

Blood profile of West African Dwarf growing bucks fed *Pleurotus ostreatus*-treated *Megathyrsus maximus* and *Brachiaria decumbens* – cassava peel-based diets

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Target audience: Ruminant farmers

Abstract

This study investigated the effects of *Pleurotus ostreatus* treated *Megathyrsus maximus* var. *ntchisii* (TMMN) and *Brachiaria decumbens* (TBD) based diets on the haematological and serum biochemistry of Twenty-four West African Dwarf (WAD) growing bucks. The bucks were randomly divided into six groups of four bucks each and offered different diets for 21 days. The diets were formulated to contain 0, 20, and 30 % dietary levels of inclusion of *P. ostreatus* TMMN (T₁, T₂ & T₃) and TBD (T₄, T₅ & T₆); 50 % concentrate each; and either 50 % or 30 % or 20 % cassava peels respectively. Blood samples were collected on the last day for analysis. Results showed that only goats on 30 % TBD diets recorded a significantly ($P < 0.05$) improved haematological red blood cells ($15.2 - 16.2 \times 10^{12}/l$), packed cell volume (33.5 – 34.8 %), haemoglobin (11.8 – 12.2 g/dl) levels, and improved serum TP (7.6 – 8.5 g/dl), glucose (70.5 – 90.4 mg/dl), and cholesterol (105.2 – 117.3 mg/dl) levels. The study revealed that the inclusion of TBD up to 30% in WAD buck diets had no deleterious effect on the haematological and serum biochemical indices of the bucks, thus maintaining the bucks in good health conditions.

Keywords: fungal treated grasses, haematological, serum biochemistry, West African Dwarf goats, total mixed ration.

Description of Problem

In tropical countries such as Nigeria, the ruminants' feeding programme is majorly dependent on forages irrespective of the season. Among the available forages, grasses such as *Megathyrsus maximus* and *B. decumbens* play a major role in several improved farming pastures and animal feeding programmes as they are extremely productive all year round due to their aggressive growth habit; adaptability to

variable environmental conditions and soil types; high dry matter yield; high leaf production; strong resilience to grazing; and lesser environmental impact on nutrient composition (1, 2). However, during the dry season, these forages are limited in nutrients especially protein and soluble carbohydrates thus unable to meet the nutritional requirements of ruminants towards maintaining them in good health conditions. Supplementation with agricultural wastes

especially cassava waste or by-products has been the norm due to its abundance caused by the increased demand for non-costly cassava as a staple food by the increased human population in developing countries (3). Cassava peel is one of the cassava by-products used in livestock diets to improve production and performance (3). However, they are also low in protein and the protein is of less quality, thus cassava-based diets will require protein to be supplemented (4). Therefore, there is a need to improve the nutrient composition of available grasses, especially protein for the grass-cassava peel-based diet to be able to achieve an efficient ruminal microbial function that supports feed degradation. This makes nutrients available for various animal functions and indirectly prevents the animals from being predisposed to health challenges.

One of the current possible methods of improving feed is the use of White rot fungi (WRF). The fungi help improve the feed by selectively degrading lignin in substrates rich in structural carbohydrates without damaging other carbohydrates (i.e. cellulose and hemicellulose) as potential sources of energy. This improvement is usually accompanied by increased feed protein and reduced feed lignin and fibre contents and a modified rumen ecosystem leading to increased feed intake and digestibility (5). Among these WRFs, *Pleurotus spp* has received a lot of attention in upgrading agro-industrial by-products and agricultural wastes for ruminant feeding at a lesser cost, and its mycelium is regarded safe as no case of fungal pathogenicity towards animal feeding has been recorded (5). Researchers have also used *P. ostreatus* to improve the nutritional quality of different agricultural wastes as well as used cassava by-products in ruminant diets in recent years (4, 6, 3). However, the effect of fungal-treated grasses with or without cassava by-products in

ruminant diets has not yet been reported. Therefore, the feeding of *P. ostreatus* treated grasses in ruminant diets might be a way forward of making more nutrients available for various animal functions coupled with good health status.

Blood analysis is one of the tools used in assessing animal health conditions because it plays a major role in animals' physiological, and nutritional status. It stands as a fast and readily available method for determining the health status of animals when undergoing feeding trials (7, 8). The blood is known to show the responses of the animals to pathological, environmental, and nutritional stresses (9). The haematological and serum biochemical indices are used to detect if animals are within or deviated from the normal ranges of blood profile found in healthy animals. Although several factors such as age, sex, breed, genetics, location, stress, housing, management, reproductive status, nutrition, and other factors have been identified to have an influence on the haematological and serum biochemical indices of animals (9). However, the search for a safe, cheap, and readily available feed for improved ruminant production and health led to this research that was designed to evaluate the effects of the varying proportion of TMMN and TBD- cassava-based diets on the haematology and serum biochemistry of WAD goats.

Materials and Methods

Animal care

The procedures and protocols used in the care and management of the experimental animals followed the ethical approval and principles of (10).

Description of the study site

This study was carried out at the Pasture and Range Management paddock unit, Directorate of University Farm

(DUFARMS), Federal University of Agriculture, Abeokuta. The location is 148m above sea level and falls within latitude 7° 13' 24N and longitude 3° 26' 14E (Google Earth, 2021). The climate is humid and it is located in the derived savannah zone of South Western Nigeria. It receives a mean annual precipitation of 1,037mm, a mean annual temperature of 33.8°C, and relative humidity of 82%.

Collection and preparation of experimental diets

The grasses used for this study were harvested from the experimental plots, DUFARMS, FUNAAB, Abeokuta, Nigeria in October, during the late dry season. About 500 kg of each grass was harvested by cutting from 2cm above the ground level, chopped to 3 - 5cm length, and sun-dried. After which each of the grasses was filled into a big drum (3/4 of the drum), soaked for 24hr, and afterward drained to remove excess water. A known amount (2.5 kg) of each of the moistened grasses was weighed into several transparent individual bags until exhausted from the drum, the content with the bags was then sterilized for one hour as reported by (11), and allowed to cool down. Following that, they were inoculated with *P. ostreatus* spawn (4% w/w) that was purchased from the Forestry Research Institute of Nigeria, Ibadan, Oyo, Nigeria. The bags' ends were slightly tightened and sealed with tape to create an airtight environment and kept on racks in the inoculation room that was left dark at room temperature and 100% relative humidity for twenty days inside closed doors in an inoculation room. The inoculation room used was thoroughly cleaned and disinfected with Izal (26 % v/v) mixed with water for decontamination, after which the floor was mopped and the doors cleaned before putting the bags. On the 21st day of inoculation, the

bags already colonized by *P. ostreatus* mycelium showing whitish growth were removed from the inoculation room, autoclaved to terminate the mycelia growth, and then thinly spread on a sheet to dry the degraded materials. The materials were stored in bags at room temperature until when needed. The dried cassava peels used were purchased from a local Garri Processing Unit in Eleweran, Abeokuta, and crushed with a hammer mill to about 5mm in size. The dried treated grasses were then incorporated into a total mixed ration (TMR) containing different ratios of 0% (T₁), 20% (T₂), 30% (T₃) for TMMN, and 0% (T₄), 20% (T₅), 30% (T₆) for TBD; concentrate (50 %): and dried cassava peels (50 % or 30% or 20% to make each grass up to 100%) as shown in Table 1. The concentrate was made from groundnut cake (12kg), Palm kernel cake (34kg), wheat offal (22kg), rice bran (28kg), bone meal (2kg), premix (1kg), and salt (1kg).

Experimental design and Animal Management

Twenty-four (24) WAD growing bucks between 6 -7 months of age with an average weight of 7 kg ± 0.99 were purchased from Kuto market, Abeokuta, Ogun State, Nigeria. Before the commencement of the experiment, the goats were treated against endoparasites and ectoparasites with Albendazole 10 % (given orally at 0.1 mg/kg BW) and Diazinole solution (by dipping animals) respectively. For a preliminary period of 10 days, the animals were offered concentrate mixed with cassava-peel (50: 50) at 3.5 % BW in the morning and each half of the group received either 1kg of chopped and wilted *M. maximus* var. ntchisi or *B. decumbens* as basal diets. Subsequently, the goats were randomly distributed into six experimental groups (n = 4), kept in individual metabolic cages (1.5 m x 1 m)

with good ventilation, feeders, and drinkers, and fed their allocated experimental diets (Table 1) for a period of 21 days. The animals received their allocated TMR diets twice daily in equal amounts at 08.00 and 16.00 h at 3.5% of their body weight on a DM basis. Clean water was provided to the animals' ad-libitum on a daily basis.

Blood sampling and collection

On the last day of the experiment, blood samples (10 ml) were collected from each animal via jugular vein puncture. The blood sample was divided into two parts of 5 ml each for determination of haematological and serum biochemical indices according to the methods described by (12). The first five (5) ml of the blood was placed into labelled vacutainers containing ethylene diamine tetraacetic acid (EDTA) and stored on ice during transit to the laboratory for determination of haematological indices (Red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), haemoglobin (Hb), Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV), neutrophils, lymphocytes, monocytes, basophils, and eosinophils). The second five (5) ml was discharged into labelled vacutainers without EDTA, kept in the box during transit, and on kaylite holders set at 37°C for coagulation in the laboratory. The content was separated by centrifugation at 3000rpm for 15 mins and stored at -20°C for determination of serum biochemical indices (Total protein (TP), globulin (GL), Albumin (Al), Glucose, cholesterol, urea, and creatinine (Cr), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)).

Chemical analyses

Triplicate samples of TMMN and TBD, experimental diets, and cassava peel were analyzed for dry matter (DM), crude protein

(CP), organic matter (OM), and ash following the (13) procedures. Neutral detergent fibre (NDF), Acid detergent fibre (ADF), and Acid detergent lignin (ADL) were determined by the methods described by (14).

Statistical analysis

The data obtained in this study were subjected to analysis of variance (ANOVA) using the completely randomized design (CRD) on Minitab 16 software. Tukeys post-hoc test was used to separate means and they were declared significant at $P < 0.05$.

Results and Discussion

Chemical composition of experimental diets

The chemical composition of the experimental diets is presented in Table 1. An increase in the inclusion levels of TMMN and TBD in the diets led to an increase in the CP, Ash, NDF, ADF, and ADL contents of the diets. This was similar to the findings of (3) when an increase in the inclusion level of *Pleurotus tuber-regium*-treated cassava root sievate in the diets led to an increase in CP and Ash contents of the diets but different in terms of reduced fibre and lignin content recorded. The difference may be attributed to the chemical content of the substrate used as grasses are more fibrous and lignified than cassava by-products. The CP value of the diets was above the minimum CP value (7-8% DM) capable of supporting normal rumen function needed for increased feed intake and nutrient digestibility (15). The higher CP in T₃ and T₆ diets than other diets can be attributed to more fungal biomass (i.e. mycelium) added during biodegradation. The values of NDF, ADF, and ADL contents of the diets were well below the levels of 65 %, 45 %, and 8 % regarded as low quality (16). Therefore the diets are capable of meeting the nutritional needs of the goats.

Table 1: Ingredient proportion and chemical composition of diets containing *P. ostreatus* treated *M. maximus* var. ntchisi and *B. decumbens*

Items (%)	TMMN diets			TBD diets			Ingredients			
	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	TMMN (%)	TBD (%)	Cas-P (%)	Con (%)
TMR										
Cassava peel	50.0	30.0	20.0	50.0	30.0	20.0	-	-	-	-
Treated grass	-	20.0	30.0	-	20.0	30.0	-	-	-	-
Concentrate	50.0	50.0	50.0	50.0	50.0	50.0	-	-	-	-
Chemical composition (%)										
DM	90.6	90.8	90.9	90.6	90.5	90.5	91.8	91.9	89.1	92.4
Ash	9.6	11.0	11.7	9.6	11.0	11.8	12.7	12.3	5.7	9.6
CP	8.5	8.9	9.4	8.5	10.7	11.2	6.2	9.8	4.5	16.2
NDF	48.7	56.5	57.2	48.7	53.1	54.2	73.4	65.9	22.8	34.1
ADF	24.6	34.9	35.2	24.6	31.2	31.8	55.5	51.3	14.0	28.1
ADL	4.2	4.9	5.3	4.2	4.6	4.7	7.2	6.7	2.9	4.2

TMR, total mixed ration; TMMN, treated *M. maximus* var. ntchisi; TBD, treated *B. decumbens*; Cas-P, cassava peel; Con, concentrate; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

Haematological parameters

The haematological indices of WAD growing buck-fed diets containing different levels of TMMN and TBD are presented in Table 2. The RBC, PCV, Hb, MCV, and MCH values were significantly ($P < 0.05$) influenced by the diets while other parameters measured were not influenced by the diets. An increase in the inclusion levels of the treated grasses in the diets increased the RBC, PCV, Hb, MCV, and MCH values while it reduced ($P > 0.05$) the WBC values. In comparison with goats on the control diets, only goats on 30% TBD diets had improved RBC, PCV, Hb, and WBC levels while those on TMMN diets showed reduced RBC, PCV, Hb, and WBC levels. In general, the data obtained indicate that the haematological indices measured were within the normal ranges for clinically healthy goats (17) which marks that the animals' physiological, nutritional, and health status are not negatively affected by the diets (18). There are no data about the effect of feeding *P. ostreatus*-treated grasses on ruminants' haematology and serum biochemistry. However, there is information

on the use of *Pleurotus* species with agricultural waste in ruminant diets, which this research discussed.

The significantly increased RBC, PCV, and Hb values in this study can be attributed to the CP contents of the diets as a marginal increase in CP contents of diets are one of the contributory factors to high RBC, Hb, and PCV values (6). The increased RBC, PCV, and Hb values with an increase in inclusion levels of treated grasses in the diets support the existing findings that the inclusion of *Pleurotus* species treated substrates in small ruminants' diets supports increased RBC, PCV, and Hb concentrations (19, 20, 6, 3). This is an indication that the diets are not making the animals anaemic or under erythropoiesis depression, do not cause respiratory disorder, decreased parasitic challenge, and increased nutrient utilization (7).

The highest RBC, PCV, and Hb values in animals fed 30% TBD –cassava peel-based diet indicate that the diet supported better animals' health status than others on other diets. The PCV and Hb ranges reported in this study are higher than ranges of 26.95

– 34.19 % and 8.42 – 10.70 g/dl (20); 24.20 – 30.80 % and 8.50 – 11.40 g/dl (6); and 24.0 – 32.33 % and 8.00 – 10.80 g/dl (8) reported for PCV and Hb respectively in small ruminants fed different *Pleurotus* treated agricultural wastes. The RBC ranges were higher than the ranges of $6.88 - 7.28 \times 10^{12}/L$ (19) and $9.07 - 13.44 \times 10^{12}/L$ (3) for elk deer fed *P. eryngii* spent mushroom substrate-based diets and WAD goats fed *Pleurotus tuber regium* treated cassava root sievate based diets respectively. This indicate that the supplementation of fungal-treated grasses in ruminant diets might have a greater influence on the RBC, PCV, and Hb levels than with fungal-treated agricultural wastes.

The WBC resists diseases by generating antibodies that help increase the animal immune system (21). The tendency of the diets to influence normal ranges of WBC in the animals indicates that none of the diets contains metabolites that suppress the immune system of the animals. The increased WBC level in goats on TBD diets corroborates the existing findings where *Pleurotus* mushroom inclusion in ruminant diets had an enhancing effect on the immune system and may connote improved nutrient utilization (6). This indicates that TBD diets may have the tendency of altering the immune system of the goats through monocyte activation towards the maintenance of good health. Although the WBC values obtained between the TBD group and the control were similar. Lymphocytes and monocytes are regarded as major components of WBC because they are needed for the immune system. Lymphocytes deal with the cell-mediated and humoral immune responses and monocytes are macrophages precursors (7).

The reduced lymphocyte values in animals fed 30% TMMN-based diets might have contributed to the reduced WBC values.

The diets maintained the animals' MCHC, MCV, and MCH within the normal values found in healthy goats (17), which indicates that all of the animals are not anaemic and the feed is of good nutritional quality. The MCV and MCH ranges of this study are lower than the ranges of 39.68 – 42.60 fl and 12.25 – 13.14 pg (19); 38.40 – 38.86 fl and 9.28 – 10.46 pg (20); and 22.85 – 24.80 fl and 6.93 – 7.36 pg (6) for MCV and MCH concentrations in small ruminants fed different *Pleurotus* treated agricultural wastes. This indicates that the treated grasses inclusion in the goat diets is capable of maintaining the animals within the normal MCV and MCH values than the use of treated agricultural wastes which produced values higher than the normal values.

Serum biochemical indices

The serum biochemical indices of WAD growing bucks fed diets containing different levels of TMMN and TBD are presented in Table 3. The TP, glucose, cholesterol, urea, and ALT were significantly ($P < 0.05$) influenced by the diets while other parameters were not. An increase in the inclusion levels of the treated grasses in the TMR diets increased the TP, glucose, cholesterol, and urea values. In comparison with goats on the control diets, the 30% inclusion of both treated grasses in the diets improved the TP and glucose levels of the animals with goats on the 30% TBD diet producing higher TP and glucose values; TMMN diets increased the goats' urea level; and 30% TBD in the diet increased the goats' cholesterol level.

Table 2: Haematological parameters of WAD goats fed total mix ration containing *P. ostreatus* treated *M. maximus* var. *ntchisi* and *B. decumbens*.

Blood parameters	Treated <i>M. maximus</i> var. <i>ntchisi</i> diets			Treated <i>B. decumbens</i> diets			SEM	P value	Normal ranges (17)
	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)			
RBC (X10 ¹² /l)	15.4 ^{ab}	12.3 ^b	14.7 ^{ab}	15.2 ^{ab}	15.2 ^{ab}	16.2 ^a	0.81	0.05	8.0–18.0
PCV (%)	34.3 ^{ab}	24.0 ^{bc}	30.8 ^{abc}	33.5 ^{abc}	22.8 ^c	34.8 ^a	2.28	0.003	22.0–38.0
Haemoglobin (g/dl)	12.0 ^a	8.2 ^b	10.5 ^{ab}	11.8 ^{ab}	7.9 ^b	12.2 ^a	0.79	0.001	8.0 – 12.0
WBC (x10 ⁹ /l)	12.8	12.1	9.3	11.7	15.6	12.3	2.35	0.60	4.0 – 13.0
MCHC (g/ dl)	35.2	34.1	34.1	35.1	34.7	35.7	0.69	0.51	30.0 – 36.0
MCV (fl)	22.3 ^a	19.4 ^{ab}	20.9 ^a	22.2 ^a	15.0 ^b	21.5 ^a	1.21	0.004	16.0 – 25.0
MCH (pg)	7.8 ^a	6.6 ^{ab}	7.2 ^{ab}	7.8 ^a	5.2 ^b	7.7 ^a	0.45	0.01	5.2 – 8.0
Lymphocytes (%)	64.0	60.5	56.3	65.8	54.8	60.8	3.31	0.20	50.0 – 70.0
Neutrophils (%)	35.0	36.3	39.8	31.0	41.8	37.0	2.87	0.18	30.0 - 48.0
Monocytes (%)	2.0	1.8	2.0	2.5	2.3	2.0	0.42	0.85	2.0 – 9.0
Eosinophils (%)	1.0	1.5	1.8	0.8	1.3	0.8	0.33	0.24	1.0 – 8.0

^{a,b,c} Means within the same row with different superscripts are significantly ($P < 0.05$) different. SEM, standard error of means; RBC, red blood cells; PCV, packed cell volume; WBC, white blood cells; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MCH, Mean corpuscular haemoglobin.

Serum biochemical indices are used to measure the nutritional status of animals. The TP levels obtained in this study are within the ranges of 6.4 -7.8 g/dl reported by (17) for a clinically healthy goat. However, the TP ranges are higher than the ranges of 6.83 – 7.08 g/dl (6) and 6.17 – 7.66 g/dl (3) for WAD goats fed *Pleurotus*-treated agricultural wastes. This might indicate that the grass protein is of superior quality to that of the agricultural wastes. The higher TP level in goats on 30% TBD might indicate better quality protein utilization, protein absorption, protein retention in the body, and high CP content of diets (20). The animals' similar albumin (ALB) and globulin (GLO) contents were higher than the normal ranges of 2.7 – 3.9 g/dl for albumin and within the normal ranges of 2.7 – 4.1 g/dl for globulin (17) found in a healthy goat. The ALB values were also higher than the ranges of 2.51 – 3.02 g/dl (20) and 3.2 – 3.3 g/dl (6) for WAD goats fed *Pleurotus*-treated

agricultural wastes. The high albumin level is an indication that the diets are able to meet the protein requirements of rumen microbes (3) but protein loss might be envisaged. The normal globulin suggests better quality protein. The glucose obtained in this study was higher than the ranges of 50.0 – 75.0 mg/dl reported by (17) and the animals are not surviving on energy reserves. Creatinine is used to measure the level of animal dependence on energy reserves as it is directly related to building muscle mass; a way the body tries to utilize excess energy in the blood (22). The animals in this study have normal creatinine values which indicates that the animals are not depending on energy reserves. Despite being slightly high, the glucose values are lower than the ranges of 116.28 – 174.24 mg /dl reported by (19) for elk deer fed *P. ostreatus* spawn-based diets and 108.4 – 158.0 mg /dl reported by (23) for Sika doe and suckling fawn fed *P. ostreatus* substrate based diets

but higher than the ranges of 51.48 – 56.16 mg/dl reported by (3) for WAD goats fed *P. ostreatus* treated cassava root sievate based diets. The differences can be linked to the kind of energy source in the diets.

The cholesterol levels were within the normal ranges of 80.0 -130.0 mg/dl reported by (17) for clinically healthy goats. Although the results were higher than the ranges of 95.30 – 96.0 mg/dl (6), 31.5 – 52.2 mg/dl (23), and 38.7 – 56.3 mg/dl (3) for WAD goats fed Pleurotus- treated agricultural wastes. The high cholesterol levels might be linked to the high availability of glucose in the blood which might have been converted to fatty tissues in the form of cholesterol. The blood urea of the animals is slightly lower than the normal ranges of 34.5 – 40.1 mg/dl reported by (17) in healthy goats. Low urea level is an indication of better protein utilization, quality protein, and low

catabolism of body protein (21) which is evident in TBD diets. The urea levels in this study are lower than those ranges of 89.28 – 113.04 mg/dl reported by (23) for WAD Sika doe and suckling fawn fed *P. ostreatus* spawn-based diets and 13.32 – 16.72 mg/dl reported by (3) for WAD goats fed *P. ostreatus* cassava root sievate based diets. This indicates that treated grasses are of better quality than treated agricultural wastes. The similar creatinine, AST, ALT, TBIL and DBIL levels in the animals are still within the normal ranges of 0.5 – 1.8 mg/dl for creatinine needed for efficient energy production; 67 – 170 U/L and 15.3 – 52.3 for AST and ALT respectively which indicates no liver dysfunction; and 0.1 – 0.9 mg /dl and 0.1 – 0.4 mg/dl for TBIL and DBIL respectively which indicate good protein quality of the diet (17).

Table 3: Serum biochemical indices of WAD goats fed a total mixed ration containing *P. ostreatus* treated *M. maximus* var. *ntchisi* and *B. decumbens*.

Blood parameters	Treated <i>M. maximus</i> var. <i>ntchisi</i> diets			Treated <i>B. decumbens</i> diets			SEM	P value	Normal ranges (17)
	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)			
Total protein (g/dl)	7.4 ^a	6.7 ^b	7.8 ^{ab}	7.6 ^{ab}	7.3 ^{ab}	8.5 ^a	0.41	0.09	6.4-7.0
Albumin (g/dl)	4.2	4.0	4.6	4.7	4.6	4.9	0.21	0.07	2.7-3.9
Globulin (g/dl)	3.1	2.7	3.1	2.9	2.7	3.6	0.30	0.27	2.7-4.1
Glucose (mg/dl)	63.5 ^{ab}	58.0 ^b	79.1 ^{ab}	70.5 ^{ab}	82.5 ^{ab}	90.4 ^a	6.50	0.02	50.0-75.0
Cholesterol (mg/dl)	100.5 ^{ab}	87.9 ^b	98.7 ^{ab}	105.2 ^{ab}	102.7 ^{ab}	117.3 ^a	4.72	0.01	80.0-130.0
Urea (mg/dl)	33.2 ^{ab}	35.3 ^a	37.0 ^a	33.6 ^{ab}	26.8 ^b	27.0 ^b	1.83	0.003	10.0-20.0
Creatinine (mg/dl)	1.5	1.7	1.7	1.2	0.9	0.9	0.31	0.21	1.0-1.8
AST (U/l)	77.0	59.3	63.5	81.3	71.0	82.5	6.92	0.14	167.0-513.0
ALT (U/l)	23.0	22.3	26.8	27.8	23.5	29.5	3.92	0.73	-
Total bilirubin (mg/dl)	0.7	0.5	0.7	0.8	0.8	0.8	0.11	0.35	-
Direct bilirubin (mg/dl)	0.3	0.2	0.2	0.2	0.3	0.3	0.05	0.65	0.0-0.1

^{a,b,c} Means within the same row with different superscripts are significantly ($P < 0.05$) different. SEM, standard error of means; AST, aspartate aminotransferase; ALT, alanine transaminase.

Conclusions and Applications

It can be concluded from this study that:

- i. *P. ostreatus* treatment enhanced the nutrient composition of *M. maximus* var. *ntchisi* and *B. decumbens*.
- ii. The feeding of TBD diets up to 30%

greatly maintained the animal's haematological levels within normal ranges than TMMN diets as well as maintained the serum biochemical indices levels. Although the serum indices of animals on both the TBD

- and TMMN diets appear comparable.
- iii. The feeding of 30% TBD as a supplementary diet in ruminant feeding is seen to be safe and capable of maintaining the animals in good health status.

Competing interest

The authors declare that they consent to this study and have no conflict of interest.

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