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ORIGINAL RESEARCH ARTICLE

Effect of varying concentrate level on voluntary intake and haematochemical indices of goats fed *Piliostigma thonningii* basal diet

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ABSTRACT

The effects of feeding different levels of concentrate supplement to Red Sokoto goats (body weight (BW), 6.64±0.28), fed basal tannin-containing *Piliostigma thonningii* foliage on voluntary intake, haematological and biochemical parameters, were evaluated. Fifteen goats were stratified into three groups of homogenous BW and randomly assigned to one of three treatment diets in a completely randomized experiment in a 70-d assay. One concentrate ration was formulated and fed at varying levels of 1.5 or 2.5% BW and *ad libitum*. Intakes of dry matter and crude protein, and serum urea nitrogen and glucose concentrations increased ($P < 0.05$) with increasing level of concentrate supplementation. Intake of condensed tannins was highest and lowest ($P < 0.05$) for 2.5% BW and *ad libitum* concentrate allowance respectively. Red blood cell counts were higher ($P < 0.05$) for *ad libitum* concentrate feeding than for 1.5 and 2.5% BW concentrate allowances. Serum total protein and albumin were intermediate for 2.5% BW concentrate feeding but higher ($P < 0.05$) for *ad libitum* concentrate allowance than for 1.5% BW concentrate supplementation. Values of all blood and serum measurements were within normal ranges for goats. The results indicate that low or high concentrate supplementation level did impair the health status of the animals, and tannin-containing *Piliostigma thonningii* foliage can used as a basal forage without inducing tannic acid toxicity, metabolic disorders and compromising the nutritional and health status of goats. However, for superior health and nutritional status, *ad libitum* concentrate allowance may be preferred.

Keywords: Concentrate allowance, Condensed tannins, Goat, intoxication, *Piliostigma thonningii*

INTRODUCTION

There is an increasing interest in utilization of tree leaves for feeding ruminants due to their evergreen nature and better nutritive value (high protein profile) than grasses, which are unavailable throughout the year (Olafadehan *et al*., 2014a). However, tree leaves contain anti-nutritional factors, especially condensed tannins (CT), which may impair the performance and health of animals. Condensed tannins in tree or shrub fodders, if consumed in excess by animals, can produce intoxication. *Piliostigma thonningii* is a leguminous plant that belongs to the family Caesalpiniacea. Several authors (Tona 2011; Olafadehan *et al*., 2014a, Okunade *et al*., 2016) have confirmed *P. thonningii* as a tannin-containing tree fodder. One of the insured ways of abating the intoxicating effect or toxicity of CT in tree leaves is by feeding supplementary concentrates due to their diluting effect on the toxicants (Olafadehan *et al*., 2014a). Whereas many previous studies have evaluated the effect of supplementary concentrate feeding on both productive and reproductive performance of ruminant stock, only a few have considered the effects of varying level of concentrate supplementation on the health of the animals. Feeding varying levels of supplementary concentrate may affect animals' welfare due to variation in the quantity of available feed, nutrients and energy, and plane of nutrition. It thus becomes essential to investigate the effect of varying concentrate supplementation level on the health of animals consuming tannin-containing basal forage. Metabolic distress, one of the means for measuring wellbeing in animals, is a situation arising from metabolic imbalances in the blood, which can result in discomfort, impaired feed intake and sickness eventually (Broom, 1991; Ohl and Van der Staay, 2012). Blood metabolite profile can assist in appreciation of the animals' welfare status, especially in relation to their nutritional and health status. For example, the blood protein profile, which relates to the proportions of the various fractions of albumin and globulins, provides farmers with a welfare indicator for their animals (Gobindram *et al*., 2016). Alteration of serum protein profile may occur in the case of liver disorders, acute inflammation and other physiological disorders. It was hypothesized that tannin-containing *P. thonningii* foliage can be used as a basal diet to a supplementary concentrate diet fed at varying

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levels without negatively affecting feed intake and welfare of growing goats. From the foregoing, the study was conducted to assess the blood profile of goats fed tanniniferous *Piliostigma thonningii* basal diet supplemented with varying levels of concentrate.

MATERIALS AND METHODS

Study site, animals and management

The experiment was carried out at the goat unit of the University of Abuja Teaching and Research Farm. The study site is situated between latitude 8º 55'N and 9º 00'E and longitude 7º 00'N and 7º 05'E. The mean annual rainfall and temperature range from 1100 to 1650 mm and 25.8 to 35.1ºC respectively. Relative humidity is about 60% during raining season and 30% during dry season.

Fifteen healthy young intact Red Sokoto male goats of about 6 to 7 months of age, with a pre-experimental body weight (BW) of 6.64 ± 0.28, were used for the assay. Prior to the commencement of the study, the animals were dewormed with levamisole at the dosage of 1 mL/10 kg BW and dipped against ectoparasites with diazintol. They were also given intramuscular injection of antibiotics (oxytetracycline) LA and vitamin B complex at the rate of 1 mL/10 kg BW respectively. The animals were weighed, stratified into three homogeneous groups and housed in individual open and well-ventilated pens. Goats in each group were randomly assigned to one of three experimental diets. Fresh leaves of *Piliostigma thonningii* were harvested, sun cured, chopped into smaller sizes and fed *ad libitum* daily at 11:00 h. One concentrate diet was formulated and animals were fed for 70 d under three feeding regimes comprising varying concentrate level of 1.5 or 2.5% BW and *ad libitum*. The concentrate ration was formulated to contain 30% maize, 20% corn bran, 15% groundnut cake, 30% dried brewers grains, 2% limestone, 2% salt and 1% vitamin-mineral premix. Feeding of supplementary concentrate was once daily at 09.00 h and water was freely available. Adjustments were made to the supplemental concentrate offered based on the changes in BW of the animals to ensure strict compliance with planned feeding levels. Feed samples were collected weekly for DM determination and 10-week samples were pooled for chemical analysis. The feeding trial lasted for 12 weeks, with two weeks of adjustment period.

Blood sampling and analysis

Blood samples were collected once at the end of the feeding period by jugular vein puncture using hypodermic syringes into Vacutainer tubes. One set of the blood samples (5 mL) was collected into plastic tubes containing ethylene diamine tetra acetic acid (EDTA) for the determination of haematological parameters. The other set of blood samples (5 mL) was collected into EDTA-free plastic tubes, allowed to coagulate at room temperature and centrifuged for 5 min x 3000 g. The supernatant sera were decanted and deep-frozen for biochemical analysis.

Blood samples containing EDTA were shipped in an icepacked cooler to the laboratory and analysed within 6 h of collection. Packed cell volume (PCV) and haemoglobin (Hb) concentrations were determined as described by Dacie and Lewis (2001). Red blood cell (RBC) and total white blood cell (WBC) as well as the differential WBC counts were determined using the improved Neubauer haemocytometer counting chamber (BS748:1982; British Standard Institution; London, UK) after appropriate dilution. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from RBC, Hb and PCV values using appropriate formulae by Dacie and Lewis (2001).

Serum total protein, albumin and globulin values were obtained by the buiret method (Reinhold, 1953), and serum urea and creatinine by modified methods of Varley *et al*. (1980). The routine flame photometric technique of Varley *et al*. (1980) was used to determine the serum electrolytes: sodium (Na^+) and potassium (K^+) concentrations. Glucose and serum enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were determined using a commercial test kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) and finally measured using the UV Spectrophotometer (SEAC, Florence, Italy). Other serum electrolytes calcium (Ca^{2+}), phosphate (PO_4^{3-}) and magnesium (Mg^{2+}) were determined using atomic absorption spectrophotometer.

Chemical analysis

The chemical composition of the basal *Piliostigma thonningii* foliage and concentrate was determined according to the procedures of AOAC (1995). Fibre fraction analysis was by the methods of Van Soest *et al*. (1991). Condensed tannins were determined by the methods of Makkar (2003).

Data analysis

Data were analysed using general linear model of analysis of variance procedure for the completely randomized design using SPSS Base 17 computer software (SPSS software products, USA), and differences among means were separated using the Duncan's multiple range test of the same software at the level of $P \leq 0.05$. The statistical model is: $Yij = \mu + Ci + \mathcal{E}ij$, where Yij = dependent variables; μ = population mean; Ci = effect of varying concentrate level and $\mathcal{E}ij$ = residual error.

RESULTS

Chemical composition of the basal forage and experimental diet

The crude protein (CP), organic matter (OM) and ether extract of the supplementary concentrate were higher than that of the basal *P. thonningii* foliage, but the fibre fractions of the foliage were higher than that of the concentrate. While the *P. thonningii* foliage was a tannin-containing fodder, the concentrate had no CT.

Voluntary intake

Feed and CP intakes were affected ($P < 0.05$) by diets; the rank order was: 1.5% BW <2.5% BW <*ad libitum* concentrate level. Intake of CT was higher ($P < 0.05$) for 2.5% BW concentrate allowance compared to other diets.

Table 1: Chemical composition of the basal forage and supplementary concentrate

Item	Concentrate mixture	*Piliostigma thonningii* foliage
Dry matter	90.92	95.11
In % dry matter		
Crude protein	17.50	16.10
Organic matter	90.92	89.12
Ether extract	7.89	4.02
Ash	9.08	10.88
Neutral detergent fibre	33.04	42.21
Acid detergent fibre	19.19	31.07
Lignin	5.83	10.87
Condensed tannins	ND	4.20

ND: not detected

Table 2: Voluntary intake of goats fed varying concentrate level

Intake (g/d)	Concentrate level (% body weight)			SEM
	1.5	2.5	Ad libitum	
Dry matter	353.58^{c}	486.68^{b}	575.68^{a}	7.43
Crude protein	58.76^{c}	81.63^{b}	98.57^{a}	0.57
Condensed tannins	9.35^{b}	11.45^{a}	6.53^{c}	0.44

Means in the same row with different superscript letters differ significantly ($P < 0.05$).

Haematological parameters

All the studied haematological indices were not ($P > 0.05$) affected by diets, except for RBC which was lower ($P < 0.05$) in goats fed concentrate at 1.5 and 2.5% BW compared to those fed concentrate *ad libitum.*

Serum protein and enzyme

Serum enzymes, globulin and albumin/globulin (A/G) were not affected ($P > 0.05$) by level of concentrate allowance but serum total protein and albumin were greater ($P < 0.05$) for *ad libitum* concentrate allowance than for 1.5% BW concentrate level.

Table 3: Haematological indices of goats fed varying concentrate level

	Concentrate level (% body weight)			
	1.5	2.5	*Ad libitum*	SEM
Haematocrit (%)	27.67	28.0	30.0	0.97
Haemoglobin (g/dL)	90.0	86.67	90.0	7.20
Red blood cell (10^{12}/L)	11.66[b]	11.72[b]	13.29[a]	0.33
White blood cell (10^9/L)	12.1	12.8	11.	0.70
MCH (fmol)	7.70	7.37	6.73	0.62
MCHC (%)	29.98	30.93	32.46	1.76
Mean corpuscular volume (fL)	23.76	23.90	22.60	1.17
Neutrophils (%)	42.17	36.17	39.33	4.63
Lymphocytes (%)	59.50	60.50	58.53	1.77
Neutrophils/lymphocytes	0.71	0.60	0.67	0.11
Eosinophils (%)	3.60	3.50	3.0	0.36
Basophils (%)	1.33	1.32	1.67	0.46
Monocyte (%)	2.60	1.90	1.70	0.31

Means in the same row with different superscript letters differ significantly ($P < 0.05$).
MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

Serum metabolite and electrolyte

Whereas serum glucose and urea nitrogen increased ($P < 0.05$) with increasing level of concentration supplementation, serum electrolytes (Ca^{2+}, PO_4^{3-}, Na^+, K^+ and Mg^{2+}) were similar ($P > 0.05$) among the diets.

Table 4: Serum proteins and enzymes of goats fed varying concentrate level

	Concentrate level (% body weight)			SEM
	1.5	2.5	*Ad libitum*	
Total protein (g/L)	6.95[b]	7.11[ab]	7.25[a]	0.17
Albumin (g/L)	3.20[b]	3.40[ab]	3.40[a]	0.09
Globulin (g/L)	3.75	3.71	3.85	0.15
Albumin/globulin	0.85	0.92	0.88	0.17
Alkaline phosphatase (U/L)	14.0	12.7	12.2	1.07
Alanine aminotransferase (U/L)	12.0	13.2	14.3	1.13
Aspartate aminotransferase (U/L)	92.0	94.0	97.5	17.4

Means in the same row with different superscript letters differ significantly ($P < 0.05$).

Table 5: Serum metabolites and electrolytes of goats fed varying concentrate level

	Concentrate level (% body weight)			SEM
	1.5	2.5	*Ad libitum*	
Creatinine (mmol/L)	1.67	1.71	1.87	0.12
Cholesterol (mmol/L)	5.77	5.53	5.33	0.45
Urea N (mmol/L)	5.67[c]	6.71[b]	7.57[a]	0.32
Glucose (mmol/L)	56.0[c]	63.2[b]	70.5[a]	2.60
Calcium (mmol/L)	1.68	170	2.10	0.25
Phosphorous (mmol/L)	4.77	5.47	5.50	0.34
Potassium (mmol/L)	4.93	5.03	5.40	0.30
Magnesium (mmol/L)	2.10	2.25	2.08	0.19
Sodium (mmol/L)	137	140	142	2.40

Means in the same row with different superscript letters differ significantly ($P < 0.05$).

DISCUSSION

The chemical composition of *P. thonningii* foliage compares favourably with the previously reported composition but the CT concentration is much higher than the previous values (Olafadehan *et al*., 2015, Tona, 2011, Ighodaro *et al*., 2012). The CT content is slightly above the moderate level of 2 - 4% at which tannins are beneficial (Min *et al*., 2003) but below the concentration of >5% at which tannin toxicity is induced and animals adversely affected (Frutos *et al*., 2004).

The increasing DM and CP intakes with an increasing level of concentrate allowance is obviously due to the increasing availability of diets. Feed intake is a function of feed availability, ceteris paribus. Higher DM and CP intakes have been reported in sheep fed high concentrate level compared to those fed low level of concentrate supplementation (Yirga *et al*., 2011). However, Tripathi *et al*. (2006) reported higher DM intake for lambs fed concentrate at 2.5% BW compared to those fed concentrate at 1.5% BW and *ad libitum*. The linearly increasing CP intake with increasing level of concentrate supplementation is in tandem with the reports of Tripathi *et al*. (2006). Intake of CT reflects the pattern of the tanniniferous foliage intake, in agreement with earlier reports (Olafadehan *et al*., 2014a, b) where nutrient intake was stated to be a function of the feed intake and concentration of the nutrient in the feed.

Madubuike and Ekenyem (2006) indicate that haematological characteristics of livestock suggest their physiological disposition to plane of nutrition. All the haematological and serum biochemical parameters were within the normal physiological ranges for goats (Sirois, 1995; Daramola *et al*., 2005; Merck, 2012). Since blood metabolites provide an immediate indication of an animal's nutritional status, lack of diet effect on virtually all the haematological metabolites except RBC indicates the good nutritional status of the goats. Therefore, the lowest feed and CP intakes and highest CT consumption of goats fed supplemental concentrate at the low of 1.5% BW did not impair the nutritional and health status of the goats. The results demonstrate that goats can be fed tannin-containing basal fodder and supplemented with concentrate at a low level of 1.5% BW without jeopardizing their nutritional and health status or producing intoxication. The normal and non-significant haematocrit and haemoglobin values indicate absence of anaemia, in agreement with earlier reports (Olafadehan, 2011; Olafadehan *et al*., 2014a). Higher RBC value of *ad libitum* concentrate feeding shows that the diet supported improved erythropoiesis and oxygen carrying capacity of the blood relative to the other concentrate levels (Olafadehan, 2011; Olafadehan *et al*., 2014a; Okunade *et al*., 2016). This is possibly due to the superior plane of nutrition of goats on this diet. However, the normal values for all the goats ruled out the possibility of haemolytic anaemia and depression of erythropoiesis (Olafadehan, 2011; Olafadehan *et al*., 2014a). The normal values of the erythrocytic indices (MCH, MCHC and MCV) further confirm that all the animals were not anaemic. Similarly, the normal values and similar WBC and its differential counts among the diets connote that the immune system of the goats were not jeopardized, and also confirm the absence of intoxication or ill health due to tannic acid toxicosis. The neutrophil to lymphocyte ratio, a biomarker of stress and inflammation (Gross and Siegel, 1983), was similar among the diets indicating absence of stress and inflammation even in the goats fed the low concentrate level and consuming highest amount of CT. Reduction in neutrophils/lymphocyte ratio in lambs fed tanniniferous sericea lespedeza has been associated with the CT and its anti-inflammatory nature (Mota *et al*., 1985; Archaya *et al*., 2015).

In the current study, the metabolic response of the animals was a major concern and it was observed that in all of the measured metabolic parameters there was no significant difference among the diets, except for serum proteins, urea N and glucose. This is a promising result as it indicates that even at the lowest level of concentrate supplementation, feed and CP intakes, and highest consumption of CT, metabolic discomfort or health challenge could plausibly be excluded. Higher total serum protein of the *ad libitum* concentrate group is due to higher protein intake and thus availability for utilization. Rivero and Salem (2015) attributed lower serum total protein to lower protein intake. Blood albumin is an indicator of protein status in the body in that serum albumin is reduced with loss of protein (Sykes and Field, 1972). Albumin level, a good indicator of nutritional status (Hoaglund *et al*., 1992; Hoffman *et al*., 2001), is one of the plasma proteins known as acute phase proteins (APP), whose levels change whenever the animal is under external or internal challenges like trauma, inflammation and stress (Murata *et al*., 2004). Depending on whether the level increases or decreases, the various APP are termed as 'positive' or 'negative'. In ruminants, APP are used as biomarkers of disease conditions (Ceciliani *et al*., 2012), and albumin is considered as a negative APP because it decreases in cases of health disorders (Ceciliani *et al*., 2012). In this study, it was found that the diets had some effects on the serum protein fractions with *ad libitum* concentrate-fed animals having higher albumin levels and indicating better nutritional status. Similar albumin/globulin (A/G) ratio indicates proper protein utilisation, and absence of liver dysfunction, mild infection and nutritional stress because low A/G ratio is suggestive of low protein utilisation efficiency and liver dysfunction; and high A/G ratio indicates mild infection and nutritional stress (Farver, 1997; El-Sherif and Assad, 2001; Ndlovu *et al*., 2009). El-Sherif and Assad (2001) reported that ewes in lactation have higher A/G ratio than dry ones due to higher nutritional stress. Normal values for serum enzymes, which are liver enzymes, indicate absence of hepatic injury or malfunctions and heat stress (Olafadehan *et al*., 2014a; Acharya *et al*., 2015; Rivero and Salem, 2015). Reduced neutrophil to lymphocyte ratio and high AST activity have been implicated for an alleviation of potential of heat stress (Acharya *et al*., 2015).

Blood urea nitrogen (BUN) is a good indicator of protein utilization in the ruminants. Urea concentration in plasma may increase due to overfeeding, resulting in higher protein intake level and subsequent reflection in the level of urea excretion, or due to underfeeding, when animal mobilises the body reserves and tends to recycle the nitrogen and minimises its excretion in urine (Nozière *et al*., 2000). In this trial, BUN increased when the goats were fed *ad libitum* concentrate allowance probably due to high protein availability and intake. It appears that the diet supplied more than enough protein resulting in an excessive production of ruminal NH_3-N more than the amount that could be incorporated into microbial protein synthesis. Generally, BUN tends to decrease in ruminants fed diets with low protein content or with severe liver disease (Mahgoub *et al*., 2008). It appears that protein intake is positively correlated with BUN and serum proteins levels.

Serum creatinine can be affected by diet. Its concentration in serum provides a measure of glomerular filtration rate in ruminants. This parameter is an indicator of renal failure; small increases in creatinine may be seen with progressively compromised renal function (Turner *et al*., 2005; Smith, 2007). The normal creatinine values therefore implies that there were no impaired glomerular filtration and renal failure. Increasing protein intake had no effect on plasma creatinine concentration but it can be positively related to the turnover of the protein pool in ruminants (Turner *et al*., 2005). The normal and similar values of cholesterol among the diets indicates that the goats were neither hyper nor hypocholesterolaemic. Higher but normal glucose level of goats fed *ad libitum* concentrate may be due to higher feed intake and invariably energy intake of the goats, in consonance with earlier findings (Olafadehan *et al*., 2014a). However, the normal range indicates that the depressed and increased serum glucose of goats fed concentrate at 1.5% BW and *ad libitum* respectively was not due to hypoglyceamia or hyperglycaemia respectively. Since all serum electrolytes were not affected by diets, the normal and similar serum electrolytes of the goats show that the diets did not have deleterious effects on these serum indices and can thus be fed without impairing the availability and absorption of the serum electrolytes. The results also suggest absence of serum electrolytes imbalance, and normal metabolic activities and cells functions.

CONCLUSION

The normal values of the studied haematochemical indices indicate feeding supplementary concentrate at a level as low as 1.5% BW of the goats when tanniniferous *Piliostigma thonningii* foliage was used as a basal diet did not negatively impact the nutritional and health status of the animals under the present experimental conditions. However, higher feed and CP intake, red blood cell, serum proteins and glucose of goats supplemented with *ad libitum* concentrate allowance demonstrate the superior nutritive value and ability of the diet to promote better nutritional and health status.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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