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Immunometabolic Responses and Adaptive Physiology of Yankassa Ram Lambs Fed Diets Containing *Pleurotus ostreatus*-Mediated Solid-State Fermented *Saccharum officinarum* Scrapings

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Abstract

Sheep production systems in sub-Saharan Africa are largely constrained by seasonal fluctuations in feed availability and persistent shortages in both the quantity and quality of forages throughout the year. These limitations adversely affect growth performance and reproductive efficiency in sheep. In addition, the escalating cost of conventional feed ingredients used in ration formulation has intensified the search for alternative, affordable, and locally available feed resources that do not compete directly with human food systems. Particular attention has been directed toward the utilization of lignocellulosic agro-industrial residues, which often constitute environmental pollutants when improperly disposed of. The present study investigated the effects of replacing corn bran with biodegraded sugarcane scrapings (BSS) treated with *Pleurotus ostreatus* on the physiological and immunological responses of growing sheep. Twenty-one clinically healthy Yankassa ram lambs (7-8 months old; mean body weight 10.33 ± 0.42 kg) were randomly allotted to three dietary treatments in a completely randomized design: 0% (T1), 15% (T2), and 30% (T3) inclusion levels of BSS over a 14-week feeding period. The study specifically evaluated immune and physiological indices, including serum immunoglobulins (IgA, IgM, and IgG), triiodothyronine (T3), total antioxidant capacity (TAC), and major antioxidant enzymes-superoxide dismutase (SOD),

catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). The results demonstrated that dietary inclusion of BSS did not significantly influence these immunological or oxidative stress parameters, and all measured values remained within normal physiological ranges across treatments. The solid-state biodegradation of sugarcane scrapings enhanced their nutritive value, and incorporation at 15% supported adequate nutrient intake and maintained physiological equilibrium without impairing immune competence. Furthermore, the 30% inclusion level proved safe and did not compromise health status or adaptive responses in the sheep.

Keywords
Solid state fermentation; *Pleurotus ostreatus*; sugarcane scrapings; immune status; small ruminant welfare

1. Introduction

Feed shortage and seasonal fluctuations in forage quantity and quality remain major constraints to sheep production in sub-Saharan Africa, limiting growth, reproductive performance, and physiological adaptation, particularly in smallholder production systems [1-6]. Coupled with the rising cost of conventional feed ingredients due to food-feed competition and market instability, these challenges have increased the need for affordable, locally available, and sustainable alternative feed resources [7, 8].

Lignocellulosic agro-industrial residues are promising alternatives because they are abundant, inexpensive, and can be converted into valuable livestock feed, thereby reducing environmental pollution and supporting circular bioeconomy initiatives [5, 9-18]. Sugarcane (*Saccharum officinarum*) scrapings are one such residue, but their utilization is constrained by low crude protein content, high fibre, and poor digestibility [7, 13, 19, 20]. Solid-state fermentation with *Pleurotus ostreatus* enhances the nutritional value of these residues by degrading lignin, improving nutrient availability, and increasing digestibility [11, 12, 14-18, 21-28].

Although the benefits of biologically treated agro-residues on animal performance have been widely reported, information on their effects on immune function and oxidative status, particularly in growing ram lambs, remains limited [29-31]. Assessing immunological and oxidative stress biomarkers provides a more comprehensive evaluation of the health implications of alternative feed resources than production performance alone [1].

Therefore, this study evaluated the effects of dietary inclusion of *Pleurotus ostreatus*-biodegraded sugarcane scrapings on the oxidative blood profile and immune status of growing Yankassa ram lambs to determine its suitability as a sustainable alternative feed resource for sheep production under tropical conditions. While the application of *P. ostreatus* in upgrading lignocellulosic feed resources is well documented [1, 2, 26, 32-36], the novelty lies in evaluating the effects of *Pleurotus ostreatus*-biodegraded sugarcane scrapings on immunological and oxidative stress biomarkers in growing Yankassa ram lambs. This important area has received limited attention despite the widespread use of biologically treated agro-residues in ruminant nutrition.

2. Materials and Methods

2.1 Experimental Location

The experiment was conducted at the Co-farms Greenaid Revolution Research Farm in Abuja, Nigeria. Located between latitudes 8°55' N and 9°00' E and longitudes 7°00' N and 7°05' E, the position is 456 meters above sea level. The area has an annual temperature of 25.8-42°C and 1100-1650 mm of precipitation [37-40].

2.2 Preparation and Biodegradation of Sugarcane Scrapings

Pleurotus ostreatus was obtained from a reputable commercial producer in Nigeria. At the same time, fresh sugarcane scrapings (SS) were collected from local sugarcane processors, chopped into 1-2 cm lengths, and air-dried at room temperature (25-30°C). Before inoculation, the SS was thoroughly mixed with distilled water to obtain a moisture content of approximately 67%. The initial substrate pH was adjusted to approximately 6.0-6.5, which is within the optimum range for the growth of *P. ostreatus*. The moistened substrate was transferred into previously cleaned and sanitized containers and sterilized by autoclaving at 121°C for 15 min on two successive occasions, with cooling intervals between sterilization cycles, to eliminate competing microorganisms. After cooling under aseptic conditions, the sterilized substrate was inoculated with *P. ostreatus* at a substrate-to-inoculum ratio of 25:1 (w/w). The inoculated substrate was incubated under solid-state fermentation conditions at 30 ± 2°C and 90-100% relative humidity for 21 days. The fermentation room temperature was monitored daily and maintained within the specified range to ensure optimal mycelial colonization. At the same time, the substrate pH remained within the desirable range (6.0-6.5) throughout the fermentation period. Sterilization of the substrate before inoculation eliminated competing microorganisms, thereby facilitating rapid colonization and biodegradation of the sugarcane scrapings by *P. ostreatus*. At the end of the 21-day fermentation period, the biodegraded sugarcane scrapings (BSS) were dried to constant weight, packaged, and stored until incorporation into the experimental diets.

2.3 Diets, Management, and Experimental Animals

For the study, twenty-one Yankassa ram lambs with an average initial body weight of 10.33 ± 0.42 kg and aged 7-8 months were procured from a local livestock market. The animals were housed individually in 1.2 m^2 pens within a 6 m × 8 m × 4 m well-ventilated corral. Two weeks before stocking, the housing facility and surrounding environment were thoroughly sanitized using Hypo® (containing sodium hypochlorite, caustic soda, and demineralized water) followed by Morigad® antiseptic solution to ensure strict biosecurity.

Upon arrival, the lambs were subjected to a two-week quarantine and prophylactic health management protocol. Preventive treatments included intramuscular administration of long-acting oxytetracycline hydrochloride at 1 mL per 10 kg body weight, oral administration of Vitalyte® as an anti-stress supplement, subcutaneous vaccination against peste des petits ruminants (1.0 ml at $10^{2.5}$ $TCID_{50}$ PPR virus) in the neck region, and subcutaneous injection of Avomec® at 0.5 mL per 25 kg body weight for endo- and ectoparasite control. Throughout the experimental period, the animals were housed under permanent shade in well-ventilated pens that were not overcrowded, allowing

adequate air movement and effective cross-ventilation. The housing system was designed to minimize heat accumulation and provide a comfortable environment under the prevailing tropical climatic conditions. Animals were monitored daily for clinical signs of heat stress, including excessive panting, open-mouth breathing, and lethargy, and no such signs were observed during the study.

Three experimental diets were formulated to contain graded inclusion levels of *Pleurotus ostreatus*-biodegraded sugarcane scrapings (BSS) at 0% (control), 15%, and 30% of the diet in Table 1 below. The inclusion levels were selected to evaluate the response of growing Yankassa ram lambs to progressive replacement of the conventional energy source (corn bran) with the biodegraded sugarcane scrapings while maintaining nutritionally balanced diets. All diets were formulated to be as nearly isoenergetic and isonitrogenous as practicable and to satisfy the nutrient requirements of growing sheep, based on the recommendations of the NRC [41]. Diet formulation considered the chemical composition of the individual feed ingredients to ensure that adequate dietary energy, crude protein, minerals, and vitamins were supplied across treatments, thereby allowing any observed responses to be attributed primarily to the dietary inclusion level of BSS rather than nutrient deficiencies. The lambs were offered feed daily at 5% of their body weight on a dry matter basis, and feed allowances were adjusted periodically according to changes in body weight throughout the 12-week feeding trial conducted between January and March.

Table 1 Ingredient composition of the experimental diets.

Ingredient (kg)	Diet		
	T1 (control)	T2	T3
Yellow Corn	25.00	25.00	25.00
Corn bran	30.00	15.00	0.00
BSS	0.00	15.00	30.00
GNC	17.00	17.00	17.00
Cowpea husk	25.00	25.00	25.00
Salt	0.50	0.50	0.50
Limestone	2.00	2.00	2.00
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00

GNC, groundnut cake; BSS, biodegraded sugarcane scrapings.

Animals were randomly assigned to the experimental diets after the adaptation period. Feed allowances were adjusted during the trial to ensure minimal refusals while maintaining ad libitum intake within the predetermined feeding level. Feed was provided twice daily at 08:00 and 16:00 hours, and clean drinking water was provided ad libitum throughout the experimental period.

2.4 Determination of Immunological and Oxidative Stress Biomarkers

Blood samples were collected by jugular venipuncture into both plain and heparinized tubes. Blood collected into plain tubes was allowed to clot at room temperature and centrifuged at 3,000 rpm for 15 min to obtain serum. The harvested serum was transferred into sterile cryovials and stored at -20°C until analysis of immunoglobulins, triiodothyronine (T3), cortisol, malondialdehyde (MDA), and total antioxidant capacity (TAC). Blood collected into heparinized tubes was centrifuged

to separate erythrocytes, which were washed three times with physiological saline (0.9% NaCl), lysed with distilled water to obtain erythrocyte hemolysates, and stored at -20°C until determination of erythrocyte antioxidant enzyme activities.

Immune function was evaluated by determining serum immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) using commercially available Stanbio Laboratory diagnostic kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's instructions and the procedures described by Elghalid et al. [42] and Anaso [15, 16]. The assays were based on specific antigen-antibody immunochemical reactions, and absorbance was measured spectrophotometrically using a UV-Visible spectrophotometer. Immunoglobulin concentrations (IgG, mg/100 mL; IgA and IgM, mg/mL) were calculated from calibration curves generated with manufacturer-supplied standards. Commercial quality-control sera of known concentrations were included in each analytical batch to verify assay accuracy, precision, and repeatability.

Serum triiodothyronine (T3; nmol/L), cortisol (ng/mL), malondialdehyde (MDA; nmol/L), and total antioxidant capacity (TAC; nmol) were determined using commercially available Stanbio Laboratory assay kits following the manufacturer's protocols and standardized analytical procedures [16, 17, 43-49]. Malondialdehyde was quantified by the thiobarbituric acid reactive substances (TBARS) method as an indicator of lipid peroxidation. In contrast, TAC was measured by assessing the cumulative antioxidant capacity of serum against a standardized oxidant system. Serum T3 and cortisol concentrations were quantified using enzyme immunoassay procedures supplied with the commercial kits.

Erythrocyte antioxidant enzymes, including superoxide dismutase (SOD; U/mL erythrocyte suspension), catalase (CAT; U/g Hb), glutathione peroxidase (GPx; U/g Hb), and glutathione reductase (GR; IU/10^{11} RBC), were determined spectrophotometrically in erythrocyte hemolysates using Stanbio Laboratory reagent kits according to the manufacturer's instructions. The assays were based on the catalytic activity of each enzyme toward its specific substrate, and enzyme activities were calculated from the change in absorbance over time using the corresponding calibration factors provided with the assay kits.

All assays were performed under the analytical conditions recommended by the manufacturer. Calibration curves were prepared using supplied standards before sample analysis, and internal quality-control materials were analyzed with each batch to ensure analytical precision and accuracy. Absorbance readings were obtained using a UV-Visible spectrophotometer, and analyte concentrations or enzyme activities were calculated according to the manufacturer's instructions and expressed in the respective reporting units. All samples were analyzed in duplicate, and the mean values were used for statistical analysis.

2.5 Data Analysis

Data were first examined for completeness and screened for outliers before statistical analysis. The assumptions of normality and homogeneity of variance were evaluated using the Shapiro-Wilk and Levene's tests, respectively. Variables that satisfied these assumptions were subjected to one-way analysis of variance (ANOVA) under a completely randomized design using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA).

The statistical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observed value of the dependent variable, μ is the overall mean, T_i is the fixed effect of the i-th dietary treatment (0%, 15%, or 30% biodegraded sugarcane scrapings), and e_{ij} is the random experimental error.

When a significant treatment effect was detected, treatment means were separated using Duncan's Multiple Range Test (DMRT). Statistical significance was declared at $P < 0.05$. Exact P-values are reported in the Results where appropriate.

3. Results and Discussion

3.1 Phytochemical Analysis of the Unfermented and Fermented Sugarcane Scrapings

The phytochemical composition of untreated sugarcane scrapings (USS) and *Pleurotus ostreatus*-biodegraded sugarcane scrapings (BSS) is presented in Table 2. Biodegradation reduced the concentrations of all measured secondary metabolites, including saponins, phytate, oxalate, condensed tannins, and flavonoids, demonstrating the effectiveness of *P. ostreatus* in improving the nutritional quality of sugarcane scrapings. Plant secondary metabolites may exert beneficial or anti-nutritional effects depending on their concentration [26, 38, 50]. The reductions observed in the present study agree with previous reports that white-rot fungal fermentation lowers anti-nutritional factors in lignocellulosic feed resources through the action of extracellular ligninolytic and cellulolytic enzymes [1, 3, 7, 11, 13-19, 51-53]. In ruminants, the detoxification capacity of rumen microorganisms further enhances the utilization of such biologically treated feed resources [12, 54-56].

Table 2 Phytochemical analysis of unfermented and fermented sugarcane scrapings.

Parameters (g/100 g DM)	Sugarcane scrapings	
	USS	BSS
Saponins	8.67	7.83
Phytate	1.96	1.72
Oxalate	0.20	0.10
Condensed tannins	2.12	1.57
Flavonoids	6.40	4.39

USS, untreated sugarcane scrapings; BSS, biodegraded sugarcane scrapings.

Although saponins, oxalates, phytates, tannins, and flavonoids influence rumen fermentation, mineral utilization, and antioxidant activity, their concentrations in BSS remained below levels considered detrimental to sheep [7, 57-60]. At moderate concentrations, saponins and tannins may improve rumen fermentation, nitrogen utilization, immune function, and methane mitigation without compromising animal health [11-13, 19, 31, 61-65]. Likewise, the reductions in phytate and oxalate may improve mineral bioavailability, while the lower flavonoid concentration indicates that biodegradation did not compromise the antioxidant suitability of the substrate [7, 66, 67].

3.2 Chemical Composition of the Experimental Diets

Table 3 summarizes the proximate and fibre fractions of the experimental diets formulated for Yankassa ram lambs. Dry matter (DM) values ranged from 91.90 to 93.78%, with a slight reduction observed at the highest inclusion level of biodegraded sugarcane scrapings (BSS) in T3. Although crude protein (CP) appeared marginally lower in the control diet (T1), all diets were deliberately formulated to be isonitrogenous. Ether extract (EE), crude fibre (CF), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, and cellulose increased progressively as corn bran was replaced with BSS. Conversely, hemicellulose and non-fibre carbohydrates (NFC) declined consistently with increasing BSS inclusion.

Table 3 Chemical composition of the experimental diets.

Components	Diets		
	T1 (control)	T2	T3
Dry matter (%)	92.90	93.78	91.90
In % DM			
Crude protein	15.60	15.70	15.79
Ether extract	5.68	5.70	5.72
Crude fibre	12.72	15.40	17.89
Crude ash	6.14	7.94	8.35
Organic matter	93.66	91.86	91.45
NFC	33.49	29.64	27.14
NDF	38.59	40.52	42.50
ADF	20.85	23.00	25.14
Lignin	2.79	3.11	4.35
Cellulose	17.96	19.79	20.69
Hemicellulose	17.64	17.42	17.26

Diet T1, 0% biodegraded sugarcane scrapings; Diet T2, 15% biodegraded sugarcane scrapings; Diet T3, 30% biodegraded sugarcane scrapings inclusion; ADF, acid detergent fibre; NDF, neutral detergent fibre; NSC, non-structural carbohydrates.

The relatively high DM content across treatments is nutritionally desirable, as it indicates low moisture concentration and a greater density of nutrients. Dry matter represents the nutritive fraction of feed exclusive of water [3, 68, 69]. The DM values obtained were comparable to those reported for sugarcane peel-based and fungus-treated agro-residue diets in small ruminant feeding trials, with minor variations attributable to differences in substrate composition and processing techniques [70, 71].

The isonitrogenous nature of the diets and CP levels approximating the 15% requirement for growing sheep [41] indicate that *Pleurotus ostreatus*-mediated biodegradation substantially enhanced the protein value of sugarcane scrapings to levels comparable with corn bran. Improvements in CP following fungal fermentation are widely documented. They are typically attributed to fungal biomass synthesis, microbial protein enrichment, and nitrogen immobilization within the fungal matrix during aerobic solid-state fermentation [7, 11-13, 25, 56, 72, 73].

The progressive increase in EE with higher BSS inclusion suggests a relatively greater lipid contribution from the biodegraded substrate compared with corn bran. Nevertheless, EE concentrations remained within physiologically acceptable limits that are unlikely to impair rumen microbial activity or fermentation dynamics in ruminant [74]. The slight decline in organic matter (OM) with increasing BSS inclusion reflects the comparatively higher OM content of corn bran relative to the treated residue.

Crude fibre increased from T1 to T3, consistent with greater incorporation of BSS, a lignocellulosic by-product. Although fungal biodegradation reduced fibre complexity, the treated material retained higher fibre fractions than corn bran. Similar trends have been reported in sheep and goat diets incorporating fibrous agro-residues [70]. Correspondingly, NDF, ADF, and lignin values increased with greater BSS inclusion, reflecting the inherent structural carbohydrate content of the substrate. Importantly, NDF concentrations remained below the 65% threshold commonly associated with depressed intake and digestibility in ruminants [1, 13]. Comparable responses have been observed when conventional concentrates were partially replaced with fermented fibrous materials in small ruminant diets [75].

Neutral detergent fibre serves as an indicator of dietary bulk. It is frequently used to predict voluntary dry matter intake, whereas ADF represents the less digestible cell wall components that can limit feed utilization [14, 76]. In the present study, cellulose increased with greater BSS inclusion, while hemicellulose declined. Both are structural polysaccharides of the plant cell wall and are partially degradable in the rumen ecosystem [76]. Elevated hemicellulose concentrations have been associated with reduced voluntary intake in some cases.

The decline in NFC with increasing BSS inclusion highlights the need to maintain balanced fermentable carbohydrate levels in sheep diets. Adequate NFC supports optimal rumen microbial growth and energy supply, whereas excessive NFC intake can disrupt ruminal pH homeostasis and predispose animals to metabolic disturbances such as acidosis [3, 7, 77, 78].

3.3 Immune and Oxidative Stress Responses of Rams

Table 4 shows that dietary inclusion of *Pleurotus ostreatus*-biodegraded sugarcane scrapings (BSS) at 15 and 30% had no significant effect ($P > 0.05$) on the measured immunological, endocrine, or oxidative stress biomarkers of growing Yankassa ram lambs. Serum concentrations of immunoglobulins (IgA, IgM, and IgG), triiodothyronine (T3), malondialdehyde (MDA), cortisol, total antioxidant capacity (TAC), and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) were comparable among treatments. These results indicate that replacing corn bran with BSS at dietary inclusion levels of up to 30% did not significantly affect the evaluated biomarkers under the conditions of the present study.

Table 4 Immune and oxidative stress responses of rams-lambs.

Parameter	T1	T2	T3	SEM
Immunoglobulin G (mg/dL)	3898.22	3873.00	3865.23	102.10
Immunoglobulin A (mg/mL)	0.35	0.32	0.28	0.07
Immunoglobulin M (mg/mL)	2.72	2.54	2.49	0.39
Triiodothyronine (nmol/L)	1.94	1.76	1.73	0.49
Malondialdehyde (nmol/L)	0.82	0.87	0.88	0.08
Superoxide dismutase (U/mL)	787.50	773.20	741.42	30.07
Catalase (U/g Hb)	1580.00	1570.77	1560.47	22.83
Glutathione peroxidase (U/g Hb)	173.60	163.50	159.90	8.09
Cortisol (ng/mL)	13.71	14.01	14.51	0.97
Total antioxidant capacity (mmol/L)	1.57	1.43	1.18	0.20
Glutathione reductase (IU/10^{11} RBC)	1.66	1.58	1.48	0.20

T1, T2 and T3 denoting treatments one, two and three.

Immunoglobulins are widely used indicators of humoral immune status [1, 79-83]. Likewise, T3 is involved in regulating growth, metabolism, and thermoregulation in ruminants [84-88], whereas MDA, SOD, CAT, GPx, GR, TAC, and cortisol are commonly used biomarkers for assessing oxidative status and physiological responses in animals [89-93]. The comparable values observed across treatments indicate that inclusion of BSS did not result in measurable changes in these biomarkers during the experimental period.

The absence of significant treatment effects may reflect the suitability of the fermented sugarcane scrapings as a replacement for corn bran at the inclusion levels evaluated. Solid-state fermentation with *P. ostreatus* has been reported to degrade lignin and reduce anti-nutritional components through the action of ligninolytic enzymes, thereby improving the feeding value of lignocellulosic materials [11-13, 23, 27, 94]. However, the present findings do not demonstrate improvements in immune function or oxidative status; rather, they indicate that dietary inclusion of BSS up to 30% was not associated with significant changes in the measured immunological and oxidative stress biomarkers under the conditions of this study [14].

4. Conclusion

The present study demonstrated that dietary inclusion of *Pleurotus ostreatus*-biodegraded sugarcane scrapings (BSS) as a partial or complete replacement for corn bran had no adverse effects on the evaluated immunological and oxidative stress indicators of growing Yankassa ram lambs. Across all dietary inclusion levels (0-30%), serum concentrations of immunoglobulins (IgA, IgM, and IgG), triiodothyronine (T3), cortisol, malondialdehyde (MDA), total antioxidant capacity (TAC), and antioxidant enzyme activities remained comparable among treatments, indicating that dietary BSS inclusion did not alter these measured physiological biomarkers under the conditions of the present study.

4.1 Limitations Recommendations

A limitation of the present study is the relatively modest sample size (n = 7 per treatment), which, although comparable to similar controlled sheep nutrition studies, may have limited the statistical power to detect subtle physiological and immunological differences among treatments. Future studies with larger sample sizes and formal a priori power analyses are recommended to validate these findings further.

Author Contributions

The author did all the research work of this study.

Competing Interests

The author has declared that no competing interests exist.

AI-Assisted Technologies Statement

AI-assisted language editing was used solely to improve grammar, clarity, and readability. All scientific content, data analysis, interpretation, and final editorial decisions were undertaken and verified by the authors, who take full responsibility for the manuscript.

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