

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 Buiret 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 aminotransferase (AST), Alanine amino transferase (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

RESULTS

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 (i.u/l)	7.42	7.54	6.95	0.31
ALP (i.u/l)	30.31 ^b	61.34 ^a	61.96 ^a	1.57
ALT (i.u/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.

DISCUSSION

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 i.u/l) and (25.0 - 70 i.u/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 (i.u/l) reported [7] for clinically healthy ewe.

2. 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.

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