

Blood Profile of West African Dwarf Growing Bucks Fed Fungal Treated Megathyrsus Maximus and Brachiaria Decumbens Based Diets.

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Abstract

Fungal treated grasses can be used as a supplementary feed for ruminants during dry seasons. A total of 24 West African dwarf (WAD) growing bucks with the average weight of 7 kg were fed with fungal treated either *Brachiaria decumbens* (TBD) or *Megathyrsus maximus* var. *ntchisii* (TMMN) to investigate their effects on the animals' blood profile. The goats were allocated to six dietary treatments in a completely randomized design. The diets were offered as a total mix ration (100%) with one of the two treated forages at one of three levels (0%, 20%, 30%). Additionally, each diet contained concentrate (50%) and cassava peels (either 50% or 30%, or 20%) to a total of 100%. These diets were fed to the animals for 21 days with 14 days adaptation and the last 7 days for data collection. It appeared that the diets significantly ($P < 0.05$) influenced some haematological and serum biochemical parameters. The fungal treated BD and MMN at 30% inclusion maintained the animals within the expected ranges of blood profiles, indicating no apparent threat to goat wellbeing. However, animals consuming TBD diet showed better health than those fed TMMN. Therefore, 30% inclusion of TBD can be used as an alternative feedstuff in ruminant diets during the dry season.

Introduction

Small ruminants especially goats play a major role in the economic development of most tropical countries (Ogunbosoye et al., 2018). During the dry season, these animals majorly depend on less expensive feeds such as the remains of roots and tubers especially cassava (*Manihot* spp.) peel; agro-industrial by products and crop residues; and some available grasses (Franzel et al., 2014). However, these feeds are of low quality and unable to meet their nutrient requirements for better ruminant production and performance. Thus, these feeds will require an upgrade or supplementation to achieve better ruminant production.

Some of the current possible supplements used in ruminants feeding are the white rot fungal (WRF) improved feeds. 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 with increased feed protein and reduced feed lignin and fibre contents and modified rumen eco-system leading to increased feed digestibility (Sharma and Arora, 2015). Among these WRF, *Pleurotus* spp have received a lot of attention in upgrading agro-industrial by-products and crop residues for ruminant feeding, and its mycelium is regarded safe as no case of fungal pathogenicity towards animal feeding has been recorded (Sharma and Arora, 2015).

However, there is little research on the use of fungal improved grasses in ruminant diets, despite the fact that these grasses are of better nutritive value than many agro-industrial by products and crop residues (Daley et al., 2010). Among these available grasses are *Andropogon* spp; *Pennisetum* spp; *Brachiaria* spp, and *Megathyrsus* spp etc. and they have been found to be promising as possible improved dry season feeds due to their persistence, hardiness and heat tolerance (An and Reynolds, 1989). Therefore,

improving such grasses will be a way forward towards solving the problem of poor performance of ruminants' production enterprise especially during the dry season. Therefore, to assess the efficacy of feeding fungal treated grasses on animal health; immunity and nutrient utilization, this study investigated the dietary effect of fungal treated *B. decumbens* and *M. maximus* var. *ntchisi* on the blood profiles of WAD growing bucks.

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 NENT (2016).

Study Site Description

This study was carried out at Pasture and Range management paddock unit, Directorate of University Farm (DUFARMS), Federal University of Agriculture, Abeokuta. The location is 148m above the sea levels, 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, mean annual temperature of 33.8°C and relative humidity of 82%.

Fungal Specie Selection And Spawn Source

The white rot fungi (*Pleurotus ostreatus*) and the grasses were selected from a pre-study by Idowu et al. (2022). The fungus showed the highest potential in improving the nutritive value of BD and MMN, among the four grasses investigated. The pure spawn was purchased from the Forestry Research Institute of Nigeria, Ibadan, Oyo, Nigeria.

Collection And Preparation Of Experimental Diets

The grasses were harvested by cutting around 2cm above the soil surface from the experimental plots, Directorate of University Farms (DUFARMS), FUNAAB, Abeokuta, Nigeria in October, the late dry season. They were chopped to 3–5cm length, and appropriate amounts were packed in cotton bags, soaked with clean water for 24hr and thereafter left for one hour to drain the excess water. The moistened individual grasses were then sterilised for one hour as reported by Fazaeli et al. (2004). Each grass cooled down before inoculation with 4% *P. ostreatus* spawn (w/w) and re-packing about 2.5 kilogram of each grass in a sterilized transparent plastic bag. The bags were kept on racks in dark at room temperature and 100% relative humidity for twenty days. They were autoclaved to terminate the mycelia growth, and then sun dried for several days and stored in bags at room temperature. The dried cassava peels used were purchased from a local Garri Processing Unit in Eleweran, Abeokuta, and crushed with hammer mill to

about 5mm size. The dried inoculated grasses were then incorporated into a total mixed ration (TMR) at different ratios of 0% (diet 1), 20% (diet 2), 30% (diet 3) for *B. decumbens* and 0% (diet 4), 20% (diet 5), 30% (diet 6) for *M. maximus var. ntichisi*. Dried cassava peels were added to the ration to bring up the amount of either grasses or cassava peels to 50% and the concentrate made up the remaining 50% of the TMR (Table 1). The various chemical compositions of the six TMR diets, concentrate, TMMN and TBD are shown in Table 1. The concentrate (100kg DM) was made up of 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 $7 \text{ kg} \pm 0.99$ were randomly distributed into six groups ($n = 4$). The goats were dewormed of endoparasites with Albendazole 10% and dipped in Diazinole solution to control ectoparasites. They were also injected with oxytetracycline (L.A 20%), 10mg/kg of body weight to prevent infections. The goat were then kept in individual metabolic cages (1.5 m x 1 m) with good ventilation and fed with the concentrate, half of the group received additional freshly cut MMN and the other half received additional freshly cut BD for a period of 10 days before the commencement of the experiment. Subsequently, they were fed their allocated experimental diets (Table 1) for a period of 21 days which included fourteen (14) days for acclimatization to the experimental conditions, and 7 days of data collection. The animals received their allocated diets twice daily in equal amount at 08.00 and 16.00 h at 5% of their body weight on DM basis and this was changed with changes in the animal weight during transition from adaptation to trial period. Clean water was provided to the animals' ad-libitum.

Blood Sampling And Collection

On the last day of the data collection, ten ml of blood samples was collected from each animal via jugular vein-puncture using a syringe and 5 ml placed into labelled vacutainers containing ethylene diamine tetracetic acid (EDTA) for haematological analysis, while the remaining 5 ml was discharged into labelled vacutainers without EDTA to obtain serum. The tubes with EDTA were stored on ice within 1 hour of transit to the laboratory for determining haematological parameters. Non-EDTA tubes were kept in a box during transit to the laboratory where they were kept on kaylite holders at 37°C for coagulation for serum collection after centrifugation at 3000rpm for 15 mins and stored at -20°C for biochemical analyses. The haematological and serum biochemical indices were estimated as described by Adelusi *et al.* (2018).

Statistical analysis

The effects of feeding fungal treated grasses on the mean concentration of blood haematological and serum biochemical indices were determined by subjecting the data obtained 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 six experimental diets offered to the growing WAD bucks over 21 days are presented in Table 1. The crude protein (CP), Neutral detergent fibre and acid detergent fibre contents, and lignin increased as the inclusion level of each grass increased in the diets which is a reflection of the chemical composition of the various grasses and other ingredients used. The obtained CP is above the minimum CP value (7–8%DM) capable of supporting normal rumen function involved in increased feed intake and nutrients digestibility (Van Soest, 1994). The NDF, ADF and ADL contents of the diets were lower than the values of 65% (NDF), 45% (ADF), and 8% (ADL) found in feeds regarded as low quality (Van Saun, 2006), therefore the diets can be regarded as high quality.

Table 1

Ingredient proportion and chemical composition of diets containing fungal treated *M. maximus* var. *ntchisi* var. *ntchisi* and *B. decumbens*

Items (%)	TMMN diets			TBD diets			Ingredients			
TMR	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	TMMN (%)	TBD (%)	Cas- P (%)	Con (%)
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 <i>M. maximus</i> var. <i>ntchisi</i> ; TBD, treated <i>B. decumbens</i> ; 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 red blood cell (RBC; $10^{12}/\text{l}$), packed cell volume (PCV;%), haemoglobin (Hgb; g/dl), mean corpuscular volume (MCV; fl), and mean corpuscular haemoglobin (MCH; pg) were significantly ($P < 0.05$) influenced by the dietary treatments while the diets had no significant ($P > 0.05$) influence on the white blood cells (WBC; $10^9/\text{l}$), mean corpuscular haemoglobin concentration (MCHC; g/dl); eosinophils (%), neutrophils (%), lymphocytes (%) and monocytes (%). Bucks fed diet T6 showed increased RBC, PCV, and Hgb contents than other bucks. The variation in the responses of different animals to the dietary treatments indicates that the diets had influence on the animals. The RBC, PCV, Hgb, WBC, MCV, and MCH results obtained in this study were within the ranges of $8\text{--}18 \times 10^{12} / \text{l}$; 22–38%; 8–12 g/ dl; and a bit higher than $4\text{--}13 \times 10^9 / \text{l}$; 16–25 fl; and 5.2–8.0 pg reported for RBC, PCV, Hgb, WBC, MCV and MCH respectively in a clinically healthy goat and sheep (Radostits et al., 2000; Merck, 2016). The normal RBC values observed in this study implied that the animals were not anaemic; showed no erythropoiesis depression; and were capable of transporting nutrient and oxygen around the cardiovascular system. Increased RBC is complemented with more capability in carrying more amount of the respiratory pigment (Hgb) which are responsible for high oxygen carrying capacity in the blood needed by animal to withstand several stresses that cause respiratory disorder (Soul et al., 2019). Therefore, high RBC in a diet are expected to have more Hgb which corroborates with the findings of this study as observed in the animals on the T6 diet. The PCV is the percentage of RBCs in the circulating blood and high PCV indicates decreased parasitic challenge and increased nutrient utilization (Okpanachi et al., 2019). In this study, increase in the inclusion levels of both treated grasses in the diets led to higher PCV. This might be attributed to the CP contents of the diets as increased CP contents of diets are or were one of the contributory factors to high PCV value (Radostits et al., 2000). White blood cells (WBC) resist diseases by generating antibodies that helps increase the animal immune system (Anyia et al., 2018). The tendency of the diets to produce similar WBCs in the animals that are still within the normal ranges indicates that none of the diets contains metabolites that suppresses the immune system of the animals. The diets maintained the animals MCHC, MCV and MCH within the normal values found in healthy goats (Merck, 2016), which indicate that all of the animals are not anaemic. The animals on all of the diets produced similar Lymphocytes, monocytes, neutrophils and eosinophils that are within or slightly below the normal ranges of 55.4–67.8%; 2.1–4.5%; 25.3–41.9%; and 1–8% found in healthy goats respectively (Opara et al., 2010). This suggests that diets are capable of boosting the immune system (Soul et al., 2019).

Table 2

Haematological parameters of WAD goats fed total mix ration containing fungal treated *M. maximus* var. *ntchisi* and *B. decumbens*.

	Treated <i>M. maximus</i> var. <i>ntchisi</i> diets			Treated <i>B. decumbens</i> diets				
Blood parameters	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	SEM	P value
Red blood cells ($\times 10^{12}/\text{l}$)	15.4 ^{ab}	12.3 ^b	14.7 ^{ab}	15.2 ^{ab}	15.2 ^{ab}	16.2 ^a	0.81	0.05
Packed cell volume (%)	34.3 ^{ab}	24.0 ^{bc}	30.8 ^{abc}	33.5 ^{abc}	22.8 ^c	34.8 ^a	2.28	0.003
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
White blood cells ($\times 10^9/\text{l}$)	12.8	12.1	9.3	11.7	15.6	12.3	2.35	0.60
MCHC (g/ dl)	35.2	34.1	34.1	35.1	34.7	35.7	0.69	0.51
MCV (fl)	22.3 ^a	19.4 ^{ab}	20.9 ^a	22.2 ^a	15.0 ^b	21.5 ^a	1.21	0.004
MCH (pg)	7.8 ^a	6.6 ^{ab}	7.2 ^{ab}	7.8 ^a	5.2 ^b	7.7 ^a	0.45	0.01
Lymphocytes (%)	64.0	60.5	56.3	65.8	54.8	60.8	3.31	0.20
Neutrophils (%)	35.0	36.3	39.8	31.0	41.8	37.0	2.87	0.18
Monocytes (%)	2.0	1.8	2.0	2.5	2.3	2.0	0.42	0.85
Eosinophils (%)	1.0	1.5	1.8	0.8	1.3	0.8	0.33	0.24
a,b,c Means within the same row with different superscripts are significantly ($P < 0.05$) different. SEM, standard error of means; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MCH, Mean corpuscular haemoglobin.								

Serum Biochemical Indices

The results of the serum biochemical indices obtained in this study from WAD bucks fed different experimental diets are presented in Table 3. Total protein (g /dl), glucose (mg/dl), cholesterol (mg/dl), urea (mg/dl), and Alanine transaminase (ALT; U/L) were significantly ($P < 0.05$) influenced by the diets. The diets produced similar ($P > 0.05$) effects on the albumin (g/dl), globulin (mg/dl), creatinine (mg/dl), aspartate aminotransferase (AST; U/L), total bilirubin (T. bil; mg/dl) and direct bilirubin (D. bil; mg/dl) of the animals. Bucks on T6 diets showed better performance in terms of increased total protein, glucose, and cholesterol as well as lowered urea content than other bucks. The differences obtained in this study

showed that the animals varied in their immunity levels. The total protein values obtained in this study are higher than the normal range of 6.4–7.8 g/dl reported by Merck (2016) to be found in a healthy goat. High total protein might indicate better quality protein utilization, protein absorption, protein retention in the body, and high CP content of diets (Anyia et al., 2018) which is evident in animals on T6 diet. The animals' similar albumin and globulin contents were higher than the normal range of 2.7–3.9 g/dl for albumin and within the normal range of 2.7–4.1 g/dl for globulin (Merck, 2016) found in a healthy goat. High albumin might indicates that the goats are prone to haemorrhage (Okpanachi et al., 2019), while normal globulin suggest better quality protein. The glucose obtained in this study was higher than the normal ranges of 50.0–75.0 mg/dl while the cholesterol content was within the normal range of 80–130 mg/dl (Merck, 2016) found in clinically healthy goats. This suggest that the animals are not depending on reserved energy in body tissues and are not pre-disposed to diabetic hyperglycaemia. The blood urea of the animals are slightly lower than the normal range of 34.5–40.1 mg/dl reported by Merck (2016) in healthy goat. Low urea is an indication of better protein utilization, quality protein and low catabolism of body protein (Anyia et al., 2018) which is evident in TBD diets compared to TMMN diets. The diets led to similar creatinine, AST, ALT, t.bil and d.bil contents in the animals. However the ranges of all these parameters are still within the normal ranges of 0.5–1.8 mg/dl for creatinine needed for an 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 t.bil and d.bil respectively which indicate good protein quality of the diet (Merck, 2016).

Table 3

Serum biochemical indices of WAD goats fed total mixed ration containing fungal treated *M. maximus* and *B. decumbens*.

	Treated <i>M. maximus</i> var. <i>ntichisi</i> diets			Treated <i>B. decumbens</i> diets				
Blood parameters	T1 (0%)	T2 (20%)	T3 (30%)	T4 (0%)	T5 (20%)	T6 (30%)	SEM	P value
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
Albumin (g/dl)	4.2	4.0	4.6	4.7	4.6	4.9	0.21	0.07
Globulin (g/dl)	3.1	2.7	3.1	2.9	2.7	3.6	0.30	0.27
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
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
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
Creatinine (mg/dl)	1.5	1.7	1.7	1.2	0.9	0.9	0.31	0.21
AST (U/l)	77.0	59.3	63.5	81.3	71.0	82.5	6.92	0.14
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
^{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

In conclusion, the addition of TBD up to 30% of the diet did not pose any threat to the animals' health as the parameters measured were within the normal blood ranges of a clinically healthy animals, although the total protein albumin and glucose seemed higher than the normal ranges. Therefore, the use of treated *B. decumbens* can be suggested as a good feedstuff for ruminant feeding during the dry season and investigation requiring the use of higher inclusion levels of TBD is recommended.

Declarations

Competing interest

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

Statements and Declarations

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Authors Contributions

All authors contributed to the paper. Idowu O.J. and Chaudhry, A. S. conceptualised and designed the study. Tanimowo, R.O, Akinyemi, S. B; Agboola F.O, and Ogunsiji R.T., sourced and prepared the materials, executed the experiment and collected data. Idowu O.J. carried out the data analysis, presentation, and manuscript preparation. Adelusi, O.O; Chaudhry, A.S; and Ojo, V.O.A revised the manuscript. All authors read and approved the final manuscript.

Data Availability

The data set generated is available from the corresponding author as a supplementary document.

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