plot in Figure 12 shows the relationships among the duodenum, jejunum, and ileum. The distributions across treatments overlap considerably, indicating that the fermented banana peel powder had minimal influence on the relative weights of small intestinal segments. This pattern is further reflected in the boxplots. As shown in Figure 21, the relative weight of the duodenum varied slightly among the treatments, with T0 and T2 tending to have slightly higher median values. Moreover, the data in Figure 22 indicate that the jejunum weight was relatively stable across treatments, although T3 exhibited a somewhat broader range of values.

With respect to the ileum, the differences among treatments were modest (Figure 23), with T1 showing a slightly higher median than the other groups did. However, the overlapping interquartile ranges again suggest that treatment effects were minimal. The pair plot presented in Figure 13 illustrates the relationships between the cecum and immune organs (spleen and thymus). The

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distributions of these organs across treatments were largely similar, indicating comparable immune organ development among the groups.

Boxplot analyses support these observations. As shown in Figure 24, the spleen weight varied slightly across treatments, with T3 tending to show a marginally higher median. Similarly, Figure 20 indicates some variation in the bursa of Fabricius, with T3 exhibiting a broader distribution. However, the differences among treatments remained relatively small overall. The correlation matrix presented in Figure 14 provides further insight into the relationships among organ weights. Moderate positive correlations were observed between certain intestinal segments, particularly between the jejunum and ileum. In contrast, correlations between digestive organs and immune organs were generally weak, suggesting that these systems developed relatively independently under the experimental conditions.

Table 4. Effects of fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on the relative internal organ weights of broiler chickens.

Parameters (% Live BW)	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Bursa of fabricius	0.04	0.04	0.04	0.04	<0.01	0.47	0.12	0.28
Duodenum	0.42	0.41	0.43	0.37	0.09	0.16	0.15	0.12
Gizzard	1.60	1.52	1.47	1.53	<0.01	0.64	0.40	0.45
Liver	1.94	1.86	1.88	1.85	0.03	0.81	0.40	0.68
Ileum	0.77	0.81	0.79	0.71	0.02	0.24	0.24	0.12
Jejunum	0.93	1.01	0.95	0.95	0.02	0.57	0.89	0.66
Abdominal Fat	1.11	0.93	1.03	1.13	0.08	0.93	0.90	0.81
Spleen	0.09	0.08	0.07	0.09	<0.01	0.53	0.86	0.34
Proventriculus	0.80	0.37	0.35	0.38	0.01	0.80	0.94	0.65
Cecum	0.38	0.39	0.37	0.34	<0.01	0.51	0.20	0.31
Thymus	0.31^{b}	0.33^{b}	0.21^{a}	0.21^{a}	0.02	0.02	0.01	0.03

T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A: analysis of variance; L: linear regression effect; Q: quadratic regression effect.

3.4. Hematological Indices

The hematological parameters of the broiler chickens are summarized in Table 5. Dietary supplementation with fermented banana peel powder produced with Averrhoa bilimbi L. filtrate significantly affected erythrocyte, haematocrit, haemoglobin, the heterophil-to-lymphocyte (H/L) ratio, leukocyte and lymphocyte counts, platelet distribution width (PDW), and thrombocyte counts ($P < 0.05$). In contrast, the mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), red blood cell distribution width coefficient of variation (RDW-CV), and red blood cell distribution width standard deviation (RDW-SD) were not influenced by the dietary treatments ($P > 0.05$).

The distribution of these hematological variables across treatments is illustrated in Figures 28–38. As shown in Figures 28 and 29, the hemoglobin concentration and hematocrit exhibited noticeable variation among the treatments. Similarly, the leukocyte and lymphocyte counts (Figures 30 and 31, respectively) varied across treatment groups, indicating potential modulation of immune status. The platelet-related parameters also showed variability, particularly thrombocyte counts (Figure 30) and PDW (Figure 37). Regression analysis indicated linear and/or quadratic responses to increasing levels of fermented banana peel supplementation for several hematological variables, particularly hematocrit, leukocyte count, PDW, and thrombocyte count ($P < 0.05$). Moreover, erythrocyte indices, including the MCV (Figure 32), MCH (Figure 33), MCHC (Figure 34), RDW-CV (Figure 35), RDW-SD (Figure 36), and MPV (Figure 36), remained relatively stable across treatments.

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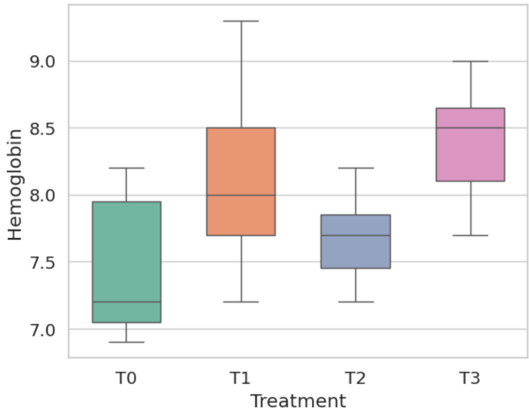

Figure 28. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens hemoglobin.

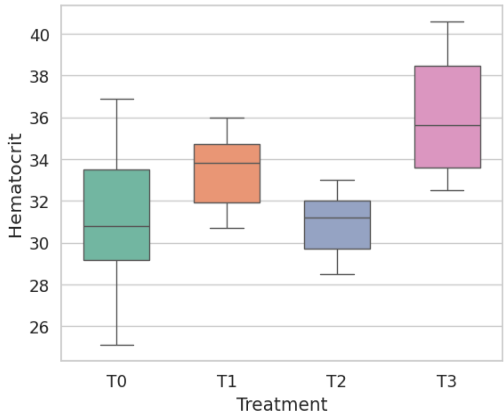

Figure 29. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens hematocrit.

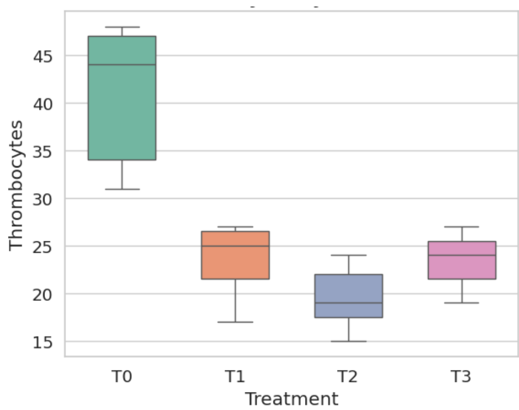

Figure 30. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens thrombocytes.

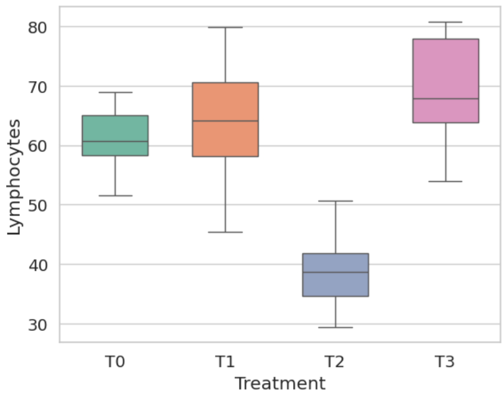

Figure 31. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens lymphocytes.

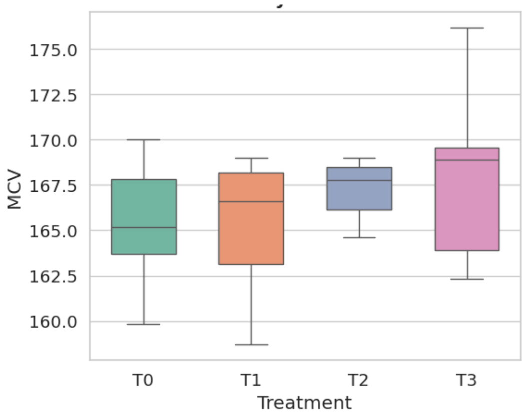

Figure 32. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens mean corpuscular volume.

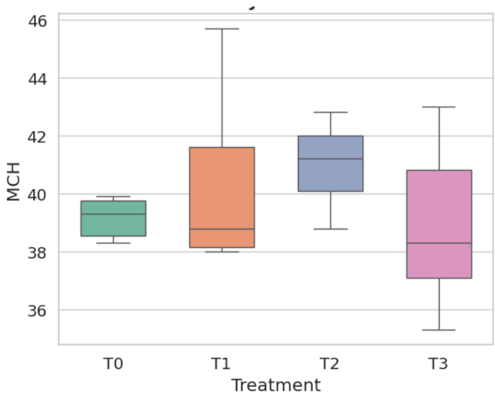

Figure 33. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens mean corpuscular hemoglobin.

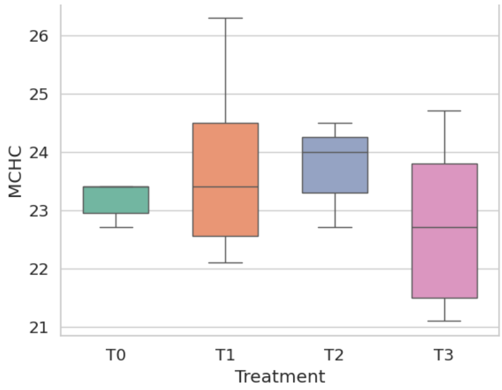

Figure 34. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens mean corpuscular hemoglobin concentration.

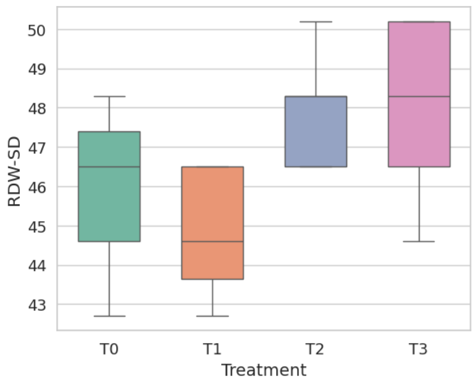

Figure 35. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens red blood cell distribution width–standard deviation.

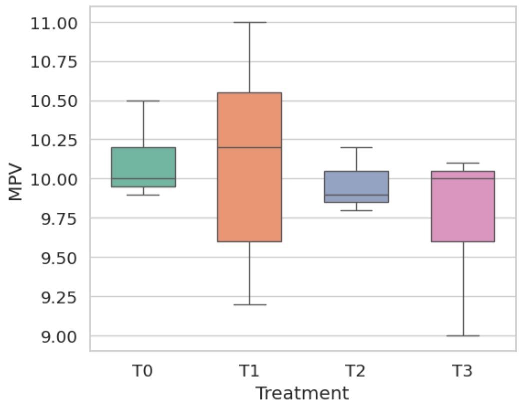

Figure 36. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens mean platelet volume.

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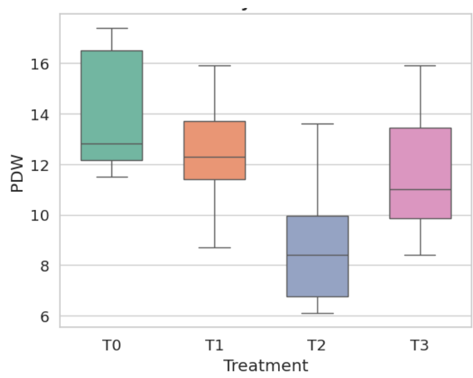

Figure 37. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens mean platelet distribution width.

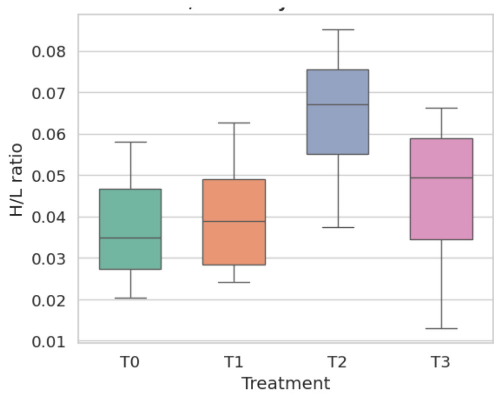

Figure 38. Boxplot of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on broiler chickens heterophil-to-lymphocyte ratio.

Table 5. Effects of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on the hematological indices of broiler chickens.

Parameters	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Erythrocytes (10^6/μL)	1.89[a]	2.01[ab]	1.84[a]	2.16[b]	0.04	0.01	0.07	0.08
Hematocrit (%)	31.16[a]	33.39[ab]	30.8714[a]	36.11[b]	0.65	0.01	0.03	0.05
Hemoglobin (g/dL)	7.47[a]	8.13[b]	7.6714[ab]	8.39[c]	0.12	0.02	0.03	0.10
H/L ratio	0.04[a]	0.04[a]	0.06[b]	0.05[a]	<0.01	0.03	0.14	0.10
Leukocytes (10^3/μL)	63.41[b]	66.51[b]	41.3429[a]	72.68b	2.88	<0.01	0.92	0.04
Lymphocytes (10^3/μL)	61.14[b]	63.86[b]	38.83[a]	69.43[b]	2.63	<0.01	0.86	0.06
MCH (pg)	39.64	40.29	41.6429	38.91	0.54	0.35	0.87	0.14
MCHC g/dL	23.54	23.70	24.2714	22.73	0.30	0.37	0.50	0.31
MCV (fL)	165.44	166.59	167.7571	168.17	0.85	0.69	0.27	0.50
MPV (fL)	10.10	10.10	9.9571	9.77	0.08	0.41	0.11	0.23
Heterophils (10^3/μL)	2.27	2.66	2.51	3.26	0.19	0.19	0.06	0.09
PDW (%)	14.14[b]	12.44[a]	8.79[b]	11.70[b]	0.59	0.01	0.03	0.01
RDW-CV (%)	9.74	9.51	9.80	9.89	0.09	0.53	0.39	0.48
RDW-SD (fL)	46.73	45.93	47.80	48.07	0.55	0.52	0.23	0.45
Thrombocytes (10^3/μL)	52.14[b]	24.86[a]	19.57[a]	26.29[a]	4.03	0.01	0.02	<0.01

a–c Values within the same row bearing different superscripts indicate significant differences among treatment means (P < 0.05). μL—microliterT0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A: analysis of

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variance; L: linear regression effect; Q: quadratic regression effect. H/L ratio: heterophil-to-lymphocyte ratio; MCH: mean corpuscular hemoglobin concentration; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; PDW: platelet distribution width; RDW-SD: red blood cell distribution width–standard deviation.

3.5. Serum Biochemical Indices

The effects of dietary fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on serum biochemical indices are presented in Table 6. No significant differences in the serum albumin level, creatinine level, globulin level, lipid profile (HDL, LDL, or total cholesterol level), total protein level, liver enzyme activity (SGOT or SGPT), triglyceride level, or uric acid level (P > 0.05) were detected among the treatments. Regression analysis also revealed no linear or quadratic responses of serum biochemical parameters to increasing levels of fermented banana peel supplementation.

Table 6. Effects of fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on the serum biochemical indices of broiler chickens.

Parameters	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Albumin (g/dL)	1.27	1.13	1.18	1.17	0.03	0.46	0.40	0.43
Creatinine (g/dL)	0.07	0.05	0.07	0.06	0.01	0.62	0.94	0.99
Globulin (g/dL)	1.84	1.82	2.00	1.59	0.08	0.26	0.57	0.34
HDL (mg/dL)	87.14	69.01	79.57	79.71	2.69	0.11	0.63	0.21
LDL (mg/dL)	30.39	23.79	27.76	25.06	2.19	0.74	0.55	0.76
Total cholesterol (mg/dL)	124.97	107.44	115.17	111.83	4.11	0.51	0.39	0.49
Total protein (g/dL)	3.10	2.95	3.28	2.76	0.09	0.26	0.41	0.47
SGOT (g/dL)	245.23	248.65	216.44	240.87	11.96	0.79	0.68	0.84
SGPT (g/dL)	3.68	4.11	2.80	3.30	0.37	0.67	0.47	0.77
Triglycerides (mg/dL)	41.23	30.37	35.85	35.26	1.78	0.20	0.87	0.93
Uric acid (mg/dL)	7.01	7.97	10.91	8.57	0.69	0.24	0.23	0.24

T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A: analysis of variance; L: linear regression effect; Q: quadratic regression effect. HDL: high-density lipoprotein; LDL: low-density lipoprotein; SGOT: aspartate aminotransferase (AST); SGPT: alanine aminotransferase (ALT).

3.6. Intestinal Histomorphometry

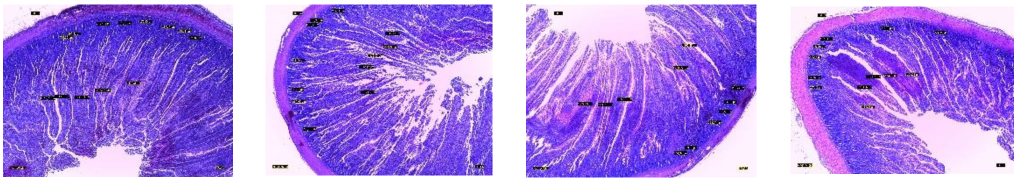
The villus height, crypt depth, and villus height–to–crypt depth (VH/CD) ratio of the broiler chickens are presented in Table 7. No significant differences in the villus height, crypt depth, or VH/CD ratio in the duodenum, jejunum, or ileum were detected among the dietary treatments (P > 0.05). Regression analysis further indicated the absence of linear or quadratic responses of these intestinal morphometric parameters to increasing levels of fermented banana peel powder supplementation. The intestinal histomoprhometry can be shown in Figures 39–41.

Table 7. Effects of fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on intestinal histomorphometry in broiler chickens.

Parameters	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Duodenum								
Villus height (μm)	1124.68	1254.30	1426.45	1235.48	43.29	0.09	0.19	0.07
Crypth depth (μm)	195.01	195.69	208.77	192.93	7.40	0.88	0.92	0.86
VH/CH ratio	6.09	6.43	7.10	6.59	6.55	0.71	0.43	0.58

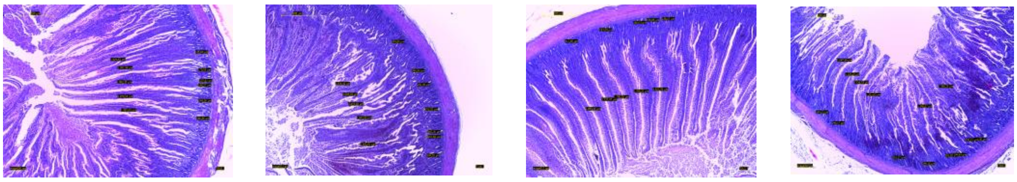
Jejunum								
Villus height (µm)	1284.65	1312.73	1477.92	1247.54	56.48	0.51	0.91	0.53
Crypth depth (µm)	220.47	247.05	228.99	223.58	0.86	0.83	0.92	0.76
VH/CH ratio	6.15	5.75	6.98	5.64	6.13	0.66	0.94	0.85
Ileum								
Villus height (µm)	793.69	1007.80	1004.13	942.04	46.09	0.32	0.29	0.18
Crypth depth (µm)	172.76	186.84	197.79	197.85	9.31	0.76	0.31	0.56
VH/CH ratio	4.94	5.71	5.15	4.93	5.18	0.75	0.81	0.67

T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A—analysis of variance; CD—crypt depth; L—linear regression effect; Q—quadratic regression effect; VH—villus height; VH/CD ratio—villus height to crypt depth ratio.



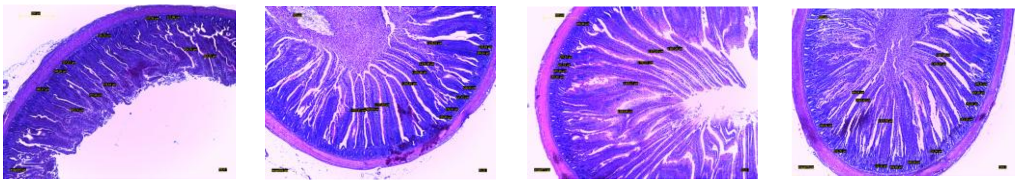
T0 T1 T2 T3

Figure 39. Histopathological image of the duodenum segment of broiler chickens fed diets supplemented with fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.



T0 T1 T2 T3

Figure 40. Histopathological image of the jejunum segment of broiler chickens fed diets supplemented with fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.



T0 T1 T2 T3

Figure 41. Histopathological image of the ileum segment of broiler chickens fed diets supplemented with fermented banana peel powder produced with *Averrhoa bilimbi* L. filtrate.

3.7. Intestinal Bacterial Populations

The effects of dietary fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on intestinal bacterial populations are presented in Table 6. No significant differences were detected among the treatments for lactic acid bacteria (LAB), coliforms, or lactose-negative enterobacteria (LNE) in the ileum and cecum (P > 0.05). Regression analysis revealed no linear or quadratic responses of intestinal bacterial populations to increasing levels of fermented banana peel supplementation.

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Table 8. Effects of fermented banana peel powder produced with Averrhoa bilimbi L. filtrate on intestinal bacterial populations in broiler chickens.

Parameters (log cfu/g).	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Ileum								
LAB	9.11	9.63	9.66	8.87	0.19	0.39	0.69	0.21
Coliform	5.48	5.55	5.55	5.64	0.05	0.71	0.25	0.52
LNE	5.48	5.59	6.10	5.91	0.12	0.21	0.59	0.18
Cecum								
LAB	10.26	10.44	10.43	10.40	0.06	0.74	0.48	0.55
Coliform	7.40	6.02	6.83	6.64	0.23	0.21	0.49	0.36
LNE	6.04	6.12	5.79	5.93	0.17	0.92	0.48	0.55

T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T4: basal diet supplemented with 1% fermented banana peel powder. SEM: standard error of the mean; A– analysis of variance; cfu—colony forming unit; g—gram; L– linear regression effect; Q—quadratic regression effect. LAB—lactic acid bacteria; LNE—lactose-negative *enterobacteria*.

3.8. Intestinal pH

The effects of dietary fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on intestinal pH values are presented in Table 9. Dietary treatments significantly affected the duodenal, jejunal, and ileal pH values ($P < 0.05$). Jejunal pH exhibited significant linear and quadratic responses to increasing levels of fermented banana peel powder ($P < 0.01$). Similarly, ileal pH was influenced by dietary supplementation, with both linear and quadratic effects ($P \leq 0.05$). In contrast, the caecal pH was not significantly affected by the dietary treatments ($P > 0.05$).

Table 9. Effects of fermented banana peel powder produced with *Averrhoa bilimbi L.* filtrate on intestinal pH values in broiler chickens.

Parameters	T0	T1	T2	T3	SEM	*P* Value		
						A	L	Q
Duodenum	6.12^{b}	5.93^{a}	6.14^{b}	6.19^{b}	0.03	0.02	0.18	0.07
Jejunum	5.92^{a}	6.18^{b}	6.27^{b}	6.29^{b}	0.04	<0.01	<0.01	<0.01
Ileum	5.65^{a}	6.14^{ab}	6.06^{ab}	6.42^{b}	0.10	0.04	0.01	0.03
Cecum	6.18	6.22	6.22	6.27	0.04	0.93	0.51	0.81

a–c Values within the same row bearing different superscripts indicate significant differences among treatment means ($P < 0.05$).T0: basal diet without additives; T1: basal diet supplemented with 0.25% fermented banana peel powder; T2: basal diet supplemented with 0.5% fermented banana peel powder; T3: basal diet supplemented with 1% fermented banana.

4. Discussion

The results of this study demonstrated that dietary supplementation with fermented banana peel powder produced from *Averhoa bilimbi* L. fruit filtrate modulated growth performance, carcass yield, hematology, serum biochemical indices, gut morphology and the intestinal environment in broiler chickens in a manner that suggests nutrient utilization and physiological resilience. These findings are consistent with growing evidence that fermented feed additives can enhance growth performance and intestinal function in broiler chickens through coordinated effects on nutrient digestibility, microbial ecology, and host metabolism.

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4.1. Growth Performance and Carcass Traits

The dietary supplementation of fermented banana peel powder markedly improved key growth performance by increasing body weight gain and improving the feed conversion ratio (FCR). The significant linear and quadratic responses observed indicate that moderate inclusion levels optimized performance, whereas higher inclusion resulted in diminishing returns. This pattern aligns with the dose–response relationship commonly reported for feed additives in the nutritional status of broiler chickens.

Similarly, recent studies have shown that the use of fermented or probiotic-enriched feed additives can lead to better body weight and improved feed efficiency. The effectiveness of nutrient usage efficiency can be improved through microbial activity, as compared with control diets, these formulations have been shown to increase body weight and lower FCR. Fermented feed products are often rich in organic acids, microbial metabolites and enzymatic degradation products generated during fermentation. These compounds can increase feed palatability, stimulate digestive enzyme secretion, and reduce the levels of the nutritional factors naturally present in plant-based materials. As a consequence, nutrient digestibility and intestinal absorption improve, ultimately promoting more efficient growth. Mechanistically, the fermentation begins to hydrolyse complex polysaccharides and lignocellulosic components of banana peels, which can increase the availability of fermentable substrates and bioactive compounds through mechanistic processes. Improved access to nutrients by improving digestive enzymes and intestinal microbiota leads to increased feed efficiency. Supplementation with fermented feed or probiotic-based additives can increase nutrient utilization efficiency, as broiler chickens have shown similar improvements in body weight gain and FCR when supplemented with other feeds, such as rice vinegar or maize [22–24]. Optimal carcass traits were also indicative of better growth performance. More efficient carcass yield, especially in muscle-dominant cuts, probably represents a consequence of improved partitioning of nutrients rather than energy losses due to metabolic processes. Research on the effects of fermented feed ingredients in poultry diets has also shown that higher final body weight leads to better slaughter performance but not necessarily to a lower carcass composition [25,26]. The observed increase in carcass yield in the current study may be attributed to increased protein deposition and metabolic efficiency, which are linked to improved nutritional potential.

Taken together, these effects lead to improved absorption and utilization of nutrients, which in turn leads to growth. Improvements in growth performance were also reflected in the positive carcass characteristics. Improved nutrient partitioning toward lean tissue accretion leads to increased carcass yield, especially in muscle-dominant cuts, rather than energy loss through inefficient metabolic processes. Optimal carcass traits were also indicative of better growth performance. More efficient carcass yield, especially in muscle-dominant cuts, is likely a consequence of improved nutrient partitioning rather than energy loss due to metabolic processes [27–30]. The observed increase in carcass yield in the current study may be attributed to increased protein deposition and metabolic efficiency, which are linked to improved nutritional potential [31].

Recent investigations have consistently demonstrated comparable improvements in body weight and feed efficiency following the inclusion of a fermented or probiotic-enriched feed additive [32,33]. In a study by Opazo et al. [34], the use of probiotic formulations significantly improved body weight gain (BWG) and lowered the feed conversion ratio compared with those of the control, highlighting the importance of microbial contributions to nutrient utilization efficiency. Fermented feed products are known to contain organic acid and fermentation metabolites that improve feed palatability, support digestive enzyme activity and reduce antinutritional factors, collectively promoting more efficient nutrient uptake and conversion to body mass [33,35]. The improvement in growth performance was mirrored by positive changes in carcass traits. From study Sjofjan et al. [36] using enzyme can help to reduce crude fibre in banana tuber meal which is consisted non-starch polysaccharides.

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4.2. Hematological and Immune Status

Hematological indices provide insights into the immunological and physiological status of broiler chickens. In this study, the erythrocyte count, haematocrit and haemoglobin concentration remained within the normal physiological range across all treatments, indicating that supplementation with fermented banana peels did not induce haematological stress or compromise oxygen transport capacity. Adding supplements to the serum significantly modulated the heterophil-to-lymphocyte (H/L) ratio. The H/L ratio is a reliable indicator of physiological stress in poultry, with lower values typically indicating better immune status and reduced stress levels. The decrease in the H/L ratio observed in the supplemented groups indicates that ferment-derived banana peel powder may contribute to better physiological stability and immune function. In line with Niu et al. [37] and Habibian et al. [38], a low H/L ratio is widely recognized as an indicator of reduced stress and better immune status in poultry and is often associated with improved performance and resilience to environmental challenges

Mechanistic processes involve interactions between LAB and organic acids in fermented feed and gut-associated lymphoid tissue, which is a critical component of the intestinal immune system. This may impact immune responses. Through fermentation, metabolic substances such as organic acids and microbial proteins can be produced, which may affect immune signalling pathways and decrease oxidative stress levels. The gut-immunity axis is crucial because it influences systemic immune responses through changes in the intestinal microbial environment. Additionally, the presence of beneficial microorganisms and their metabolites can activate immune cells and intestinal epithelial cells, leading to the production of balanced cytokines that enhance mucosal immunity. Similarly, the use of fermentation-extant compounds has been shown to stimulate intestinal epithelial cells and immune cells, leading to a more balanced cytokine profile and less oxidative stress and a tendency toward improved digestion. Hence, fermented feed additives may have immunomodulatory effects through complex interactions between host immunity and the gut microbiota. Moreover, these conditions contribute to modulating the immune response by interacting with gut-associated lymphoid tissue, reducing the systemic stress response and enhancing anti-inflammatory signalling [39,40]. For instance, fermentative metabolites can stimulate intestinal epithelial cells and immune cells, promoting a more balanced cytokine profile and reducing oxidative stress, a hypothesis supported by reviews of fermented feeds that highlight their immunomodulatory potential through interactions with host immunity and microbiota [41,42].

4.3. Serum Biochemical Indices and Metabolism

The use of fermented banana peel powder in supplementation did not result in any adverse effects on organ function, as serum biochemical profiles revealed normal levels of total protein and liver enzyme activities (SGOT and SGPT) during all treatments. Instead, the stability of these biochemical parameters suggested that metabolic homeostasis was preserved during the experimental period.

Fermentation-induced bioactive compounds and organic acids have been shown to regulate lipid metabolism in poultry. Waghmare et al. [43] reported that supplementation with organic acidic compounds can reduce both serum cholesterol and triglyceride levels, suggesting an increase in lipid metabolism. Although no significant changes in lipid parameters were detected in this study, the absence of any detrimental biochemical effects indicates that broiler chickens can safely use fermented banana peel supplements without harming their health. The gut–metabolism axis of poultry is evident in the connection between microbial fermentation products and host metabolism. SCFAs generated by fermentation can affect metabolic processes linked to energy consumption, lipid metabolism and nutrient partitioning. The results of this study indicate minimal changes in lipid parameters, but these findings support the notion that adding fermented banana peel to one's diet supports efficient nutrient use and therefore provides evidence of metabolic safety.

The metabolic metabolism of nutrients may be linked to fermentation metabolites through the utilization of short-chain fatty acids (SCFAs) and other organic acids as substrates in energy

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metabolism. Through the stimulation of digestive enzyme activity and a reduction in luminal pH, these compounds can improve intestinal conditions by promoting better nutrient digestion. The results of this study revealed that these mechanisms may have been responsible for the increase in growth performance and carcass traits. The relationship between fermentation metabolites and nutrient metabolism is mechanistically grounded in the capacity of short-chain fatty acidacids (SCFAs) and other organic acids to serve as substrates for energy metabolism while creating an acidic gut microenvironment that favours digestive enzyme activity and nutrient absorption, further supporting the improvement in growth performance and carcass traits [44,45].

4.4. Microbial Environment and Intestinal pH

One of the salient findings of this study was the modulation of intestinal pH in the duodenum, jejunum, and ileum following supplementation with fermented banana peels, and the observed linear and quadratic responses suggest that fermentation-derived metabolites stimulate the intestinal microenvironment. Many digestive processes are supported by a decrease in gastrointestinal pH. Increased intestinal pH can improve digestive efficiency by increasing the activity of proteolytic enzymes such as pepsin and stimulating pancreatic enzyme secretion. In addition, acidic conditions restrict the development of pathogenic microorganisms and promote the growth of advantageous bacteria such as lactic acid bacteria (LAB). However, this favourable intestinal environment is established by organic acids, such as lactic acid, which are produced during fermentation.

Lower pH results in increased pepsin activity and pancreatic enzyme secretion, which increase protein digestion and nutrient availability. Lactic acid fermentation is associated with a reduction in intestinal pH and the establishment of favourable conditions for beneficial microbial communities, including lactic acid bacteria. This occurs in the presence of organic acids during fermentation. Organic acids produced during fermentation, such as lactic acid, are known to reduce the luminal pH and create an environment that suppresses harmful bacteria while supporting beneficial communities such as lactic acid bacteria (LAB), even if the overall count did not change significantly in this study. Reviews on fermented feed underscore that the combination of decreased intestinal pH, elevated organic acid, and increased LAB population contributes to a favourable gut ecosystem, improving digestive efficiency and the sanitary status of the gut [33]. Although the bacterial counts did not significantly differ between treatments, changes in intestinal pH may have occurred without major changes in the size of the microbes. Research on the microbiome is increasingly acknowledging the physiological effects of metabolic activity and microbial interactions as independent of significant changes in bacterial abundance. This phenomenon is becoming more common. These findings emphasize the importance of the gut ecosystem, where microbial metabolites, intestinal pH, and host digestive processes interact to regulate nutrient usage and health. Accordingly, Katu et al. [33] the positive effects of fermented banana supplementation may be offset by improvements in intestinal conditions that promote microbial stability and digestion.

These beneficial microbes aid in maintaining gut health and preventing the spread of pathogens. Studies on fermented feed additives have revealed that the effects of reduced intestinal pH, higher organic acid concentrations, and enhanced beneficial microbial activity are responsible for improved digestive efficiency and intestinal health [33,41]. Additionally, acidic conditions promote antimicrobial growth by inhibiting the proliferation of pathogenic bacteria. Studies that investigate the impact of organic acids and fermented feed products on poultry nutrition have revealed similar mechanisms [46,47].

4.5. Intestinal Histomorphometry

Although previous studies investigating probiotic and fermented feed supplementation predicted changes in intestinal morphology, the current study did not reveal any significant differences between treatments. Despite the results of previous studies on fermented feed additives, the results of the current study revealed no significant differences in intestinal histomorphological parameters such as villus height or crypt depth between treatments. However, this was not the case.

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Even so, there may be some indications of functional improvement in intestinal health despite the lack of morphological changes. Through biochemical and microbial mechanisms, digestion can be enhanced by increasing the activity of digestive enzymes, improving intestinal barrier function, and modulating the intestinal microenvironment [48–50]. The intestinal morphology may not exhibit any quantifiable structural changes, making these functional improvements unattainable.

The dietary treatments did not significantly affect parameters such as the villus height or the proportion of crypt depth to height. These observations may be influenced by several factors, such as the type of fermentation medium utilized, the level of inclusion of the additive, and the age of the broiler chickens during testing [48–50]. However, none of these observations were definitive. Importantly, the lack of morphological changes does not necessarily mean that intestinal health is improving. Enhanced intestinal efficiency may be achieved through biochemical and microbial mechanisms, such as enhanced enzyme activity or changes in the intestinal microenvironment, without the production of quantifiable structural changes. In the past, studies on fermented fruit byproducts revealed varying effects on intestinal morphology, with some studies indicating an increase in villus height and others showing no significant differences. This variation demonstrates how substrate composition, fermentation conditions and dietary inclusion levels play a role in determining the physiological responses of the gastrointestinal tract [51]. Importantly, banana peel represents a fibre-rich substrate containing phenolic compounds and fermentable carbohydrates that may act as prebiotic components, supporting beneficial microbial activity in the gut [52,54]. Fermentation further enhances these functional properties by generating bioactive metabolites that contribute to intestinal health and nutrient utilization [55,56].

5. Conclusions

In summary, by adding fermented banana peel powder produced from *Averrhoa bilimbi L.* fruit filtrate to the diet, broiler chickens were able to achieve better growth performance, better feed efficiency, and a better intestinal physiological environment without any negative effects on hematological or serum biochemical parameters. These outcomes imply that the fermented product is physiologically benign and can facilitate the efficient utilization of nutrients. In terms of mechanics, the results of this study may account for the positive effects obtained through synergistic interactions among bioactive compounds, organic acids and fermentation-derived metabolites, which affect the gut–metabolism–immunity angle. Enhanced intestinal pH, enhanced digestive processes, and improved physiological stress indicators are all factors involved in the maintenance of systemic physiological stability and the improvement of digestive efficiency through fermentation with fermented banana peel supplementation. The utilization of banana peel, a valuable underlying material in circular agriculture, is essential for the benefit of agricultural waste. The conversion of fruit-processing residues into functional feed additives not only enhances feed resource efficiency but also contributes to environmentally sustainable poultry production systems. *A. bilimbi* filtrate can be used to produce fermented banana peel powder, which has significant potential as a natural and eco-friendly feed additive that enhances broiler productivity and supports sustainable livestock production and the efficient use of unused hay and cellulose products.

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Institutional Review Board Statement: All experimental procedures involving animals, including animal handling, experimental treatments and sample collection, were conducted carefully in accordance with

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established animal welfare and ethical guidelines. The experimental protocol was reviewed and approved by the Animal Ethics Committee of the Faulty of Animal and Agricultural Sciences, Universitas Diponegoro, Ethics approval No. 61-12/A-32/KEP-FPP.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AGPs	Antibiotic growth promoters;
ANOVA	Analysis of variance;
BWG	Body weight gain;
$CaCO_3$	Calcium bicarbonate
CD	Crypt depth;
CFU	Colony forming unit;
Co	Cobalt;
CRD	Completely randomized design;
FCR	Feed conversion ratio;
BW	Body weight;
Fe	Ferum;
g	Grams;
H&E	Hematoxylin and eosin
Kcal	Kilocalorie;
HDL	High-density lipoprotein;
H/L	Heterophil-to-lymphocyte;
IBD	Infectious Bursal Disease;
IU	International units;
LDL	Low-density lipoprotein;
L	Linear;
LAB	Lactic acid bacteria;
LNE	Lactose-negative enterobacteria;
H/L	Heterophil to lymphocyte ratio;
MCH	Mean corpuscular hemoglobin;
MCHC	Mean corpuscular hemoglobin concentration;
MCV	Mean corpuscular volume;
mg	Miligrams;
Mn	Mangan;
MPV	Mean platelet volume;
MRS	de Man, Rogosa, and Sharpe;
NaCl	Natrium chloride;
ND	Newcastle Disease;
PDW	Platelet distribution width;
pH	Potential hydrogen;
Q	Quadratic regression effect;
RDW-CV	Red cell distribution width–coefficient of variation;
RDW-SD	Red cell distribution width–standard deviation;
SCFA	Short-chain fatty acids;
Se	Selenium;
SEM	Standard error of the mean;
SGOT	Serum glutamic oxaloacetic transaminase;
SGPT	Serum glutamic pyruvic transaminase;
VH	Villus height;

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VH/CH Villus height to crypt depth ratio;
Zn Zinc

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