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Immunological Status and Oxidative Stress Responses of Kano Brown Buckling Goats Fed Diets Containing Pleurotus Ostreatus–Biodegraded Sugarcane (Saccharum Officinarum) Scrapings

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ABSTRACT

Ruminant production in sub-Saharan Africa is constrained by inconsistent availability and poor quality of feed resources, which adversely affect animal productivity and reproductive performance. The rising cost of conventional feed ingredients has intensified the search for affordable and sustainable alternatives, particularly agro-industrial residues such as sugarcane scrapings. This study evaluated the effects of replacing corn bran with Pleurotus ostreatus-biodegraded sugarcane scrapings (BSS) on the physiological and immunological responses of growing goats. Twenty-one healthy Kano Brown bucklings (6–7 months old; 9.40 ± 0.39 kg body weight) were randomly assigned to three dietary treatments containing 0% (T1), 15% (T2), and 30% (T3) BSS in a completely randomized design over 16 weeks. Parameters assessed included immunoglobulins (IgA, IgM, and IgG), triiodothyronine (T3), total antioxidant capacity (TAC), and antioxidant enzymes comprising superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, malondialdehyde (MDA) and cortisol. Dietary inclusion of BSS had no significant effect on immunological or oxidative stress indices, with all values remaining within normal physiological ranges. The biodegradation process improved the nutritive value of sugarcane scrapings, while inclusion levels up to 30% maintained physiological stability and immune competence without adverse effects. The findings demonstrate that biodegraded sugarcane scrapings can serve as a safe, sustainable, and cost-effective non-conventional feed resource for small ruminant production systems.

Contents

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1. Introduction

Feed scarcity and the pronounced seasonal variability in both quantity and quality of available forages remain the most critical constraints limiting ruminant productivity in sub-Saharan Africa [1, 2]. These challenges exert profound negative effects on growth performance, reproductive efficiency, and overall physiological resilience of livestock, particularly under smallholder and semi-intensive production systems [3, 4]. In West Africa, feed costs alone account for approximately 60–70% of total livestock production expenses, and reliance on conventional feed ingredients such as maize and wheat offal has become increasingly unsustainable due to competition with human food systems, import dependence, and price volatility [5–8].

The escalating cost and limited availability of conventional feedstuffs have therefore intensified the search for alternative, non-competitive, locally available, and low-cost feed resources capable of sustaining animal productivity without compromising health or product quality [9–14]. Among such alternatives, lignocellulosic agricultural residues have attracted increasing attention because of their abundance and underutilization, despite their frequent classification as environmental pollutants when improperly managed [14–19]. Efficient valorization of these residues aligns with circular bioeconomy principles by converting agricultural waste into value-added animal protein while reducing environmental degradation [3, 20].

Sugarcane (*Saccharum officinarum*) is widely cultivated across northern Nigeria, and its processing generates substantial quantities of coproducts, including sugarcane scrapings or peels. This residue is readily available, inexpensive, and often discarded, contributing to environmental pollution and waste-management challenges [14, 19]. However, the practical use of sugarcane scrapings in livestock feeding is constrained by their inherently low crude protein content, high fiber fractions, and extensive lignification, which collectively limit digestibility and nutrient availability [21–25].

To overcome these limitations, biological upgrading techniques particularly solid-state fermentation (SSF) using lignocellulolytic fungi have emerged as sustainable and effective strategies for improving the nutritive value of fibrous residues. White-rot fungi such as *Pleurotus ostreatus* possess a unique enzymatic system capable of degrading lignin through the secretion of ligninolytic enzymes including laccase, manganese peroxidase, and lignin peroxidase, thereby enhancing carbohydrate accessibility and microbial fermentability [17, 26–32]. Through this process, SSF increases crude protein content via microbial biomass synthesis, reduces structural carbohydrates, and improves palatability and digestibility, making treated residues more suitable for ruminant diets [5, 33–38].

Beyond improvements in nutrient composition, biologically treated lignocellulosic feeds may exert broader physiological effects, including modulation of metabolic status, antioxidant defense, immune competence, and reproductive function. However, most previous studies on solid-state fermented roughages have focused primarily on growth performance and nutrient utilization, with limited attention to immune response, oxidative stress balance, and adaptive physiological indices, particularly in male ruminants whose reproductive efficiency is closely linked to nutritional and oxidative status [16].

Against this background, the present study was designed to evaluate the effects of *Pleurotus ostreatus*-biodegraded sugarcane scrapings on oxidative blood profile and immune status of Kano Brown bucks. By integrating nutritional, immunological, and physiological endpoints, this study seeks to provide evidence-based insights into the suitability of biodegraded sugarcane scrapings as a sustainable feed resource capable of supporting both productivity and adaptive health in tropical small ruminant systems.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted at the Teaching and Research Farm of the University of Abuja, located in Abuja, Nigeria. The study area lies within latitude 8°55′–9°00′ N and longitude 7°00′–7°05′ E, at an altitude of approximately 456 m above sea level. The region is characterized by a tropical climate with annual rainfall ranging from 1,100 to 1,650 mm and ambient temperatures ranging from 25.8 to 42 °C [39–43].

2.2. Preparation and Biodegradation of Sugarcane Scrapings

Pleurotus ostreatus was bought from a reputable commercial producer in Nigeria, while fresh SS was collected from local sugarcane processors, chopped into 1-2 cm lengths, and air-dried at room temperature (25–30°C). Before the Pleurotus inoculation for solid state fermentation, the SS was combined with distilled water in a 1:1 ratio in each previously cleaned, dried, and sanitized container to reduce the moisture content to 67%. To get rid of any living microorganisms, the SS were autoclaved twice, once at 121°C for 15 minutes each time, with cooling intervals in between. Before being infected with *P. ostreatus* spores in a 25:1 ratio, the prepared SS were cooled in an aseptic atmosphere. After that, the inoculation room was kept at 30°C and 100% relative humidity until mycelia developed. Autoclaving the material prevented mycelia growth and biodegradation of the biodegraded SS (BSS) after a 21-day period of inoculation and SSF. The BSS were packaged and stored until they were needed for feeding after being dried to a constant weight.

2.3. Diets, Management, and Experimental Animals

For the experiment, 21 brown male goats weighing 9.44 ± 0.39 kg and aged between 6 and 7 months were bought from an open market. Within a 6 m × 8 m x 4 m corral, the goats were kept in individual 1.2 m2 cages. Two weeks prior to their arrival, the pen and its immediate surroundings were thoroughly cleaned using Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad). During their two-week quarantine, the animals received preventative treatments. These included an intramuscular injection of a long-acting oxytetracycline HCl at 1 mL/10 kg BW, an oral anti-stress drug called Vitalyte®, a subcutaneous injection of the live PPR vaccine (1 mL at 102.5 of the TCID50 PPR virus) at the neck region, and a subcutaneous injection of an anti-parasitic drug called Avomec® at 0.5 mL/25 kg BW. Three complete meals were created using varying SS 0 (control), 15, and 30% inclusion ratios. The meals that catered to the requirements of growing goats were designed in accordance with the NRC's [44] recommendations. Buck juveniles were fed at 5% of their body weight (based on dry matter; DM) and were balanced for body weight throughout a 16-week period (December to March). After then, one of the experimental diets detailed in Table 1 was given to the goats at random. The amount of feed given to the goats was changed during the trial to ensure that some was left behind. Feeding was done twice a day between 8:00 and 16:00. Every day, they had unrestricted access to clean water.

Table 1 Ingredient composition of the experimental diets

Ingredient (kg)	Diet		
	T1	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.

2.4. Chemical Analysis

Phytochemical analysis was carried out on treated and untreated sugarcane scrapings using standard as described by Sofowora [45]. Percentage composition of saponins, phytate, oxalate and condensed tannins were carried out according to procedures outlined by Harborne [46] and Boham and Kocipai-Abyazan [47]. Samples of treated and untreated sugarcane scrapings and the experimental diets were ground to pass a 1 mm sieve in a Wiley mill and stored for further analysis. Proximate analyses of crude protein, ash, ether extract and crude fiber in the experimental samples were carried in accordance with the procedures of AOAC International [48]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methods of Soest et al. [49]. NDF was analyzed using sodium sulphite and amylase and expressed with residual ash. Acid detergent lignin was determined by solubilization of cellulose with sulphuric acid. Concentrations of hemicellulose and cellulose were calculated mathematically as the differences between NDF and ADF and ADF and lignin respectively. Non-fiber carbohydrate (NFC) was calculated according to Hinders [50, 51] using the following formula:

$$NFC = 100 - (CP + EE + ash + NDF)\% \qquad (1)$$

2.5. Blood Collection and Analysis

On the final day of the experimental trial, blood samples were collected from all goats assigned to the respective dietary treatments for the determination of immune, endocrine, and oxidative stress biomarkers. Blood sampling was conducted in the morning prior to feeding and water administration in order to minimize postprandial and diurnal variations in serum biochemical parameters.

Approximately 10 mL of blood was aseptically collected from the jugular vein of each animal using sterile disposable needles and vacutainer tubes. Samples intended for serum analysis were collected into plain tubes without anticoagulants and allowed to clot at room temperature for 30–45 min. Thereafter, the samples were centrifuged at 3,000 rpm for 15 min to obtain clear serum, which was carefully harvested into sterile cryovials and stored at −20 °C pending laboratory analyses.

The harvested serum samples were subsequently used for the determination of immunological indices including immunoglobulins A (IgA), G (IgG), and M (IgM); endocrine biomarkers such as triiodothyronine (T3) and cortisol; and oxidative stress parameters comprising total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR).

2.5.1. Immune function

Immune function was evaluated through the quantification of serum immunoglobulin classes IgA, IgG, and IgM using commercially available ELISA diagnostic kits (Stanbio Laboratory Inc., Boerne, TX, USA; Cat. Nos. SIgA-96, SIgG-96, and SIgM-96), following the procedures described by Elghalid et al. [52] and Anaso [36, 37]. Serum samples and standards were incubated in antibody-coated microplates at 37 °C for 30 min, followed by washing and addition of enzyme conjugates. Absorbance was measured using a microplate spectrophotometer at 450 nm after substrate development. Calibration curves were generated using manufacturer-provided standards, and concentrations were expressed in mg/mL or mg/100 mL as appropriate.

2.5.2. Oxidative stress markers

Oxidative stress and endocrine biomarkers assessed included triiodothyronine (T3), cortisol, total antioxidant capacity (TAC), antioxidant enzyme activities comprising superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), as well as malondialdehyde (MDA), which was evaluated separately as a biomarker of lipid peroxidation and oxidative membrane damage. Analyses were conducted using standardized commercial assay kits (Stanbio Laboratory Inc., USA; Cayman Chemical, Ann Arbor, MI, USA; Randox Laboratories Ltd., UK) according to manufacturer instructions and previously validated protocols [53–56].

Serum T3 and cortisol concentrations were determined by ELISA at an absorbance wavelength of 450 nm following incubation at 37 °C for 60 min. Total antioxidant capacity was measured spectrophotometrically at 593 nm using the ferric reducing antioxidant power (FRAP) method. Malondialdehyde concentration was quantified via the thiobarbituric acid reactive substances (TBARS) assay at 532 nm after incubation in a boiling water bath for 15 min.

Superoxide dismutase activity was assayed based on inhibition of epinephrine auto-oxidation at 480 nm, while catalase activity was determined by monitoring hydrogen peroxide decomposition at 240 nm. Glutathione peroxidase and glutathione reductase activities were measured at 340 nm using NADPH oxidation kinetics. For all assays, reagent blanks, calibration standards, and quality-control sera were included to ensure analytical accuracy, calibration precision, and repeatability. Data Analysis

The obtained data was subjected to and met normal distribution conditions. Subsequent results were subjected to analysis of variance (ANOVA) in a completely randomized design using the SPSS BASE 23 (SPSS software products, USA). Duncan multiple range test (DMRT) of same software was used to test the significant difference between the means at (p≤0.05 level of significance. The sample size (n = 7 per treatment) was selected based on comparable animal nutrition and physiology studies involving phytogenic feed additives in ruminants, where similar replication levels provided sufficient statistical sensitivity for detecting treatment effects.

3. Results

3.1. Phytochemical analysis of the unfermented and fermented sugarcane scrapings

Table 2 presents the phytochemical composition of untreated sugarcane scrapings (USS) and *Pleurotus ostreatus*–biodegraded sugarcane scrapings (BSS). Both materials contained measurable amounts of major secondary metabolites, including saponins, phytate, oxalate, condensed tannins, and flavonoids. However, the concentrations of these phytochemicals were consistently higher in the untreated sugarcane scrapings compared with the biodegraded counterpart. Specifically, saponin content decreased from 8.67 to 7.83 g/kg DM, phytate from 1.96 to 1.72 g/kg DM, oxalate from 0.20 to 0.10 g/kg DM, condensed tannins from 2.12 to 1.57 g/kg DM, and flavonoids from 6.40 to 4.39 g/kg DM following fungal biodegradation. These reductions clearly indicate the efficacy of *P. ostreatus* in modifying the phytochemical profile of sugarcane scrapings.

Table 2 Phytochemical analysis of unfermented and fermented sugarcane scrapings

Parameters (g/kg 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

3.2. Chemical composition of the experimental diets

Table 3 presents the proximate and fiber composition of the experimental diets. Dry matter (DM) content ranged from 91.90 to 93.78%, with a marginally lower value observed in T3. Although crude protein (CP) was slightly reduced in T1, all diets were formulated to be isonitrogenous. Ether extract (EE), crude fiber (CF), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and cellulose increased progressively with increasing replacement of corn bran by biodegraded sugarcane scrapings (BSS). In contrast, hemicellulose and non-fiber carbohydrates (NFC) declined consistently as the level of BSS inclusion increased.

Table 3 Chemical composition of the experimental diets

Components	**Diets**		
	T1	**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	93.86	92.06	91.65
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 fiber; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates

3.3. Immune and oxidative stress responses of goats

Table 4. presents the immunological, endocrine, and oxidative stress responses of growing Kano Brown buckling goats fed diets containing graded inclusion levels of *Pleurotus ostreatus*–biodegraded sugarcane scrapings (BSS).

Table 4 Immune and oxidative stress responses of goats

Parameter	**T1**	**T2**	**T3**	**SEM**
Immunoglobulin G (mg/100 mL)	3998.22	4015.37	4031.45	102.50
Immunoglobulin A (mg/mL)	0.30	0.31	0.29	0.04
Immunoglobulin M (mg/mL)	3.72	3.68	3.65	0.27
Triiodothyronine (nmol/L)	1.99	2.03	2.01	0.18
Malondialdehyde (nmol/L)	0.92	0.89	0.90	0.05
Superoxide dismutase (nmol/mL)	887.55	895.31	901.47	15.07
Catalase (U/gHb)	1680.00	1692.77	1701.47	21.85
Glutathione peroxidase (U/gHb)	183.60	186.50	188.90	4.59
Cortisol (ng/mL)	14.71	14.48	14.36	0.42
Total antioxidant capacity (nmol)	1.08	1.11	1.13	0.06
Glutathione reductase (IU/10^{11} RBC)	1.56	1.61	1.64	0.14

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

The dietary treatments comprised 0% (T1), 15% (T2), and 30% (T3) BSS arranged in a completely randomized design. Across all measured indices, dietary incorporation of BSS did not result in significant differences ($P > 0.05$), indicating that immune competence, endocrine regulation, and oxidative homeostasis were maintained irrespective of treatment. This suggests that replacing maize bran with BSS, even at full substitution, did not impose physiological or metabolic stress on the animals.

4. Discussion

4.1. Phytochemical Analysis of the Unfermented and Fermented Sugarcane Scrapings

Plant secondary metabolites are recognized for their therapeutic, protective, and anti-nutritional effects in feed resources [56, 57]. The reduced phytochemical concentrations observed in biodegraded sugarcane scrapings (BSS) agree with previous studies showing that fungal fermentation decreases phenolics, saponins, and related anti-nutritional compounds in agro-industrial by-products [2, 8, 19, 39, 40, 54, 55, 58–60]. This reduction is primarily linked to microbial extracellular enzymes, including ligninases, cellulases, and oxidoreductases, which degrade resistant plant metabolites and anti-nutritional factors [14, 61]. Rumen microorganisms may further detoxify low levels of these compounds without impairing fermentation or animal performance [16, 17, 62], consistent with findings by Nwafor et al. [63] and Olafadehan et al. [18].

Saponins, although reduced after biodegradation, remained at moderate concentrations capable of beneficially modulating rumen fermentation, immunity, and methane reduction without adverse effects [14, 31, 64–66]. Oxalate and phytate levels in BSS were below critical thresholds reported for ruminants, suggesting minimal risk of mineral chelation or metabolic interference [8, 67–69]. Similarly, condensed tannins remained below levels associated with depressed feed intake, while still offering potential benefits such as improved nitrogen utilization and methane mitigation [19, 40, 41, 70–72]. Flavonoid concentrations were also relatively low, indicating limited risk of metabolic disturbances while retaining antioxidant potential [73].

4.2. Chemical composition of the experimental diets

The high dry matter (DM) content of the diets indicates low moisture and greater nutrient concentration, which is nutritionally advantageous for ruminants [8, 74]. Although slightly lower than values reported for sugarcane peel and fungi-treated cassava peel diets [75, 76], the variations likely reflect differences in ingredients and processing methods. The isonitrogenous diets met the crude protein requirement for growing goats [44], confirming that biodegradation of sugarcane scrapings with *Pleurotus ostreatus* improved protein quality to levels comparable with corn bran. Similar increases in crude protein following fungal treatment have been widely reported [2, 8, 14, 17–19, 31, 32, 34, 59, 77–80], mainly due to fungal biomass accumulation and microbial protein synthesis during fermentation.

Ether extract increased with higher BSS inclusion, indicating greater lipid fractions in BSS than corn bran, although levels remained within acceptable limits for rumen function [81]. Crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin also increased progressively with BSS inclusion due to the fibrous nature of sugarcane scrapings, despite partial fiber degradation during fermentation. Nevertheless, NDF values remained below critical levels associated with reduced intake and digestibility [19], consistent with reports by Olafadehan and Adebayo [82]. Increased cellulose and reduced hemicellulose contents further reflected changes in structural carbohydrates following biodegradation [38, 49]. In contrast, non-fiber carbohydrates declined with increasing BSS inclusion, emphasizing the need to maintain balanced NFC levels to sustain rumen microbial activity and prevent metabolic disorders such as acidosis [2, 8, 83].

4.3. Immune and oxidative stress responses of goats

Humoral immune indices, including IgA, IgM, and IgG, were unaffected by dietary treatments, indicating that Pleurotus ostreatus–biodegraded sugarcane scrapings (BSS) did not impair adaptive immune function or humoral defense mechanisms in goats. Immunoglobulins are essential for mucosal protection, pathogen neutralization, and long-term immunity [84–87]. The stable immune responses observed are particularly important under tropical production conditions where nutritional and environmental stressors often suppress immunity [55, 56, 88].

Similarly, serum triiodothyronine (T3) concentrations remained stable across treatments, suggesting that endocrine regulation, metabolic balance, and thermoregulatory functions were maintained despite replacement of maize bran with BSS [89–92]. Oxidative stress indices, including superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, total antioxidant capacity, malondialdehyde, and cortisol, were also comparable among treatments, indicating preserved antioxidant defense systems, redox balance, and absence of dietary stress or oxidative damage [93–99].

These favorable physiological responses may be attributed to fungal biodegradation, during which P. ostreatus ligninolytic enzymes improve fibre digestibility, nutrient availability, and detoxification of lignocellulosic residues [17, 19, 28, 29, 31]. Consequently, goats fed up to 30% BSS maintained normal metabolic, immunological, and oxidative status throughout the feeding trial.

5. Conclusion

The present study demonstrated that replacing corn bran with Pleurotus ostreatus–biodegraded sugarcane scrapings (BSS) had no adverse effects on the immunological, endocrine, or oxidative status of growing Kano Brown goats. Humoral immune indices, including IgA, IgM, and IgG, remained within normal physiological ranges across all inclusion levels, indicating preserved immune competence and systemic defense. Similarly, stable serum triiodothyronine concentrations confirmed that metabolic regulation and endocrine homeostasis were maintained.

Comparable activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, total antioxidant capacity, malondialdehyde, and cortisol further indicated that BSS-based diets did not induce oxidative stress, impair redox balance, or trigger physiological stress responses. These findings suggest that goats effectively maintained antioxidant protection and cellular integrity even at the highest BSS inclusion level.

Importantly, solid-state fermentation with P. ostreatus improved the nutritive quality and digestibility of sugarcane scrapings through selective lignin degradation, transforming the residue into a safe, nutrient-enriched, and sustainable feed resource suitable for climate-smart and circular livestock production systems.

Conflict of Interest

The authors have no conflicts of interest to declare.

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