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Body weight, scrotal parameters and semen characteristic of Kano Brown Buck Kids fed *Pluerotus ostreatus* solid state fermented sugarcane scrapings

Emmanuel ANASO [1*], Olurotimi OLAFADEHAN [1], Ayoola John SHOYOMBO [2], Emeka Solomon FIDELIS [3]

[1] Department of Animal Science, University of Abuja, Abuja, Nigeria
[2] Department of Animal Science, Landmark University, Omu-Aran, Kwara State, Nigeria
[3] Department of Agricultural Economics, University of Abuja, Abuja, Nigeria

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*CONTACT

dranasoeub@gmail.com

ABSTRACT

The study evaluated the effect of substitution of *Pleurotus ostreatus* biodegraded sugarcane scrapings (BSS) for corn bran on the growth performance and reproductive potential of Kano Brown bucks. Twenty-one healthy buck kids (6 – 7 months of age, with an average initial BW of 9.44 ± 0.39 kg) were stratified into three treatments and fed: (1) a total mixed diet containing no BSS (0% BSS; control), (2) the control diet containing 15% BSS substituting 50% corn bran and (3) the control diet containing 30% BSS substituting 100% corn bran on dry matter basis (DM) for 12 weeks in a completely and fully randomized design. Intakes of DM, crude protein and organic matter, and sperm concentration varied in the order: 15% BSS > 0% BSS >30% BSS ($p<0.05$). Final BW, semen volume, initial fructose, scrotal length (SL) and scrotal circumference (SC) were greater in 15% BSS diet than 0 and 30% BSS diets. Semen pH and color, sperm progressive motility, viability and abnormalities, and live spermatozoa were not affected by diets. Whereas testosterone level was greater in 0 and 15% BSS diets, libido was lower in 30% BSS diet. Final BW was positively correlated with SC ($p=0.030$; $r = 0.510$) and SL ($p = 0.048$; $r = 0.472$). It was concluded that up to 30% biodegraded sugarcane scrapings can be used in a complete diet for bucks without negatively impacting final body weight and semen quality, though 15% BSS was more impactful and recommended.

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ORCID> 0009-0001-6886-8557 (EA), ORCID> 0009-0002-6949-3205 (OO), ORCID> 0000-0003-2937-7779 (AJS), ORCID> 0000-0003-4276-2735 (ESF)

1. Introduction

Ruminant production in sub-Saharan African is principally constrained by dearth and unsteady quantity and quality of the year-round feed availability which affect both the growth (productive) and sexual (reproductive) performance of the animal (Olafadehan and Adewumi, 2009). This problem of soaring cost of conventional feedstuffs used in ration formulation necessitates quest by animal nutritionists for alternative, non or less competitive, available and cheap feedstuffs, particularly lignocellulosic residues constituting environmental hazard due to improper disposal (Olafadehan et al., 2014).

Production of sugarcane, an extensively cultivated plant in the northern Nigeria, is associated with concomitant production of a variety of its coproducts among which sugarcane scraping or peel is an important abundantly available coproduct (Anaso et al., 2021; Olafadehan et al., 2021). In spite of the availability, it has a limited utilization in livestock due to its low crude protein (CP) and lignified nature (Anaso et al., 2021).

By improving the CP and dissolving the lignin barrier, solid state fermentation (SSF) with fungus of lignocelluloses has been utilized to increase the nutritional value. This has changed the lignocellulosic structure and made the carbs accessible for more effective conversion (Isroi et al., 2011). There have been reports of white rot fungus producing ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, and effectively mineralizing lignin into carbon dioxide and water (Sanchez, 2010; Isroi et al., 2011), Furthermore, using white rot fungus in solid state fermentation of lignified roughages is an environmentally benign method of using them instead of physical or chemical treatment. Whereas most of the previous studies on the utilization of solid-state fermented lignocelluloses in the diets of ruminants focused on the productive performance, information on the reproductive potential or fertility, particularly of male animals, is rarely available. A thorough assessment of the animals' testicular metrics, especially scrotal length and circumference, as well as their semen qualities, may provide some light on how well the food treatment of the animals satisfies their needs for adequate reproduction. The objective of the current study was to assess the body weight, scrotal parameters, and reproductive performance of bucks that were fed diets that included sugarcane scrapings that had been biodegraded by *Pleurotus ostreatus*.

2. Materials and methods

2.1. Experimental location

The University of Abuja Teaching and Research Farm in Abuja, Nigeria, served as the site of the experiment. Situated at an elevation of 456 m, the location is situated between latitudes 8º 55'N and 9º 00'E and longitudes 7º 00'N and 7º 05'E. The place experiences 1100–1650 mm of precipitation and 25.8–42°C of yearly temperature.

2.2. Preparation and biodegradation of sugarcane scrapings

While fresh SS was gathered from nearby sugarcane processors, chopped into 1-2 cm lengths, and air-dried at room temperature (25–30°C), *Pleurotus ostreatus* was purchased from a respectable commercial producer in Nigeria. The SS was mixed with distilled water in a 1:1 ratio in each previously cleaned, dried, and sterilized container to bring the moisture content down to 67% prior to the *Pleurotus* inoculation for solid state fermentation. The SS were autoclaved twice, once at 121°C for 15 minutes each time, with cooling intervals in between to eradicate any living microbes. The prepared SS were cooled in an aseptic environment before being infected with P. ostreatus spores in a 25:1 ratio. The inoculation room was then maintained at 30°C and 100% relative humidity until mycelia formed. Following a 21-day period of inoculation and SSF, the mycelia growth and biodegradation of the biodegraded SS (BSS) were stopped by autoclaving the material. After being dried to a consistent weight, the BSS were bagged and kept until they were required for feeding.

2.3. Experimental animals, management and diets

Kano Brown male goats (n =21; 6 to 7 months of age; 9.44 ± 0.39 kg body weight) for the experiment were purchased from an open market.

The goats were housed in separate 1.2 m^2 cages within a 6 m x 8 m x 4 m pen. The pen and its immediate surrounds were carefully cleansed with Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad) two weeks before their arrival. Prophylactic treatments were given to the animals during their two-week quarantine. These included an oral anti-stress medication called Vitalyte®, a subcutaneous injection of the live PPR vaccine (1 mL at 102.5 of the TCID50 PPR virus) at the neck region, a subcutaneous injection of an anti-parasitic medication called Avomec® at 0.5 mL/25 kg of body weight (BW), and an intramuscular injection of a long-acting oxytetracycline HCl at 1 mL/10 kg BW. Using different levels of SS 0 (control), 15 and 30% inclusion levels, three whole diets were developed (Table 1). The NRC's (2007) recommendations were followed when designing the diets that addressed the needs of growing goats. During a 12-week period (from January to March), buck kids were fed at 5% of their body weight (based on dry matter; DM) and were balanced for body weight. The goats were then randomly assigned to one of the experimental diets. As the trial went on, the amount of feed provided to the goats was adjusted to make sure some was left over. Feeding took place twice a day, at 8:00 and 16:00. They had unlimited access to clean water every day.

Table 1. Ingredient and chemical composition (% DM) of the experimental diets

	Level of BSS inclusion, %		
	0	15	30
Ingredient			
Maize	25	25	25
Corn bran	30	15	0
BSS	0	15	30
Groundnut cake	17	17	17
Cowpea husk	25	25	25
Salt	0.5	0.5	0.5
Limestone	2	2	2
Premix	0.5	0.5	0.5
Chemical composition			
Crude protein	15.7	15.8	15.9
Ether extract	5.78	5.80	5.82
Organic matter	93.8	92.0	91.6
Non-fibre carbohydrate	33.6	29.7	27.2
Neutral detergent fibre	38.7	40.6	42.6
Acid detergent fibre	21.0	23.1	25.2

BSS: Biodegraded Sugarcane Scrapings

2.4. Feed intake and body weight

Feed intake was calculated by deducting the weight of the feed supplied the day before from the weight of the leftover feed. The calculation of nutrient intake involved multiplying the animal's feed intake (measured in dry matter) by the nutrient's (measured in dry matter) content in the diets. The individual initial body weight (BW) was measured at the start of the experiment, and the individual final BW was determined right before the goats were fed at the end of study using a standard goat weighing scale (UmaTech Scales)

2.5. Testicular and semen parameters

Scrotal length (SL) was measured weekly with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Akpa et al. (2012). Scrotal circumference (SC) is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa et al., 2006). It was measured by using measuring tape.

Semen was collected from all the bucks from each treatment through electro-ejaculation, using an automatic electro-ejaculator (Autojact, Neovet) with 12 V and 5 A as outlined by Zemjanis (1970). The ejaculates were obtained by the automatic method with animals standing in a chute followed by a sequence of 1-35 stimuli adding up a total of 30 s to 5 min. The semen ejaculatory volume was determined immediately in the collection vial graduated in milliliter (mL). The samples were kept in water bath at 37°C, and evaluations were made in sequence according to CBRA (1998) manual.

2.6. Chemical analysis

Following AOAC (2000) protocols, diet samples were analyzed for their proximate elements. Neutral and acid detergent fiber were determined according to the method described by Van Soest et al. (1991). Semen samples was evaluated immediately for volume through direct reading of millimeter graduation of the collection vial, and the result was expressed in milliliter (mL). Semen pH was determined by dipping a litmus paper into the ejaculate and monitoring the corresponding color changes (Anaso et al., 2023). Semen appearance was determined by visualization of consistency of ejaculates and classified as: creamy marble, creamy, thick milky, milky and watered (Anaso et al., 2023). Semen initial fructose evaluation was carried out immediately after collection according to Mann (1948). Smear of each semen sample was prepared, air dried, labelled and kept for further examination. The progressive motility was determined by placing 10 µL of semen into 1 mL of Tris dilution buffer, hydroxymethylaminomethane, (3.0 g), sodium citrate (2.0 g) and fructose (1.0 g). Then a 10 µL-aliquot of the diluted semen sample was placed between preheated slide and coverslip (37 °C) and evaluated under optical microscope (100 x magnification). The progressive motility was expressed in percentage. The concentration of the spermatozoa was determined using a hemocytometer that were crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. The total number of smaller squares on the hemocytometer was 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot et al., 1997). Prior to counting, formaldehyde was used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The hemocytometer was mounted into the microscope and an absorbable tube as used to pipette a drop of the solution into the hemocytometer chamber. The absorbable tube was blown before pipetting to avoid air bubbles in the absorbable tube. The result obtained was recorded as the sperm cell concentration for the sample.

Sperm morphology was determined from 95 slide smears stained with Giemsa at 7.5% (Doles Laboratory), diluted in distilled water and immersed in this solution for two h. Afterwards, the slides were kept upright until they dried completely and viewed under the microscope to get the normal and abnormal sperm percentage. Normal spermatozoa and spermatozoa abnormalities were classified according to principles used for rabbits by Barth and Oko (1989). The result was expressed in percentage. The live to dead sperm ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosine stain immediately after collection. A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosine solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labelled. This was done for each sample, and they were later mounted on the microscope for counting the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained (Hancock, 1951). Testosterone concentration in serum was measured by radioimmunoassay via assay kits (Siemens, Mexico, D.F). Libido (reaction time to does) was determined by exposing bucks to estrogenized doe. A stop-watch was used to take time for mounting without intromission and ejaculation and duly recorded as described by Angel-Garcia et al. (2015).

2.7. Data analysis

The obtained data was subjected to and met normal distribution conditions. Subsequent results for body weight, scrotal parameters and semen characteristic 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 ($p \leq 0.05$ level of significance.

The statistical model is shown below:

$Yij = \mu + tij + eij$

Where:

Yij = the general response to the specific parameter under investigation,

μ, the general mean peculiar to each observation,

tij = the fixed effect of the dietary treatment on the observed parameters, and

eij = the random error term for each estimate

Linear relationships between BW and SL and BW and SC were estimated by simple regression analysis while multiple regression was used to estimate the contribution of both SL and SC to variation in the final BW. The statistical model for the multiple regression was:

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$

Where:

Y = final BW; X_1 = SL; X_2 = SC; β_0 = the intercept; β_1 and β_2 are the slopes and ε is the error. The R^2 and P values were used to express the degree of the significances.

3. Results and discussion

3.1. Chemical composition of experimental diets

Replacement of corn bran with BSS, a poor-quality roughage, slightly alter the chemical composition of the diets, implying that SSF of SS enhanced its nutritive value to almost similar level to that of the corn bran (Table 1). Previous studies (Akinfemi, 2010; Khattab et al., 2013) reported enhanced nutritive value in form of increased CP and reduced fiber contents of *Pleurotus ostreatus* fermented lignocellulosic materials.

3.2. Feed intake and body weight

Feed intake was affected ($p<0.05$) by the diets in the order: 15% BSS > 0% BSS > 30% BSS (Table 2). There was no ($p>0.05$) difference in the initial BW among the three experimental groups. Final BW (16.1 kg > 14.6 kg > 13.6 kg) indicates that 15% BSS in treatment 2 was greater ($p<0.05$) than 0 and 30% BSS.

Table 2. Feed intake and bodyweight of Kano Brown goats fed biodegraded sugarcane scrapings (BSS) meal

Parameter	Level of BSS inclusion, %			SEM
	0 (n*=7)	15 (n=7)	30 (n=7)	
DM intake, g/d	354^{b}	374^{a}	319^{c}	3.39
Crude protein intake, g/d	55.6^{b}	59.1^{a}	50.7^{c}	0.53
Organic matter intake, g/d	332^{b}	344^{a}	291^{c}	3.16
Initial body weight, kg	9.50	9.50	9.40	0.38
Final body weight, kg	14.6^{b}	16.1^{a}	13.6^{b}	1.17

BSS. Biodegraded Sugarcane Scraping. n*: Animal numbers. ^{abc} Means in the same row without common letter are different at P<0.05

The DM intake of ruminants is a function of many factors such as chemical composition, characteristics and palatability of feeds (Olafadehan and Okoye, 2017). The higher feed intake of 15% BSS suggests superior palatability, nutritive value, digestibility and ruminal fermentation at this inclusion level (Olafadehan et al., 2014; Olafadehan and Adebayo, 2016; Kholif et al., 2021). The optimum feed intake of 15% BSS diet would have undoubtedly ensured availability of energy and various nutrients for body activities like body weight gain and physiological and reproductive functions (Olafadehan and Adewumi, 2009;2010). The insignificant difference in initial body weight of the goats indicates the effectiveness of the randomization of the animals which further justified the use of complete randomized design. The higher final BW of animals in 15% BSS diet is a direct response to the improved feed as well as the nutrient intake and hence availability, utilization, assimilation and conversion to the body weight. This result indicates a synergy between the nutritional components of both corn bran and BSS which had equal combinations in 15% BSS diet unlike in either sole corn bran-based diet (0% BSS) or sole BSS based diet (30% BSS).

However, similar final BW of 0 and 30% BSS diets plausibly implies that BSS can completely replace corn bran in a practical goat ration without compromising the final BW of the animals.

3.3. Semen quality and testicular parameters

Semen color was the same (creamy) for the diets. Semen pH, sperm viability, progressive motility, abnormalities and live spermatozoa were not ($p>0.05$) affected by diets (Table 3). Semen volume was higher ($p<0.05$) in 0 and 15% BSS than in 30% BSS. Semen concentration was affected ($p<0.05$) in the order: 15% BSS > 0% BSS > 30% BSS. The testosterone level and libido test (measured as the reaction time when exposed to female) followed the same trend and were higher ($p<0.05$) for 15 and 30% BSS diet relative to 0% BSS diet.

Table 3. Semen quality and testicular parameters of goats fed biodegraded sugarcane scrapings (BSS) meal

Parameter	Level of BSS inclusion, %			SEM
	0 (n*=7)	15 (n=7)	30 (n=7)	
Semen color	Creamy	Creamy	Creamy	
pH	6.38	6.43	6.68	0.07
Ejaculatory volume, mL	0.29^{b}	0.33^{a}	0.27^{b}	0.00
Progressive motility, %	84.0	84.3	84.0	3.64
Sperm viability, %	82.0	83.0	80.0	3.34
Sperm concentration, x 10^{6}	322^{b}	397^{a}	312^{c}	2.98
Live spermatozoa, %	80.0	85.0	80.0	3.88
Sperm abnormalities, %	12.0	10.0	15.0	5.19
Testosterone, ng/mL	3.05^{a}	3.06^{a}	2.87^{b}	0.36
Libido, seconds	13.3^{b}	12.4^{b}	14.9^{a}	0.63
Initial fructose, mg/dL	803^{b}	830^{a}	780^{b}	2.95
Scrotum length, cm	9.14^{b}	9.43^{a}	9.11^{b}	1.15
Scrotum circumference, cm	16.6^{b}	17.0^{a}	16.4^{b}	0.97

abc: Means in the same row without common letter are different at P<0.05. n*: Animal numbers

Evaluation of semen quality is essential because of the imperativeness of a good semen quality in achieving adequate fertility in farm animals. In fact, semen quality and sexual behavior are the essential standards that determine male reproductive efficiency. The parallel results for semen pH, sperm viability, progressive motility, abnormalities and live spermatozoa indicate that BSS can be included in buck diets without negatively impacting these semen traits. The bucks' comparable semen colors are consistent with earlier studies on goats and rams (Oyeyemi et al., 2011; Ososanyo et al., 2013), which noted a creamy color feature. Translucent semen generally indicates low concentration, blood stains and strange colors indicate poor quality or contamination, and creamy white semen often indicates acceptable quality. The unaffected semen's pH did not match Osinowo's (2016) reported value of 6.9. The 15% BSS had greater sperm and semen EV concentrations, indicating that the diet had a significant impact on spermatogenesis and that the goats' nutritional status was enhanced while on this diet. The improved nutritional status may be attributed to the increased supply of nutrients for spermatogenesis process arising from increased the enhanced feed and nutrient intake (particularly protein intake). Though not reported in this study, the diet might have also possibly improved nutrient digestibility (Gado et al., 2015) and ruminal microbial growth and production of microbial protein (Gado et al., 2009). Enhanced nutrient digestibility has been reported to promote nourishment of the sertoli cells and seminal fluid that nurse the germ cells (Gado et al., 2015). The increase in sperm concentration and volume, of goats on 30% BSS, therefore, signals the possibility of high fertility during service or insemination (Oyeyemi and Okediran, 2007). The unaffected sperm progressive motility was within the above the minimum motility of 50% (Oyeyemi et al., 2000) and 65% (Osinowo, 2016) required for satisfactory fertility. Osinowo (2016) asserts that strong, progressive motility is an essential measure of sperm viability and sperm level, which might be high or low. Motile cells are usually innately viable, and viability is crucial in the determination of non-motile cells that are alive or dead. The marginal effect of the diets on of the sperm viability suggests diets did not adversely impact spermatogenesis (Sumalatha, 2010) and livability of the sperm cells.

It appears BSS is a nutritionally adequate feedstuff can be included in the diets of bucks up to 30% without compromising semen characteristics and fertility because low-quality or inadequate diet has been linked to cases of low-live sperm count. (Irkham et al., 2017).

The similar sperm abnormalities across the treatment groups did not surpass the earlier reported permissible limit in rams fed pineapple waste-based diets (Ososanya et al., 2013). Generally, high levels of sperm abnormalities indicate poor quality semen. Therefore, it could be inferred that nutritional treatments did not cause any negative effects on semen quality in the current study. Testosterone, produced from the leydig cells of the testes, is vital for spermatogenesis and male characteristics (Sekoni et al., 2010) and its vascular distribution throughout the body is a major contributory factor to the libido of males (Sajjad et al., 2007). Though the testosterone was higher for 0 and 15% BSS diets than for 30% BSS diet, the levels were within the range of 2.10 – 10.8 ng/mL for White goats in Türkiye (Polat et al., 2011) and 0.1 – 10 ng/mL for Creole bucks (Delgadillo et al., 1999), suggesting that testosterone concentration was unaffected by the 30% BSS threshold level in the present investigation. Given its favorable association with other semen characteristics, testosterone has been identified as a critical component in the formation of superior quality semen, Cornwall (2009) attributed low sperm quality to low testosterone levels. Given that testosterone has been shown to increase male sexual behavior, it is possible that the higher libido (lower reaction time to doses) in the 0 and 15% BSS is related to testosterone levels (Gado et al., 2015). The increased initial fructose of 15% BSS suggests that the diet increased the amount of nutrient and energy for spermatozoa (Wilke et al., 2009; El-Gindy et al., 2020). Generally, libido (sex drive) is an important component of male fertility.

Scrotal length and circumference are important indicators when observing animals for breeding soundness. Higher SC and SL of goats fed 15% BSS diet indicate that the diet may improve the reproductive performance and breeding soundness of the bucks. Earlier studies (Azizunnesa et al., 2013) attributed increased scrotal circumference and growth rate to nutritional plane, implying that 15% BSS diet is perhaps nutritionally superior to the other diets. Both SC ($p=0.030$; $r = 0.051$) and SL ($p = 0.048$; $r = 0.472$) had a notably positive correlation with the final BW (Table 4), in consonance with previous findings (Olafadehan et al., 2015). However, multiple regression of final BW on SC and SL indicates that final BW was insignificantly ($p>0.05$) positively correlated ($r = 0.513$) with SC (X1) and SL (X2) with 26.3% ($R^2 = 0.263$) variation in final BW attributable to these testicular parameters. However, SC might have had a higher contribution ($R^2 = 0.260$) to the final BW than SL ($R^2 = 0.222$). The multiple regression equation is $Y = 4.17 + 0.099XI + 0.581X2$. Akpa et al. (2012) reported a positive and significant correlation between testicular dimensions and body measurements and implied that males with greater scrotal sizes may also have larger body morphology better suited for reproduction as seen in this experiment.

Table 4. Linear relationships between body weight (Y) and testicular parameters (X) of goats

Dependable variables	Regression equation	R	R^2	SEM	P-value
Scrotal length	Y = 10.6 + 0.449X	0.472	0.222	0.210	0.048
Scrotal circumference	Y = 2.97 + 0.708X	0.510	0.260	0.298	0.030

4. Conclusions

- The final body weight, testicular characteristics, semen quality, and fertility of bucks can all be positively impacted by substituting their diet with biodegraded sugarcane scrapings instead of maize bran.

- For enhanced and superior final body weight, testicular parameters, semen quality and fertility, 15% BSS, replacing 50% of corn bran, is recommended in the diet of bucks.

Compliance with Ethical Standards

Conflict of Interest

The author declares no conflict of interest.

Authors' Contributions

Emmanuel ANASO: Writing and editing. **Olurotimi OLAFADEHAN:** Statistical analysis and major editing. **Ayoola John SHOYOMBO:** Minor editing. **Emeka Solomon FIDELIS:** Minor editing.

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Data availability

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Consent for publication

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