

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

Haematology and Serum biochemical indices of

-West African Dwarf ewes

ABSTRACT (this section must be rewritten to summarize the results obtained. In the present status, it is not clear if neem fruit can be used in the feeding of WAD ewes without affect seriously the haematological and biochemical parameters.)

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 (T1, T2 and T3) were formulated to contain water-washed neem fruit at graded levels of inclusion; 0% (control), 5% and 10%. Twelve WAD sheep between 102—15 months old were used. The experimental animals were divided into 3 groups of 4 sheep, per group and randomly assigned to different the 3 dietary treatments. Variations in values of obtained for haemohematological and serum biochemical parameters globin, Red Blood Cell (RBC), Mean Cell Volume (MCV), Mean Cell haemoglobin (MCH), White Blood Cell (WBC) and Lymphocyte of WAD sheep fed experimental diets were determined 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, cserum creatinine, serum glucose and serum alkaline phosphatase levels in serum offer WAD sheep fed showed diets 1, 2 and 3 respectively were significantly ($p < 0.05$) differences. 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 phoshatase. 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 (sort by alphabetical order)

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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 pointed 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 West African Dwarf (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 two 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 to experimental feeds. 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

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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** ~~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: Ingredients Gross (g/100g) and chemical composition (%) of eExperimental diets (g/100g)

DIETS			
Ingredient (%)	Treatment 1	Treatment 2	
Treatment 3			
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
Parameter (%; specify dry or wet weigh ???)			
	T1	T2	T3
SEM			
Crude protein	10.65	10.76	10.97
Crude fibre	19.90	19.79	19.69
Ether extract	4.15	4.20	4.18
Ash	7.50	7.69	7.59
Nitrogen free extract	57.80	57.56	57.57
Neutral detergent fibre	53.83	54.05	54.17
Acid detergent fibre	33.56	33.98	33.93
Acid detergent lignin	4.26	4.77	4.68

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2.2 Blood sample collection

Blood samples were collected from the jugular veins of the ewes at the day 28th day, into a sterilized glass tube containing EDTA (cEthylene-dDiamine-tTetra-aAcetic aAcid) 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 Biuret method [11] and [12], while albumin was determined using the BCG (Bromocresol green) method [13]. The gGlobulin was obtained by subtracting aAlbumin from tTotal pProtein. Serum cCholesterol, tTriglycerides were measured using appropriate laboratory kits [14; 15]. aspartate aminotransferase (AST), aAlanine amino transferase (ALT) and aAlkaline 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	
	1	2	3	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

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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 (x10 ⁶ /mm ³)	4.81 ^{ab}	5.84 ^a	4.08 ^b	0.22
MCV (fl)	70.75 ^a	49.95 ^e	58.39 ^b	1.05
MCHC (%)	33.33	33.33	33.33	0.003
MCH (pg)	23.58 ^a	16.65 ^e	19.46 ^b	0.32
WBC (x10 ⁹ /mm ³)	6.81 ^a	6.59 ^{ab}	6.38 ^b	0.28

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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

abe—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

abe 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.

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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 10.00g/dl to 7.67g/dl, and s for T1 to T3. Significant differences ($p \leq 0.05$) 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 MCV (28.4–31.6L) and MCH (9.20–9.80 pg) values observed in this study were above the normal range reported for healthy ewes 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] those reported for animal of temperate climate could be attributed to climate and breed differences. The mean cell haemoglobin concentration (MCHC) of animals fed on T-2 and T-3 were not significantly ($p \geq 0.05$) different from the value of control group. The 33.33% obtained across the treatments for MCHC was within the 30.0–34.4% reported [7] for clinically healthy ewes. The MCHC values have

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been reported to be the most accurate and absolute values that indicate anaemic condition in animals [19 and 20].

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 (COMMENT: this sentence seems contradictory). The haemoglobin (Hb) and red blood cell values show significant differences among dietary treatments and this was not in agreement with the findings [21 and 20] that nutrition affects the blood profiles of animals (COMMENT: this sentence seems contradictory). 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 for immune function [23]. The results of the WBC in the present study clearly shows that the ewe fed on 10% water-washed neem fruit supplemented diets were stressed hence the significant reduction in leukocyte counts.

Table 2: Haematological response of West African Dwarf ewes fed water-washed neem fruit supplemented diets. (COMMENT: include an additional column with the standard range recommended for clinically healthy ewes)

Parameter	T1	T2	T3	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 – 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

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The MCV and MCH values observed in this study were above the normal range (28.4–31.6 fL) 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 possess 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.

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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 34. The serum-urea, creatinine and serum-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 were observed, in serum urea and creatinine levels and a corresponding decrease in the serum glucose level were observed. This result agrees with others authors, who reported [24] that animals will normally fall back on the stored energy in the muscles when there is reduction in blood glucose level [24]. 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 two parameters were within the normal range reported [7] for a normal healthy ewes [7]. 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 triglycerides values obtained in this study were within the normal range reported for healthy ewes [7]. The values for both the 2 parameters were raised 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) 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 [7]. The non-significant decrease in serum AST activity joined and to the increase in serum ALT activity of the animal fed T2 and T3 treatments 2 and 3 diets could indicate an improvement in liver function due to a potential hepatoprotective activities of neem fruit [29 and 20]. The serum alkaline phosphatase (ALP) activity did not show values were significant difference among dietary treatments, and values were not within the normal range (IU/l) reported [7] for clinically healthy ewe [7].

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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	0.25
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	42.80	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.

2.4. CONCLUSION

The results obtained from this study have shown that the inclusion of water-washed neem fruit at either 5 % or 10% ~~affected~~^{depressed} the haematological and blood biochemical parameters measured in West African Dwarf ewes.

Include the following aspects in the conclusion of the manuscript

Comment: Could be considered within a normal range the fluctuation observed in the haematological profile and serum enzyme activities?

Comment: Specify if the inclusion of 5% or 10% of water washed-neem fruits is recommended in the feeding of WAD ewes or not.

Comment: Can inclusion of 5% or 10% of neem fruit produce physiological stress in animals?

However, an inclusion level of 5% or 10% is safe and economical in diets with no visible adverse effect for ewe production (omit this sentence, results of production were not provided in this study by authors).

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The fluctuation observed in the haematological profile and serum enzyme could be normal.

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