

Influence of Water – washed *Neem* fruits (*Azardirachta indica A. juss*) on

Haematology and Serum biochemical indices of

West African Dwarf ewes

ABSTRACT

A 4-week feeding trial was conducted to investigate the effect of supplementation of water-washed neem fruit (*Azardirachta indica A.juss*) in diets on haematological and serum biochemical indices of West African Dwarf (WAD) sheep. Three diets were formulated to contain water-washed *neem* fruit at graded levels of inclusion; 0% (control), 5% and 10%. Twelve WAD sheep between 12 – 15 months old were used. The experimental animals were divided into 3 groups of 4 sheep per group and randomly assigned to the 3 dietary treatments. Variations in values obtained for haemoglobin, Red Blood Cell (RBC), Mean Cell Volume (MCV), Mean Cell haemoglobin (MCH), White Blood Cell (WBC) and Lymphocyte of WAD sheep fed diets 1, 2 and 3 respectively. Haematological parameters measured were significantly ($p<0.05$) different while other parameters were similar among the treatment. The serum urea, serum creatinine, serum glucose and serum alkaline phosphatase for WAD sheep fed diets 1, 2 and 3 respectively were significantly ($p<0.05$) different. Other serum biochemical indices measured were similar among the treatments. The results obtained from this study showed that the inclusion of water-washed *neem* fruit at 5 % or 10% depressed the haematological parameters measured. Considering the serum biochemical parameters measured, all the values reported fell within the normal physiological range except for the serum alkaline phosphatase. The fluctuation observed in the haematological parameters and serum biochemical indices could probably be physiological responses to the anti nutritional factors present in the *neem* fruit.

Key words: Water-washed; *neem* fruit; haematology; serum biochemistry; sheep

1. INTRODUCTION

Nutrition has been an indispensable aspect of livestock production. Meeting the nutritional needs as well as the requirements of livestock, conventional and established feedstuffs have been implicitly utilized in feeding livestock. Although plethora of research findings on the excellent performance of these conventional feedstuffs fed to livestock have been extensively documented but competition between man and livestock for cereals and cereal product has made these ingredients uneconomical for continuous use as livestock feed. In order to reduce cost of feed which constitutes a significant fraction of total cost of production, focus has been on the search for cheaper and readily available feedstuffs with little or no competition with man's dietary demands [1] and [2].

Until recently, search for **feed alternatives** led to a compendium of non-conventional feed resources available for feeding livestock. Among the promising non-conventional feedstuffs is *neem* (*Azadirachta indica*).

Neem is a tropical evergreen tree, native to several countries; its seed/fruit contains a high protein content ranging between 300 to 400g/kg, and anti-nutrients (i.e. Triterpenoids, Azadirone, Nimbin, and Salanin) responsible for its pungent smell as well as its bitter taste. Interestingly, the products from the neem plant such as; leaves, fruits, seeds, kernels, oil and cake have demonstrated some health benefits in human subjects [3]. On the other hand, prolonged consumption of neem over a period of time could lead to kidney damage [4]. In spite of its anti-nutrients, possibility of neem as animal feed source and its toxicological effects have been widely reported for monogastrics [3]; [5]. The possibility of **neem fruit as an** animal feed source to elucidate normal responses from monogastrics can be attributed to reduction in level of anti-nutrients by processing such as washing.

Emphasis has been on performance responses of monogastrics (specifically rabbits and broiler chickens) to **diets supplemented with neem fruit. However, there is** little or no documentation on the implications of feeding water-washed *neem* fruit on blood indices of WAD sheep. Hence, the objective of this study is to evaluate the haematological and serum biochemical responses of WAD sheep to water-washed *neem* fruit based diets.

1. MATERIALS AND METHODS

Preparation of samples

Neem fruits were harvested from matured tree during the late rainy season period (September – October). The fruits were sundried and the sun-dried fruits were then soaked in water twice within 24 hours for about 4 times within 2 days, then, decantation at the end of every 24 hours. **Thereafter**, the fruits were sundried **and milled after 48th hour**.

2. 1 Experimental procedure

A total of twelve West African Dwarf (WAD) **ewes aged 10 months** were purchased and allowed two weeks adaptation to the experimental pens. Routine management practices outlined for sheep production [6] were carried out. Sequel to the start of experiment, ewes **were offered experimental diets for two weeks** to ensure acclimatization of the ewe to the experimental diets. At the beginning of the experiment, **ewes** were randomly allotted to three treatments with four ewes per treatment housed individually. Weighed amount of the experimental diets offered to **the ewes** comprised of guinea grass (*Panicum maximum*), the basal forage at 70% of the ration, while the supplementary feed (cassava peels, soybean meal, corn bran, water-washed neem fruits, and molasses) supplied the balance of 30% Table 1. The addition of water-washed neem fruit was done at graded levels of 0%, 5% and 10% for treatments 1, 2 and 3 respectively in replacement of corn bran in the diet. Water was made available throughout the duration (28 days) of the feeding trial.

Table 1: Gross composition of Experimental diets (g/100g)

DIETS			
Ingredient (%)	Treatment 1	Treatment 2	Treatment3
Cassava peels	60.00	60.00	60.00
Soybean meal	20.00	20.00	20.00
Corn bran	10.00	5.00	0.00
Neem fruit	0.00	5.00	10.00
Molasses	10.00	10.00	10.00
Total	100.00	100.00	100.00

2.2 Blood sample collection

Blood samples were collected from the jugular veins of the ewes at the 28th day, into a sterilized glass tube containing EDTA (Ethylene Diamine Tetra Acetic Acid) and another glass tube without anti-coagulant for haematological and serum biochemical assays respectively. Blood samples for serum assay were centrifuged and serum was decanted and freeze stored at -10⁰C until analysis.

2.3 Haematology

The packed cell volume (PCV) and haemoglobin (Hb) were determined using micro haematocrit method and cyanmethaemoglobin method respectively [7]. Erythrocyte count (RBC) and leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution [8]. Other blood corpuscular constants (MCV, MHC and MCHC) were calculated.

2.4 Serum biochemistry

Serum glucose was determined by the O-Toluidine method using acetic acids [9]. Serum urea was determined by urease method and creatinine by Folin-wu filtrate methods [10]. Serum total protein was determined by Buiuret method [11] and [12], while albumin was determined using the BCG (Bromocresol green) method [13]. The Globulin was obtained by subtracting Albumin from Total Protein. Serum Cholesterol, Triglycerides were measured using appropriate laboratory kits [14; 15]. Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphate (ALP) activities were determined using spectrophotometric methods [16 and 17].

2.5 Statistical Analysis

Data obtained were subjected to analysis of variance [18] and where significant difference occurred means were separated using Duncan Multiple range test of the same package.

Table 2: Chemical Composition of the Experimental Diets

Nutrient (%)	Treatment	Treatment	Treatment	SEM
	1	2	3	
Crude protein	10.65	10.76	10.97	7.00
Crude fibre	19.90	19.79	19.69	37.00
Ether extract	4.15	4.20	4.18	0.90
Ash	7.50	7.69	7.59	10.00
Nitrogen free extract	57.80	57.56	57.57	45.10
Neutral detergent fibre	53.83	54.05	54.17	77.00
Acid detergent fibre	33.56	33.98	33.93	60.00
Acid detergent lignin	4.26	4.77	4.68	40.00

Table 3: Haematological response of West African Dwarf ewes fed water-washed neem fruit supplemented diets.

Parameters	Treatment 1	Treatment 2	Treatment 3	SEM
PCV (%)	29.67	26.50	23.00	0.81
Hb (g/dl)	10.00 ^a	8.83 ^{ab}	7.67 ^b	0.27
RBC ($\times 10^6/\text{mm}^3$)	4.81 ^{ab}	5.84 ^a	4.08 ^b	0.22
MCV (fl)	70.75 ^a	49.95 ^c	58.39 ^b	1.05
MCHC (%)	33.33	33.33	33.33	0.003
MCH (pg)	23.58 ^a	16.65 ^c	19.46 ^b	0.32
WBC ($\times 10^9/\text{mm}^3$)	6.81 ^a	6.59 ^{ab}	6.38 ^b	0.28
NEUT (%)	49.00	43.25	45.25	1.04
LYMPH (%)	48.50 ^b	55.25 ^a	53.00 ^{ab}	0.93
MONO (%)	1.50	0.50	1.00	0.24
EOSI (%)	1.00	1.00	0.75	0.27

abc – Means along the same row with different superscripts are significantly ($p < 0.05$) different.

SEM-Standard error of mean, PCV- packed cell volume, Hb-haemoglobin, RBC-Red blood cell, MCV-Mean cell volume, MCHC- Mean cell haemoglobin concentration, MCH- Mean cell haemoglobin, WBC- White blood cell, NEUT-Neutrophils, LYMPH- Lymphocytes, MONO-Monocytes, EOSI-Eosinophils

Table 4: Serum biochemistry of West African Dwarf ewes fed water-washed neem fruit supplemented diets.

Parameters	Treatment 1	Treatment 2	Treatment 3	SEM
Total protein (g/dl)	5.34	5.88	6.09	0.21
Albumin (g/dl)	2.72	3.84	2.96	0.21
Urea (mg/dl)	15.31 ^b	15.42 ^b	19.27 ^a	0.25
Creatinine (mg/dl)	0.73 ^b	1.04 ^a	1.08 ^a	0.02
AST (μ.i/l)	7.42	7.54	6.95	0.31
ALP (μ.i/l)	30.31 ^b	61.34 ^a	61.96 ^a	1.57
ALT (μ.i/l)	14.57	13.98	14.42	0.19
Cholesterol (mg/dl)	59.51	64.16	64.81	1.21
Triglyceride (mg/dl)	48.87	42.80	47.34	1.83
Glucose (mg/dl)	70.66 ^a	58.61 ^b	57.51 ^b	1.35

abc – Means along the same row with different superscripts are significantly ($p < 0.05$) different.

SEM-Standard error of mean, AST-Aspartate amino transferase, ALT-Alanine amino transferase, ALP-alkaline phosphatase.

3. RESULTS AND DISCUSSION

3.1 Haematological indices of WAD ewes fed water – washed neem fruit

Presented in Table 3 is the haematological response of ewes fed water – washed neem fruit supplemented diets.

Table 3: Haematological response of West African Dwarf ewes fed water-washed neem fruit supplemented diets.

Parameters	Treatment 1	Treatment 2	Treatment 3	SEM
PCV (%)	29.67	26.50	23.00	0.81
Hb (g/dl)	10.00 ^a	8.83 ^{ab}	7.67 ^b	0.27
RBC (x10 ⁶ /mm ³)	4.81 ^{ab}	5.84 ^a	4.08 ^b	0.22
MCV (fl)	70.75 ^a	49.95 ^c	58.39 ^b	1.05
MCHC (%)	33.33	33.33	33.33	0.003
MCH (pg)	23.58 ^a	16.65 ^c	19.46 ^b	0.32
WBC (x10 ⁹ /mm ³)	6.81 ^a	6.59 ^{ab}	6.38 ^b	0.28
NEUT (%)	49.00	43.25	45.25	1.04
LYMPH (%)	48.50 ^b	55.25 ^a	53.00 ^{ab}	0.93
MONO (%)	1.50	0.50	1.00	0.24
EOSI (%)	1.00	1.00	0.75	0.27

abc – Means along the same row with different superscripts are significantly ($p < 0.05$) different.

SEM-Standard error of mean, PCV- packed cell volume, Hb-haemoglobin, RBC-Red blood cell, MCV-Mean cell volume, MCHC- Mean cell haemoglobin concentration, MCH- Mean cell haemoglobin, WBC- White blood cell, NEUT-Neutrophils, LYMPH- Lymphocytes, MONO-Monocytes, EOSI-Eosinophils

The values obtained in this study for PCV were 29.67%, 26.50% and 23.0% for T₁, T₂ and T₃ respectively. Hb values ranged from 10.00g/dl to 7.67d/dl for T₁ to T₃. Significant differences ($P = .05$) were obtained with increasing levels of water – washed *neem* fruit in the diet. Also, the values obtained for RBC were 4.81. The haematological values obtained in this study including control or T₂ and T₃ alone (Table 3) were outside the standard range recommended for clinically healthy ewes [7]. The mean cell haemoglobin concentration (MCHC) of animals on T₂ and T₃ were not significantly ($P = .05$) different from the control group. The 33.33% obtained across the treatments for MCHC was within the 30.0 - 34.4% reported [7] for clinically healthy ewes. The MCHC values have been reported to be the most accurate and absolute values that indicate anaemic condition in animal [19 and 20].

The MCV and MCH values observed in this study were above the normal range (28.4 – 31.6fL) and (9.20 – 9.80 pg) respectively reported for healthy ewe in temperate climate [7]. The higher mean cell volume (MCV) and the higher mean haemoglobin (MCH) values recorded in the present study when compared to [7] in the temperate climate could be attributed to climate and breed differences.

The non significant value of packed cell volume (PCV) of the ewe on water-washed *neem* fruit supplemented diets relative to the control group was an indication that the animals were not anaemic. The PCV values of the ewe on the test diets were not significantly different from the control group and this confirms the report [21] and [20] that nutrition affects blood profile of animals. The haemoglobin (Hb) and Red blood cell shows significant differences in the values obtained from the ewe across the treatments and this was not in agreement with the findings [21 and 20] **that nutrition affects the blood profiles of animals**. The values of the white blood cell (WBC) and their differential counts were within the normal range reported for healthy ewe [7] except for neutrophils. White blood cell in animal possesses phagocytic function [22 and 20] and differential WBC counts were used as an indication of stress response and sensitive biomarker crucial to immune function [23]. The results of the WBC in the present study clearly shows that the ewe on 10% water-washed *neem* fruit supplemented diets were stressed hence the significant reduction in leukocyte counts.

3.2 Serum biochemical indices

Results of serum biochemical indices of ewes fed water-washed *neem* fruit supplemented diets in this study are presented in Table 4. The serum urea, creatinine and serum glucose of animals were significantly ($P = .05$) influenced by the dietary treatment. In the study, increases were observed in serum urea and creatinine levels and corresponding decrease in the serum glucose level. This result agrees with [24] that animal will normally fall back on the stored energy in the muscles when there is reduction in blood glucose level. The urea and creatinine concentration in the blood were used as kidney function test [25 and 20]. The non significant value of serum total protein and albumin of the ewe on water-washed *neem* fruit supplemented diets can be compared with the earlier report of protein retained in animals [26 and 20] and all the values obtained for the 2 parameters were within the normal range reported [7] for a normal healthy ewe. This result contradicts the depression in plasma protein concentration that was reported [27] using Murrah Milch buffaloes on the replacement of concentrate mixture with 15 and 20 parts of *neem* seed cake and [28] in growing goats on

replacement of concentrate mixture with 15 – 25 parts of water-washed neem seed kernel cake. The serum cholesterol and triglyceride values obtained in this study were within the normal range reported for healthy ewe [7]. The values for the 2 parameters were found to be increasing with the increasing dietary levels of water-washed *neem* fruits fed to the WAD ewe. This observation probably may suggest an increase in the lipid mobilization. The serum Aspartate amino transferase (AST) and serum Alanine amino transferase (ALT) values observed in this study were above the normal range (40.0 - 123 μ .i/l) and (25.0 - 70 μ .i/l) respectively reported for healthy ewe [7]. The non significant decrease in serum AST and increase in serum ALT, activities of the animal on treatments 2 and 3 could indicate an improvement in liver function due to hepatoprotective activities of *neem* [29 and 20]. The serum alkaline phosphatase (ALP) values were significant and were not within the normal range (μ .i/l) reported [7] for clinically healthy ewe.

4. CONCLUSION

The results obtained from this study have shown that the inclusion of water-washed *neem* fruit at either 5 % or 10% depressed the haematological parameters measured. However, an inclusion level of 5% or 10% is safe and economical in diets with no visible adverse effect for ewe production. The fluctuation observed in the haematological profile and serum enzyme could be normal.

REFERENCES

1. Egbunike GN. What is Animal Science and how can Nigerian get out of malnourishment. In: Proc. 2nd Ann. Confr. Animal Sci. Assoc. Nigeria Sept. 16-17, 1997. Ikeja Lagos (1997): 1-12.
2. Egbunike GN, Ikpi AE. Can agro industrial by products and crop residues save the Nigerian Livestock Industry. In: proc. PANESE.ARNAB workshop on utilization of Research results in Forage and Agricultural by product material as animal feed Resources in Africa (Dzowela BH, Said AN, Wendem-Angenehu Asrat and Kategile J JA, editors. Lilongwe. (1988): 477 – 487.
5. National Research Council. *Neem*: A tree for solving global problems. National Academy Press, Washington DC. (1992).
6. Sokunbi OA, Egbunike GN, Salako AO, Bobadoye AO. Biochemical and hematological response of weanling female pigs fed diets containing sun cured *neem* (*Azadirachta indica* A. Juss) leaf meal. Trop. Anim. Prod. Invest. 2002(5):1-8
7. Baiden RY, Rhule SWA, Otsyina HR, Sottie ET, Amaleke G. Performance of West African Dwarf sheep and goat fed varying levels of cassava pulp as a replacement for cassava peels. Livestock Research for Rural Development 2007(19)35.
8. Mitruka BM, Rawnsley HM. Clinical, Biochemical and Hematological reference values in normal experimental animals Masson publishing New York. 1977.
9. Schalms OW, Jane NC, Carol EJ. 1975. Veterinary Haematology. 3rd ed. Lea and Febiger, Philadelphia 1975:15 – 18.
10. Cooper GR, McDaniel V. Standard method in Serum Enzyme activity. Clinical Chemistry 1970(6): 159
11. Toro G. Ackermann PG. Practical clinical chemistry. Little, Brown and Company, Boston. 1975.
12. Reinhold JG. Standard methods of clinical chemistry. M. Reiner, editor. Academic Press, New York. 1953:188.
13. Kohn RA, Allen MS. Enrichment of Proteolytic activity relative to Nitrogen in Preparation from the rumen for in vitro studies. Journal of Animal Feed Science and Technology. 1995(52):1-14.
14. Peter T, Biamonte GT, Doumas BT. Protein (total protein) in serum, urine and cerebrospinal fluid; albumin in serum. In: Selected methods of clinical chemistry, Faulkner WS, Meites S, editors. Washington, DC. American Association for clinical chemistry. 1982 (9).
15. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of the LDL Cholesterol in plasma without using Preparative ultracentrifuge. Journal of Clinical Chemistry 1972(18):499-502.
16. Gowenlock AH, McMurray JR, MacLauchlan DM. Varley Practical Clinical Biochemistry 6th edition. CAC Publishers and Distributors, New Delhi. 1988:477-549.
17. Rej R, Hodder M. Aspartate Transaminase. In: Methods of Enzymatic Analysis. 3rd Ed. (Bergmeyer HU, Bergmeyer J, Grassl M editors. Weinheim. Verlag-Chemie 1983(3): 416-433
18. McComb RB, Bowers GN, Rosen S. Alkaline Phosphatase. Plenum Press, New York. 1988.
19. S.A.S. Statistical Analytical Software Institute Inc., SAS Online Doc. New Cary. SAS Institute Inc., 1999(8).

20. Thompson RB. A short textbook of hematology. 7th ed. Garden City Press Ltd, Hertfordshire, UK. 2006: 217.
21. Ogbuewu IP, Okoli IC, Iloeje MU. Evaluation of toxicological effects of leaf meal of an ethno medicinal plant-neem on blood chemistry of pubertal Chinchilla Rabbit does. Rep. Opin., 2010(2): 29-34.
22. Talebi AS, Asri-Rezaei R, Rozeh –Chai, Sahraei R. Comparative studies on haematological values of broiler strains. International Journal of Poultry Science 2005; 4(8):573-579.
23. Campbell TW, Coles EH. Avian clinical pathology. In: Coles EH, editor. Veterinary clinical pathology. 4th ed. Saunders Co., Philadelphia, Pennsylvania, USA; 1986.
24. Graczyk S, Pliszczak-Król A, Kotonski B, Wilczek J, Chmielak Z. Examination of haematological and metabolic changes mechanisms of acute stress in turkeys. Electronic Journal of Polish Agricultural Universities 2003(6):1-10.
25. Esonu BO, Emenalom OO, Udedibie ABI, Herbert U, Ekpore CF, Okoli IC, Ihukwumere FC. Performance and blood chemistry of weaner pigs fed raw Mucuna beans (Velvet bean) meal. Tropical Animal Production Investment 2001(4): 49-54.
26. Davis ME, Burndt WD. Renal methods for toxicology. In: Hayes AW, editor. Principles and methods of toxicology, 3rd edition. New York Raven, USA; 1994:871-894.
27. Akintola SO, Abiola SS. Blood chemistry and Carcass yield of cockerel fed melon husk diets. Tropical Journal of Animal Science, 1988(2):39.
28. Gangopadhyay P. Studies on biochemical constituents of blood within neem seed cake into ration of Milch, Murrah buffaloes. Indian J. Animal Health 1981(2): 61 – 63.
29. Verma AK, Sastry VRB, Agrawal DK. Feeding water-washed *neem* (*Azadirachta indica* A. Juss) seed kernel to growing goats. Small Ruminant Research 1995(15): 105-114.
30. Chattopadhyay RR, Chattopadhyay RN, Maitra SK. Effects of *Neem* on hepatic glycogen in rats. Indian Journal of Pharmacology 2000(25): 174-175.

