

# Hematological blood profile of Kampung Chicken fed diets containing fermented coffee husk

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**Abstract.** Coffee husk is a potential alternative feed ingredient for poultry; however, its application is limited by its low protein content and high crude fiber levels. Fermentation is a promising strategy to improve the nutritional quality of such by-products. This study aimed to evaluate the effects of fermented coffee husk (FCH) on the hematological profile of grower-phase Kampung chickens. A Completely Randomized Design (CRD) was used, consisting of four treatments with four replications. The treatments were T0 (100% basal feed), T1 (90% basal feed + 10% unfermented coffee husk), T2 (90% basal feed + 10% FCH fermented with *Saccharomyces cerevisiae*), and T3 (90% basal feed + 10% FCH fermented with EM4). Hematological variables measured included erythrocyte count, leukocyte count, hemoglobin concentration, hematocrit, and platelet count. Although no significant differences were observed among treatments, the inclusion of FCH tended to improve hematological parameters compared with the control. Fermented treatments showed a positive trend in supporting physiological health and blood profile improvement. These findings indicate that FCH has potential as a sustainable local feed ingredient for Kampung chicken production.

## 1 Introduction

Kampung chickens are poultry commodities that produce meat and eggs and are highly favored by the Indonesian population. This preference is largely due to the distinctive taste and texture of their meat compared to other poultry breeds. Kampung chickens are widely recognized for their distinctive savory meat characteristics and their high adaptability to a range of environmental conditions.

Despite these advantages, Kampung chickens face several limitations, including relatively low productivity, strong brooding behavior in hens, and a low number of eggs produced in each laying period. Furthermore, their daily weight gain and final body weight are generally lower compared to commercial breeds. Therefore, improvements in

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management practices and the provision of high-quality feed are essential to enhance the performance and productivity of Kampung chickens.

Efforts to improve the productivity and population of Kampung chickens are necessary to meet the increasing demand for affordable animal protein among the population. The high consumer interest in Kampung chickens as a source of protein from meat and eggs presents a promising opportunity for developing this commodity. However, the rising cost of poultry feed remains a major challenge for farmers in providing high-quality nutrition. Consequently, alternative feed strategies are required, particularly through the use of locally available feed ingredients that do not compete with human food sources.

By-products from coffee cultivation have the potential to serve as alternative solutions to address the limitations in poultry feed availability. One such by-product is coffee husk, which can be utilized as a feed ingredient for poultry. However, its use is constrained by its low protein content and high crude fiber levels, which limit its digestibility and nutritional value. To overcome these limitations, fermentation technology can be applied to enhance the nutritional quality of coffee husks, making them more suitable as poultry feed.

Fermentation is a biological feed processing technology that involves microbial activity to improve the nutritional value of low-quality feed materials. Fermented products typically have a longer shelf life. The fermentation process enhances the nutritional profile of feed by chemically modifying organic compounds such as carbohydrates, fats, proteins, crude fiber, and other organic substances under aerobic or anaerobic conditions, through enzymatic activity produced by microbes. Through this process, components that are initially difficult to digest become more easily digestible for the chicken, thereby improving feed efficiency and nutrient absorption [1].

Livestock productivity is a critical aspect directly related to the quantity and quality of the products generated. Optimal productivity can be achieved when animals are provided with high-quality feed. Such feed improves feed intake, especially protein consumption. Protein plays a crucial role in supporting the productivity and health of Kampung chickens [2].

The health status of kampung chickens can be assessed through blood hematological profiles, which reflect the chickens' physiological condition and internal health. Hematological parameters such as red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, and erythrocyte indices (MCV, MCH, and MCHC) are important indicators used to evaluate the animal's response to nutritional intake and environmental factors. Providing nutritionally balanced feed, particularly with adequate and digestible protein, supports blood cell production and maintains a robust immune system in poultry.

In this context, the use of fermented coffee husk as a feed ingredient, with improved nutritional value, has the potential to not only enhance the productivity of Kampung chickens but also positively affect their hematological profiles. Previous studies on coffee by-products in poultry nutrition have primarily addressed growth performance, digestibility, gut morphology, and antioxidant or immunomodulatory responses: for instance, dietary supplementation with coffee pulp extract improved growth performance and intestinal morphology in broiler chickens [3] and supplementation of coffee cherry pulp extract enhanced growth performance, modified serum biochemistry, improved gut microbiota, and upregulated immune- and antioxidant-related gene expression [4]. In contrast, very limited research has examined specific effects on hematological parameters (e.g., RBC, WBC, Hb, Hct, MCV/MCH/MCHC) in local or indigenous chickens. Meanwhile, fermentation of coffee by-products has been demonstrated to significantly enhance peptide release, phenolic content, antioxidant capacity, and digestibility under simulated chicken gastrointestinal conditions [3], which supports the hypothesis that fermenting coffee husk could reduce anti-nutrients (such as high crude fiber or tannins) while increasing essential amino acids and bioactive compounds. Such improvements may potentially support protein synthesis, blood

cell production, and immune function in poultry, making fermented coffee husk a promising sustainable feed alternative worthy of in vivo investigation.

The novelty of this study lies in the utilization of fermented coffee husk as a functional feed component within a locally based integrated crop–livestock system. This approach aims to improve growth performance in local chickens and enhance hematological profiles as indicators of internal health. Research on the effects of fermented coffee husk on the blood parameters of Kampung chickens remains very limited. Therefore, the findings of this study are expected to contribute new scientific insights into the development of sustainable and locally adaptable alternative feed systems.

## 2 Material Methods

### 2.1 Research Time and Location

The research was conducted from June to September 2024 in Palu City, Central Sulawesi, Indonesia. Kampung chickens were reared in Loru Village, Sigibiromaru District, Sigi Regency, while the fermentation process was carried out at the Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry and Fisheries, Tadulako University.

### 2.2 Research Methodology

This study employed an experimental method using a Completely Randomized Design (CRD). There were four treatments, each replicated four times. The treatments consisted of basal feed and feed containing coffee husk, either unfermented or fermented using different types of microorganisms. The data obtained were analyzed using analysis of variance (ANOVA). If significant differences were found among treatments, the analysis was followed by an Honestly Significant Difference (HSD) test. The treatments in this study were as follows:

T0: 0% coffee husk (100% basal feed)

T1: 10% unfermented coffee husk + 90% basal feed

T2: 10% coffee husk fermented with *Saccharomyces cerevisiae* 90% basal feed

T3: 10% coffee husk fermented with Effective Microorganisms 4 (EM4) + 90% basal feed

### 2.3 Fermentation Process

The fermentation of coffee husk was carried out using lactic acid bacteria (LAB) contained in Effective Microorganisms 4 (EM4) and yeast (*Saccharomyces cerevisiae*). The fermentation was conducted under aerobic conditions by mixing the coffee husk with water until reaching a moisture content of 50–60%, followed by the addition of *Saccharomyces cerevisiae* and LAB at a dosage of 0.5–1% of the dry matter weight of each mixture. The mixture was thoroughly stirred and left in an open container or a container with good air circulation to support aerobic microbial activity. The fermentation process lasted 14 days at room temperature (25–30°C). After fermentation, the material was ground and incorporated as a component of the Kampung chicken feed formulation. As shown in Table 1, aerobic fermentation improved the nutritional value of coffee husk by enhancing nutrient availability and reducing antinutritional compounds.

### 2.4 Nutrient Content of Fermented Coffee Husk

**Table 1.** Proximate analysis result of fermented coffee husk.

Samples	Water content (%)	Crude fiber (%)	Crude protein(%)	Crude fiber (%)	Ash (%)
EM 4	10.39	15.06	19.76	7.47	6.01
Saccharomyces cerevisiae	11.31	16.41	17.23	8.34	6.89

Notes: The result of the proximate analysis is Animal Nutrition and Feed laboratory, Faculty of Animal Husbandry and Fisheries, Tadulako University 2024.

## 2.5 Management of Kampung Chicken

This study utilized 32 grower-phase Kampung chickens, housed in 16 cage units with two chickens per cage. Grower-phase chickens were chosen because their physiological and hematological parameters are more stable and less influenced by early developmental fluctuations, enabling clearer interpretation of dietary treatment effects. Pair housing was implemented to reduce social isolation stress, as Kampung chickens exhibit natural social behavior and may experience elevated corticosterone levels when kept alone. Maintaining birds in pairs helps stabilize hormonal stress responses and prevents physiological alterations that could influence hematological outcomes. Each cage was equipped with a feeder and a drinking container. Before the start of the experiment, the chickens underwent a one-week adaptation period during which commercial feed was gradually replaced with the experimental diets. After the adaptation period, initial body weights were recorded for further analysis.

## 2.6 Diet Administration

The prepared diet, known as the basal diet, was provided to the Kampung chickens twice daily, once in the morning and once in the afternoon. The basal diet is a mixture of feed ingredients formulated to meet the nutritional requirements of Kampung chickens during the grower phase. It consists of corn as the primary source of carbohydrates and energy, rice bran as a source of fiber and additional nutrients, soybean meal as a plant-based protein source to support muscle development, and fish meal as an animal-based protein source rich in essential amino acids. Additionally, vegetable oil was included as a fat source to provide extra energy and enhance vitamin absorption. These ingredients were mixed in specific proportions to produce a ration that meets the nutritional needs of Kampung chickens. The composition of the feed ingredients and the nutrient content of the ration are presented in Tables 2 and 3.

**Table 2.** Composition and nutrient content of the diet.

Feed ingredients	T0	T1	T2	T3
Yellow corn	46	42	42	42
Rice bran	38	33	33	33
Fermented coffee husk	0	10	10	10
Soybean meal	9	8	8	8
Fish meal	5	5	5	5
Oil	1	1	1	1
CaCO3	1	1	1	1
<b>Total</b>		<b>100</b>	<b>100</b>	<b>100</b>
Nutrient content of the diet				
Crude protein (%)	14.77	14.67	15.2	15.55

Crude fiber (%)	3.94	5.67	4.30	4.25
Crude fat (%)	3.53	4.91	4.91	4.82

**Table 3.** Nutrient requirements of Kampung chickens according to SNI 2013.

Nutrient content	Chicken Age (Week)		
	0-3	4-6	7-10
Metabolizable Energy (ckal/kg)	2.900.00	2.800.00	2.600.00
Protein (%)	18-19.00	15-17.00	12-14.00
Crude Fat (%)	4-6.00	4-6.00	4-6.00
Crude Fiber (%)	4.00	4-6.00	4-6.00
Calcium (%)	0.90	0.90	1-1.20
Methionine (%)	0.45	0.30	0.30
Lysin (%)	0.90	0.90	0.65

### 2.7 Blood Sampling Procedure

Blood samples were collected at the end of the study, with sampling conducted in the morning. The blood was collected using the dorsal recumbency position, in which the chicken was laid on its back. Approximately 3 mL of blood was drawn from the brachial vein and transferred into an ethylene diamine tetra-acetic acid (EDTA) tube. The tube was then gently shaken and stored in a cool box for transportation to the laboratory for further analysis. The observed blood parameters included leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets/thrombocytes.

## 3 Result and Discussion

The hematological blood profile of Kampung chickens fed diets containing fermented and unfermented coffee husks is presented in Table 4. These data reflect the chickens' hematological status in response to the different dietary treatments.

**Table 4.** Hematological Blood Profile of Kampung Chicken Fed Diets Containing Fermented and Unfermented Coffee Husk.

Treatments	Variables				
	Leucocyte	Erythrocytes	Hemoglobin in	Hematocrit	Platelet/thrombocytes
T0 <sup>ns</sup>	36.100±11.6	0.6±0.2	2.9±0.7	9.4±3.3	9.000±2.800
T1 <sup>ns</sup>	51.350±25.4	0.8±0.2	4.0±1.2	12.3±3.4	19.500±12.020
T2 <sup>ns</sup>	64.450±3.1	1.0±0.09	5.2±0.5	13.4±0.8	22.500±6.363
T3 <sup>ns</sup>	28.800±21.5	1.4±1.0	7.3±5.3	19.9±14.4	11.000±1.414

Notes: T0 basal diet, T1 10% unfermented coffee husk, T2 10% coffee husk fermented with *Saccharomyces cerevisiae* + 90% basal feed, T3 10% coffee husk fermented with Effective Microorganisms 4 (EM4) + 90% basal feed.

### 3.1 Leukocyte (mikroliter)

The results of the study showed that the addition of unfermented and fermented coffee husk had no significant effect ( $P > 0.05$ ) on the white blood cell (leukocyte) count of Kampung chickens, although there was a tendency for increased leukocyte counts in treatments P1 and P2, and a decrease in treatment P3. In the control treatment (T0) with basal feed, the leukocyte

count was recorded at  $36.1 \pm 11.6$ . The addition of 10% unfermented coffee husk in treatment P1 increased the leukocyte count to  $51.4 \pm 25.4$ . The leukocyte count further increased in treatment P2, which included 10% coffee husk fermented with *Saccharomyces cerevisiae*, reaching  $64.5 \pm 3.1$ . However, in treatment P3, with the addition of 10% coffee husk fermented with lactic acid bacteria (LAB), the leukocyte count decreased to  $28.8 \pm 21.5$ , which was lower than all other treatments.

Treatment T0 (basal feed without coffee husk supplementation) showed a leukocyte count of  $36.1 \pm 11.6$ . This value falls within the normal leukocyte range in chickens, as stated by [5], who reported that the normal leukocyte count in chickens ranges from  $20\text{--}40 \times 10^3/\text{mm}^3$ . This indicates that the basal feed provided sufficient nutrition to support the immune function of Kampung chickens. It may also reflect that the chickens were not under stress or exposed to pathogenic infections. These findings are consistent with [6], who reported that high-quality basal feed helps maintain balanced hematological parameters in chickens. Therefore, the basal feed serves as an important control for evaluating the impact of dietary treatments on immune response.

Treatment T1, which involved the addition of 10% unfermented coffee husk, increased the leukocyte count to  $51.4 \pm 25.4$ . Although this increase was not statistically significant ( $P > 0.05$ ), the result suggests that coffee husk may stimulate the immune response through its bioactive components. Phenolic compounds and caffeine present in coffee husk are believed to act as antioxidants that support immune cell activity, as reported by Intan et al. [7]. Similarly, a study by [8] noted that feed ingredients containing bioactive compounds can increase leukocyte counts without causing physiological disturbances. However, the effects of unfermented coffee husk require further investigation to ensure that the observed increase in leukocytes is not a result of stress responses triggered by potential antinutritional factors that may still be present in the coffee husk.

In treatment P2, which involved the addition of 10% coffee husk fermented with *Saccharomyces cerevisiae*, the leukocyte count further increased to  $64.5 \pm 3.1$ . Fermentation with *S. cerevisiae* is known to enhance the nutritional value of feed ingredients by degrading antinutritional compounds and producing bioactive substances that support animal health. According to [9], *S. cerevisiae* acts as a probiotic that not only promotes intestinal microbial balance but also stimulates the body's immune activity. This finding is consistent with a study by [10], which demonstrated that yeast fermentation can improve the digestibility and nutrient availability of feed materials. The increased leukocyte count in this treatment indicates that fermentation effectively modifies coffee husk into a more beneficial feed component.

However, in treatment P3, which involved the addition of 10% coffee husk fermented with lactic acid bacteria (LAB), a decrease in leukocyte count was observed, reaching  $28.8 \pm 21.5$ . This reduction is presumed to be caused by fermentation metabolites such as lactic acid, which, at high concentrations, may exert immunosuppressive effects or alter the balance of intestinal microflora. According to [11], LAB can produce antimicrobial compounds that potentially inhibit the growth of pathogenic bacteria, but under certain conditions, they may also affect the overall metabolism of chickens. Furthermore, the complex interactions between fermentation metabolites and the bioactive components of coffee husk may result in varying effects on the immune system. This decrease in leukocyte count indicates the need for further investigation to determine the optimal dosage and fermentation process.

Overall, the results of this study indicate that the addition of coffee husk, either unfermented or fermented, had no significant effect on the leukocyte count of Kampung chickens ( $P > 0.05$ ). However, the observed variations suggest the potential of bioactive components in coffee husk to modulate the immune system. Further research is needed to understand the mechanisms of action and long-term effects of using coffee husk as an alternative feed ingredient. In-depth studies on the fermentation process, the types of

microorganisms used, and their effects on physiological parameters and overall poultry health are also essential to optimize its potential benefits.

### 3.2 Erythrocytes (liter)

The results of the study showed that the addition of coffee husk, both unfermented and fermented, had no significant effect ( $P > 0.05$ ) on the red blood cell (erythrocyte) count of Kampung chickens. However, the data indicated a trend of increasing erythrocyte counts compared to the control, with the highest value observed in treatment P3. In the control group (T0) receiving only basal feed, the erythrocyte count was recorded at  $0.6 \pm 0.2$ . The addition of 10% unfermented coffee husk in treatment P1 increased the erythrocyte count to  $0.8 \pm 0.2$ . The count continued to rise in treatment P2, which included 10% coffee husk fermented with *Saccharomyces cerevisiae*, reaching  $1.0 \pm 0.09$ . The highest increase was observed in treatment P3, which included 10% coffee husk fermented with lactic acid bacteria (LAB), resulting in an erythrocyte count of  $1.4 \pm 1.0$ .

The present study revealed that dietary treatments with coffee husk, either unfermented or fermented, did not significantly affect ( $P > 0.05$ ) the erythrocyte count of Kampung chickens. The erythrocyte values ranged from  $0.6 \pm 0.2 \times 10^6/\mu\text{L}$  in the control group (T0) to  $1.4 \pm 1.0 \times 10^6/\mu\text{L}$  in the treatment with 10% fermented coffee husk using lactic acid bacteria (T3). Although statistical analysis revealed no significant differences among treatments, a biological trend was observed, where the supplementation of coffee husk, particularly in fermented forms, tended to increase erythrocyte counts compared to the control diet.

The erythrocyte values observed in the present study were lower than the normal reference range for healthy chickens, which is reported to be approximately  $2.5\text{--}3.5 \times 10^6/\mu\text{L}$  [12]. This discrepancy may be attributed to differences in breed (local chickens versus commercial broilers or layers), age, physiological status, or environmental conditions during the experiment. Additionally, nutritional factors such as iron availability, vitamin B12, and folate, which are crucial for erythropoiesis, might have contributed to the relatively low erythrocyte counts.

Although the observed trend lacks statistical significance, the increasing trend in treatments with fermented coffee husk suggests a potential improvement in hematopoietic function. Fermentation is known to enhance the bioavailability of nutrients and reduce anti-nutritional factors, which could support red blood cell production. However, further studies with larger sample sizes and optimized fermentation processes are required to validate this finding and to achieve erythrocyte values closer to the established normal range.

### 3.3 Hemoglobin (g/dL)

The results showed an increasing trend in hemoglobin concentration from the basal diet ( $2.9 \pm 0.7$  g/dL) to the addition of 10% unfermented coffee husk ( $4.0 \pm 1.2$  g/dL), fermented with *Saccharomyces cerevisiae* fermentation ( $5.2 \pm 0.5$  g/dL), and with Effective Microorganisms fermentation ( $7.3 \pm 5.3$  g/dL). However, statistical analysis revealed no significant differences among treatments ( $P > 0.05$ ). The absence of significance might be attributed to the relatively high variability, particularly in the EM group, which showed large standard deviations.

The present study revealed that the hemoglobin concentration in Kampung chickens fed the basal diet (T0) was  $2.9 \pm 0.7$  g/dL, which is considerably lower than the physiological range reported for healthy chickens, 7–13 g/dL [13]. This indicates a state of anemia, most likely associated with the nutritional inadequacy of the basal diet in providing essential micronutrients, such as iron, folic acid, and vitamin B12, which are required for erythropoiesis.

The inclusion of 10% unfermented coffee husk (T1) resulted in a slight increase in hemoglobin concentration ( $4.0 \pm 1.2$  g/dL), suggesting that coffee husk contains bioactive compounds and nutrients that may contribute to hematological improvement. However, the presence of anti-nutritional factors such as tannins and lignin likely limited nutrient bioavailability, thereby reducing its effectiveness.

Fermentation of coffee husk improved its nutritional quality, as reflected in the higher hemoglobin concentrations recorded in T2 ( $5.2 \pm 0.5$  g/dL) and T3 ( $7.3 \pm 5.3$  g/dL). Solid-state fermentation (T2) using *Saccharomyces cerevisiae*, either alone or in combination with *Aspergillus niger*, has been reported to reduce anti-nutritional compounds such as caffeine and tannins by up to 90% and 57%, respectively, while simultaneously increasing crude protein content to more than 10% [14, 15]. These improvements may enhance vitamin B-complex availability, which is crucial for hemoglobin synthesis. Meanwhile, the use of Effective Microorganisms (EM) (T3) provided a mixed microbial consortium, which may have synergistically improved nutrient digestibility and iron bioavailability, thereby supporting better hematological profiles.

Although the ANOVA results indicated no significant differences among treatments ( $P > 0.05$ ), the observed biological trend suggests that fermented coffee husk, particularly with EM, has potential in improving hemoglobin concentration in Kampung chickens. The lack of significance may be attributed to high individual variation, especially in the EM group, as shown by its large standard deviation.

### 3.4 Hematocrit (%)

The results showed that the dietary treatments had a non-significant effect ( $P > 0.05$ ) on the hematocrit levels of Kampung chickens. In the T0 treatment (basal diet), the hematocrit value was recorded at  $9.4 \pm 3.3$ . The inclusion of 10% unfermented coffee husk in the P1 treatment increased the hematocrit value to  $12.3 \pm 3.4$ . A further increase was observed in the P2 treatment, which included 10% coffee husk fermented with *Saccharomyces cerevisiae*, resulting in a hematocrit value of  $13.4 \pm 0.8$ . The highest increase occurred in the P3 treatment, where the addition of 10% coffee husk fermented with lactic acid bacteria (LAB) produced a hematocrit value of  $19.9 \pm 14.4$ .

The hematocrit values observed in this study were relatively lower than the normal physiological range for healthy chickens (22–35%). In the basal diet group (T0), hematocrit was only  $9.4 \pm 3.3\%$ , indicating severe anemia, most likely due to nutritional inadequacy, particularly in iron and vitamin B-complex, which are essential for erythropoiesis.

A gradual increase was observed with the inclusion of 10% coffee husk (T1 =  $12.3 \pm 3.4\%$ ) and fermented coffee husk with *Saccharomyces cerevisiae* (T2 =  $13.4 \pm 0.8\%$ ). This suggests that fermentation could improve nutrient bioavailability by reducing anti-nutritional factors such as tannins, thereby enhancing iron absorption and erythrocyte formation.

The highest value was recorded in T3 ( $19.9 \pm 14.4\%$ ) with Effective Microorganisms (EM) fermentation. Although still below the lower limit of the normal range, this treatment showed a substantial improvement compared to the basal diet. However, the high standard deviation indicates large individual variability, which might have contributed to the lack of statistical significance in the ANOVA analysis. This variability could be associated with differences in feed intake, health status, or physiological response of individual chickens.

Overall, while the treatments did not restore hematocrit values to the normal range, the biological trend suggests that fermented coffee pulp, particularly with EM, has the potential to improve hematological status in native chickens. Further studies with larger sample sizes and optimized fermentation processes are recommended to validate these findings.

### 3.5 Platelets (thrombocytes)

The results of the study showed that dietary treatments had no significant effect ( $P > 0.05$ ) on platelet count in Kampung chickens. Treatments T1 and T2 exhibited a clear increasing trend compared to the control, with the highest value observed in treatment T2. However, a reduction in platelet count was recorded in treatment T3, which remained higher than the control but lower than treatments T1 and T2. In treatment T0 (basal diet), the platelet count was recorded at  $9 \pm 2.9$ . The addition of 10% coffee by-product without fermentation in treatment T1 increased the platelet count to  $20 \pm 12.0$ . A further increase was observed in treatment T2, which included 10% coffee by-product fermented with *Saccharomyces cerevisiae*, reaching  $23 \pm 6.4$ . In contrast, treatment T3, with the addition of 10% coffee by-product fermented with LAB, showed a decrease to  $11 \pm 1.4$ , lower than treatments T1 and T2 but still slightly higher than the control.

The platelet values recorded in this study fell below the typical levels found in healthy chickens. The T0 group receiving the basal diet showed an average platelet count of  $9.000 \pm 2.800/\mu\text{L}$ , which may reflect a thrombocytopenic condition potentially related to insufficient nutritional support for hematopoiesis.

A marked increase was observed with the inclusion of 10% coffee pulp, both unfermented (T1 =  $19.500 \pm 12.020/\mu\text{L}$ ) and fermented with *Saccharomyces cerevisiae* (T2 =  $22.500 \pm 6.363/\mu\text{L}$ ). These values are closer to the physiological range and suggest that coffee pulp supplementation may enhance platelet production. Fermentation with yeast likely improved nutrient availability, particularly B vitamins and minerals, which are essential for thrombocyte formation.

Interestingly, platelet counts drastically dropped in the EM-fermented coffee pulp group (T3 =  $1.000 \pm 1.414/\mu\text{L}$ ). This severe reduction may indicate a negative impact of EM fermentation on blood cell production or an adverse physiological response in chickens. Possible explanations include the production of excessive secondary metabolites during EM fermentation, impaired feed palatability leading to reduced nutrient intake, or immunological reactions that suppressed thrombocyte production. The high variability in earlier groups and the extreme reduction in T3 highlight the need for careful evaluation of microbial fermentation strategies in poultry diets.

Overall, while unfermented and *Saccharomyces*-fermented coffee husk showed promising trends toward normalizing thrombocyte counts, EM fermentation appeared detrimental under the conditions of this study. Further investigations with larger sample sizes and controlled microbial compositions are required to confirm these findings.

## 4 Conclusion

The inclusion of unfermented and fermented coffee husk tended to improve the hematological profile of Kampung chickens, although no significant differences were observed among treatments. Fermentation enhanced the nutritional quality of coffee husk, supporting improvements in erythrocytes, hemoglobin, hematocrit, leukocytes, and platelet values. Fermented coffee husk shows potential as a sustainable alternative feed ingredient for Kampung chicken production. Further studies are required to determine optimal inclusion levels, fermentation methods, and long-term physiological effects.

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