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# Haematological and Biochemical Parameters of Uda Lambs Fed Graded Levels of Alkali -Treated Neem Kernel Cake.

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**ABSTRACT:** The study was conducted to evaluate the effect of feeding alkali- treated neem kernel cake (ATNKC) on haematological and biochemical parameters of Uda lambs. It was conducted at the Teaching and Research Farm of Usmanu Danfodiyo University, Sokoto with 20 male Uda lambs. The experimental animals were allotted (n=5) to diets A, B, C, D and E with 0%, 5%, 10%, 15% and 20% levels of inclusion of ATNKC, respectively. The experiment lasted for 84days. Blood samples were collected at the end of the experiment for analyses of haematological and biochemical parameters. Haematological and biochemical parameters of the experimental animals on control and test diets were normal. The mean values for per cell volume (PCV), haemoglobin concentration (Hb) and red blood cell (RBC) in treatments E, D, C and B were not significantly (P>0.05) different from treatment A which served as the control. However, the white blood cell value in treatment A ($11.67x10^9$l) was similar to the values in treatments E ($9.70 x10^9$l), B ($9.67 x10^9$l ) and C($9.53 x10^9$l) but significantly (P<0.05) different that of treatment D($8.90 x10^9$). The values for neutrophil, eosinophil and basophil in the control treatment were not significantly (P>0.05) different from the test treatment except lymphocytes and monocytes. For biochemical parameters, the values to total protein, albumin, globulin, SGPT, total bilirubin and conjugated bilirubin in the control and test treatment did not show any significant (P>0.05) difference except in SGOT and unconjugated bilirubin. Urea nitrogen concentration, Creatinine and potassium values did not show any significant (P>0.05) difference between the control and test treatments. It was recommended in the study that alkali treated neem kernel cake can be safely included in feed of sheep up to 20% levels for lambs.
**Keywords:** Uda lambs; Alkali- treated neem kernel cake; haematological and biochemical parameters

## INTRODUCTION

The ruminants, which feed mainly on forages and crop residues are affected by seasonality and experience seasonal weight fluctuation between the wet and dry periods of the year (Dayo *et al.*, 2009). Seasonal availability of production inputs such as feed, water and quality pasture constitutes constraint to livestock production (PCOL, 2003). According to Adegbola (1982), the scarcity of energy and protein feedstuffs during dry season is a major setback to ruminant livestock production in the tropics. During this period, the available forages are dry, protein content of which is very low and there is marked decrease in voluntary intake and digestibility by the animal (Oyenuga, 1968; Steinbach, 1997).

The commonest protein supplements 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) (Maigandi, 2001). The prices of GNC and CSC have been rising, thereby increasing the cost of production (Maigandi, 2001). Researchers therefore considered the use of alternative source of feed ingredients in order to reduce the cost of production. One promising material considered is the neem kernel cake. The neem kernel cake is a by- product of neem tree (*Azadirachta indica*). The neem tree is planted widely in semi-arid parts of Nigeria as shelterbelts and windbreaks to reduce soil erosion and desert encroachment. Neem tree is also grown on marginal lands where it does not compete with food crops (Sokumbi and Egbunike, 2000).

Neem Seed Cake (NSC) is a non-conventional feed ingredient that shows great potential for livestock feeding (Nath *et al.*, 1974; Bawa *et al.*, 2005). It has been noted as a rich protein source with 34-38% crude protein level (Bawa *et al.*, 2007). However, feeding neem seed cake in its

raw form to livestock is generally discouraged due to the presence of bitter triterpenoids (Musalia *et al.*, 2000) which make it unpalatable. The main objective of the study is to determine the haematological characteristics and biochemical parameters of Uda sheep fed ATNKC diet.

## MATERIALS AND METHODS

The study was conducted at the Livestock Teaching and Research Farm of the Usmanu Danfodiyo University, Sokoto. The farm is located within the main campus of the University at about 10 km North of Sokoto metropolis in Wammako Local Government of Sokoto State. The study was conducted in July and August of 2009.

Twenty (20) entire male Uda lambs were purchased from village markets in Sokoto state for the experiment. The animals were balanced for weight with 18.50kg in treatments A, C, D and E; and 18.45kg in treatment B before the commencement of the experiment. The lambs were below one year of age because the milk teeth (incisors) have not been replaced by permanent ones. The lambs were quarantined for two weeks. The lambs were dewormed with Banminth IIR dewormer (12.5g/kg body weight), sprayed against ectoparasites using triatic and treated with oxytetracycline (a broad-spectrum antibiotic) administered by intramuscular injection. The animals were managed intensively and group-fed with cowpea and wheat offal before the commencement of the experiment.

The ingredients used for preparing the experimental feeds were alkali treated neem kernel cake, cotton seed cake, rice milling waste, cowpea husk, wheat offal, cowpea haulms, salt and bone meal.

The ripe neem fruits were dried by spreading them in the sun for fifteen days. The dried ripe neem fruits were dried, soaked in water for three days and then depulped. The depulped seeds were washed and sun dried for a period of ten days. The dry seeds were decorticated, further dried for five days, crushed and the oil removed manually to produce the neem kernel cake. The neem kernel cake was treated with Sodium Hydroxide (NaOH) by soaking the cake in water (w/v 1:1.5) in which 20g NaOH/kg cake wt/wt was dissolved for 24 hours. This was followed by sun drying and grinding.

Five complete experimental diets were formulated. Treatment A which was the control diet was without neem kernel cake. Treatments B, C, D and E consisted of 5, 10, 15 and 20% inclusion levels of alkali treated neem kernel cake, respectively. The gross compositions of the experimental diets are shown in Table 1.

**Table 1:** Gross Composition (%) of the experimental diets

| Ingredients | Diet (%) | | | | |
|---|---|---|---|---|---|
| | A (Control) | B | C | D | E |
| ATNKC | 0 | 5 | 10 | 15 | 20 |
| Maize | 13 | 15 | 13 | 13 | 12 |
| Cowpea Haulms | 12 | 10 | 10 | 10 | 7 |
| Cotton Seed Cake | 30 | 25 | 20 | 12 | 6 |
| Wheat Offal | 25 | 19 | 20 | 18 | 19 |
| Cowpea Husk | 13 | 15 | 15 | 20 | 19 |
| Rice Milling Waste | 5 | 9 | 10 | 10 | 15 |
| Bone Meal | 1 | 1 | 1 | 1 | 1 |
| Salt | 1 | 1 | 1 | 1 | 1 |
| Total | 100 | 100 | 100 | 100 | 100 |

Diet A: 0% level of ATNKC inclusion; Diet B: 5% level of ATNKC inclusion; Diet C: 10% level of ATNKC inlusion; Diet D: 15% level of inclusion of ATNKC; Diet E: 20% level of inclusion of ATNKC.

Experimental animals were housed individually in a pen measuring 2m×1m. A Completely Randomized Design (CRD) was used in the experiment. The lambs were divided into five treatment groups of four animals per group.

They were balanced for body weight for the treatment groups and fed *ad libitum* with experimental diets in the morning and evening for 84 days.

The feeding pens were cleaned and disinfected a week before the commencement of the experiment. Each pen was provided with feed and water troughs big enough to allow for sufficient feeding and drinking without waste. The feed and water troughs were cleaned every morning before feeding. Water was provided *ad libitum*.The animals were weighed weekly between 8.00am and 9.00am after overnight fasting throughout the period of the experiment. Daily records of feed intake were kept throughout the 12 weeks of feeding. Feed offered and leftover were weighed in the morning of the following day.

Blood samples were collected from three randomly selected animals from each of the groups at the 84$^{th}$ day of the experiment. The blood samples were collected from the jugular vein (Coles, 1986). Bleeding was done early in the morning before feeding and an average of 10ml of blood was collected from each animal. About 3ml of the sample was placed in EDTA (anti coagulant) bottle for hematological analysis. The remaining 7ml was placed in a universal bottle and allowed to stand for about two hours at room temperature. The universal bottle was thereafter centrifuged at 700×g for 15 minutes. The serum was separated, decanted and stored in a deep freezer for analysis of blood biochemical parameters test.

Thoroughly- mixed representative samples of the five experimental diets, and faecal samples were analysed for proximate composition as outlined by AOAC (2000).Whole blood samples in EDTA bottles were analyzed for haemoglobin (Hb) content using cyanomethemoglobin method (Coles, 1986). Packed cell volume (PCV), erythrocyte and leucocytes counts were also determined according to the methods described by Coles (1986).

The blood urea concentration was estimated by Nessler's reaction (Tannins and Maylor, 1968). Total proteins were estimated by the Biuret method as described by Henry and Stobel (1957). Albumin was determined by BromoCresol Green Method (Grant, 1987) while globulin was determined by differences between total protein and albumin.

The bilirubin was determined by Colometric method based on the method described by Jendrassik and Grof (1938). Creatinine was determined by Jaffe reaction (Sarre and Nierenkrankheifen, 1959). GOT and GPT were determined by Kinetic technique (Giorgio and Giorgio, 1982) while Sodium and Potassium were determined by Flame Photometric technique (Cole, 1986).

The data generated were subjected to analysis of variance (ANOVA) using Completely Randomized Design (CRD) according to Steel and Torrie (1980). Where significant differences between the treatment means were indicated, Duncan's Multiple Range Test (DMRT) was used to separate the means (Duncan, 1955) using the Statistical Package for the Social Sciences (SPSS, version 16, 2007).

## RESULTS AND DISCUSSION

### Proximate Composition of Experimental Diets

The chemical compositions of the experimental diets used in the trial are shown in Table 2.

**Table 2:** Proximate composition (%) of experimental diets.

| Parameter | Diet | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Dry matter | 95.50 | 95.00 | 94.00 | 94.50 | 95.20 |
| Crude protein | 16.50 | 16.40 | 16.55 | 16.50 | 16.56 |
| Ether extract | 8.40 | 8.40 | 7.10 | 7.15 | 8.25 |
| Crude fibre | 20.10 | 22.0 | 19.50 | 20.00 | 19.50 |
| NFE | 41.20 | 41.90 | 44.15 | 44.00 | 43.39 |
| Ash | 13.80 | 11.30 | 12.70 | 12.35 | 12.30 |

From the table, it can be observed that the dry matter of the experimental diets ranged between 94.0 to 95.50% while the crude protein varied from 16.40% in treatment B to 16.56% in treatment E. Ether extract ranged from 7.10% in treatment C to 8.40% in treatments A and B while the value of crude fibre varied between 19.50% in treatment C and E to 22% in treatment B. Treatment B contained the lowest ash content of 11.30% while treatment A contained the highest value of 13.80%. For Nitrogen free extract content, treatment C had the highest value of 44.15% .The crude protein level obtained in the present study was highest in treatment E with 16.56% and lowest in B with 16.40%. This falls within the crude protein requirement of 15-18% for growing lambs with weight range of 10-30kg (Church, 1978; ARC, 1990). The crude fibre level in this study ranged from 19.50% to 22%. This is adequate for the requirement of growing sheep as reported by Ganovsk and Ivanov (1982) when they estimated the crude fibre requirement of ruminants to be 22% to 25%. Jana (1997) reported that alkali treatment improves the nutritive value of NSC.

**Haematological Characteristics of Growing Lamb Fed Varying Levels of ATNKC**

The values of the haematological parameters of the experimental animals are shown in Table 3. The values for packed cell volume for all the treatments were not significantly different (P>0.05) from each other. Haemoglobin concentration and Red blood cell did not show any significant differences (P>0.05) among the treatments. For white blood cells, treatment A was higher but was not significantly different (P>0.05) from other treatments, except treatment D. However, treatments E, B, C and D were similar (P>0.05) in white blood cells values.

Values of the packed cell volume (PCV) and haemoglobin concentration did not differ between the control diet and test diets significantly (P>0.05). The values for all the treatments were within range of PCV (24-45%) and Hb (8-16g/dl) of growing sheep reported by Coles (1986). The result is also comparable to the reported range of 38-45% PCV by Swenson (1990) and; Dacie and Lewis (1991). The values obtained for PCV and Hb show that the experimental diets were adequate for the nutritional requirements, and the test diet did not portend any danger to the animals. The RBC for this study did not show any significant differences between the control and test diets. The WBC obtained for the control diet was comparable with the test diets, though it was slightly higher than the other treatments. The values of RBC was comparable to the reported range of $11x10^{12}$/l by Frandson (1981) for sheep, $7.38\text{-}13.62x10^{12}$/l for West African goat by Aina and Akinsoyinu (1996) and 12.0x10/l by Heath and Olusanya (1988) for sheep. It also falls within the range reported by Maigandi (2001) and Aruwayo *et al.* (2007). The RBC and WBC counts obtained in the study indicated that Sodium hydroxide treated neem kernel cake in animal feed can be tolerated to 20% inclusion level.

**Table 3:** Means for Haematological Characteristics of Growing Lamb fed the Experimental Diets

| Parameter | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | ±SE |
| Packed cell volume (%) | 34.17 | 31.83 | 32.17 | 32.33 | 32.40 | 1.01 |
| Haemoglobin concentration (g/dl) | 9.37 | 9.73 | 10.43 | 10.50 | 9.53 | 0.51 |
| Red blood concentration ($10^{12}$l) | 8.83 | 8.40 | 8.73 | 8.90 | 8.82 | 0.10 |
| White blood cell ($10^{9}$l) | $11.67^{a}$ | $9.67^{ab}$ | $9.53^{ab}$ | $8.90^{b}$ | $9.70^{ab}$ | 0.68 |

Means not followed by the same superscripts are significantly different (P<0.05) along the row.

From Table 4, it could be seen that the values of lymphocytes count was higher in treatment B (66.0%) but not significantly different (P> 0.05) from treatments A (65.33%) and C (58.67%). Treatments A and C were similar (P>0.05) to treatments D (56.0%) and E (55.67%). There were no significant differences (P>0.05) between treatment means in Neutrophil, Eosinophil and Basophil counts. Monocyte count was higher in treatment D (3.0%), but similar (P>0.05) to treatments C (2.0%), A and C with 1.67% each.

The differential counts of animals in all the treatment groups were within the normal ranges. Lymphocyte count for the control and test diet correspond with the report of Coles (1986). The neutrophil, eosinophil, basophil and monocyte

were all within the normal range of 10-50% neutrophil, 40-75% lymphocytes, 1-5% monocytes, 1-8% eosinophil and 0-3% basophil reported by Coles (1986) and compare with Heath and Olusanya (1988) report of 30% neutuphils, 62% lymphocytes, 25% monocytes, 5% eosinophil and 0.5% basophil. Though basophil presence in the blood is an indication of a disease condition (Jain, 1993). The level obtained did not portend any danger since it is still within the reported range of 0-3% (Coles, 1986). Eosinophil was also reported to be an indication of parasitic infestation, their low level in the blood which fell within the reported range of 1-8% (Coles, 1986) and 5% (Heath and Olusanya, 1988) indicate lack of parasitic infection. The values of differential counts are possible indicators of health problems in an animal (Frandson, 1981). Therefore, the differential counts value obtained in this study show that the experimental animals were in good health. This indicates that neem bitterness and toxicity may be inactivated by alkali treatment as reported by Nagalakshmi *et al*. (1996). The result was also supported by the report of Reddy (1992) that processing NKC with alkali converted it to a wholesome vegetable protein supplement for growing buffalo calves.

**Means for Serum Biochemical Parameters in lambs fed the Experimental Diets**

From Table 5, total proteins of all the treatments were not significantly (P>0.05) different from each other. The same trend was observed for albumin and globulin. SGOT value in treatment D (63.53U/L) was similar to treatments A, E, and B, but significantly (P<0.05) higher than treatment C. Treatments A, E, and B were not significantly (P>0.05) different from treatment C. For SGPT, there were no significant differences (P>0.05) in the values between treatment means. Total and Conjugated bilirubin conjugate were not significantly (P>0.05) different in all the treatments. Unconjugated bilirubin in treatment D (0.036) was similar to treatments A, B and C, but significantly (P<0.05) higher than that of treatment E. Treatments A, B, C and E were similar.

**Table 4:** Means for Differential counts of WBC (%) in growing lambs fed the Expereimental Diets for 84 days

| Parameters (%) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | ±SE |
| Lymphocytes | $65.33^{ab}$ | $66.0^{a}$ | $58.67^{ab}$ | $56.0^{b}$ | $55.67^{b}$ | 2.94 |
| Neutrophil | 25.0 | 27.33 | 34.0 | 33.0 | 34.67 | 3.16 |
| Monocytes | $1.67^{ab}$ | $1.0^{b}$ | $2.0^{ab}$ | $3.0^{a}$ | $1.67^{ab}$ | 0.56 |
| Eosinophils | 6.67 | 4.0 | 5.0 | 7.67 | 5.67 | 1.18 |
| Basophil | 2.0 | 1.67 | 1.0 | 1.50 | 2.67 | 0.43 |

Means not followed by the same superscripts are significantly different (P<0.05) along the row.

**Table 5:** Liver Function Test of the Growing Lambs Fed Varying levels of ATNKC for 84days

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | ±SE |
| Total protein (g/dl) | 6.35 | 6.37 | 6.23 | 6.38 | 6.50 | 0.26 |
| Albumin (g/dl) | 2.90 | 2.77 | 3.0 | 3.17 | 3.10 | 0.18 |
| Globulin (g/dl) | 3.44 | 3.60 | 3.23 | 3.21 | 3.40 | 0.13 |
| SGOT (U/L) | $55.07^{ab}$ | $54.0^{ab}$ | $51.57^{b}$ | $63.53^{a}$ | $54.70^{ab}$ | 3.25 |
| SGPT (U/L) | 16.0 | 16.50 | 16.10 | 17.57 | 17.0 | 0.53 |
| Total bilirubin (mg/dl) | 0.095 | 0.094 | 0.084 | 0.185 | 0.082 | 0.035 |
| Conjugated biliribin (mg/dl) | 0.067 | 0.075 | 0.065 | 1.28 | 0.066 | 0.24 |
| Unconjugated bilirubin (mg/dl) | $0.028^{ab}$ | $0.019^{ab}$ | $0.019^{ab}$ | $0.036^{a}$ | $0.016^{b}$ | 0.005 |

Means not followed by the same superscripts are significantly (P<0.05) different along the row.
ATNKC: Alkali Treated Neem Kernel Cake.

The urea and creatinine levels were not significantly (P>0.05) different between the treatments. For Sodium level, treatment B (151.33mmol) was not significantly different (P>0.05) from treatments A and E, but significantly (P<0.05) higher than treatments C and D. However, treatments A and E were not significantly (P>0.05) higher from treatments C

and D. Potassium levels were not significantly (P>0.05) different in all the treatments.

Total protein, albumin and globulin did not show any significant differences and compared with the report of total protein of 5.81gm/dl, albumin of 2.96gm/dl and globulin of 2.85gm/dl by Coles (1986) and the report of Aina and Akinsoyinu (1996); and Maigandi (2001). This implied that the test diets were able to supply adequate amount of protein needed to maintain normal serum protein levels. This was in accordance with the report of Coles (1986). Ranjna (1999) reported that low albumin is associated with low protein intake.

The SGOT and SGPT values in the control and test diets were comparable. These values were within the range of 14-123u/l for SGOT and 15-44u/l for SGPT reported by Boyd (1984). These indicate that inclusion of ATNKC is not toxic to the liver. Ranjna (1999) reported that SGPT and SGOT are excellent markers of liver damage caused by exposure to toxic substances. This agrees with Gangopadhay *et al.* (1981), who reported that incorporation of NSC up to 20% did not alter GOT and GPT activities in the blood. The total, Conjugated and Unconjugated bilirubin values in the study conformed to the report of Coles (1986) and Boyd (1984). The results indicate that our experimental diets did not have any debilitating effect on the liver.

The urea nitrogen level in the control and test diets were not significantly (P>0.05) different from each other and were all within the normal range reported for sheep by Boyd (1984) and Coles (1986). The result of this study was comparable to the report of Maigandi (2001). The normal values obtained in the study portends that the test diet provided adequate protein for the animals. This agrees with Coles (1986) that low dietary protein may result in decrease urea nitrogen. This equally showed that ATNKC up to 20% level of inclusion did not interfere with the renal function. The creatinine levels obtained in the study are within the normal range reported by Boyd (1984). The urea levels in conjunction with creatinine levels indicate normal liver.

The Sodium and Potassium levels in the control and test diets were within the range of 142-160mmol/l for Sodium and 4.3-6.3mmol/l for potassium (Boyd, 1984). Our result compares with the report of Borjesson (2000) who reported values of 153mmol/l and 4.7mmol/l for Sodium and Potassium, respectively. The outcomes of all the values obtained for renal function in the study show that ATNKC inclusion up to 20% level did not interfere with the renal functions of the animals. Khan (1994) reported that inclusion of ATNKC in diet of rabbits did not alter sensitive transaminases and urea nitrogen levels.

**Table 6:** Renal Function Test of Growing Lambs Fed Varying Levels of ATNKC

| Parameter | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | ±SE |
| Urea Nitrogen concentration (mmol/l) | 4.60 | 5.23 | 5.67 | 5.28 | 5.06 | 0.48 |
| Creatinine (mg/dl) | 1.21 | 1.25 | 1.41 | 1.41 | 1.44 | 0.12 |
| Sodium (mmo/L) | $148^{ab}$ | $151.33^{a}$ | $145.0^{b}$ | $143.33^{b}$ | $147^{ab}$ | 1.69 |
| Potassium (mmo/L) | 5.03 | 4.60 | 4.80 | 5.10 | 4.93 | 0.16 |

Means not followed by the same superscripts are significantly different (P<0.05) along the row.

## CONCLUSION

The haematological and biochemical levels in the study were within recommendation. This implies that the test diets were not harmful and supplied nutrients needed by the animals; therefore, they are fit for animal consumption.

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