

Haemato-biochemical parameters of lambs fed tannin containing *Daniellia oliveri* seed meal based diet

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ABSTRACT

This study investigated the effect of replacement of conventional protein source groundnut cake (GNC) with toasted-decorticated *Daniellia oliveri* seed meal (DSM) on haemato-biochemical parameters of lambs. Twenty intact 6-month-old lambs were used in an 84-day experiment. The animals were randomly divided into four treatments containing 0, 25, 50 and 75% DSM replacement levels for GNC. Five animals were randomly allotted to one of dietary treatments in a completely randomised design. The concentrates were formulated to have similar ($P>0.05$) CP of 181.0 g/kg DM on average. All the other nutrients in the formulated supplements were significantly different ($P<0.05$). There were increases in levels of organic matter (924.9–961.00 g/kg DM), non-fibre carbohydrates (238.03–329.36 g/kg DM) as well as condensed tannins, as the level of inclusion of DSM in the concentrate diet increased. Packed cell volume, white blood cell, lymphocytes, serum urea and glucose linearly increased ($P<0.05$) but mean corpuscular haemoglobin concentration linearly decreased ($P<0.05$) while other haematological and serum parameters were similar among diets. Blood parameters were within established ranges for healthy sheep. The results indicate that tannins containing *Daniellia oliveri* seed meal can be incorporated up to 75% without compromising body immunity system and health of the animals.

Keywords: Sheep, *Daniellia oliveri* seed, Chemical composition, Haematology, Serum biochemical parameters

INTRODUCTION

In Nigeria and most of the developing countries where livestock is raised on natural fodders, the usual practice among livestock holders is to supplement livestock diets with non-conventional plant protein rich ingredients such as browse leaves and wild legume seeds. This practice is due to prohibitive cost of conventional plant protein-rich ingredients such as groundnut cake, soybean meal and cotton seed cake due to their various competitive uses. This makes them unaffordable for many local farmers who are the major custodians of livestock in the tropic. More attention is now focused on alternative wild (or lesser known) legume seeds not only because of their abundance and cheapness, but also their adequate nutritional value, especially amino acids and minerals profiles (Ragab et al. 2010), crude protein (19-35% on DM basis) and starch contents (over 40% DM), despite the presence of anti-nutritional factors which are heat labile and can be reduced or removed completely by thermal processing methods.

One of such abundantly available wild legume seeds in the Guinea savannah of Nigeria is *Daniellia oliveri* seed. *Daniellia oliveri* is a leguminous tree belonging to the *Leguminosae* family and *genus Caesalpinaceae*. The seeds, which have no preference as a human food or in industrial use till now could, therefore, serve an alternative feed ingredient for livestock production. Raw *Daniellia oliveri* seed contained 44.60% carbohydrate, 6.35% crude fibre, 33.40 % crude protein, 28.32% lipid and 4.30% ash on dry matter basis (Adubiaro et al., 2011). However, the presence of anti-nutritional substances, such as phytate, oxalate, tannins and saponins, in the seed hinders its optimal utilisation by animals (El-Mahmood et al., 2008). The seed of this multipurpose legume tree contains low to moderate concentration of phenols (Adubiaro et al., 2011; Okunade and Olafadehan, 2017) and may be strategically used to replace the conventional protein sources without detrimental effect on the physiology and health of animals. The present study aimed at investigating haemato-biochemical parameters in sheep fed tannins containing *Daniellia oliveri* seed meal based diet.

MATERIALS AND METHODS

Animal management, experimental design and diets

Twenty healthy Yankasa ram lambs (6 months old and body weight (BW) of 13.7 ± 0.1 kg) were stratified into four groups of five animals of similar BW each. All the ram-lambs were fed with experimental diets during for the two

weeks of acclimation when they were intramuscularly injected and dewormed with 1 mL/10 kg BW of oxytetracycline and levamisole respectively and sprayed against external parasites with diazuntol. Each group was randomly assigned to one of the four experimental diets in a completely randomised design (CRD) experiment which lasted for 12 weeks. Four iso-nitrogenous concentrate diets were formulated to meet nutrient requirement of growing ram lambs (Table 1), using toasted-decorticated *D. oliveri* seed meals (DSM) to replace GNC at 0, 25, 50 and 75%, and the treatments were designated DSM0, DSM50, DSM100 and DSM150 respectively. Each of the experimental animals were offered 1 kg of concentrate once daily at 09:00 h, while the basal diet, threshed sorghum tops (TST), and clean water were offered *ad libitum*.

Collection of blood samples

Blood (10 mL) was collected from the jugular vein of sheep at the end of the experiment for haematological and serum biochemical indices analysis. Blood samples (5 mL) were collected into vials containing ethylenediaminetetra-acetic acid (EDTA) for the determination of haematological parameters. The vials were immediately capped and the content mixed gently for about a minute by repeated inversion. Another 5 mL blood samples for serum biochemical analysis were collected in bottles without EDTA.

Chemical analysis

Samples of the experimental diets were analysed for proximate constituents according to the method of AOAC (2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by methods of Van Soest *et al.* (1991). Cellulose and hemicellulose were estimated as differences between ADF and lignin and NDF and ADF respectively. Condensed tannins (CTs) were determined as reported by Polshettiwar *et al.* (2007). Haemoglobin content (Hb) and packed cell volume (PCV) as stated in the method of Jain (1986). Red blood cell (RBC) and differential total white blood cell (WBC) counts were carried out using the Neubauer haemocytometer after appropriate dilution. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin and mean corpuscular volume (MCV) were calculated as described by Jelalu (2014). Serum urea nitrogen, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphate (ALP), glucose, cholesterol and serum total protein and its components (albumin and globulin) were determined using the methods of Jelalu (2014). Mineral elements were determined with atomic absorption spectrophotometer (Model 490, Gallenkamp and Co. Ltd., London).

Statistical analysis

Data were subjected to one way of ANOVA in completely randomised design using version 6 of SAS software (SAS Institute, 2012). Significant difference among individual means was separated by Duncan's procedure of the same software. Mean differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of experimental diets

Chemical composition (g/kg DM) of experimental diets is presented in Table 1. All the nutrients in the supplements were significantly different ($P < 0.05$) except for CP.

Table 1. Ingredient and chemical composition of the experimental diets (g/kg DM)

Nutrient	Diet				SEM	DMS	TST
	DSM0	DSM50	DSM100	DSM150			
Maize offal	450	450	450	450			
Cowpea husk	255	250	250	250			
Brewers dried grain	50.0	50.0	50.0	50.0			
Groundnut cake	200	150	100	50.0			
<i>D. oliveri</i> seed meal	0.0	50.0	100	150			
Urea	5.00	10.0	10.0	10.0			
Dicalcium phosphate	10.0	10.0	10.0	10.0			
*Vit-Mineral Premix	10.0	10.0	10.0	10.0			
Sulphur powder	10.0	10.0	10.0	10.0			
Table salt	10.0	10.0	10.0	10.0			
<i>Chemical composition</i>							
Dry matter	847.1 ^c	842.8 ^d	872.9 ^a	867.5 ^b	0.83	957.0	978.0
Organic matter	924.90 ^d	948.10 ^c	956.0 ^b	961.00 ^a	0.83	959.5	900.0
Crude protein	181.34	180.50	181.28	181.11	0.28	390.0	6.0
Ether extract	60.92 ^a	53.71 ^b	50.33 ^c	37.52 ^d	0.27	66.8	48.0
Non fibre carbohydrate	238.03 ^d	283.64 ^c	298.23 ^b	329.36 ^a	7.48	250.0	440.0
Neutral detergent fibre	443.61 ^a	431.40 ^b	426.20 ^c	413.06 ^d	1.73	263.3	695.7
Acid detergent fibre	255.04 ^a	223.22 ^b	200.40 ^c	182.30 ^d	2.14	172.2	580.0
Condensed tannins	ND	25.0 ^b	26.0 ^b	30.2 ^a	1.20	29.5	6.55

^{abc} means within a row not bearing a common superscript letter significantly differ ($P < 0.05$)

*supplied the following per kg of complete diet:

Vitamin A 4,000,000 IU; Vitamin D3 2,000,000 IU; Vitamin E 7,000 IU; Vitamin B2 4,000 mg; Nicotinic acid 15,000 mg; Calcium D-pentothenate 8,000 mg; Biotin 40 mg; Vitamin B12 10 mg;

Mn 20,000 mg; Fe 50,000 mg; Zn 100,000 mg; Cu 10,000 mg; Iodine 750 mg; Co 3,000 mg. TST= threshed sorghum tops. DSM= toasted-decorticated *Daniellia oliveri* seed. DSM added at 0 (DSM0), 50 (DSM50), 100 (DSM100) and 150 g/kg (DSM150) replacing 0, 25, 50 and 75% of GNC respectively, ND = Not determined.

There were increases in levels of CTs, as the level of DSM in the concentrate diet increased. However, CTs content in DSM diets was lower than the level of 55 g/kg CT which can hamper nutrient utilization in ruminants (Bunglavan and Dutta, 2013).

Haematological parameters

There were variations ($P < 0.05$) in the haematological parameters except for white blood differentials (lymphocytes, monocytes, neutrophils, basophils and eosinophil) as the inclusion level of DSM meal increased in the diet (Table 2). All haematological parameters in this study were within the normal ranges. Several rearing methods, environmental factors and nutrition have been established to often cause variation in the concentration of many haematological and serum metabolite profiles of farm animals. Etim et al. (2014) opined that haematological components are valuable in monitoring feed toxicity, especially with feed constituents that affect the blood profile and consequently the health status of farm animals. In the present study, the experimental sheep, particularly those placed on DSM150 which had the highest concentration of CTs, did not show clinical signs of ill health or tannin toxicity throughout the experimental period. This validates the observations of Okunade et al. (2016) when they fed tannin-containing forages as supplement to low quality roughage to goats. The depressed of PCV, Hb, RBC, erythrocyte differentials and WBC concentrations in sheep fed DSM150 may be the result of the higher CTs concentration in this diet. This validates the previous reports (Okunade et al. 2016) of effect of high CTs content on blood parameters. Haemoglobin (Hb) is a physiological vehicle for transportation of oxygen to tissues of animals for oxidation of digested feed so as to release energy for the other body functions as well as transport CO_2 out of the body of animals (Olafadehan et al. 2014). The Hb levels alongside erythrocyte indices (MCV, MCH and MCHC) were within the normal physiological ranges (9- 15 g/dL, 22.9 - 29.40 fL, 8 - 12 pg and 31 -38 g/dL respectively) for Ovine (Jelalu, 2014), indicating that none of the animals suffered from hypochromic microcytic anaemia, ischaemia, anoxia, depressed respiratory capability and any circulatory problem (Etim et al. 2014). Decreases in Hb with increasing DSM meal level depicts reduction in utilisation of iron for production of Hb as a result of increasing CTs level in DSM diets (Okunade et al. 2016). Packed cell volume is a blood toxicity reduction index, CTs concentration in all the DSM diets (DSM50, DSM100 and DSM150) were not high enough to induce tannin toxicity in the animals placed on the diets. The normal PCV values obtained in this study indicates the absence of normocytic anaemia and polycythemia

which is reportedly a reflection of abnormal PCV and can only be detected by a decreased number of RBC (Cullis, 2011). The RBC values are comparable to the range of $9-13 \times 10^6 \mu\text{L}$ reported for Omani sheep fed diets containing phenol and CTs (Mahgoub et al., 2014).

Table 2. Haematological parameters of ram lambs fed *Daniellia oliveri* seed meal based diets

Parameter	Diet				SEM	P-value
	DSM0	DSM50	DSM100	DSM150		
Packedcell volume (%)	31.35 ^a	29.97 ^{ab}	29.32 ^b	27.00 ^c	0.74	<0.001
Haemoglobin (g/dL)	11.13 ^a	11.04 ^a	9.87 ^b	8.65 ^c	0.60	<0.001
Red blood cell ($10^6 \mu\text{L}$)	10.16 ^a	10.07 ^{ab}	9.95 ^b	9.27 ^c	0.35	<0.001
MCHC (%)	34.54 ^b	36.76 ^a	33.57 ^b	32.07 ^c	2.44	<0.001
MCH (%)	11.15 ^a	10.95 ^a	9.92 ^b	9.32 ^c	0.35	<0.001
MCV (fL)	31.72 ^a	29.73 ^b	29.44 ^b	29.09 ^b	0.91	<0.001
White blood cells (10^6)	12.23 ^a	11.56 ^{ab}	10.28 ^{bc}	8.97 ^c	0.31	<0.001
Lymphocytes (%)	63.82	63.94	63.24	64.00	0.39	0.085
Monocytes (%)	1.98	2.26	1.96	2.30	0.27	0.760
Neutrophils (%)	32.76	32.05	31.98	31.80	0.36	0.121
Basophils (%)	0.39	0.44	0.43	0.32	0.10	0.144
Eosinophils (%)	2.67	2.69	2.74	2.71	0.08	0.170

^{abc} means within a row not bearing a common superscript letter significantly differ ($P < 0.05$)

DSM added at 0 (DSM0), 50 (DSM50), 100 (DSM100) and 150 g/kg (DSM150) replacing 0%, 25%, 50 and 75 % of GNC respectively.

MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Though RBC differentials provide information about the average cell size and haemoglobin concentration, an increased MCV (macrocytosis) is an indication of regenerative response, while microcytosis (decreased MCV) is often noticed in metabolic disorders due to iron deficiency in diet. An increased MCHC indicates the presence of haemolysis or decreased as in the case of reticulocytosis and iron deficiency anaemia. Total WBC count in all the experimental animals was within the physiological range of $4-13 \times 10^9/\text{L}$ for healthy sheep (Bello and Tsado, 2014). Higher WBC value observed in animals fed DSM0 (control diet) was not due to leucocytosis, inflammation or any other physiological disorder, but it implies that the animals maintained improved immune system than animals on other diets (Olafadehan et al., 2014). This indicates that the dietary treatments did not affect the immune system (Etim et al., 2014).

Serum biochemical parameters

There were dietary treatment effects ($P < 0.001$) on urea nitrogen, serum glucose and cholesterol concentrations, while serum magnesium

concentration increased linearly ($P < 0.001$) with increased inclusion level of Daniellia seed meal (Table 3). All other serum metabolites, including enzymes alkaline phosphate (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and major serum minerals, such as calcium, phosphorus, potassium and sodium, were not affected ($P > 0.001$) by treatments. Recently it was reported that serum biochemistry is a useful tool in the clinical diagnosis of various systemic states and growth performance of lambs (Salem et al., 2017). Serum urea concentration is an aftermath of proteolysis in the rumen. Serum urea concentration in the present study is in agreement with the normal range reported by Njidda et al. (2014) for healthy sheep. Similar variation in blood urea nitrogen level in sheep fed with tanniferous leaf meal based multi-nutrient blocks has been reported (Singh et al. 2015). Low level of blood urea nitrogen in the DSM diets indicates better protein quality compared with the DSM0 (control) diet (Das et al. 2011). Condensed tannin prevents rapid protein degradation by rumen microbes and absorption of rumen protein, consequently furnished the lower gut with adequate rumen undegradable protein for better absorption and utilization. The lower serum urea nitrogen of DSM diets, therefore, indicates the superiority of their protein relative to the control diet (DSM0). The urea levels of the serum being within the normal reference range implies that liver and kidney were not overworked and the likelihood of organ damage and the anti-nutritional and feed toxic effect can be disregarded; thus, the DSM diets which were tannin-containing diets could be termed safe for sheep diet. Serum glucose level observed in the present study is similar to the normal reference value reported by Olafadehan et al. (2014), but slightly fell below the range of 3.50 – 4.45 mmol/L observed by Aljameel and Abdulkarim (2017) who fed graded levels of *Parkia biglobosa* (African Locust Bean) fruit pulp to Uda rams. Significant differences in energy intake and growing ability among the treatments might have resulted in variation in glucose concentration among the animals. Glucose is a relatively good indicator of energy balance. The higher glucose recorded for sheep offered DSM150 diet could be due to the higher dietary energy intake and better growth rate of sheep fed this diet. Lower glucose level is an indication of hypoglycaemia (Olafadehan et al., 2014). Cholesterol values in the current study were within the reference range value of 1.7- 3.00 mmol/L reported by Merck (2012) and 1.8 – 2.5 mmol/L by Njidda et al. (2014) for yearling northern Nigeria sheep breed. This observation suggests that the lower cholesterol level in animals offered (DSM150) was not due to hypocholesterolemia or tannin toxicity (Olafadehan et al., 2014). The fatty acid composition present in meat poses serious concern to the health of meat consumers (Vannice and Rasmussen, 2014). Red meat lipids are widely criticized for their saturated fatty acid richness (Geay et al., 2002).

Table 3. Serum biochemical parameters of ram lambs fed *Daniellia oliveri* seed meal based diets

Parameter	Diet				SEM	P-value
	DSM0	DSM50	DSM100	DSM150		
Urea nitrogen (mmol/L)	7.78 ^a	6.83 ^b	6.52 ^b	5.86 ^c	0.26	<0.001
Alkaline phosphatase (UI/L)	40.20	34.89	37.52	38.19	3.85	0.830
Alanineaminotransferase (UI/L)	20.10	19.89	19.78	18.98	0.12	0.264
Aspartate aminotransferase (UI/L)	87.00	86.30	79.63	81.34	3.42	0.121
Glucose (mmol/L)	1.92 ^d	2.09 ^c	2.55 ^b	3.00 ^a	0.05	<0.001
Cholesterol (mmol/L)	3.20 ^a	3.00 ^b	2.86 ^b	2.73 ^c	0.01	<0.001
Total protein (g/L)	71.00	69.90	70.06	69.71	0.54	0.112
Albumin (g/L)	34.02	33.92	32.95	33.02	0.53	0.076
Globulin (g/L)	36.80	36.57	37.12	36.07	0.37	0.690
Albumin: globulin ratio	0.91	0.92	0.89	0.88	0.03	0.140

^{abc...}Means in the same row with different superscripts differ significantly ($p < 0.05$).

DSM added at 0 (DSM0), 50 (DSM50), 100 (DSM100) and 150 g/kg (DSM150) replacing 0%, 25%, 50 and 75 % of GNC respectively.

Sera minerals/electrolytes are involved in most of the body's daily functions such as proper nerve conduction, heart and skeletal muscle contraction and maintenance of proper hydration status and blood pH (Robert et al., 2012). All the serum electrolytes (Table 4) were within the established ranges of 8.55 – 9.05 mg/dL, 2.2 – 2.8 mg/dL, 5.0 – 7.3 mEq/L, 3.9 – 5.4 mEq/L and 139 – 152 mEq/L for Ca, Mg, P, K and Na respectively (Kaneko et al., 2008; Merck, 2012).

Table 4. Serum electrolytes of ram lambs fed *Daniellia oliveri* seed meal based diets

Parameter	Diet				SEM	P-value
	DSM0	DSM50	DSM100	DSM150		
Calcium (mg/dL)	9.02	8.93	9.33	8.94	0.32	0.916
Phosphorus (mg/dL)	6.67	6.29	7.37	7.46	0.82	0.770
Potassium (mEq/L)	5.05	4.66	4.65	4.83	0.29	0.248
Sodium (mEq/L)	150.0	140.40	141.00	138.33	4.50	0.163
Magnesium (mg/dL)	2.41 ^c	2.65 ^b	2.97 ^a	3.00 ^a	0.09	<0.001

^{abc...}Means in the same row with different superscripts differ significantly ($p < 0.05$).

DSM added at 0 (DSM0), 50 (DSM50), 100 (DSM100) and 150 g/kg (DSM150) replacing 0%, 25%, 50 and 75 % of GNC respectively.

The occurrence of the serum electrolyte levels within the normal range is an indication that the diets did not obstruct the availability and absorption of major serum minerals and may not interfere with the normal renal function of

the animals (Olafadehan et al., 2023). Although all of these clinical manifestations were absent in all the experimental animals, DSM150 furnished the animals with more adequate Mg than other diets.

CONCLUSION

Toasted-decorticated *Daniellia oliveri* seed meal can partially replace groundnut cake up to 75% in the diet of sheep without any detrimental effect on their welfare. Based on the feeding value of *Daniellia* seed meal in the current study, further research on complete replacement of processed *Daniellia oliveri* seed meal for groundnut cake should be conducted.

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