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BODY THERMOREGULATION AND SERUM METABOLIC PROFILE OF KANO BROWN BUCKS FED *Pluerotus ostreatus* BIODEGRADED SUGARCANE SCRAPINGS

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Abstract: The study assessed if feeding of *Pleurotus ostreatus* biodegraded sugarcane scrapings (BSS) would have detrimental effects on body thermoregulation and serum metabolic profile of goats. Twenty-one healthy male Kano Brown bucks (6 – 7 months of age; 9.44 ± 0.39 kg mean body weight) were stratified based on their BW into three treatment groups containing 0 (T1), 15 (T2) and 30% (T3) of BSS in a completely randomised design. Serum total protein, albumin, globulin and albumin:globulin ratio were greater (P<0.05) in T2 than in T1 and T3. Serum urea was higher (P<0.05) in T3 relative to T1 and T2. While serum glucose was greater in BSS diets, cholesterol and alanine transaminase were higher in the T1 than in BSS diets (P<0.05). Alanine phosphatase decreased in the order: T1 > T2 > T3 (P<0.05). Serum creatinine, aspartate transaminase, total bilirubin, sodium, potassium, calcium and bicarbonate, rectal temperature, earlobe temperature, heart rate and respiratory rate were not influenced (P>0.05) by dietary treatments. Values of all serum metabolic indices and body vital signs were within normal ranges for goats. Results show that *Pleurotus ostreatus* biodegraded sugarcane scrapings can be used up to 30% in the diets of goats without negatively impacting their body thermoregulation, metabolic welfare and health.

Key words: sugarcane scrapings, solid state fermentation, *Pleurotus ostreatus*, metabolic welfare, vital signs, goats

Introduction

Lignocellulosic biomass from agricultural residues are abundantly available with about 73.9 Tg yearly production in the world *(Kim and Dale, 2014)*. The disposal of these wastes, which are often left on the field, constitutes a problem for the local producing agro-industries and thus causes environmental degradation. However, if properly harnessed and processed, these wastes have a great potential as livestock feed. Sugarcane scrapings, the bark of cane sugar stem after peeling, is one of such lignocellulosic materials in Nigeria. Like other lignocellulosic materials, its use in livestock feed is constrained by the poor nutritive value attributable to low protein and large contents of cellulose, hemicellulose and lignin. Lignin is, however, the major culprit because linkages between lignin and cellulose and hemicellulose inhibit accessibility of rumen microbial enzymes to the cell wall contents and, thus lock up appreciable amounts of potential energy and nutrients *(Tengerdy and Szakacs, 2003)*. Therefore, even ruminants with their effective digestive system for fibre degradability cannot extract sufficient energy and protein from lignocelluloses. Biological treatment such as solid state fermentation/biodegradation with white rot fungi, particularly *Pleurotus ostreatus*, has been used to improve the nutritional value of poor-quality roughages and develop unconventional ingredients at competitive prices *(Isroi et al., 2011; Atuhaire et al., 2016)*. Although there are many reports on the use of white rot fungi biodegraded lignocelluloses in ruminants, there is, however, a paucity of information on the effect of feeding these biodegraded materials on the body thermoregulation and blood metabolic profile of goats in the tropics. Examination of blood metabolic profile of ruminants fed fungal solid state fermented fibrous materials may be necessary to ascertain if the welfare of the animals is compromised or not. Blood metabolic profiles have been used for diagnosis and prognosis of diseases in animals and are also useful to assess the welfare condition of animals or to understand if some changes in a diet can affect animal physiology and health *(Olafadehan, 2011a; Olafadehan et al., 2014)*. The current study aimed to evaluate the body thermoregulation and serum metabolic profile of Kano Brown bucks fed diets containing *Pleurotus ostreatus* biodegraded sugarcane scrapings in their diets.

Materials and Methods

Experimental site

The experiment was conducted at the University of Abuja Teaching and Research Farm, Federal Capital Territory, Nigeria. The site is at 456 m altitude and lies between latitude 8º 55' N and 9º 00' E and longitude 7º 00' N and 7º 05' E. It has a tropical climate with temperature and annual rainfall ranging from 25.8 to 42°C and 1100 to 1650 mm respectively.

Substrate preparation and biodegradation process

Fresh sugarcane scrapings (SS) were collected from local processors of sugarcane, chopped into 1-2 cm lengths and air-dried at ambient temperature (25 – 30°C). The white rot fungus, *Pleurotus ostreatus*, used for the solid state fermentation of the SS was obtained from a reputable commercial producer in Nigeria. Prior to the inoculation with the *Pluerotus* for solid state fermentation, the inoculation containers were sterilised by being washed thoroughly and dried for 10 minutes at 100°C, while SS were autoclaved twice at 121°C for 15 minutes with cooling between cycles to eliminate any active microorganisms. Thereafter, the moisture content of the SS was adjusted to 67% by mixing the SS and distilled water in ratio 1:1.

After cooling under aseptic condition, the prepared SS were inoculated with the *P. ostreatus* spores in ratio 25:1 and kept in the inoculation room maintained at 30°C and 100% relative humidity until mycelia were formed. After 21 days of inoculation and solid state fermentation, the biodegraded SS (BSS) was autoclaved to terminate mycelia growth and biodegradation. The BSS were then dried to constant weight, bagged and kept until needed for feeding.

Experimental animals, management and diets

Twenty-one healthy intact Kano Brown male goats, about 6 to 7 months of age with an average initial body weight (BW) of 9.44 ± 0.39 kg, were used for the study. Two weeks prior to the arrival of the goats, the pens and its immediate surroundings were thoroughly disinfected with antiseptic (Morigad). The animals were quarantined for two weeks and administered prophylactic treatment comprising subcutaneous vaccination with *Peste Des Petits* Ruminants (PPR) live vaccine diluted at 2 ml per 10 kg BW, subcutaneous injection with Avomec® at 0.5 ml/25 kg of the BW for the control of endo and ecto parasites and injection with a long acting oxytetracycline HCl at 1 ml/10 kg BW. Goats were housed in individual cages measuring 1.2 m^2 each and kept in open sided pens measuring 3 m x 4 m x 4 m. Three complete diets were formulated, using varying levels of BSS meal: no sugarcane scrapings (control; T1), 15 (T2) and 30% (T3) inclusion levels to replace corn bran at 0, 50 and 100 % respectively on dry matter (DM) basis. Table 1 shows the ingredient and chemical composition of the experimental diets formulated to meet the requirements of growing goats according to the recommendations of *NRC (2007)*. Goats in each treatment group were randomly assigned to one of the experimental diets and fed at 5% of their BW on DM basis for a period of 12 weeks. Adjustments were made to feed given to the goats as the experiment continued to ensure collection of orts. Feeding was twice in a day at 08:00 h and 16:00 h. Clean water was supplied daily *ad libitum*. Feed intake was determined by subtracting the weight of the left over feed from the weight of the feed offered the previous day. Nutrient intake was determined by multiplying the feed intake (in DM) of an animal by the nutrient (in DM) from the chemical composition of the diets.

Body thermoregulation

Rectal temperature (RT), earlobe temperature (ET), respiratory rate (RR) and heart rate (HR) of each goat were measured twice a week at 11:00 h. Rectal temperature was measured using digital thermometer. The sensory tip was disinfected with an antiseptic, lubed with petroleum jelly (Vaseline) and then inserted into the rectum of individual animal, at the display of a constant temperature indicated by “C L0” on the thermometer’s mini digital screen, at a uniform depth of 1.5 cm. After the beeping sound of the alarm signal of the digital thermometer, the thermometer was removed from the rectum and the displayed body temperature was recorded. The ET was also measured by placing the digital thermometer in the earlobe at the display of a constant temperature indicated by “C L0” on the thermometer’s mini digital screen. The earlobe was then folded around the thermometer to ensure adequate contact and avoid temperature interactions. The digital thermometer was removed from the earlobe after the beeping sound of the alarm signal and the temperature recorded. RR was determined by counting the number of abdominal movement per minute using the seconds hand in an analogue wrist watch for one minute and the counts recorded. HR was determined using a stethoscope placed on the left side of the ribs (the anatomical location of the heart). The normal sound of the heart was the loob dope sound which indicated a complete heart beat for a minute using the seconds hand in an analogue wrist watch for one minute and the rates recorded appropriately.

Blood collection

Blood samples (5 ml) were taken from the jugular vein of each goat in the morning before feeding on the last day of the experiment into anti-coagulant free vacutainer tubes placed in ice-packed flasks and taken immediately to the laboratory for serum biochemical analysis. Serum for biochemical indicators was harvested by centrifugation of complete blood at 3000 rpm for 15 minutes in a laboratory centrifuge (NOP-350R, NOP medical instruments, Punjabi, India) at 4°C. The serum was analysed within four hours of collection.

Chemical analysis

Samples of the experimental diets were analysed for their proximate constituents in accordance with the procedures of *AOAC (2000)*. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the procedures of *Van Soest et al. (1991)*. NDF was analysed using sodium sulphite and amylase and expressed with residual ash. Acid detergent lignin was determined by solubilisation 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-structural carbohydrate (NSC) was calculated using the following formula NSC= 100 – (CP + EE + ash + NDF) %. Serum total protein (TP), albumin, glucose, urea, creatinine, cholesterol, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), calcium, sodium, potassium and bicarbonate were analysed by calorimetric methods using Bio Maxima reagent sets (Lublin, Poland) in a Metrolab 5.0, Norway, Oslo. Globulin was calculated as the difference between TP and albumin. Albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin values.

Data analysis

Data were subjected to analysis of variance in a completely randomized design using the SPSS (23.0). Duncan multiple range test of the same software was used to test the significant difference between the means at $P \leq 0.05$ level of significance. The statistical model is:

$Yij = \mu + ti + ei$

where Yij = the general response to the specific parameter under investigation, μ = the general mean peculiar to each observation, ti = the fixed effect of the dietary treatments on the observed parameters, and ei = the random error term for each estimate.

Results and Discussion

Chemical composition of experimental diets

The parallel CP of the diets implies that solid state fermentation of sugarcane scrapings upgraded the CP to a similar level to that of the corn bran being replaced. Earlier studies *(Belewu, 2008; Bento et al., 2014)* reported increased CP of fungal biodegraded lignocellulosic materials. The NSC decreased while the structural carbohydrates, except for hemicellulose, increased with increasing replacement of BSS for corn bran.

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

Ingredient	Treatment		
	T1	T2	T3
Maize	25	25	25
Corn bran	30	15	0
Biodegraded sugarcane scrapings	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			
Organic matter	93.76	91.96	91.55
Crude protein	15.70	15.80	15.89
Non-structural carbohydrate	33.59	29.74	27.24
Neutral detergent fibre	38.69	40.62	42.60
Acid detergent fibre	20.95	23.10	25.24
Acid detergent lignin	2.89	3.21	4.45
Cellulose	18.06	19.89	20.79
Hemicellulose	17.74	17.52	17.36

T1, 0% biodegraded sugarcane scrapings; T2, 15% biodegraded sugarcane scrapings; T3, 30% biodegraded sugarcane scrapings inclusion

Serum biochemistry

Although some of the serum metabolites were altered by feeding BSS diets, all the measured parameters were within the optimum ranges for healthy goats *(Merck Veterinary Manual, 2010)*, suggesting absence of metabolic disorder or health issue *(Olafadehan, 2017)*. Serum TP, albumin and A/G ratio were higher (P<0.05) in T2 than in other diets, indicating enhanced protein intake, availability, metabolism, absorption and utilisation, liver and kidney function, and nutritional and health status of the goats *(Olafadehan, 2011, 2017; Shoby et al., 2020)*. Whereas albumin, a negative acute phase protein *(Ceciliani et al., 2012)*, is a good index of health and nutritional status of an animal *(Olafadehan, 2017)*, A/G ratio reflects protein utilisation efficiency and the state of the liver, kidney and health of the goats *(Farver, et al., 1997; El-Sherif and Assad, 2001)*. Globulin concentration was greater in BSS diets than in control diet (P<0.05). Since globulin is produced in the liver and by immune system, the increased globulin of the BSS diets reveals enhanced liver function and immune response of the goats on these diets. Nevertheless, since the TP, albumin, globulin and A/G ratio of the treatments were within the established ranges, the results indicate adequate protein intake and utilisation, uncompromised nutritional and health status, and absence of liver and kidney dysfunctions, immunosuppression and metabolic disorders in the goats *(Olafadehan, 2017)*. Serum urea N was higher (P<0.05) in T3 than in T1 and T2.

The increased SUN in T3 compared to in T1 suggests higher ruminal proteolysis of BSS protein relative to corn bran protein.

In ruminant animals, serum glucose and creatinine are the most significant indices of energy metabolism. That these indicators of energy status in animals were within the physiological ranges indicate unimpaired energy availability and utilization. However, the higher ($P<0.05$) serum glucose of the BSS diets shows the glycogenic potential of the BSS as an ingredient in ruminant diets and suggests high nutritional and energy status *(Kholif et al., 2021a; Olafadehan, 2011a)*, organic matter digestibility *(Abd El Tawab et al., 2020b; Azazz et al., 2020)* and ruminal propionate concentration *(Olafadehan et al., 2018; Kholif et al., 2021b, c)*. Since nutrient digestibility and ruminal fermentation are not measured in the present study, more studies are required to further elucidate the effect of replacement of corn bran with BSS in the diets of growing goats on these parameters. Serum creatinine and total bilirubin were not ($P>0.05$) affected by the treatments. Normal creatinine concentrations also indicate proper renal function, as a direct relationship has been established between serum creatinine and kidney function *(Prvulovic et al., 2012)*. Bilirubin values were within the reference ranges for goats, indicating the absence of haemolysis. According to *Olafadehan (2011b)* bilirubin is an insoluble molecule derived from the breakdown of haemoglobin in the spleen. Whereas AST was not affected, ALP, ALT and cholesterol were lower ($P<0.05$) in BSS diets relative to the control diet. Though BSS diets reduced ALP and ALT concentrations, values were within the normal ranges, suggesting normal liver condition and function and absence of liver pathological lesions *(Olafadehan et al., 2014)*. The reduced serum cholesterol of BSS diets suggests that altered fat metabolism and absorption, although the normal values indicate the absence of hyper or hypocholesterolemia *(Olafadehan, 2011a; 2017)*, bile obstruction *(Silanikove and Tiomkin, 1992)* and liver dysfunction and fat malabsorption *(Zubcic, 2001)*.

Table 2. Serum biochemical indices of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM	RV
	T1	T2	T3		
Total protein (g/L)	67.81^{b}	70.92^{a}	68.44^{b}	2.03	61 - 75
Albumin (g/L)	37.30^{b}	38.91^{a}	36.12^{b}	1.71	23 - 36
Globulin (g/L)_	30.51^{b}	32.02^{a}	32.33^{a}	0.63	27 - 44
Albumin/globulin ratio	1.22^{a}	1.22^{a}	1.12^{b}	0.05	1.0 – 2.0
Urea nitrogen (mmol/L)	5.06^{b}	5.18^{b}	5.56^{a}	0.10	4.5 - 9.2
Creatinine (mmol/L)	109	113	103	22.0	60 - 135
Glucose (mmol/L)	3.07^{b}	4.01^{a}	3.77^{a}	0.16	2.7- 4.2
Cholesterol (mmol/L)	3.14^{a}	2.58^{b}	2.47^{b}	0.10	1.7 - 3.5
Alkaline phosphatase (U/L)	209.52^{a}	205.51^{b}	200.68^{c}	1.23	61- 283
Alanine transaminase (U/L)	16.23^{a}	15.56^{b}	15.32^{b}	0.15	15- 52
Aspartate transaminase (U/L)	201.21	200.83	200.79	0.33	66 - 230
Total bilirubin (mmol/L)	1.88	2.05	3.40	0.68	1.7 - 4.3

Means with the different superscripts along the row are significantly (P < 0.05) different.
T1, 0% biodegraded sugarcane scrapings; T2, 15% biodegraded sugarcane scrapings; T3, 30% biodegraded sugarcane scrapings; RV, reference value as stated by *Merck Veterinary Manual (2010)*

Serum minerals

Serum sodium (Na), potassium (K), calcium (Ca) and bicarbonate were similar (P>0.05) across the three experimental groups.

Serum mineral concentrations were within the normal ranges for goats *(Merck Veterinary Manual, 2010)*. The normal serum K levels of the goats implies non-interference of the diets with K availability and absorption *(Olafadehan et al., 2014)*. *Busher (1990)* showed a positive relationship between low serum bicarbonate and chronic kidney disease; therefore, the normal serum bicarbonate level indicates the absence of incidence of chronic kidney disease. The within the physiological range Ca levels suggest absence of hyperparathyroidism, hypervitaminosis D, multiple myoloma and neoplastic disease or osteomalacia, rickets and renal failure reportedly engendered by calcuim imbalance in ruminants *(Olafadehan et al., 2014)*. *Cheesbrough (2004)* explained that maintenance of serum Na levels, as obtained in this study, suggests that the experimental diets were able to maintain cellular tonicity fluid balance and pH, regulate metabolic processes as well as involved in regulation of neural and muscular functions.

Normal values of the serum metabolic indices indicate no hepatic and renal damage, revealing that BSS can fed to goats with uncompromised metabolic welfare.

Table 3. Major serum minerals of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM	RV
	T1	T2	T3		
Sodium (mmol/L)	141.64	148.00	147.76	9.18	137 – 152
Potassium (mmol/L)	4.07	4.48	4.52	0.12	3.8 - 5.7
Calcium (mmol/L)	2.84	2.85	2.89	0.18	2.3 - 2.9
Bicarbonate (mmol/L)	23.41	24.93	23.83	0.11	20 – 27

T1, 0% biodegraded sugarcane scrapings inclusion; T2, 15% biodegraded sugarcane scrapings inclusion and T3, 30% biodegraded sugarcane scrapings inclusion; RV; reference values as stated *Merck Veterinary Manual (2010).*

Body thermoregulation

All the vital signs (RT, ET, HR and RR) were not (P>0.05) affected by diets and were with the reference ranges of 38.5 – 39.7°C, 70 – 90 bpm and 16 – 34 cpm for RT, HR and RR respectively for healthy goats *(Merck Veterinary Manual, 2010)*. The results indicate the diets did not compromise normal goat vital signs. Generally, normal vital signs of an animal depend on recent activity, feed and water consumptions and the physiological state of the animals *(Gado et al., 2016)*. The normal values obtained for the vital signs are a confirmation of the fact that the diets did not affect the body physiology and health of the animals, suggesting that that BSS can safely be used as a feedstuff in the diets of goats without posing any nutritional or health challenge because lower or higher values than the normal ranges indicate physiological or health problem.

Table 4. Body thermoregulation of goats fed biodegraded sugarcane scrapings diets

Parameter	Treatment			SEM
	T1	T2	T3	
Rectal temperature (℃)	38.73	38.45	38.69	0.17
Earlobe temperature (℃)	37.68	37.63	37.71	0.19
Heart rate (bpm)	83.63	82.31	83.11	0.91
Respiratory rate (cpm)	22.67	23.47	23.63	0.75

T1, 0% biodegraded sugarcane scrapings inclusion; T2, 15% biodegraded sugarcane scrapings inclusion and T3, 30% biodegraded sugarcane scrapings inclusion

Conclusion

Inclusion of biodegraded sugarcane scrapings in the diets of goats posed no harm to the animal body physiology, thermoregulation, serum metabolic profile and health. Biodegraded sugarcane scrapings can thus be included up to 30% to completely replace corn bran in the diets of goats without affecting goat metabolic welfare and health condition.

Termoregulacija tela i metabolički profil seruma kano smeđih jarčeva hranjenih strugotinama šećerne trske biorazgrađenim sa *Pluerotus ostreatus*

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Rezime

Cilj ove studije je bio da se proceni da li bi ishrana strugotinama šećerne trske (BSS), koje su biorazgrađene korišćenjem *Pleurotus ostreatus*, imalo štetne efekte na telesnu termoregulaciju i metabolički profil seruma koza. Dvadeset i jedan zdrav mužjak rase smeđi kano (Kano Brown) (6 – 7 meseci starosti; 9,44 ± 0,39 kg srednje telesne mase) je raspoređen na osnovu njihove telesne mase u tri tretmana koji su sadržavali 0 (T1), 15 (T2) i 30% (T3) BSS-a u potpuno slučajnom dizajnu.

Ukupni serumski protein, albumin, globulin i odnos albumin:globulin su bili veći (P<0,05) u T2 nego u T1 i T3. Urea u serumu je bila viša (P<0,05) u T3 u odnosu na T1 i T2. Dok je glukoza u serumu bila veća u BSS ishrani, holesterol i alanin transaminaza su bili viši u T1 nego kod životinja na BSS ishrani (P<0,05). Alanin fosfataza se smanjivala po redosledu: T1 > T2 > T3 (P<0,05). Prehrambeni tretmani nisu uticali na kreatinin u serumu, aspartat transaminazu, ukupni bilirubin, konjugovani bilirubin, natrijum, kalijum, kalcijum i bikarbonat, rektalnu temperaturu, temperaturu ušne resice, otkucaje srca i brzinu disanja (P>0,05). Vrednosti svih serumskih metaboličkih indeksa i telesnih vitalnih znakova bile su u granicama normale za koze. Rezultati pokazuju da strugotine šećerne trske biorazgrađene uz korišćenje *Pleurotus ostreatus* mogu da se koriste do 30% u ishrani koza bez negativnog uticaja na termoregulaciju njihovog tela, metaboličko blagostanje i zdravlje.

Ključne reči: strugotine šećerne trske, fermentacija u čvrstom stanju, *Pleurotus ostreatus*, metaboličko blagostanje, vitalni znaci, koze

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