Haematology and serum profile of West African Dwarf Goats fed oyster mushroom (*Pleurotus ostreatus)* treated millet and cowpea chaff based diets

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Abstract

During the dry season, ruminant feeds in tropical regions have low nutritive value, which affects their health through immune suppression, metabolic stress, and increased susceptibility to diseases. Therefore, this study was designed to investigate the effect of feeding West African Dwarf (WAD) goats with fungal-treated crop residues on their blood chemistry. The primary objective was to determine whether inclusion of bio-enhanced feeds could maintain the animals' normal health status. Dried samples of 20-day oyster mushroom (Pleurotus ostreatus)-treated millet chaff (TMC) or cowpea chaff (TCC) were incorporated at 0, 20, and 30% respectively, into a total mixed ration (100%) containing 50% concentrate and either 50, 30, or 20% cassava peel to form six diets: T1, T2, and T3 (TMC diets) and T4, T5, and T6 (TCC diets). Twenty-four WAD-does were randomly assigned to the experimental diets using a completely randomised design. After 21 days, blood samples were collected and analysed for haematological and serum biochemical parameters. Data obtained were subjected to ANOVA, and means were separated at $p < 0.05$. The inclusion of P. ostreatus-treated crop residues in the goats' diets maintained haematological and serum biochemical values within the normal physiological ranges for healthy animals. Higher inclusion levels, especially 30% TMC, showed enhanced red cell indices and protein metabolism indices and maintained energy metabolism indices while supporting immune function in the goats. This study indicates that supplemental feeding with biotreated residues, particularly TMC, has the potential to sustain normal health status in WAD goats under tropical feeding conditions.

Keywords: fungal treated substrates, haematology, serum biochemistry, total mixed ration, crop residues

Hématologie et profil sérique de chèvres West African Dwarf nourries avec des régimes à base de drèche de mil et de fanes de niébé traités par le pleurote en huître (*Pleurotus ostreatus*)

Résumé

Pendant la saison sèche, les aliments pour ruminants dans les régions tropicales ont une faible valeur nutritive, ce qui affecte leur santé par immunosuppression, stress métabolique et sensibilité accrue aux maladies. Par conséquent, cette étude a été conçue pour étudier l'effet de l'alimentation de chèvres West African Dwarf (WAD) avec des résidus de culture traités par des champignons sur leur biochimie sanguine. L'objectif principal était de déterminer si l'inclusion d'aliments bio-améliorés pouvait maintenir l'état de santé normal des animaux. Des échantillons séchés de drèche de mil (TMC) ou de fanes de niébé (TCC) traités pendant 20 jours par le pleurote en huître (Pleurotus ostreatus) ont été incorporés à 0, 20 et 30 % respectivement dans une ration mixte totale (100 %) contenant 50 % de concentré et soit 50, 30 ou 20 % de cossettes de manioc pour former six régimes : T1, T2 et T3 (régimes TMC) et T4, T5 et T6 (régimes TCC). Vingt-quatre chèvres WAD ont été assignées aléatoirement aux régimes expérimentaux selon un dispositif entièrement randomisé. Après 21 jours, des échantillons de sang ont été prélevés et analysés pour les paramètres hématologiques et biochimiques sériques. Les données obtenues ont été soumises à une ANOVA, et les moyennes ont été séparées à p < 0,05. L'inclusion de résidus de culture traités par P. ostreatus dans les régimes des chèvres a maintenu les valeurs hématologiques et biochimiques sériques dans les fourchettes physiologiques normales pour *des animaux en bonne santé. Des niveaux d'inclusion plus élevés, surtout 30 % de TMC, ont montré des indices des globules rouges et des indices du métabolisme protéique améliorés, ont maintenu les indices du métabolisme énergétique tout en soutenant la fonction immunitaire chez les chèvres. Cette étude indique que l'alimentation complémentaire avec des résidus biotraités, en particulier le TMC, a le potentiel de maintenir un état de santé normal chez les chèvres WAD dans des conditions d'alimentation tropicales.*

Mots-clés : substrats traités par des champignons, hématologie, biochimie sérique, ration mixte totale, résidus de culture

Running title: Feeding of biotreated residues for improved tropical goats' health condition

Introduction

In tropical nations, small ruminants such as sheep and goats are often preferred over cattle by rural farmers due to their adaptability to harsh climates and lower space and feed requirements (Ogunbosoye *et al*., 2018). Among these, goats are preferred over sheep because they are more resistant to drought, heat stress, and disease and can efficiently utilise low-quality fodder and available browsing plants (Kulkarni *et al*., 2022). Nevertheless, the health of these animals has often been compromised by heat stress, parasitic infections, limited high-quality feed availability, and nutrient deficiencies, especially during the dry season. During this period, ruminants depend on available forages and, primarily, on crop residues, which, although plentiful, inexpensive, and non-competitive with human food, are nutritionally deficient and can impair the immune function and overall well-being of the animals (Ibhaze *et al*., 2021). In Nigeria, millet chaff and cowpea chaff are commonly used in the feeding system, yet their high structural carbohydrate and lignin content, low crude protein, and presence of anti-nutritional compounds negatively affect animal health (Singh *et al*., 2011; Lardy *et al*., 2015). There is the need to improve their nutritive value for improved animal performance towards better health, one of the possible methods adopted to achieve this is through a biological approach using selective white rot fungus (WRF) in a solid state fermentation process.

Pleurotus spp., is a selective WRF that has been extensively studied and shown to improve the nutritional quality of these residues by increasing protein content, reducing structural carbohydrate and lignin content, and antinutritional content (Tuyen *et al*., 2013; Idowu *et al*., 2023). *Pleurotus ostreatus* is edible and safe for ruminants to consume (Wuanor and Carew 2018). While several *in vitro* and a few *in vivo* studies have demonstrated improvement in chemical composition, reduction in antinutritional content, and improvement in digestibility of agricultural wastes (Tuyen *et al*., 2013; Nayan *et al*., 2018; Wuanor and Carew 2018; Ibhaze *et al*., 2021; Jiwuba *et al*., 2022; Yuan *et al*., 2022), *in vivo* evidence on animal health outcomes, including metabolic and immune responses, remains limited (van Kuijk *et al*., 2015; Yuan *et al*., 2022).

Blood-based haematological and serum biochemical analyses provide a practical approach to examine the physiological impact of novel feed such as fungal-treated crop residues, including potential toxicities that may arise from the enhanced feed options, and protein-energy balance (Anya *et al*., 2018; Wuanor and Carew, 2018). This study was designed to examine the impact of diets containing either *Pleurotus ostreatus*-treated millet chaff and/or cowpea chaff supplemented as replacements for cassava peel in a total mixed ration on the blood haematology and serum biochemistry of WAD growing does.

Materials and Methods

Study Site Description

The study was conducted at both the Clean Laboratory and the Paddock Unit of the Pasture and Range Management Department, Directorate of University Farm (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB). FUNAAB is located at latitude 70° 13' 24" N and longitude 30° 26' 14" E of the equator (Google Earth, 2021). Annual rainfall, temperature, and relative humidity are 1037 mm, 33.80°C, and 82%, respectively. FUNAAB is in the derived savannah zone of southwestern Nigeria and is 148 metres above sea level.

Collection and preparation of experimental diets

In December 2021, the crop residues (millet chaff and cowpea chaff) and cassava peel were purchased from a neighbouring market in

Eleweran, Abeokuta, Ogun State, Nigeria. A nearby (FUNAAB venture) feed mill was used to prepare the concentrate diet after the cassava peel was crushed to a size of 5 mm. An appropriate quantity of each of the crop residue (millet chaff and cowpea chaff) was packed into ¾ of a big drum, soaked until covered with clean water for 24 hours, and then drained to remove excess water. Up until the drum was emptied, about 2.5 kg of each moistened crop residue was weighed into several clear individual bags, sterilised for an hour, cooled, and inoculated with *Pleurotus ostreatus* (PO) purchased from the Forestry Research Institute of Nigeria, Ibadan, Oyo State and spawn at 4% (w/w) and mixed following the procedure of Fazaeli *et al*. (2004). The bags' ends were slightly tightened, sealed with masking tape, and stored on racks in a dark, cleaned, and disinfected (Morigad at 0.5 mL/litre) inoculation room (Wuanor and Carew, 2018) with 100% relative humidity at room temperature (25 - 35^{0}C) for 20 days. After which, the PO mycelium-colonised bags were removed from the inoculation room, autoclaved to terminate the mycelia growth (Akinfemi, 2010), dried by spreading them out on a sheet in the open air for several days until a constant weight was achieved, and eventually stored in bags at room temperature (Fazaeli *et al*., 2004) for incorporation into a total mixed ration (TMR). The TMR was prepared for six dietary treatments: T1 (0%), T2 (20%), and T3 (30%) were treated millet chaff (TMC)-based diets, while T4 (0%), T5 (20%), and T6 (30%) were treated cowpea chaff (TCC)-based diets. All diet were formulated with concentrate fixed at 50% of the TMR. The remaining 50% consisted of cassava peel which was either used in totality (control) or partially replaced with fungal treated residue (TMC or TCC). Cassava peel was substituted with the treated residue (TMC or TCC) at two inclusion levels (20% and 30% of the TMR). (Table 1). The chemical composition of the various TMR diets, concentrates, TMC, and TCC is included in Table 1. The concentrate was made up of palm kernel cake (34%), groundnut cake (12%), rice bran (28%), wheat offal (22 %), premix (1%), bone meal (2%), and salt (1%).

Experimental Design and Animal Management

All procedures were conducted in accordance with institutional guidelines for animal care by the College of Veterinary Medicine, FUNAAB Research Ethics Committee. A one-way completely randomised design (CRD) experiment was used with six dietary treatments and four (4) WAD growing does per treatment with an average body weight of 7 kg ± 1.26. The animals were obtained from Kuto Market, Abeokuta, Ogun State, Nigeria, and the trial was conducted in the late dry season between December 2021 and February 2022. Before the trial, the goats were treated for both endoparasites and ectoparasites using Albendazole 10% (administered orally at 0.1 mg/kg body weight, BW) and by dipping in Diazoline solution, respectively. The goats were administered 10 mg/kg of BW antibiotics (Oxytetracycline). The goats were split into two groups and given concentrate mixed with cassava peel (50:50) at 3.5% BW in the morning. In the afternoon, each group received either MC or CC as their basal diet for a preliminary period of 10 days. The animals were randomly distributed into six groups, housed in separate 1.5 m x 1 m metabolic cages with optimal ventilation, feeders, and drinkers, and fed their allocated experimental diets. The animals were provided access to the experimental TMR diets for the 21 days of the experiment (Table 1). The diets on a dry matter basis were offered in equal amounts to the animals twice a day at 08.00 and 16.00 h at 3.5% BW on a DM basis. The animals have access to clean and fresh water daily.

Blood sampling and collection

After the animals were fed their respective experimental diets for twenty one days, the animals were restrained, and a licensed veterinarian with experience handling small ruminant took ten (10) mL of blood via jugular venepuncture from the goats using sterile 18-gauge needles in the morning before feeding to minimise diurnal variation. Five millilitres of the blood were transferred into labelled sterile vacutainer tubes containing an anticoagulant (ethylene diamine tetra-acetic acid-EDTA; K3 EDTA Non-Vacuum Blood Collection Tube, LabCare) for haematological analysis. The remaining five millilitres were transferred into labelled sterile plain tubes (blood collection tubes, Plastilab) without anticoagulant for serum biochemical analyses. Immediately after collection, the blood samples were taken to the laboratory. On arrival, the blood samples in the EDTA tubes were gently inverted several times to ensure that the blood was properly mixed with the anticoagulant and were analysed for haematological parameters within 2 hours after collection. The blood samples in the plain tubes without anticoagulant were put on Kaylite holders and allowed to coagulate at room temperature for 30 min. They were then centrifuged at 3000 g for 15 minutes at 21°C and stored at -20°C until analysis. The haematological parameters (red blood cells (RBCs), white blood cells (WBCs), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV), neutrophils, lymphocytes, monocytes, basophils, and eosinophils) and serum biochemical indices (total protein (TP), globulin (GLO), albumin (ALB), glucose, cholesterol, urea, creatinine (Cr), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)) were determined appropriately as described by Adelusi *et al*. (2018).

Experimental design and statistical analysis

The data obtained in this study were tested by an analysis of variance (ANOVA) using the completely randomised design of Minitab 16 statistical software, except for the chemical composition data. Differences among means were analysed using Tukey's post hoc test of Minitab 16 software, with means considered significant at $P < 0.05$.

Results

Chemical composition of experimental diets

Table 1 shows the chemical composition of the six experimental diets given to the growing WAD goats. The study found that when the inclusion levels of TMC and TCC in the diets increased, so did the contents of crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). The CP values of the diets ranged from 8.52% to 12.2%. The animals on control diets had the lowest CP content, while those on diets based on 30% TCC had the greatest CP content. The diets' NDF, ADF, and ADL values ranged from 48.2% to 54.0%, 24.6% to 33.4%, and 4.20% to 5.05%, respectively.

Table 1: Ingredient proportion and chemical composition of diets containing Pleurotus-treated millet and cowpea chaffs

Items (%)	**TMC diets**			**TCC diets**					**Ingredients**	
TMR?	T1 (0%)	T2 (20%)	T3 (30)	T4 (0%)	T5 (20%)	T6 (30%)	TMC (%)	TCC (%)	Cas-P (%)	Con (%)
Cassava peel	50.0	30.0	20.0	50.0	30.0	20.0	-	-	-	-
Treated crop residues	-	20.0	30.0	-	20.0	30.0	-	-	-	-
Concentrate	50.0	50.0	50.0	50.0	50.0	50.0	-	-	-	-
Total	**100**	**100**	**100**	**100**	**100**	**100**				
Chemical composition (%)										
Dry matter	90.6	90.6	90.9	90.6	90.5	90.5	91.3	90.4	88.9	92.4
Ash	9.58	11.1	12.3	9.58	12.8	13.7	15.0	16.1	5.81	9.57
CP	8.52	8.70	9.30	8.52	11.3	12.2	4.45	14.4	5.42	16.1
NDF	48.7	52.4	54.0	48.7	48.3	48.4	69.0	50.7	22.9	34.1
ADF	24.6	29.8	33.4	24.6	29.4	31.1	48.8	46.2	14.0	28.1
ADL	4.17	4.20	4.39	4.17	4.95	5.05	9.19	10.1	2.85	4.10

TMR, total mixed ration; TMC, treated millet chaff; TCC, treated cowpea chaff; Cas-P, cassava peel; Con, concentrate;CP, Crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin

Haematological parameters

Table 2 shows the haematological parameters of WAD goats fed TMR diets that varied in TMC and TCC levels. The RBC, PCV, Hb, WBC, Lymphocyte, neutrophil, MCHC, and MCV levels ranged from 14.5 - 18.9 x 10^{12}/L, 19.0 - 35.5%, 7.40 - 12.2 g/dl, 6.70 – 12.0 x10^{9}/L, 50.0 – 71.8 %, 26.5 – 47.0 %, 32.1 -

38.8 g/dl, and 13.4 - 22.1 fl, respectively and were significantly ($p < 0.05$) influenced by the dietary treatments. The concentrations of monocytes (1.25 – 2.0 %), eosinophils (0.50 – 1.75 %), and MCH (5.17 – 7.28 pg) were unaffected by the dietary treatment. The RBC, PCV, and Hb values increased with an increase in the inclusion levels of TMC and TCC in the diets. The group fed a 30% TCC (T6) diet had the highest RBC and Hb concentrations, while the group on a 20% TCC (T5) diet showed the lowest RBC and Hb concentrations. The PCV values in goats on TMC diets (32.5 – 35.5 g/dl) were higher than those on TCC diets (19.0 – 33.3 g/dl), with goats on T5 diets recording the least content The WBC concentrations of the goats slightly decreased ($p > 0.05$) with an increase in the inclusion levels of TMC in the diet but reduced drastically with an increase in the inclusion level of TCC in the diets, with the T6 diet recording the lowest concentration. The lymphocyte percentage increased with an increase in the inclusion levels (30%) of the TMC and TCC in the diets, especially in goats fed the T6 (30% TCC) diet, but the neutrophil percentage declined correspondingly. The MCHC concentration were lower in goats on TMC diets (32-34 g/dL) than those on TCC diets (37-39 g/dL), with goats on the T3 diet recording the least concentration. The MCV values were higher in goats on TMC diets (21.3 – 22.1 *fl*) than those on TCC diets (13.4 – 17.6 *fl*), with goats on the T5 diet recording the lowest concentration.

Table 2: Haematological parameters of West African Dwarf goats fed total mix ration containing *P. ostreatus* treated millet and cowpea chaffs

	Treated millet chaff diets			**Treated cowpea chaff diets**					
Haematological parameters	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	SEM	p value	*Merck, (2016a)
RBC ($X10^{12}$/L)	17.6^{ab}	15.4^{bc}	16.0^{abc}	16.3^{ab}	14.5^{c}	18.9^{a}	0.68	<0.001	8.0-18.0
PCV (%)	32.0^{a}	32.5^{a}	35.5^{a}	29.5^{ab}	19.0^{b}	33.3^{a}	2.37	4.75	22.0-38.0
Haemoglobin (g/dl)	12.0^{a}	11.1^{a}	11.4^{a}	11.2^{a}	7.40^{b}	12.2^{a}	0.82	0.01	8.0 – 12.0
WBC ($x10^{9}$/L)	10.5^{ab}	8.50^{bc}	8.20^{bc}	12.0^{a}	10.8^{ab}	6.70^{c}	0.69	<0.001	4.0 -13.0
MCHC (g/ dL)	37.7^{a}	34.0^{bc}	32.1^{c}	38.1^{a}	38.8^{a}	36.5^{ab}	0.78	<0.001	30.0 – 36.0
MCV (*fl*)	18.1^{ab}	21.3^{a}	22.1^{a}	18.4^{ab}	13.4^{b}	17.6^{ab}	1.67	0.02	16.0 - 25.0
MCH (*pg*)	6.80	7.28	7.10	6.93	5.17	6.43	0.58	0.17	5.2 – 8.0
Lymphocytes (%)	51.0^{d}	53.0^{cd}	61.3^{bc}	50.0^{d}	62.5^{b}	71.8^{a}	2.06	<0.001	50.0 -70.0
Neutrophils (%)	45.5^{a}	42.5^{ab}	35.5^{bc}	47.0^{a}	35.3^{bc}	26.5^{c}	2.14	<0.001	30.0 – 48.0
Monocytes (%)	2.00	1.75	1.50	2.00	1.50	1.25	0.40	0.72	2.0 - 9.0
Eosinophils (%)	1.50	1.75	1.75	1.00	0.75	0.50	0.34	0.07	1.0 – 8.0

[a,b,c,d] Means within the same row with different superscripts are significantly ($P < 0.05$) different. SEM, standard error of means; RBC, red blood cells; PCV, packed cell volume; WBC, white blood cells; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MCH, Mean corpuscular haemoglobin; * Normal ranges in healthy goats.

Biochemical indices

The serum biochemical indices of WAD goats fed TMR diets with varying TMC and TCC levels are shown in Table 3. The diets had a significant ($p < 0.05$) effect on the goats' TP (g/dL), GLOB (g/dL), glucose (mg/L), and cholesterol (mg/dL) levels. The levels of ALB, AST, ALT, urea, creatinine, TBIL, and DBIL indices remained unchanged with the dietary treatments. The concentrations of TP (5.70 - 8.10 g/dL), GLOB (1.55 - 3.45 g/dL), glucose (66.1 - 86.7 mg/dL), and cholesterol (92.7 - 122 mg/dL) increased with an increase in the inclusion levels of TMC and TCC in the diets. TP and globulin concentrations were highest in goats fed TMC diets, especially the T3 diet (30% TMC), and lowest in goats fed the T5 diet (20% TCC). Glucose and cholesterol concentrations were highest in goats fed the T6 (30% TCC) diet and lowest in goats fed the T5 diet (20% TCC), while those on TMC diets maintained an intermediate concentration. The

ALB, urea, creatinine, AST, ALT, TBIL, and DBIL concentrations ranged from 4.15 – 4.93 g/dL, 21.6 – 29.3 mg/dL, 0.82 – 1.66 mg/dL, 69.5 – 100 U/L, 20.0 – 31.3 U/L, 0.52 – 0.89 mg/dl, and 0.15 – 0.31 mg/dl, respectively.

Table 3: **Serum biochemical indices of West African Dwarf goats fed total mix ration containing *P. ostreatus* treated millet and cowpea chaffs**

	Treated millet chaff diets			Treated cowpea chaff diets					
Serum biochemical parameters	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	SEM	p value	Merck, (2016b)
Total protein (g/dL)	7.93^a	8.00^a	8.10^a	7.95^a	5.70^b	7.45^a	0.32	<0.001	6.4-7.0
Albumin (g/dL)	4.73	4.58	4.65	4.70	4.15	4.93	0.28	0.53	2.7-3.9
Globulin (g/dL)	3.20^a	3.43^a	3.45^a	3.25^a	1.55^b	2.53^{ab}	0.28	<0.001	2.7-4.1
Glucose (mg/dL)	66.1^b	68.3^{ab}	69.2^{ab}	66.5^b	68.4^{ab}	86.7^a	4.06	0.02	50.0 -75.0
Cholesterol (mg/dL)	98.9^b	94.4^b	96.9^b	102^b	92.7^b	122^a	4.37	<0.001	80.0 – 130
Urea (mg/dl)	26.7	28.5	29.3	25.8	21.6	27.1	3.66	0.74	10.0-20.0
Creatinine (mg/dL)	0.82	1.53	1.66	0.84	0.90	1.49	0.25	0.07	1.0 – 1.8
AST (U/L)	85.5	69.5	71.5	87.8	92.0	100	7.03	0.05	167-513
ALT (U/L)	26.8	20.0	22.8	28.8	29.5	31.3	2.67	0.06	-
Total bilirubin (mg/dL)	0.65	0.89	0.84	0.70	0.52	0.68	0.11	0.23	-
Direct bilirubin (mg/dL)	0.21	0.30	0.31	0.20	0.15	0.25	0.04	0.07	0.0 – 0.1

[a,b.] Means within the same row with different superscripts are significantly (P < 0.05) different. SEM, standard error of means; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Discussion

Chemical composition of the diets

The rise in chemical content as the inclusion levels of the treated substrates increase can be attributed to the chemical composition of each constituent. The crude protein (CP) levels in this study exceeded the minimal CP range of 7 - 8% DM required for optimal rumen function, leading to improved feed intake and nutritional digestibility in ruminant animals (van Soest 1994). The higher CP content of the 30% based diets (T3 and T6) suggests that the diet has a greater amount of fungal biomass, which is known to enhance the nitrogen content of biodegraded substrates (van Kuijk *et al*., 2015). The diets' NDF (Neutral Detergent Fibre), ADF (Acid Detergent Fibre), and ADL (Acid Detergent Lignin) values were significantly lower than the thresholds of 65%, 45%, and 8%, respectively, which are considered indicators of inferior quality (van Saun 2006). Thus, the diets can meet the goats' nutritional requirements.

Haematological parameters

Most of the RBC, PCV, Hb, WBC, lymphocyte, neutrophil, monocyte, eosinophil, MCHC, MCV, and MCH values were within the normal physiological ranges of 8.0–18.0 x 10^{12}/L (RBC), 22.0–38.0% (PCV), 8.0–12.0 g/dl (Hb), 4.00–13.00 x 10^9/L, 50.0–70%, 30.0–48.0%, 2.00–9.00%, 1.00–8.00%, 30.0–36.0 g/dL (MCHC), 16.0–25.0 *fl* (MCV), and 5.20–8.00 pg (MCH) found in clinically healthy goats (Merck, 2016a), which might suggest that the inclusion of *Pleurotus*-treated millet chaff and cowpea chaff maintained haematological stability in the goats (Wang *et al*., 2021; Idowu *et al*., 2023) in terms of enhanced erythropoiesis and protein metabolism while supporting immune function. The obtained values of this study were higher than the ranges of 13.0 – 16.2 x 10^{12}/L (RBC), 24.2 – 30.8 % (PCV), and 8.50 – 11.4 g/dl (Hb) for WAD goats fed *a P. tuber regium*-treated rice straw-based diet (Wuanor and Carew, 2018); 8.56 – 13.1 x 10^{12}/L (RBCs), 24.0 – 32.3 % (PCV), and 8.00 – 10.8 g/dl (Hb) for WAD goats fed treated maize cob and maize husk-based diets (Ibhaze *et al*., 2021); and 9.07 – 13.4 x 10^{12}/L (RBC), 29.1 – 35.7 % (PCV), and 9.54 – 10.9 g/dl (Hb) for WAD goats fed graded levels of *Pleurotus tuber regium*-treated cassava root sievate-based diets (Jiwuba *et al*., 2022), respectively.

The increased RBC, PCV, and Hb with an increase in the inclusion level of the treated residues indicate that, up to a 30% level of inclusion, the diets did not compromise erythropoiesis nor reduce the blood oxygen-carrying capacity of the animals (Ogunbosoye *et al*., 2018). The depressed PCV and Hb found in T5, which fall below the normal physiological range, might indicate possible depressed erythropoiesis or reduced oxygen-carrying capacity that could lead to an anaemic situation in the goats (Ibhaze *et al*., 2021) at 20% treated cowpea chaff inclusion. The increased PCV value in TMC goats compared to TCC goats might indicate that TMC diets are more stable in maintaining PCV at intermediate levels than TCC diets. The reduced WBC associated with an increased inclusion level of TCC in the diets, although still within normal physiological ranges, may suggest that further increasing the TCC inclusion level above 30% could suppress WBC production, which serves as the immune system's defence against antigens and infections in the haemopoietic and lymphoid tissues, potentially compromising disease resistance (Soul *et al*., 2019). The lymphocyte and neutrophil differentials that show a shift towards higher lymphocyte percentages and lower neutrophils in higher treated diets suggest improved immune responsiveness, likely as a result of the feed's improved nutrient profiles (Du *et al*., 2025). This also indicates that, despite the low WBC in goats on T6 diets, their elevated lymphocyte percentage suggests no detrimental effect on immunological responsiveness. These results are in agreement with the findings (Ibhaze *et al*., 2021), who reported enhanced lymphocyte proliferation and improved disease resilience when microbial-treated forages were fed to goats under stress conditions. The red blood indices (MCHC, MCV, and MCH) value fluctuations within or close to the reference range indicate differences induced in haemoglobin saturation in erythrocytes by the diets. The TMC diets, particularly the T3 diet (30% TMC), maintained the MCHC and MCV levels of the goats within the normal intermediate physiological ranges more effectively than the TCC diets. The reduced MCV in T5 (20% TCC) may signal microcytic tendencies, while the high MCV in TMC diets, especially T3, suggests improved erythrocyte development. This study indicates that TMC diets support normal red cell indices, while TCC at a lower inclusion level may exert transient stress on erythropoiesis.

Biochemical indices

The TP, GLOB, glucose, and cholesterol levels of most of the animals used in this study were within the normal physiological ranges of 6.40–7.00 g/dl, 3.50–4.50 g/dl, 50.0–75.0 mg/dl, and 80.0–130 mg/dl (Merck, 2016b), found in clinically healthy goats, respectively. The increase in the concentration of these parameters with an increase in the inclusion levels of TMC and TCC in the diets suggests that up to 30% of their inclusion in the diet had no negative effect on the animals' protein and energy metabolism (Okpanachi *et al*., 2019; Ibhaze *et al*., 2021). The higher TP and globulin concentrations in goats fed TMC diets compared to those on TCC diets suggest better protein metabolism by goats on TMC-based diets. The reduced TP and globulin concentrations of goats on the T5 diet were well below the lower limits of the normal physiological ranges; this could be attributed to insufficient utilisable protein in the diet (Okpanachi *et al*., 2019). The very high glucose and cholesterol concentration of goats on the T6 (30% TCC) diet is pointing towards improved energy metabolism, although it is slightly higher than the high limits of the normal physiological range, while the reverse is the case for those on the T5 diet (20% TCC). The intermediate glucose and cholesterol concentrations in goats fed TMC diets suggest that the diet is capable of maintaining the animals within the normal physiological ranges compared to those on TCC diets that had fluctuating concentrations. The ALB levels exceeded the normal range of 2.70–3.90 g/dl (Merck, 2016b) across all treatments. This might indicate that the elevated concentrations were not influenced by the diets but might be due to dehydration, heat stress, and high temperatures (Soul *et al*., 2019).

The urea concentration in all the animals was above the upper limits of the normal physiological range (10–20 mg/dl), while the creatinine concentration was within the normal range (1.0–1.8 mg/dl) found in healthy goats (Merck, 2016b). Despite this elevated serum concentration across all treatments, which possibly suggests increased protein catabolism or reduced renal clearance, a normal creatinine level might suggest no overt renal impairment. Therefore, the elevated urea concentration might be attributed to the goat's breed. Urea concentrations of 14.3 to 155 mg/dl was reported to be a normal physiological range found in healthy WAD goats (Daramola *et al*.,

2005) . The liver enzyme (AST and ALT) levels of all animals fell within the normal ranges of 66.0-230 U/L and 15.3-52.3 U/L, respectively (Plumb, 1999), for healthy goats, but not in the normal ranges of 167-513 U/L for AST (Merck, 2016b) for healthy goats. The differences might be attributed to the goat's breed.

The liver enzymes remained within the normal safe limits (Plumb, 1999), which indicates that no hepatocellular stress is associated with the diets (Ibhaze *et al*., 2021). Also, the normal liver enzyme concentration might indicate that the elevated ALB is not attributed to diet, as diet-elevated ALB suggests the existence of bleeding or liver illness (Soul *et al*., 2019). The levels of TBIL and DBIL fell within the established normal ranges of 0.10 – 0.90 mg/dl and 0.10 – 0.40 mg/dl (Plumb, 1999) for healthy goats, respectively. The normal TBIL and DBIL levels in all the animals suggest that they have no liver dysfunction, hepatobiliary dysfunction or haemolytic anaemia (Zaitsev *et al*., 2020).

Conclusion

The study demonstrated that the use of *P. ostreatus*-treated millet and cowpea chaffs as replacements for cassava peels at both inclusion levels in the diets supported haematological and serum biochemical profiles within the normal physiological limits for healthy goats. Higher inclusion levels, particularly 30% TMC in the diets, appeared to enhance erythropoiesis, improve immune function and maintain energy metabolism. These findings suggest that the successful application of fungal bioimproved crop residues, especially TMC, at higher inclusion levels in the diet of the goats can sustain normal health status in WAD goats under tropical feeding systems and can be adopted as a sustainable feeding strategy if the method is simplified to an adaptable, low cost and farmer friendly way.

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