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Fermented Coffee Husk Waste in Sheep Feed Rations: Effects on Physiological Responses and Hematological Profile of Local Sheep

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ABSTRACT

Coffee husk is a major by-product of coffee processing with potential as ruminant feed, but its utilization is limited by anti-nutritional compounds such as tannin, lignin, and caffeine. This study evaluated the effects of fermented coffee husk (FCH) inclusion in sheep rations on physiological responses and hematological profile. Sixteen local sheep were assigned to four dietary treatments: P0 (0% FCH), P1 (10% FCH), P2 (20% FCH), and P3 (30% FCH) in a completely randomized design. FCH supplementation significantly (P<0.05) improved physiological responses, with 30% FCH producing the lowest rectal temperature, heart rate, and respiration rate. Hematological analysis showed significant treatment effects (P<0.05), including reduced erythrocyte count, hematocrit, hemoglobin, total protein, creatinine, and neutrophil percentage, while blood glucose increased, indicating improved stress resilience and anti-inflammatory effects. These findings demonstrate that fermentation enhances the value of coffee husk as a functional feed ingredient, allowing inclusion of up to 30% in sheep rations without compromising physiological status, while reducing feed costs and supporting sustainable circular economy practices in coffee-producing regions.

Keywords: *Coffee husk; fermentation; sheep; physiological response; hematology; feed utilization*

INTRODUCTION

Coffee processing generates a large amount of agricultural by-product, and coffee husk is the most abundant waste material from the wet processing of coffee cherries. In coffee-producing regions this waste accumulates in substantial quantities, causing environmental problems such as

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soil and water pollution, greenhouse gas emissions, and land degradation. Growing global coffee production has intensified these concerns, making sustainable utilization strategies increasingly important.

The livestock sector faces a related challenge, since feed costs account for 60-70% of total production expenses in many developing countries. Rising prices of conventional feed ingredients such as corn and soybean meal have increased interest in agricultural by-products as alternative feed sources. Coffee husk, given its abundant biomass and moderate nutrient content, is a promising candidate for inclusion in ruminant diets.

Coffee husk contains approximately 9-13% crude protein, 30-55% crude fiber, and a variable energy content. Its use, however, is limited by anti-nutritional factors, including tannins (about 1.18 mg/g DM), caffeine, lignin, and other phenolic compounds that reduce palatability, digestibility, and nutrient absorption. These compounds have generally restricted the inclusion of untreated coffee husk in animal diets to around 16-20%.

Fermentation is a widely used technology for improving the nutritional value of coffee husk while reducing its anti-nutritional load. Fermentation using beneficial microorganisms such as Saccharomyces cerevisiae and Lactobacillus plantarum has been reported to (Marlida et al., 2023; Ikusika et al., 2024): Reduce tannin and caffeine concentrations by more than 50%; Increase crude protein content, from around 21% to 22%; Decrease crude fiber content, from around 26% to 24%; Reduce neutral detergent fiber (NDF) content; and Enhance digestibility and palatability. Previous research indicates that fermented coffee husk can be included in complete feed formulations at up to 30% without adverse effects on dry matter intake, weight gain, or feed conversion in sheep (Hernández-Bautista et al., 2018; Villanueva & Salinas-Rios, 2023).

Physiological indicators such as rectal temperature, heart rate, and respiration rate are widely used to assess animal health and stress status, since they reflect metabolic activity and the animal's capacity to maintain homeostasis under changing dietary and environmental conditions (Kumar et al., 2025; McManus et al., 2022). Hematological profiles-including erythrocyte, hemoglobin, hematocrit, leukocyte, and neutrophil counts-similarly provide useful information on nutritional status, immune function, and stress resistance (Fanta et al., 2024a). Transportation stress in livestock is known to elevate erythrocyte, hematocrit, and hemoglobin levels through hemoconcentration. Provision of fermented coffee husk through urea molasses multi-nutrient blocks (UMMB) has previously been reported to reduce these stress indicators, with 30% fermented coffee husk lowering erythrocytes by 16.58%, hematocrit by 18.30%, and hemoglobin by 31.72% during transport stress (Ruwandani et al., 2024; Fahriza, 2024). The following hypotheses were tested: (1) fermented coffee husk inclusion improves physiological parameters through enhanced metabolic processes; (2) fermented coffee husk inclusion positively affects hematological profile by reducing stress indicators; and (3) fermented coffee husk can be included up to 30% in sheep rations without compromising health status.

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METHODS

The study was conducted at the Animal Science Research Farm over a 25-day feeding period, preceded by a 14 day adaptation phase. The trial was carried out during the dry season under tropical climatic conditions. Sixteen (16) local sheep with an initial body weight of 15-20 kg were used. Animals were housed individually with ad libitum access to water. A completely randomized design (CRD) with four treatments and four replications was applied: P0 (control): basal diet without fermented coffee husk; P1: basal diet + 10% fermented coffee husk; P2: basal diet + 20% fermented coffee husk; P3: basal diet + 30% fermented coffee husk.

1. Fermented Coffee Husk Preparation

Coffee husk was obtained from local coffee-processing facilities and fermented using a starter culture containing Saccharomyces cerevisiae and Lactobacillus plantarum at a 1:1 ratio (Marlida et al., 2023). Fermentation was carried out for 14 days under anaerobic conditions, after which the nutritional composition of the fermented product was analyzed prior to feed formulation.

Table 1. Nutritional composition of fresh and fermented coffee husk

Parameter	Fresh Coffee Husk	Fermented Coffee Husk
Dry Matter (%)	90.00	91.20
Crude Protein (%)	9.00-10.36	19.26-22.06
Crude Fiber (%)	30.00-39.43	24.47-25.83
Tannin (mg/g DM)	1.18-2.50	0.50-0.90
Caffeine (%)	0.80-1.20	0.30-0.50

Source: adapted from Khumran et al. (2024) and Ikusika et al. (2024).

2. Diet Formulation and Feeding

The basal diet was formulated to meet the nutrient requirements of growing sheep according to National Research Council (NRC) standards, using corn, soybean meal, rice bran, molasses, urea, and a mineral-vitamin premix. Diets were formulated to be isoproteinous and isoenergetic across treatments.

Table 2. Diet composition by treatment (%)

Ingredient (%)	P0	P1	P2	P3
Fermented Coffee Husk	0	10	20	30
Corn	45	40	35	30
Soybean Meal	18	16	14	12
Rice Bran	25	22	20	17
Molasses	5	5	5	5

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Ingredient (%)	P0	P1	P2	P3
Urea	2	2	2	2
Mineral-Vitamin Premix	5	5	4	4

Sheep were fed twice daily (morning and evening) at 3% of body weight on a dry-matter basis. Feed intake was recorded daily and body weight was measured weekly.

Physiological parameters were measured on days 10, 15, 20, and 25 at 07:00 AM, before morning feeding, to avoid postprandial effects: (1) rectal temperature (°C), measured with a digital clinical thermometer inserted approximately 5 cm into the rectum for 2 minutes; (2) heart rate (beats/min), measured with a stethoscope on the left thoracic wall for 1 minute; and (3) respiration rate (breaths/min), measured by observing flank movement for 1 minute. Blood samples (5 mL) were collected from the jugular vein on day 25 using EDTA tubes for the following analyses: erythrocytes (RBC, $x10^6/\mu L$) by hemocytometer; hemoglobin (Hb, g/dL) by the cyanmethemoglobin method; hematocrit (Ht, %) by microhematocrit centrifugation; leukocytes (WBC, $x10^3/\mu L$) by hemocytometer; neutrophils (%) by differential count of blood smears; glucose (mg/dL) by the glucose oxidase method; total protein (g/dL) by the biuret method; and creatinine (mg/dL) by the Jaffe method. Data were analyzed by one-way analysis of variance (ANOVA) under the CRD model. Where significant differences were detected ($P<0.05$), means were separated using Duncan's Multiple Range Test (DMRT). All analyses were performed in SAS version 9.4.

RESULTS

1. Physiological Responses

Fermented coffee husk inclusion significantly affected all three physiological parameters ($P<0.05$). Treatment P3 (30% FCH) produced the most favorable responses, consistent with an enhanced metabolic contribution from Saccharomyces cerevisiae and Lactobacillus plantarum that improves nutrient utilization (Karimah & Yulianto, 2025).

Table 3. Physiological responses by treatment

Parameter	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	LSD (P=0.05)
Rectal Temperature (°C)	39.12 ± 0.18a	38.94 ± 0.15ab	38.82 ± 0.14b	38.68 ± 0.12b	0.22
Heart Rate (beats/min)	82.4 ± 3.8a	78.6 ± 3.2ab	75.2 ± 2.9b	72.8 ± 2.6b	5.1
Respiration Rate (breaths/min)	34.2 ± 2.8a	31.8 ± 2.4ab	29.6 ± 2.1b	27.4 ± 1.9b	3.8

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Values are means ± SD (n = 4). Within rows, means with different superscripts differ significantly (P<0.05).

2. Hematological Parameters

a. Erythrocytes, Hemoglobin, and Hematocrit

FCH inclusion had a significant effect (P<0.05) on erythrocyte, hemoglobin, and hematocrit levels. All three parameters decreased as the proportion of FCH increased, indicating improved stress resistance in treated animals.

Table 4. Erythrocyte, hemoglobin, and hematocrit levels by treatment

Parameter	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	LSD (P=0.05)
Erythrocytes (x10⁶/µL)	9.86 ± 0.42a	8.92 ± 0.38b	8.41 ± 0.35c	8.12 ± 0.31c	0.52
Hemoglobin (g/dL)	12.35 ± 0.56a	11.28 ± 0.48b	10.64 ± 0.44c	10.02 ± 0.39c	0.68
Hematocrit (%)	36.72 ± 1.85a	33.84 ± 1.62b	32.15 ± 1.48c	30.58 ± 1.35c	2.14

Values are means ± SD (n = 4). Within rows, means with different superscripts differ significantly (P<0.05).

b. Leukocytes and Neutrophils

FCH inclusion did not significantly affect leukocyte counts (P>0.05), but neutrophil percentage decreased significantly (P<0.05) as FCH inclusion increased, suggesting an anti-inflammatory effect likely related to the caffeine content of coffee husk (Hernández-Bautista et al., 2018).

Table 5. Leukocyte and neutrophil counts by treatment

Parameter	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	LSD (P=0.05)
Leukocytes (x10³/µL)	8.24 ± 0.52	7.96 ± 0.48	7.72 ± 0.44	7.48 ± 0.41	0.82 ns
Neutrophils (%)	42.6 ± 3.2a	38.4 ± 2.8b	35.2 ± 2.4bc	32.8 ± 2.1c	4.2

Values are means ± SD (n = 4). Within rows, means with different superscripts differ significantly (P<0.05); ns = not significant.

3. Blood Biochemical Parameters

FCH inclusion significantly affected blood biochemical parameters (P<0.05). Glucose increased with higher FCH inclusion, while total protein and creatinine decreased.

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Table 6. Blood biochemical parameters by treatment

Parameter	P0 (0%)	P1 (10%)	P2 (20%)	P3 (30%)	LSD (P=0.05)
Glucose (mg/dL)	62.4 ± 3.8c	67.8 ± 4.2bc	72.4 ± 4.6ab	76.8 ± 4.8a	7.2
Total Protein (g/dL)	7.18 ± 0.42a	6.52 ± 0.38b	5.94 ± 0.34c	5.42 ± 0.31c	0.54
Creatinine (mg/dL)	1.12 ± 0.08a	1.08 ± 0.07a	1.04 ± 0.06ab	0.98 ± 0.05b	0.09

Values are means ± SD (n = 4). Within rows, means with different superscripts differ significantly (P<0.05).

4. Economic Assessment

Replacing conventional ingredients with fermented coffee husk progressively reduced estimated feed cost and improved the benefit-cost ratio as inclusion level increased (Table 7).

Table 7. Estimated economic benefit of fermented coffee husk inclusion

Parameter	P0	P1	P2	P3
Feed Cost (USD/kg)	0.38	0.34	0.30	0.26
Cost Reduction (%)	-	10.5	21.1	31.6
Benefit-Cost Ratio	1.82	1.96	2.08	2.24

DISCUSSION

Increasing FCH inclusion lowered all three physiological indicators in a graded manner (Table 3). Relative to the control (P0), P3 reduced rectal temperature from 39.12 to 38.68 °C (-1.12%), heart rate from 82.4 to 72.8 beats/min (-11.65%), and respiration rate from 34.2 to 27.4 breaths/min (-19.88%). The three parameters did not respond equally: respiration rate showed the largest relative change, heart rate an intermediate one, and rectal temperature only a small one. This ordering is physiologically reasonable, since rectal temperature is tightly regulated, whereas respiration rate is the first and most flexible mechanism sheep use to dissipate heat, and both rise with metabolic and thermal load (Kumar et al., 2025; McManus et al., 2022). All measurements were taken at 07:00 before feeding, so the differences between treatments are less likely to reflect postprandial heat production than the dietary treatment itself.

The multiple-comparison results in Table 3 refine the statement in the Results that P3 produced the most favorable responses. P3 had the lowest numerical means, but P2 and P3 shared the same superscript for all three parameters, and P1 did not differ significantly from either P0 or P2. A significant benefit therefore emerged at 20% inclusion, and raising the level to 30% gave a further numerical improvement that could not be distinguished statistically. This agrees in direction

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with Karimah and Yulianto (2025), who identified 30% fermented coffee husk as the optimal level, but our data suggest that the response may already plateau at 20%, so the optimum is better described as a range of 20-30% than as a single value.

Three mechanisms may explain these responses: (1) as shown in Table 1, fermentation lowered tannin (from 1.18-2.50 to 0.50-0.90 mg/g DM) and caffeine (from 0.80-1.20% to 0.30-0.50%) and raised crude protein while lowering crude fiber, which should improve nutrient availability and reduce the heat increment of feeding (Ikusika et al., 2024; Yafetto et al., 2023); (2) the probiotic organisms may enhance rumen fermentation efficiency and thereby reduce metabolic heat production (Marlida et al., 2023); and (3) phenolic compounds and antioxidants in coffee husk may reduce oxidative stress and associated inflammatory responses (Carta et al., 2022). These mechanisms are inferred from the literature, because rumen fermentation, digestibility, and oxidative status were not measured here. In addition, the design had no group fed unfermented coffee husk, so the effect of fermentation cannot be separated from the effect of coffee husk inclusion itself, and FCH replaced corn, soybean meal, and rice bran in the diets (Table 2), so a change in the dietary energy source may also have contributed.

Erythrocytes, hemoglobin, and hematocrit decreased in parallel with FCH inclusion (Table 4). At 30%, P3 was 17.65% lower in erythrocytes (9.86 to 8.12 x10^6/µL), 18.87% lower in hemoglobin (12.35 to 10.02 g/dL), and 16.72% lower in hematocrit (36.72 to 30.58%) than P0. These reductions can be compared directly with those reported for 30% fermented coffee husk in UMMB under transport stress: 16.58% for erythrocytes, 18.30% for hematocrit, and 31.72% for hemoglobin (Ruwandani et al., 2024; Fahriza, 2024). The erythrocyte and hematocrit reductions found here are very close to the reported values, supporting a consistent effect of fermented coffee husk on these two indices, whereas the hemoglobin reduction in the present study was clearly smaller than the reported 31.72%. The reason for this difference cannot be resolved from our data; the form of the feed (complete ration versus UMMB), the experimental conditions, and the initial status of the animals are possible contributors. Hematological profiles of sheep and goats have likewise been shown to respond to the type of feed offered (Fanta et al., 2024a), which indicates that these indices are sensitive to dietary change.

Interpreting the decreases as improved stress resistance requires caution. Transport stress raises these indices through hemoconcentration (dehydration, catecholamine release, and splenic contraction), and the earlier studies measured the reductions in animals exposed to that stress. In the present study the sheep were not subjected to a transport or heat challenge, so the lower values show that blood in the treated animals was less concentrated, which is consistent with, but does not prove, a lower stress load. This reading is supported by the parallel declines in heart rate and respiration rate (Table 3) and in neutrophil percentage (Table 5). The values calculated from the Table 4 means also argue against an iron-deficiency-type anemia: mean corpuscular volume (about 37.2 vs 37.7 fL) and mean corpuscular hemoglobin concentration (about 33.6 vs 32.8 g/dL) were nearly unchanged between P0 and P3, indicating that the three indices fell proportionally rather

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than because red cells became smaller or paler. Direct measurement of iron status would still be useful, since residual tannin may bind dietary iron.

Total leukocytes did not differ significantly among treatments (8.24 to 7.48 x10^3/µL; -9.22% in P3, P>0.05), whereas neutrophil percentage fell significantly from 42.6% in P0 to 32.8% in P3 (-23.00%; Table 5). The decline was graded, with P1 already lower than P0 and P3 lower than P1. FCH therefore altered the composition of the leukocyte population without changing its total count, and there was no sign of leukopenia or immune suppression. This is consistent with Hernández-Bautista et al. (2018), who reported that coffee pulp inclusion reduced neutrophil counts without compromising overall health in sheep. Two non-exclusive explanations are plausible. First, caffeine remaining in the fermented material (0.30-0.50%; Table 1) acts as an adenosine-receptor antagonist and may dampen neutrophil migration and inflammatory signalling. Second, because a high neutrophil share is a typical component of the stress leukogram, the lower percentage fits the lower heart rate, respiration rate, and erythrocyte indices observed in the same animals, giving a coherent picture of reduced physiological stress. Neither mechanism was tested directly in this study.

Blood glucose rose with FCH inclusion from 62.4 mg/dL in P0 to 76.8 mg/dL in P3 (+23.08%; Table 6); the increase in P2 was +16.03% (72.4 mg/dL), and only P3 was significantly higher than both P0 and P1. This direction agrees with reports that improved utilization of feed nutrients supports energy availability (Fanta et al., 2024b), and it matches the lower respiration and heart rates in the same treatments, which suggest less energy spent on thermoregulation. Because molasses was fixed at 5% in all diets (Table 2), the increase is unlikely to come from molasses sugar; reduced caffeine-related inhibition of glucose utilization after fermentation is another possible explanation. The time of blood sampling relative to feeding is not stated in the Methods and should be specified, since glucose is sensitive to it.

Total protein showed the largest decline among the biochemical variables, from 7.18 g/dL in P0 to 5.42 g/dL in P3 (-24.51%), while creatinine fell from 1.12 to 0.98 mg/dL (-12.50%) and differed significantly only in P3. The modest decrease in creatinine gives no indication of renal impairment or increased muscle breakdown. The fall in total protein, however, is large, and the P3 value approaches or lies below the lower end of the reference ranges commonly cited for sheep (roughly 6-8 g/dL; the range used by the laboratory should be confirmed), so it should not be interpreted simply as reduced protein catabolism. Two explanations deserve consideration: (1) a lower supply of absorbable protein, since protein in fiber-rich, tannin-containing material may be less available even after fermentation despite isoproteinous formulation; and (2) a larger plasma volume, which would also fit the parallel fall in erythrocytes, hemoglobin, and hematocrit (Table 4). Albumin, globulin, and blood urea nitrogen were not measured and would help distinguish these explanations.

Taken together, the results indicate that fermented coffee husk can be included at 20-30% in sheep rations without signs of physiological compromise: body temperature, heart rate, and respiration rate did not rise, leukocyte counts did not fall, and creatinine did not increase. This is consistent with earlier reports that diets containing fermented coffee husk or coffee pulp at up to

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30% did not impair intake or growth in small ruminants (Hernández-Bautista et al., 2018; Villanueva & Salinas-Rios, 2023; Prawirodigdo & Sutrisno, 2014). However, feed intake and body weight were recorded but are not reported in the present Results, so the absence of a performance penalty rests on the literature rather than on our own data. The economic results (Table 7) show that estimated feed cost fell from 0.38 USD/kg in P0 to 0.34, 0.30, and 0.26 USD/kg in P1-P3 (-10.5%, -21.1%, and -31.6%), with the benefit-cost ratio rising from 1.82 to 2.24. Because P2 and P3 were statistically similar for most physiological and hematological indices, P2 (cost reduction 21.1%, benefit-cost ratio 2.08) is a conservative option, whereas P3 gives the greatest saving. Practically, this supports (1) feed-cost reduction, since coffee husk can replace part of the conventional energy and protein ingredients (Phonna et al., 2024); (2) waste valorization, converting an environmentally problematic by-product into a productive input, consistent with broader reviews of agro-industrial waste fermentation for livestock feed (Yafetto et al., 2023; San Martin et al., 2023); and (3) improved animal welfare through lower physiological stress indicators.

Several limitations qualify these comparisons. Each treatment had only four animals and the feeding period lasted 25 days; blood was sampled once, on day 25; no transport or heat challenge was applied, although the stress interpretation depends on such conditions in the reference studies; no unfermented coffee husk group was included; and the economic figures are estimates that depend on ingredient prices. Longer trials with more animals, repeated blood sampling, a stress challenge, and an unfermented comparison group are needed to confirm the mechanisms proposed here.

CONCLUSIONS

This study demonstrates that fermented coffee husk can be used as an alternative feed ingredient in local sheep rations at inclusion levels of 20-30%, without signs of physiological compromise. Inclusion of 30% fermented coffee husk produced the lowest rectal temperature (-1.12%), heart rate (-11.65%), and respiration rate (-19.88%) relative to the control, although the responses in P2 (20%) and P3 (30%) were not statistically different. Hematological analysis showed that fermented coffee husk reduced erythrocyte count (-17.65%), hemoglobin (-18.87%), and hematocrit (-16.72%) and lowered neutrophil percentage (-23.00%) without changing total leukocytes, which is consistent with a lower physiological stress load, although no stress challenge was applied. Blood glucose increased by 23.08%, while total protein and creatinine decreased by 24.51% and 12.50%, respectively; the large decline in total protein needs further investigation. Furthermore, fermented coffee husk reduced estimated feed cost by up to 31.6% while addressing environmental problems associated with coffee-processing waste. These findings support fermentation as a way to turn coffee husk from an environmental liability into a functional feed ingredient, offering smallholder farmers a cost-effective and sustainable feed alternative, pending studies with larger groups, longer duration, and reported growth performance.

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