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Haematological and biochemical parameters of Kano brown bucks fed graded levels of potash treated neem (*Azadirachta indica*) leaf meal-based diets

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Abstract

The haematological and serum profile of Kano brown bucks fed neem (Azadirachta indica) leaf meal was investigated for 63 days. Twelve growing Kano Brown bucks were randomly allotted to four dietary treatments with three animals per treatments in a completely randomized block design. Treatment A was the control diet without neem leaf meal while treatments B, C and D had 10%, 20% and 30% potash-treated neem leaf inclusion levels respectively. Blood samples were collected at the end of the experiment for analysis of haematology and biochemical parameters. The mean value of packed cell volume, haemoglobin, red blood cell counts and white blood cell counts were significantly ($p>0.05$) different across all treatments. The mean and standard deviation were 29.00, 90.10; 10.06, 0.39; 9.50, 0.55; 10.5, 1.11; 30.20, 0.08; 10.41, 0.85 and 33.02, 0.45 for PCV, Hg, RBC, WBC, MCV, MCH and MCHC respectively. Mean corpuscular volume was significantly ($p<0.05$) higher for animals fed 10% inclusion of the tested material ranging from (25.50 - 36.00 fl). Eosinophils, monocytes and basophils were not significantly different across all treatments while, lymphocytes and neutrophils were statistically significant ($p<0.05$) across all treatments with mean values of 43.29% and 56.75% respectively. The serum sodium ranged from (145.00 - 150.00 mmol/L), potassium ranged from (4.975 - 5.150 mmol/L) across all treatments. The creatinine value ranged from (85.50 - 103.5 mmol/L) total protein, albumin and globulin showed significant ($p<0.05$) difference across all treatments. The standard deviation was 0.003, 0.01, 0.02 and 0.18 respectively. Aspartate Aminotransferase, Alanine Aminotransferase and Alkaline Phosphatase were significantly ($p<0.05$) different across all treatments. The study revealed that potash-treated neem leaf meal could be used as feed for small ruminants, without any deleterious effect on performance and blood profile. It is concluded that dietary inclusion of potash treated neem leaf meal at 10 and 20% improved haemoglobin, packed cell volume and white blood cell counts.

Keywords: Kano brown buck, Neem leaf meal, haematology and feed supplement.

Introduction

Ruminant animal production in Nigeria being popular among rural farmers is widely distributed in the urban and peri-urban areas representing about 63.7% of the total grazing domestic animals in Nigeria (Lakpini *et al*., 2000). Ruminants account for major supply of national meat supply with the average Nigerian consuming only 3.245 g of animal protein per day, as against the 34 g recommended (Shuaib *et al* 1999). A major obstacle to ruminant livestock production in terms of meeting the demand and supply of meat in most tropical ecosystems is the seasonal fluctuation in forage availability and quality due to the modal rainfall patterns. During the dry season, forage yields and quality reportedly decline drastically for both cultivated pastures (Olanite *et al*., 2004) and natural pastures (Adjorlolo *et al*., 2014).

The increase in human population further worsens the situation because of the pressure placed on the available land for grazing by other agricultural and non - agricultural activities. During this period,

the available forages are dry, protein content very low with marked decrease in voluntary intake and digestibility by the animals (Oyenuga, 1986). The nutritional constraints of ruminants have been increased by competition between Man and the animals for the scarce grains and the protein concentrates feed making it difficult to meet up with nutritional requirements of the animals at affordable cost. The commonest protein supplement for livestock feed in Nigeria in periods of low yield and availability of poor quality herbage are groundnut cake (GNC) and cotton seed cake (CSC).

The prices of GNC and CSC products have been rising thereby increasing the cost of production. Ruminant nutritionists have therefore considered the use of alternative sources of feed ingredients in order to reduce the cost of production.

One of the promising feed resource is the neem (*Azardirachta indica)* leaves though, there is a paucity of information on the effects of anti-nutritional factors in neem leaves on ruminants, several studies (Lu, 1988; Bais *et al*., 2002a; Dhaliwal *et al* 2004) have been carried out showing the potential of the leaves as feed ingredients.

Forage biomass yields have been shown to decline drastically in the dry season. Other studies have indicated sharp changes in forage quality during the dry season. For instance, for forage legumes, crude protein content as low as 5-7% has been reported during the dry season (Peters, 1997). Similarly, Fujihara *et al.* (2004) reported decreases in crude protein and increases in neutral detergent fibre of some forage legumes as the season changed from wet to dry. Without appropriate supplementation, ruminants on range tend to lose weight during the dry season and in some cases reproductive wastage occurs Fujihara *et al.* (2004).

Reproductive wastage, coupled with retarded or negative growth during the dry season greatly reduces productivity in ruminants. This makes supplementation of ruminant diets, especially during the dry season, imperative. Such supplements need to have high enough crude protein to elevate dietary crude protein intake to levels that can support moderate production during the dry season.

Use of fodder tree and shrub leaves as dry season supplements has yielded several promising results. Since crude protein is a major liming nutrient in grasses during the dry season (Peters 1992), tree leaves which are known to retain high crude protein content well into the dry season, become an important source for grazing ruminants.

Neem leaves can therefore be a potentially valuable alternative feed resource for small - holder ruminant producers. However, there is a widely held perception that neem leaves are not accepted by ruminants (Nanang *et al.* 1997) because of their bitter taste. Some reports indicate a contrary view. Leaves of the neem tree are reported to be fed to ruminants in India and other parts of Asia during the dry season (Shukla and Desai, 1988).

In Ghana, a survey in the Telensi-Nabdam District of the Upper East Region by Ansah and Nagbila (2011) reported that 18.8% of farmers interviewed used neem leaves and fruits as fodder. According to Bais *et al.* (2002b) neem leaves compared favourably with *Albizzia lebbek* leaves in dry matter intake and digestible crude protein content when fed as sole diets. The acceptance of neem leaves by ruminant livestock despite the bitter taste may be due to feed insufficiency during periods of drought. It is also possible that ruminants get accustomed to the taste over time.

The study was therefore carried out to determine the optimum level of inclusion of neem leaves for small ruminant feed as supplements and to evaluate the effect on

hematological and biochemical profile of growing Kano brown bucks feed varying levels of neem leaves.

Materials and methods

Study area

The study was carried out at the livestock teaching and research farm Department of Animal Science Faculty of Agriculture, Bayero University, Kano located in the Sudan savannah zone of north-western Nigeria, between latitude $10^0$33' and $12^0$27' north of the equator and longitude $7^0$34' and $9^0$29' east of the green which meridian. The vegetation type is composed of trees, grasses and shrubs. Common tree species of the area includes *Adansonia digitata, Balanites aegyptiaca, Faidherbia albida, Khaya senegalensis* etc. the wet season is from May to September and dry season from April to October. Annual rainfall and temperature ranges between 787 and 960mm and 21 $^{\circ}$C – 39 $^{\circ}$C, respectively (KNARDA, 2001).

Experimental animals and dietary treatments

Twelve growing Kano brown bucks with average weight of 11.5 kg and the average age of the bucks is between 12 – 18 months were used for the experiment which lasted for 12 weeks. The animals were given a prophylactic treatment prior to commencement of the experiment using oxyteracycline 1mL/10kg, multivitamin 1mL/10kg, levozon plus 5mL/10kg of body weight against internal parasites. They allowed 14 days adaptation period after which they were placed on the experimental diets.

Collection of feed materials

The dried leaves of ***Azadirachta indica*** were collected from trees around Bayero University, new campus and treated with 2% potash to detoxify the neem leaves before use. Other ingredients comprising wheat offal, cowpea husk, cotton seed cake, palm kernel cake, sesame residues, groundnut cake and salt were purchased from livestock feed Market in Kano state.

Formulation of experimental dies

Four experimental diets were formulated using 0%, 10%, 20% and 30% inclusion levels of the dried potash-treated neem (***Azadirachta indica)*** leaves (Table 1). The formulated experimental diets were fed to the 12 growing Kano brown bucks. The 0% level of inclusion served as control where animals were not offered diet containing the treated neem leaves.

Table 1: Gross composition (%) of experiment diet with graded levels of neem leaf meal fed Kano brown bucks

Feed Ingredients	Experimental Diets			
	A (0%)	**B (10%)**	**C (20%)**	**D (30%)**
Potash-treated neem leaves	0	10	20	30
Wheat offal	22	15	12.5	10
Rice bran	17	10	10	10
Cowpea husk	15	15	12.5	10
Cotton seed cake	15	15	15	12.5
Palm kernel cake	15	15	15	12.5
Sesame seed	15.5	19.5	14.5	14.5
Salt	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Calculated CP (%)	20.50	20.50	20.54	20.72

Experimental design

A randomized complete block design was used with number of animals representing replication and experimental diets serving as treatments. Three animals were allocated to each dietary treatment and group fed. The animals were balanced for weight prior to commencement of the trial to ensure that each treatment had same mean initial liveweight.

Analytical analyses of samples

The treated neem leaves and formulated diets were analyzed for chemical composition comprising dry matter, ash, ether extract (EE) and crude fibre (CF) and crude protein using the procedure outline by AOAC (2005)**.** Fibre constituents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as described by Van Soest *et al*. (1991).

Blood collection

About 10mL of blood were collected via jugular vein puncture using a syringe for haematology, serum chemistry and electrolytes analyses. Also, 4mL of the blood was drained into tubes containing Ethylene-diamine tetra-acetate (EDTA) for haematological test, another 4mL was deposited into plain tubes for serum biochemistry while the remaining 2 ml were drained in a glucose tube for glucose analysis. The blood samples were labeled appropriately and stored in iced block containers and then taken to the Aminu Kano Teaching Hospital Chemical laboratory for analyses.

Haematological study

The haematological parameters determined were the packed cell volume, haemoglobin, red blood cell count, white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations, these are called full blood count and they were automatically determined by using micro haematocrit method (Mitruka and Rawnsley, 1977). The erythrocytes, leukocytes, monocytes, eosinophils and basophils count known as WBC differentials were determined by using model F coulter electric cell counter (Mitruka and Rawnsley, 1977).

Serum biochemistry determination

The blood samples in the plain tubes were centrifuged at 3500 g for 15 minutes to separate the serum from the blood and the serum was removed from the plasma by using pipette into plain tubes for serum biochemistry. The serum biochemical parameters determined were sodium, potassium, chloride, $HCO^{-3,}$ PO^{2-}_{4} urea, creatinine, glucose, total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The sodium, potassium and chlorine concentrations were determined by calorimetric method (Accurex biochemical kits). The blood urea was determined using randox kit by urease-berthelot calorimetric method. The creatinine was determined by spectrophotometric determination of alkaline creatinine picrate reaction method (Taussky, 1954). Cholesterol was determined by spectrophotomeetric method using Liebermann Buchard reagent (Abel *et al*., 1958).The glucose was analyzed by chromatographic method (Abel *et al*., 1958).

The total protein was determined by spectrophotometric determination of coloured complex with cu^{2+} in alkaline solution method (Henry *et al*., 1959). Albumin and globulin were determined using modified bromocresol green colorimetric method (Tietz, 1994). The alanine transaminase (ALT), aspartate transaminases (AST) and alkaline phosphatase (ALP) were determined by spectophotometric linked reaction method (Henry *et al*., 1960).

Statistical analysis

Data collected were subjected to analysis of variance using the SAS (2000) statistical package. Means were considered significant at 5% level of significance, using the Tukey test.

Results

Table 2: Chemical composition of test materials and experimental diets containing graded level of neem leaf meal diets

Parameters			Dietary Treatments				
	Dry neem Leaves	Potash-treated neem leaves	A (0%)	B (10%)	C (20%)	D (30%)	LSD
Ash	5.15^{c}	5.58^{b}	4.43^{d}	4.40^{d}	5.63^{b}	6.15^{a}	0.39
Dry matter	93.85^{c}	95.45^{a}	95.15^{a}	94.90^{b}	93.67^{c}	92.4^{d}	0.44
Crude protein	8.65^{c}	8.12^{c}	19.16^{a}	18.99^{ab}	18.05^{b}	18.05^{b}	1.00
Crude fibre	13.75^{e}	12.28^{f}	17.46^{d}	18.29^{c}	19.67^{b}	20.98^{a}	0.72
Ether extract	5.15^{c}	5.58^{b}	4.43^{d}	4.40^{d}	5.63^{b}	6.15^{a}	0.39
Nitrogen free extract	67.31^{a}	68.45^{a}	53.70^{a}	52.54^{b}	53.25^{b}	48.67^{c}	1.81
Acid detergent fibre	40.35^{a}	26.65^{c}	27.75^{c}	26.65^{c}	31.70^{b}	21.70^{d}	2.51
Neutral detergent fibre	50.85^{b}	65.65^{a}	46.05^{c}	49.80^{b}	51.15^{d}	49.05^{b}	2.60

^{abcdef}Means in the same row bearing different superscripts differed significantly (P<0.05)

Result from Table 2 showed significant (P<0.05) differences in all variables. However, diet with 30% inclusion level had the highest mean value of ash content while 10% inclusion level diet had least mean value. Similarly, experimental diet containing 30% of the test material had the highest mean value of dry matter while 0% inclusion level had the least mean value. The results further revealed the control-based diet (0%) had highest crude protein while 20% and 30% had similar crude protein contents. Crude fibre and ether extract contents increased with increase in the test material, thus 30% inclusion level had the highest while control diet had the least. Nitrogen free extract content showed the control diet to have the highest mean value while 30% inclusion level had the lowest value. Result for neutral detergent fibre showed that 20% inclusion level had the highest while the control-based diet had the least.

Result with respect to the form of test material revealed significant (p<0.05) differences in all the variables except for the crude protein and nitrogen free extract contents where they were statistically similar.

Table 3 presents the effect of dietary treatment on the haematology of the experimental animals. There were significant (p<0.05) differences in all parameters evaluated except for eosinophil, monocyte, neutrophils, basophil, mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) which were statistically similar (p<0.05) across the treatments. The result also revealed that WBC for experimental animals fed 20% inclusion level had the highest mean value 13.00g/dL followed by those offered the control diet with 11.00g/dL, while animals fed 20% and 30% inclusion levels were statistically similar (p<0.05). The WBC was decreasing with increase in the test material and the lymphocyte count followed similar pattern with the WBC counts. The result further revealed the haemoglobin and RBC to decrease with increase in the increase test material.

Table 3: Mean haematological indices of Kano brown bucks fed graded levels of potash- treated neem leaves diets

	Treatments				
Parameters	A (0%)	B (10%)	C (20%)	D (30%)	LSD
PCV %	25.50^{b}	36.00^{a}	27.50^{b}	27.00^{b}	2.41
Hb (g/dl)	10.50^{a}	11.00^{a}	10.00^{a}	8.57^{b}	1.09
RBC ($\times 10^{6}\mu l$)	10.00^{a}	10.00^{a}	9.50^{ab}	8.50^{b}	1.39
MCH (pg)	10.50	11.00	10.55	9.57	2.55
MCHC (g/dl)	36.31	30.56	33.70	31.53	13.68
MCV (fl)	25.50^{d}	36.00^{a}	27.50^{c}	31.81^{b}	1.95
WBC (g/dl)	11.00^{ab}	13.00^{a}	9.00^{b}	9.00^{b}	3.40
WBC differentials					
Lymphocytes (%)	58.00^{b}	56.50^{a}	56.50^{a}	50.00^{c}	1.39
Neutrophils (%)	46.50^{a}	45.50^{a}	45.50^{a}	24.50^{b}	20.41
Eosinophils (%)	1.5	1.5	1.5	1.5	1.96
Monocytes (%)	0	0	0	0	0
Basophils (%)	0	0	0	0	0

^{abcd} Means in the same row bearing different superscripts differed significantly (P<0.05).
PCV: pa cked cell vol ume, Hb: haemoglobin, RBC: red blood cell, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: White blood cell and MCV, mean corpuscular volume.

Table 4: Biochemical parameters of Kano brown bucks fed graded levels of potash-treated neem leaf meal diets

Parameters	**Dietary Treatments**				
	A (0%)	B (10%)	C (20%)	D (30%)	LSD
Sodium (mmol/L)	150.0^{a}	147.0^{b}	145.0^{c}	125.0^{d}	0.25
Potasium (mmol/L)	5.100	5.15	5.03	4.98	0.98
Phosphorus (mmol/L)	9.00^{a}	8.00^{a}	3.75^{b}	3.50^{c}	1.09
Calcium (mmol/L)	9.00^{b}	9.20^{a}	8.95^{b}	8.80^{c}	0.09
Chloride (mmol/L)	110.50^{a}	109.00^{b}	105.00^{c}	100.00^{d}	0.98
HCO_3 (mmol/L)	30.00^{a}	30.00^{a}	27.50^{b}	25.50^{c}	1.38
Urea (mmol/L)	9.25^{a}	7.40^{b}	5.70^{c}	4.95^{d}	0.61
Creatinine (mmol/L)	103.50^{a}	100.00^{a}	96.50^{a}	85.50^{c}	7.01
Glucose (mmol/L)	3.85	2.85	3.30	3.75	26.90
Total Protein (mmol/L)	63.00^{b}	62.00^{b}	51.50^{b}	66.50^{a}	1.39
Albumin (mmol/L)	43.50^{a}	42.00^{b}	42.00^{b}	42.00^{b}	0.98
Globulin (mmol/L)	21.00^{b}	18.50^{c}	9.50^{d}	42.50^{a}	1.70
AST (IU/L)	58.50^{a}	52.50^{b}	50.00^{c}	43.00^{d}	1.39
ALT (IU/L)	25.50^{a}	24.50^{a}	19.50^{a}	18.00^{b}	1.70
ALP (IU/L)	60.00^{a}	41.00^{b}	35.00^{c}	33.00^{d}	0.00

Means across the same raw differently superscripted differ significantly (p<0.05)

Table 4 presents the effect of dietary treatments on the serum electrolytes of experimental animals. Result showed significant (p<0.05) differences in all variables evaluated except potassium which was statistically similar (p>0.05) across the treatments. However, the highest value was obtained in animals fed 10% inclusion level. The sodium level was negatively correlated with the test material as it decreases with increase in the inclusion level of the test material. Chloride followed similar pattern with the sodium although highest value was obtained in the control diet. Carbonate also decreased with increase in the test material. Calcium, potassium and creatinine revealed similar relationship with other parameters as an

increasing trend was observed with increase in the inclusion level of the test material. On the contrary, phosphorus level decreased significantly with increase in the test material.

Results on blood chemistry showed significant differences in all the parameters evaluated except for glucose where the means were statistically ($p>0.05$) similar across the treatments. The urea content decreased with increase in the inclusion level of the test material but animals offered the control diet had the highest mean value. Animals fed 30% inclusion level had the highest mean value for Total protein. Albumin content was similar across the treatments including those fed control diet but differed in 30% inclusion level. Globulin was statistically similar ($p<0.05$) across the treatments.

Discussion

The crude protein values (17.5% and 18.7%) obtained being lower than the value reported by Bais *et al.* (2002); Bhowmik *et al.* (2008) but similar to values obtained by Renana *et al.* (2000) who reported crude protein of 9.7% may be due to varietal difference in the neem leaves used. The value of crude fibre obtained in the present study being higher than the value reported by Bhowmik *et al.* (2008) could be due to differences in location of the studies. Similarly, the ADF and NDF for dry neem leaves and potash-treated neem leaves being higher than the value obtained by Amanullah *et al*. (2006) could be as a result of differences in experimental sites. The ash Content obtained in the present study being lower than the value reported by Esonu *et al*. (2005) and Amanullah *et al*. (2006) could be because the materials (neem seed meal) used in the former is more succulent. The increase in crude fibre content with increase in the test material is due to increase in fibre content of the neem leaves. The ether extract content following same trend could be due to the fact that the test material is succulent. Though the CP content decreased with increase in potash-treated neem leaves inclusion ranging from 18.05 – 19.16%, the CP content of the experimental diets will meet the nutritional requirement of the animals as reported by ARC (1990).

The PCV (27.00 - 36%) obtained in the present study was higher than 25.7% obtained for red Sokoto goats by Tambuwal *et al.* (2002). Though the values obtained in the present study were not in agreement with earlier reports obtained for West African dwarf goat by Opara *et al.* (2010), it is in agreement with results obtained by Daramola *et al.* (2005) for the same breed and within the normal range for goats reported by Duncan *et al.,* (1986). The Hb (11.50-8.57g/dL) values obtained in this study was similar to the values of 11.40% g/Ll reported by Tambuwal *et al.* (2002). RCB obtained in the current study (8.50 - 10) $\times 10^6$µl) is within the reference value (8-18) $\times 10^6$µl) reported by Frase and Mays (1986) but higher than the value reported by Njidda *et al.* (2013). MCHC (36.31-30.56g/dL) in the current study is higher than the report of (19.2-26.47g/dl) for Kano brown and Borno white goats by Njidda *et al.,* (2013) but within the normal range (30-36g/dl) reported by Frase and Mays (1986) and Feldman *et al.* (2002). WBC obtained in the Present study (13.00-11.00g/dL) fell within the normal range of (4.00-13g/dL) reported by Feldman *et al.,* (2002) and lower than the report of (18.3g/dL) for Kano brown but similar (13.00g/dL) to that reported for Borno white (Njidda *et al.,* 2013).

The esoinophils obtained in the current study across all the treatments is within the reference value of (1-8%) by Feldman *et al.,* (2002) and Frase and Mays (1986).

The result of serum Na (150-125mmol/L)

obtained in the current study was is as a result of the treatment induced on the test materials which decreased it with increase in the inclusion level of the treated neem leaves, though animals offered 20 and 30% inclusion levels had similar mean values which were higher than the values reported by Njidda *et al.* (2013).

The serum K 5.150-4.975mmol/L obtained was higher in all treatments compared to the value of (4.3-4.5mmol/L) for adult goat is due to differences in the categories of animals used as well as treatment of the neem leaves with potash. Values for blood urea obtained in this study (9.25-4.95mmol/L) were higher than the report of (110.5-100mmol/L) by Njidda *et al.,* (2013) due to the potash treatment of the neem leaves.

TP obtained in the current study is lower than the report of Njidda *et al.,* (2013). ALB ranges from (43.50-42.00mmpl/L) is higher than the report Njidda *et al.,* (2013) but lower than the value reported by Aruwayo *et al* (2011) for growing lamb fed graded levels of neem seed cake treated with alkali. This could be due to difference in the treatment materials used.

Conclusion

The study revealed that potash-treated neem leaf meal could be used as feed for small ruminants, without any deleterious effect on performance and blood metabolites. It is concluded that dietary inclusion of potash-treated neem leaf meal at 10 and 20% improved haemoglobin, packed cell volume and white blood cell counts. It is therefore, recommended that small ruminants be offered potash-treated neem leaf meal up to 20% as feed supplements during the dry season and for enhanced health.

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