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Influence of Water – Washed Neem Fruits (*Azardirachta indica A. juss*) on Haematology and Serum Biochemical Indices of West African Dwarf Ewes

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Authors' contributions

Author MKA designed the protocol of the study, while authors TOO and TOF coordinated the field study. Author TOO wrote the article in consultation with other authors, who read, made corrections and added their inputs. All the authors read and agreed on the final draft.

Original Research Article

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ABSTRACT

Neem is a fast growing tree that thrives well in all parts of Nigeria. The seeds are readily available because the plant is used to control desertification. 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) ewes. Three diets were formulated to contain water-washed neem fruit at graded levels of inclusion; 0% (control), 5% and 10%. Twelve 10 month old WAD ewes were randomly assigned to 3 dietary treatments. Each treatment had 4 replicates. Variations in values of haematological and serum biochemical parameters of WAD ewes fed experimental diets were determined. Some haematological parameters (RBC and Hb counts) measured were significantly ($p<0.05$) different while PCV, WBC, some differential counts and serum biochemical parameters were not significantly different among the treatments. However, urea, creatinine, glucose and alkaline phosphatase levels in serum of WAD ewes showed significant ($p<0.05$) differences, while, other serum biochemical indices measured were not significant. Although, the result of this study showed that the inclusion of water – washed neem fruit at 10% depressed some heamatological parameters, but showed no adverse effect on the ewes. However, all the values obtained for serum biochemical indices were within the normal physiological range

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except for alkaline phosphatase. Therefore, water – washed neem fruit can be included in the diet of WAD ewes at 10 % without any deleterious effect.

Keywords: Haematology; neem fruit; serum biochemistry; sheep; water – washed.

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 [1,2].

Until recently, search for feed alternatives led to a compendium of non-conventional feed resources available for feeding livestock. Neem is a promising non-conventional feedstuff. It 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. triterpeniods, 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 [4]. The possibility of neem 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 to diets supplemented with neem fruit [4]. However, there is little or no documentation on the implications of feeding water-washed neem fruit on blood indices of West African Dwarf (WAD) ewes. Hence, the objective of this study is to evaluate the haematological and serum biochemical responses of WAD ewes to water-washed neem fruit based diets.

2. MATERIALS AND METHODS

2.1 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.

2.2 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 [5] were carried out. Sequel to the start of experiment, ewes were offered experimental diets for two weeks to ensure adaptation to experimental feeds. At the beginning of the experiment, ewes were randomly allotted to three treatments with four ewes per treatment in individual pens. 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 T1, T2 and T3, respectively, in replacement of corn bran. Water was made available throughout the duration (28 days) of the feeding trial.

Table 1. Ingredients (g/100g) and chemical composition (%) of experimental diets

	DIETS			
Ingredient (%)	T1	T2	T3	
Cassava peels	60.0	60.0	60.0	
Soybean meal	20.0	20.0	20.0	
Corn bran	10.0	5.0	0.0	
Neem fruit	0.0	5.0	10.0	
Molasses	10.0	10.0	10.0	
	T1	T2	T3	SEM
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

2.3 Blood Sample Collection

Blood samples were collected from the jugular veins of the ewes at 28th day, into 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 -10^{0}C until analysis.

2.4 Haematology

The packed cell volume (PCV) and haemoglobin (Hb) were determined using micro haematocrit method and cyanmethaemoglobin method, respectively [6]. Erythrocyte count (RBC) and leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution [6]. Other blood corpuscular constants (MCV, MHC and MCHC) were calculated.

2.5 Serum Biochemistry

Serum glucose was determined by the O-Toluidine method using acetic acids [7]. Serum urea was determined by urease method and creatinine by Folin-wu filtrate methods [8]. Serum total protein was determined by Biuret method [9], while albumin was determined using the BCG (Bromocresol green) method [10]. Serum cholesterol and triglycerides were measured using appropriate laboratory kits [11, 12], aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities were determined using spectrophotometric methods [13, 14].

2.6. Statistical Analysis

Data obtained were subjected to analysis of variance [15] and where significant difference occurred means were separated using Duncan Multiple range test of the same package.

3. RESULTS AND DISCUSSION

3.1 Haematological Parameters

The values obtained in this study for packed cell volume (PCV) were 29.67%, 26.50% and 23.0% for T1, T2 and T3, respectively (Table 2). The absence of significant difference in PCV values among treatments is an indication that the animals were not anaemic. Hb values ranged from 10.00 g/dl to 7.67 g/dl, and significant differences ($p < 0.05$) were obtained with increasing levels of water–washed neem fruit in the diet. Also, the values obtained for RBC ranged from 4.08 to 5.84 $x10^6/mm^3$. The haematological values obtained in this study including for all treatments were below the standard range recommended for clinically healthy ewes [16]. The MCV (28.4 –31.6 fl) and MCH (9.20–9.80 pg) values observed in this study were above the normal range reported for healthy ewes in temperate climate [16]. The higher mean cell volume (MCV) and the higher mean haemoglobin (MCH) values recorded in the present study when compared to [16] those reported for animal of temperate climate could be attributed to climate and breed differences. The mean cell haemoglobin concentration (MCHC) of animals fed T2 and T3 were not significantly ($p > 0.05$) different from the value of control group. The 33.33% obtained across the treatments for MCHC was within the 30.0-34.4% reported [16] for clinically healthy ewes. The MCHC values have been reported to be the most accurate and absolute values that indicate anaemic condition in animals [17,18].

The PCV values of ewes of the test diets were not significantly different from the control group and this confirms the reports [18,19], but haemoglobin (Hb) and red blood cell values showed significant differences among dietary treatments. The values of the white blood cell (WBC) and their differential counts were within the normal range reported for healthy ewes [16], except for neutrophils. White blood cell in animal posses' phagocytic function [19,20] and differential WBC counts were used as an indication of stress response and sensitive biomarker crucial for immune function [21]. The results of the WBC in this study shows that the ewes fed on 10% water-washed neem fruit supplemented diets had reduced leukocyte counts, but had no visible signs of stress.

Table 2. Haematological response of West African Dwarf ewes fed water-washed neem fruit supplemented diets

Parameter	T1	T2	T3	SEM	Standard
PCV (%)	29.67	26.50	23.00	0.81	31.5- 36.7
Hb (g/dl)	10.00^{a}	8.83^{ab}	7.67^{b}	0.27	10.7 – 12.2
RBC ($x10^{6}/mm^{3}$)	4.81^{ab}	5.84^{a}	4.08^{b}	0.22	10.3 – 12.9
MCV (fl)	70.75^{a}	49.95^{c}	58.39^{b}	1.05	28.4 – 31.6
MCHC (%)	33.33	33.33	33.33	0.003	30.0 – 34.4
MCH (pg)	23.58^{a}	16.65^{c}	19.46^{b}	0.32	9.20 – 9.80
WBC ($x10^{9}/mm^{3}$)	6.81a	6.59^{ab}	6.38^{b}	0.28	4.90 – 9.70
NEUT (%)	49.00	43.25	45.25	1.04	14.5 – 41.5
LYMPH (%)	48.50^{b}	55.25^{a}	53.00^{ab}	0.93	48.9 – 75.1
MONO (%)	1.50	0.50	1.00	0.24	0.72 – 3.28
EOSI (%)	1.00	1.00	0.75	0.27	3.60 – 12.4

abc – Mean values in 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

3.2 Serum Biochemical Indices

Results of serum biochemical indices of ewes fed water-washed neem fruit supplemented diets are presented in Table 3. The urea, creatinine and glucose serum levels of animals were significantly ($p < 0.05$) influenced by the dietary treatment. In the study, an increase in urea and creatinine levels, and a decrease in the serum glucose level were observed. This result agrees with others authors, who reported that animals will normally fall back on the stored energy in the muscles when there is reduction in blood glucose level [22]. The urea and creatinine concentration in the blood were used for kidney function [18,23]. The non significant value of serum total protein and albumin of the ewes on water-washed neem fruit supplemented diets can be compared with the earlier report of protein retained in animals [18,24] and all the values obtained for the two parameters were within the normal range reported for normal healthy ewes [16]. This result contradicts the depression in plasma protein concentration that was reported [25] using Murrah Milch buffaloes on the replacement of concentrate mixture with 15 and 20 parts of neem seed cake and [26] in growing goats on replacement of concentrate mixture with 15–25 parts of water-washed neem seed kernel cake. The serum cholesterol and triglycerides values obtained in this study were within the normal range reported for healthy ewes [16]. The values for both parameters increased with increasing levels of water-washed neem fruit in the diet. This observation probably may suggest an increase in the lipid mobilization. The serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT) activity values observed in this study were above the normal range (40.0-123 IU/l) and (25.0– 70 IU/l) respectively reported for healthy ewes [16]. The non significant decrease in AST activity and the increase in ALT activity of ewes fed 10% water – washed neem fruit could be adduced to improvement in liver function due to a potential hepato - protective activity of neem fruit [18,27]. The serum alkaline phosphatase (ALP) activity did not show significant difference among dietary treatments, and values were not within the normal range (IU/l) reported for clinically healthy ewes [16].

Table 3. Serum biochemistry of West African Dwarf ewes fed water-washed neem fruit supplemented diets

Parameter	T1	T2	T3	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}	3.84
Creatinine (mg/dl)	0.73^{b}	1.04^{a}	1.08^{a}	0.02
AST (IU/l)	7.42	7.54	6.95	0.31
ALP (IU/l)	30.31^{b}	61.34^{a}	61.96^{a}	1.57
ALT (IU/l)	14.57	13.98	14.42	0.19
Cholesterol (mg/dl)	59.51	64.16	64.81	1.21
Triglycerides (mg/dl)	48.87	42.8	47.34	1.83
Glucose (mg/dl)	70.66^{a}	58.61^{b}	57.51^{b}	1.35

abc – Mean values in 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.

4. CONCLUSION

The results obtained from this study have shown that the inclusion of 10% water-washed neem fruit had significant effect on some haematological parameters (RBC and Hb count) while PCV, WBC and some differential counts were not affected. Similarly, some serum biochemical indices (urea, creatinine and glucose) were significantly affected and cholesterol, triglycerides, aspartate amino transferase (AST) and alanine amino transferase (ALT) were within the normal range reported. Therefore, inclusion of 10% water – washed neem fruit in the diet of WAD ewes will not produce physiological stress in the animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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