RESEARCH PAPER

Synergistic Effects Of Mixed Fruit Peel-Derived Ecoenzymes On Growth Performance And Hematological Responses Of Broiler Chickens Fed Low-Protein Diets

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Abstract
This study investigated the synergistic effects of ecoenzymes derived from mixed fruit peels (orange, banana, and pineapple) on growth performance, bioactive compound content, and hematological responses of broiler chickens fed low-protein diets. The experiment was conducted in two stages: ecoenzyme production and characterization, followed by a 35-day feeding trial using 160 broilers arranged in a completely randomized design with four treatments: control (P0), low-protein diet (P1), low-protein diet + 2% ecoenzyme (P2), and low-protein diet + 4% ecoenzyme (P3). The mixed fruit peel ecoenzyme exhibited elevated total phenolic (120.63 mg GAE/g) and flavonoid content (186.7 mg QE/g), indicating enhanced antioxidant capacity. Ecoenzyme supplementation significantly improved growth performance, including feed intake, body weight gain, final body weight, and feed conversion ratio ($P < 0.05$), with the best results observed in P3. Furthermore, supplementation reduced the heterophil-to-lymphocyte (H/L) ratio, suggesting improved physiological status and reduced stress.
These findings demonstrate that multi-substrate ecoenzymes function as effective feed additives by enhancing nutrient utilization and physiological resilience under protein-restricted conditions. This study highlights the potential of ecoenzyme technology as a sustainable strategy for improving poultry production efficiency while supporting circular bioeconomy principles.

Keywords: *ecoenzyme; broiler; low-protein diet; growth performance; antioxidant; hematology*
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I. Introduction

The global poultry industry is under increasing pressure to improve production efficiency while simultaneously reducing feed costs and minimizing environmental impacts. Feed accounts for approximately 60–70% of total production costs, with dietary protein representing one of the most expensive components (Ravindran, 2013; Abdel-Wareth & Lohakare, 2021). Although protein plays a crucial role in muscle development, metabolic processes, and overall growth, reducing crude protein levels has become a strategic approach to lower feed costs and reduce nitrogen excretion, thereby improving environmental sustainability (Kumar et al., 2019; Attia et al., 2023; Santos et al., 2024). However, low-protein diets often impair growth performance, feed efficiency, and nutrient utilization due to amino acid imbalance and reduced digestive efficiency (Yulianto et al., 2021; Kiarie et al., 2024).

To overcome these limitations, the application of functional feed additives has gained considerable attention. Among these, fermentation-derived products have emerged as promising alternatives due to their ability to enhance nutrient digestibility, improve gut health, and modulate metabolic responses (Adeola & Cowieson, 2011; Gao et al., 2023). Ecoenzymes, produced through the fermentation of organic waste such as fruit peels, represent a novel and sustainable feed additive aligned with circular bioeconomy principles (Raharjo & Febrianto, 2023; Tallei et al., 2023). These products contain a complex mixture of hydrolytic enzymes, organic acids, and bioactive compounds, including phenolics and flavonoids, which may synergistically enhance digestive processes and metabolic efficiency (Widowati et al., 2021; Suleria et al., 2020; Arun & Sivashanmugam, 2015).

Despite growing interest, the application of ecoenzymes in poultry nutrition remains limited, particularly under low-protein feeding conditions. Previous studies have largely focused on single-substrate fermentation or general fermented products, without addressing the potential synergistic effects of combining multiple fruit peel substrates. Mixed fruit peels, such as orange, banana, and pineapple, differ substantially in their chemical composition, including fiber fractions, simple sugars, and phytochemical profiles (Dewi et al., 2021; Sagar et al., 2018). Their integration during fermentation may enhance nutrient diversity, bioavailability, and functional efficacy through synergistic interactions among substrates (Ismail et al., 2024).

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From a physiological perspective, the beneficial effects of ecoenzymes can be explained through several interacting mechanisms. Enzymes produced during fermentation facilitate the breakdown of complex feed components, thereby improving nutrient digestibility (Adeola & Cowieson, 2011). In addition, phenolic and flavonoid compounds exhibit strong antioxidant and antimicrobial properties, reducing oxidative stress and stabilizing gut microbiota (Suleria et al., 2020; Wang et al., 2023). Organic acids further contribute by lowering intestinal pH, enhancing enzyme activity, and inhibiting pathogenic bacteria (Gao et al., 2023; Liu et al., 2023). Collectively, these mechanisms improve nutrient utilization efficiency and support growth performance under protein-restricted conditions.

Although several studies have demonstrated the benefits of fermented feed additives in poultry production, a critical knowledge gap remains regarding the functional role of multi-substrate fermentation in determining ecoenzyme efficacy. In particular, the synergistic effects of combining different fruit peel substrates and their integrated impact on growth performance and physiological responses have not been comprehensively evaluated.

Therefore, this study aimed to evaluate the effects of ecoenzymes derived from mixed fruit peels on growth performance, feed efficiency, and hematological responses of broiler chickens fed low-protein diets. This research contributes to the development of sustainable and efficient feeding strategies by integrating waste valorization with nutritional innovation.

Novelty Statement

Unlike previous studies that primarily focused on single-substrate fermentation or general enzyme supplementation, this study provides the first evidence of synergistic effects derived from multi-substrate ecoenzyme fermentation using mixed fruit peels. The integration of biochemical characterization (phenolic and flavonoid content), growth performance, and hematological responses under low-protein dietary conditions offers a comprehensive understanding of the functional role of ecoenzymes in poultry nutrition. This approach bridges the gap between waste valorization and precision feeding strategies in sustainable animal production systems.

2. Materials and Methods

2.1. Experimental Design: This study was conducted in two sequential stages: (1) preparation and characterization of ecoenzymes derived from mixed fruit peels, and (2) evaluation of their effects on the growth performance and physiological responses of broiler chickens fed low-protein diets. The feeding trial was arranged in a completely randomized design (CRD) with four dietary treatments and four replicates per treatment.

2.2. Ethical Approval: All experimental procedures involving animals were conducted in accordance with institutional guidelines for animal care and use. The protocol was approved by the Animal Ethics Committee of Universitas Islam Kalimantan Muhammad Arsyad Al Banjari (UNISKA), Indonesia (Approval No.: 172/LPPM/UNISKA/IV/2025).

2.3. Stage 1: Preparation and Characterization of Ecoenzymes

Ecoenzymes were produced from mixed fruit peel waste consisting of orange (*Citrus sinensis*), banana (*Musa paradisiaca*), and pineapple (*Ananas comosus*). The substrates were combined with palm sugar and water at a ratio of 3:1:10 (w/v). The mixture was placed in airtight plastic containers and fermented for 21 days under ambient conditions (27–30°C) in a dark environment to facilitate microbial activity and enzymatic development.

After fermentation, the liquid fraction was filtered using muslin cloth to obtain the ecoenzyme extract. The extract was stored in airtight containers at room temperature until further analysis and use in feeding trials.

The ecoenzyme was analyzed for: (1) proximate composition (ash, crude fat, crude fiber, crude protein, and carbohydrates), (2) total phenolic content (TPC), and

(3) total flavonoid content (TFC).

Total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g sample, while total flavonoid content was measured using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g sample.

2.4. Stage 2: Feeding Trial on Broiler Chickens

Animals and Housing: A total of 160 seven-day-old broiler chicks were randomly assigned to four dietary treatments. Each treatment consisted of four replicates with 10 birds per replicate. Birds were housed in litter-floor pens under uniform environmental and management conditions. Feed and water were provided *ad libitum* throughout the experimental period. Standard husbandry practices, including sanitation and lighting programs, were applied consistently across all treatments.

Diet Formulation

The basal diet (P0) was formulated to meet standard nutrient requirements for broiler chickens, containing approximately:

Table 1. Ingredient and nutrient composition of experimental diets

Component	P0 (Control)	P1 (Low Protein)	P2 (LP + 2% Ecoenzyme)	P3 (LP + 4% Ecoenzyme)

Ingredient Composition (%)				
Corn	55.00	58.00	57.00	56.00
Soybean Meal	25.00	21.00	20.00	19.00
Rice Bran	10.00	12.00	12.00	12.00
Fish Meal	5.00	4.00	4.00	4.00
Palm Oil	2.50	2.50	2.50	2.50
Ecoenzyme	0.00	0.00	2.00	4.00
Limestone	1.00	1.00	1.00	1.00
Dicalcium Phosphate	0.80	0.80	0.80	0.80
Premix (vitamin–mineral)	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.20	0.20	0.20	0.20
Total (%)	**100**	**100**	**100**	**100**
Calculated Nutrient Composition				
Crude Protein (%)	21.50	19.00	19.00	19.00
Metabolizable Energy (kcal/kg)	3050	3050	3050	3050
Crude Fiber (%)	5.20	5.40	5.60	5.80
Crude Fat (%)	5.50	5.30	5.40	5.50
Ash (%)	6.00	6.10	6.20	6.30
Calcium (%)	0.90	0.90	0.90	0.90
Available Phosphorus (%)	0.45	0.45	0.45	0.45
Lysine (%)	1.20	1.05	1.05	1.05
Methionine (%)	0.50	0.45	0.45	0.45

The reduction in crude protein levels in P1–P3 was achieved by decreasing soybean meal inclusion and adjusting energy sources. Ecoenzyme supplementation (2% and 4%) was incorporated by partially replacing corn. Essential amino acids (DL-methionine and L-lysine) and mineral–vitamin premix were maintained constant across treatments to avoid confounding effects.

Dietary Treatments

The experimental treatments were defined as follows:

- **P0**: Basal diet with standard protein level (control)
- **P1**: Low-protein diet without ecoenzyme
- **P2**: Low-protein diet + 2% ecoenzyme
- **P3**: Low-protein diet + 4% ecoenzyme

The basal diet (P0) was formulated to meet standard nutrient requirements for broiler chickens, while the low-protein diets (P1–P3) were formulated by reducing soybean meal inclusion and adjusting energy sources. Ecoenzyme supplementation was incorporated by partially replacing corn in the diet. Essential amino acids (DL-methionine and L-lysine) and vitamin–mineral premix were maintained at constant levels across treatments to avoid confounding effects.

Measured Parameters

1. **Growth Performance :** Growth performance parameters included feed intake (g/bird), body weight gain (BWG; g/bird), final body weight (g/bird), average daily gain (ADG), and feed conversion ratio (FCR).
2. **Bioactive Compounds of Ecoenzymes:** Total phenolic content (TPC) and total flavonoid content (TFC) were measured as indicators of antioxidant capacity.
3. **Hematological Parameters:** The heterophil-to-lymphocyte (H/L) ratio was determined as an indicator of physiological stress and immune status. Blood smears were prepared and stained using Giemsa stain. Differential leukocyte counts were performed under a light microscope to determine heterophil and

lymphocyte proportions, and the H/L ratio was calculated as the ratio of heterophils to lymphocytes.

2.5. Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) based on a completely randomized design. When significant differences among treatments were detected ($P < 0.05$), Duncan's multiple range test was used to compare treatment means. In addition, linear regression analysis was performed to evaluate the relationship between ecoenzyme inclusion levels and growth performance parameters, particularly final body weight, to determine dose-dependent responses. Correlation analysis was also conducted to assess the relationship between bioactive compound content (TPC and TFC) and physiological responses (H/L ratio).

3. Results and Discussion

3.1. Nutrient Composition of Ecoenzymes from Fruit Peels

The proximate composition of ecoenzymes derived from different fruit peels (orange, banana, pineapple, and their mixture) is presented in Table 2. The results demonstrate clear variations among individual substrates, with the mixed fruit peel ecoenzyme exhibiting a more balanced and functionally advantageous nutrient profile compared to single-substrate ecoenzymes.

Table 2. Proximate composition of ecoenzyme solutions from various fruit peels

Parameter	Orange Peel	Banana Peel	Pineapple Peel	Mixed Peel
Ash (%)	7.1	7.1	5.4	7.2
Crude Fat (%)	2.9	3.8	3.1	3.6
Crude Fiber (%)	16.5	8.6	15.7	14.5
Crude Protein (%)	7.4	7.2	5.1	6.2
Carbohydrates (%)	52.4	48.5	58.6	54.7

The ash content, which reflects mineral availability, was highest in the mixed peel ecoenzyme (7.2%) and lowest in pineapple peel (5.4%). This suggests that the combination of substrates enhances mineral release during fermentation, likely due to synergistic degradation of plant cell wall structures. Increased mineral availability is essential for microbial metabolism and enzymatic activity during fermentation processes (Bampidis & Robinson, 2006; Tallei et al., 2023).

Crude fat content ranged from 2.9% to 3.8%, with banana peel exhibiting the highest value. Although relatively low, lipid fractions may act as supplementary energy sources for fermentative microorganisms and contribute to the functional characteristics of ecoenzymes (Sagar et al., 2018). Furthermore, lipid content may influence microbial growth dynamics and fermentation efficiency.

Crude fiber content was highest in orange peel (16.5%) and lowest in banana peel (8.6%), reflecting differences in structural carbohydrate composition, particularly pectin and cellulose fractions. Notably, the mixed peel ecoenzyme exhibited a reduced fiber content (14.5%), indicating partial degradation of structural polysaccharides during fermentation. This degradation process converts complex carbohydrates into simpler sugars and organic acids, thereby improving nutrient availability and digestibility (Adeola & Cowieson, 2011; Siregar et al., 2024).

Crude protein content ranged from 5.1% to 7.4%, with orange peel showing the highest value. The slightly lower protein content observed in the mixed peel ecoenzyme (6.2%) may be attributed to proteolytic activity during fermentation, where proteins are hydrolyzed into peptides and free amino acids. This transformation enhances nutrient bioavailability and improves the functional properties of ecoenzymes when used as feed additives (Ismail et al., 2024).

Carbohydrates were the dominant component across all treatments, ranging from 48.5% to 58.6%. Pineapple peel exhibited the highest carbohydrate content, likely due to its high concentration of simple sugars such as sucrose, glucose, and fructose. The relatively high carbohydrate content observed in the mixed peel ecoenzyme (54.7%) supports microbial fermentation, leading to the production of organic acids, enzymes, and other bioactive metabolites (Arun & Sivashanmugam, 2015; Tallei et al., 2023).

Overall, the mixed fruit peel ecoenzyme demonstrated a more balanced and functionally enhanced nutrient composition compared to single-substrate ecoenzymes. This finding highlights the importance of substrate diversity in fermentation systems, as combining different fruit wastes promotes synergistic interactions that improve nutrient complexity and bioactive compound availability. Such improvements are critical for enhancing digestive processes, nutrient utilization efficiency, and overall metabolic performance when ecoenzymes are applied as functional feed additives in poultry nutrition.

3.2. Phenolic and Total Flavonoid Content of Ecoenzymes

Total phenolic content (TPC) and total flavonoid content (TFC) are widely recognized as key indicators of antioxidant capacity in fermented products. These bioactive compounds play a crucial role in scavenging free radicals, reducing oxidative stress, and protecting cellular structures from oxidative damage (Suleria et al., 2020; Sagar et al., 2018; Wang et al., 2023). Therefore, the evaluation of TPC and TFC provides critical insight into the functional quality and bioactivity of ecoenzymes derived from fruit peel waste.

Table 3. Average total phenolic and flavonoid content of fruit peel ecoenzymes

Characteristic	Orange Peel	Banana Peel	Pineapple Peel	Mixed Peel
Total Phenolic (TPC, mg GAE/g)	72.7± 0.15	21.4± 0.45	11.6± 0.14	120.63± 0.14
Total Flavonoid (TFC, mg QE/g)	184.6± 0.22	8.2± 0.34	7.9± 0.25	186.7± 0.35

Among the individual substrates, orange peel exhibited the highest TPC (72.7 mg GAE/g) and TFC (184.6 mg QE/g), confirming its richness in phenolic and flavonoid compounds. This observation is consistent with previous studies indicating that citrus peels contain high concentrations of polyphenols and possess strong antioxidant activity compared to other tropical fruit residues (Suleria et al., 2020; Sagar et al., 2018). In contrast, banana and pineapple peels showed substantially lower TPC and TFC values, reflecting inherent differences in phytochemical composition and antioxidant potential.

Notably, the mixed fruit peel ecoenzyme exhibited a marked increase in both TPC (120.63 mg GAE/g) and TFC (186.7 mg QE/g), exceeding all single-substrate treatments. This result demonstrates a clear synergistic effect arising from the combination of different fruit peel substrates during fermentation. Each substrate contributes distinct phenolic compounds, and their interaction enhances both the diversity and extractability of bioactive constituents, resulting in a higher cumulative antioxidant capacity (Arun & Sivashanmugam, 2015; Ismail et al., 2024).

From a mechanistic perspective, the elevated TPC and TFC in mixed peel ecoenzymes can be attributed to enzymatic hydrolysis and microbial biotransformation processes during fermentation. Enzymes such as cellulases and pectinases degrade plant cell wall matrices, releasing bound phenolic compounds into soluble forms. Additionally, microbial metabolism can convert complex phenolic structures into simpler, more bioavailable compounds, thereby enhancing their antioxidant potential (Ismail et al., 2024; Liu et al., 2023).

The enhanced levels of phenolics and flavonoids in mixed peel ecoenzymes have important biological implications, particularly in poultry nutrition. These compounds contribute to the reduction of oxidative stress in intestinal tissues, modulation of gut microbiota, and improvement of nutrient absorption efficiency. Improved antioxidant status has been closely associated with enhanced immune response, reduced physiological stress, and improved growth performance in broiler chickens (Adeola & Cowieson, 2011; Gao et al., 2023).

Overall, these findings indicate that multi-substrate fermentation significantly enhances antioxidant capacity through synergistic enrichment of phenolic and flavonoid compounds. This functional improvement represents a key advantage of mixed fruit peel ecoenzymes and supports their application as effective natural feed additives for improving broiler performance under low-protein dietary conditions.

3.3. Effect of Ecoenzyme Supplementation on Growth Performance

This study evaluated the effects of ecoenzyme supplementation in low-protein diets on broiler growth performance over a 35-day rearing period. The results are presented in Table 4.

Table 4. Feed intake, body weight gain (BWG), final body weight, and feezconversion ratio (FCR) of broiler chickens

Treatment	Total Feed Intake (g/bird/35d)	BWG (g/bird)	Final Weight (g/bird)	FCR
P0	3600± 21.16	1160± 5.17	1200± 8.45	3.10± 0.25
P1	3200± 10.32	960± 4.22	1000± 11.45	3.33± 0.14
P2	3400± 12.24	1160± 2.22	1200± 6.45	2.93± 0.42
P3	3500± 48.5	1260± 3.12	1300± 10.45	2.78± 0.17

Reducing dietary protein levels negatively affected broiler performance, as evidenced by the lower feed intake, reduced body weight gain, and higher feed conversion ratio observed in the P1 group compared to the control (P0). Specifically, birds in P1 exhibited a substantial reduction in final body weight (1000 g) compared to P0 (1200 g), indicating impaired growth performance under protein-restricted conditions. This decline is primarily associated with insufficient amino acid availability, which limits protein synthesis, muscle accretion, and metabolic regulation of appetite (Kumar et al., 2019; Abdel-Wareth & Lohakare, 2021; Santos et al., 2024).

In contrast, ecoenzyme supplementation effectively mitigated these adverse effects. Birds receiving ecoenzymes (P2 and P3) showed significant improvements in growth performance parameters. Notably, the P3 group (4% ecoenzyme) achieved the highest final body weight (1300 g) and the lowest FCR (2.78), outperforming both the low-protein group (P1) and the control (P0). This finding indicates that ecoenzyme supplementation not only compensates for reduced protein levels but can also enhance growth performance beyond conventional feeding conditions.

A dose-dependent response was clearly observed, where increasing ecoenzyme inclusion levels corresponded to progressive improvements in growth performance. Linear regression analysis further demonstrated a positive relationship between ecoenzyme inclusion level and final body weight ($R^2 = 0.63$), indicating that higher levels of ecoenzyme supplementation are associated with increased growth efficiency. This pattern confirms that ecoenzymes act in a concentration-dependent manner, optimizing nutrient utilization under protein-restricted conditions.

From a nutritional perspective, these improvements can be attributed to enhanced nutrient digestibility. Ecoenzymes contain hydrolytic enzymes produced during fermentation, which facilitate the breakdown of complex feed components such as fiber and protein into simpler, more absorbable forms. This process increases the availability of amino acids and energy required for growth (Adeola & Cowieson, 2011; Kiarie et al., 2024). Furthermore, organic acids present in ecoenzymes likely improved gut conditions by lowering intestinal pH, thereby enhancing endogenous enzyme activity and inhibiting pathogenic microorganisms. A more stable gut environment supports nutrient absorption and reduces energy losses associated with immune activation and microbial imbalance (Gao et al., 2023; Liu et al., 2023).

Another contributing factor is the high content of phenolic and flavonoid compounds in mixed fruit peel ecoenzymes. These bioactive compounds exert antioxidant effects, reducing oxidative stress and maintaining intestinal integrity. Improved antioxidant status has been associated with enhanced nutrient utilization efficiency and improved growth performance in broiler chickens (Wang et al., 2023).

The improved feed conversion ratio observed in ecoenzyme-supplemented groups, particularly in P3, further supports the role of ecoenzymes in enhancing metabolic efficiency. Under low-protein conditions, birds typically require more feed to achieve comparable growth. However, ecoenzyme supplementation improved feed utilization efficiency, allowing birds to convert nutrients into body mass more effectively.

Importantly, the superior performance observed in the mixed fruit peel ecoenzyme treatment (P3) suggests a strong synergistic interaction among substrates during fermentation. Unlike single-substrate ecoenzymes, the combination of orange, banana, and pineapple peels provides a broader spectrum of carbohydrates, fiber fractions, and phytochemicals, which enhances microbial diversity and enzymatic activity during fermentation.

This synergistic interaction likely results in a more complex enzymatic profile, including cellulases, proteases, and amylases, which collectively improve the degradation of feed components. In addition, the diversity of phenolic compounds derived from different substrates may exert complementary antioxidant effects, thereby enhancing intestinal health and nutrient absorption more effectively than single-source ecoenzymes.

Therefore, the improved growth performance observed in this study is not solely attributable to enzyme activity or antioxidant capacity alone, but rather to the combined and interactive effects of multiple bioactive components generated through multi-substrate fermentation.

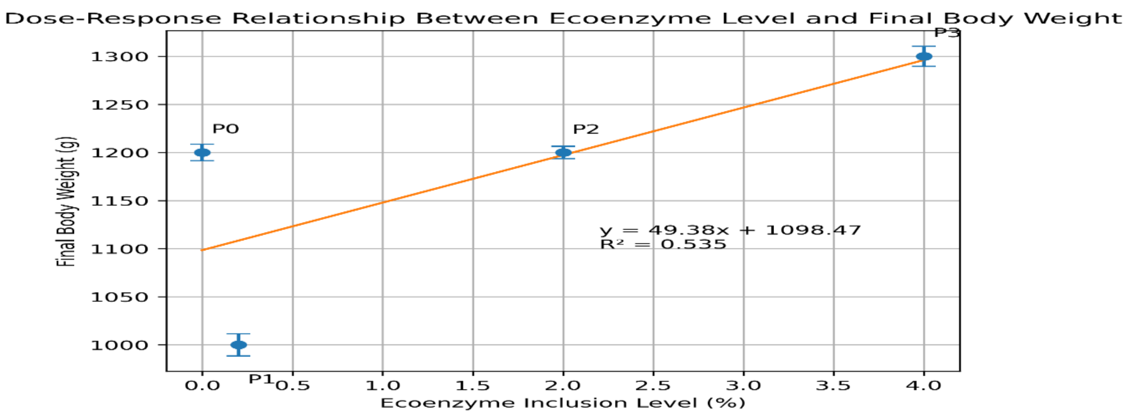

Figure 1. Relationship between ecoenzyme inclusion level and final body weight of broiler chickens. Error bars represent standard deviation. A positive dose-dependent response was observed, indicating improved growth performance with increasing ecoenzyme supplementation.

3.4. Hematological Profile of Broiler Chickens

The hematological parameters of broiler chickens under different dietary treatments are presented in Table 5.

Table 5. Hematological profile of broiler chickens

Parameter	Hematocrit (%)	Hemoglobin (%)	Heterophils (%)	Lymphocytes (%)	H/L Ratio

P0	28.11[a]	18.05[a]	4.50	9.00	0.49[a]
P1	27.11[b]	17.41[b]	4.25	8.50	0.50[a]
P2	26.98[c]	16.90[c]	3.75	9.50	0.42[a]
P3	26.43[c]	16.67[c]	3.00	7.75	0.38[b]

Different superscripts within the same column indicate significant differences ($P < 0.05$).

Hematocrit and Hemoglobin

Hematocrit and hemoglobin values showed a decreasing trend with reduced dietary protein levels, with the highest values observed in the control group (P0) and the lowest in P3. Specifically, hematocrit decreased from 28.11% (P0) to 26.43% (P3), while hemoglobin declined from 18.05% to 16.67%. This reduction is likely associated with decreased protein availability, which plays a critical role in erythropoiesis and hemoglobin synthesis (Kumar et al., 2019; Santos et al., 2024).

Despite this decline, all hematological values remained within the normal physiological range for broiler chickens, indicating that ecoenzyme supplementation did not induce pathological conditions. The relatively stable hematological profile suggests that improved nutrient digestibility and bioavailability associated with ecoenzyme supplementation may partially compensate for reduced protein intake, thereby supporting metabolic processes, including blood formation (Adeola & Cowieson, 2011; Kiarie et al., 2024).

Heterophils, Lymphocytes, and H/L Ratio

The heterophil-to-lymphocyte (H/L) ratio is widely recognized as a sensitive indicator of physiological stress and immune status in poultry (Gross & Siegel, 1983; Maxwell, 1993). In the present study, the highest H/L ratio was observed in the P1 group (0.50), indicating that low-protein diets without supplementation induced physiological stress.

This finding is consistent with previous studies demonstrating that nutritional stress and oxidative imbalance can elevate glucocorticoid secretion, leading to increased heterophil counts and reduced lymphocyte activity, thereby increasing the H/L ratio (Wang et al., 2023; Liu et al., 2023).

In contrast, ecoenzyme supplementation significantly reduced the H/L ratio, with the lowest value observed in P3 (0.38). This result indicates that ecoenzymes effectively alleviated physiological stress and improved immune balance. The magnitude of reduction from P1 (0.50) to P3 (0.38) further confirms the biological relevance of ecoenzyme supplementation under protein-restricted conditions.

Correlation analysis further revealed that total phenolic and flavonoid contents were positively associated with growth performance and negatively correlated with the H/L ratio. This indicates a functional relationship between antioxidant capacity and physiological status, where improved oxidative balance contributes to enhanced growth efficiency and reduced stress levels.

The reduction in H/L ratio observed in this study also suggests that ecoenzyme supplementation mitigates nutritional stress associated with low-protein diets. Protein deficiency is known to impair immune competence and increase physiological stress in broilers; therefore, the ability of ecoenzymes to counteract these effects highlights their dual role as both digestive enhancers and immunomodulatory agents.

Mechanistic Interpretation: Antioxidant and Immunomodulatory Effects

The improvement in hematological parameters, particularly the reduction in H/L ratio, can be attributed to the antioxidant and immunomodulatory properties of ecoenzymes. Ecoenzymes derived from mixed fruit peels contain high levels of phenolic and flavonoid compounds, which reduce oxidative stress by neutralizing reactive oxygen species (ROS). Oxidative stress is a major factor impairing immune function and increasing susceptibility to disease in poultry (Wang et al., 2023).

The reduction of oxidative stress contributes to stabilization of immune cell function, decreased heterophil proliferation, and maintenance of lymphocyte activity. In addition, bioactive compounds and organic acids produced during fermentation improve gut health and microbiota balance, which play a critical role in immune regulation. A healthier intestinal environment reduces pathogen load and systemic stress, thereby contributing to a lower H/L ratio (Liu et al., 2023; Kiarie et al., 2024).

Integration with Growth Performance

The improved hematological profile observed in ecoenzyme-supplemented groups is consistent with the enhanced growth performance reported in this study. Birds in P3 not only exhibited the lowest H/L ratio but also achieved the highest final body weight and the most efficient feed conversion ratio.

This relationship highlights the close interaction between physiological stress, immune function, and production performance. Reduced stress levels allow nutrients and energy to be utilized more efficiently for growth rather than being diverted to stress responses, thereby improving overall production efficiency.

Overall, these findings provide strong evidence that multi-substrate fermentation represents a more effective strategy than single-substrate approaches in producing functional feed additives. The synergistic interaction among different fruit peel substrates enhances enzymatic complexity and bioactive compound

availability, which collectively improves nutrient utilization efficiency and physiological resilience.
This concept represents a shift from conventional single-additive approaches toward multifunctional feed systems that integrate nutritional, metabolic, and immunological benefits into a single strategy for sustainable poultry production.

4.Conclusion

Ecoenzyme supplementation derived from mixed fruit peels significantly improved growth performance and reduced physiological stress in broiler chickens fed low-protein diets. The 4% inclusion level produced the most optimal results, demonstrating improved feed efficiency and immune balance. These findings highlight the potential of ecoenzymes as a sustainable and functional feed additive for optimizing broiler productivity under protein-restricted conditions.
These findings provide a scientific basis for the application of ecoenzymes in sustainable poultry production systems.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Author Contributions

All authors contributed to the study design, data analysis, and manuscript preparation.

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