

Effects of *Balanites Aegyptiaca* (Del) Seed Cake on Haematological and Serum Biochemical Indices of Growing Rabbits

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Abstract: Feeding trial was conducted to evaluate the effect of *Balanites aegyptiaca* seed bio meal (BASM) as a substitute for groundnut cake in the diet of growing rabbits. Five experimental diets were formulated representing T1, T2, T3, T4 and T5. T1 (0% BASM) was served as the control diet, while T2, T3, T4 and T5 contained 25, 50, 75 and 100% BASM respectively. A total of 100 weaner rabbits of mixed breeds were purchased from National Animal Production Research Institute (NAPRI), Zaria, Nigeria. The rabbits were fed the control diet during the one week of adjustment period. They were given vitality as anti-stress and were dewormed using ivermectin. At the end of one week of adjustment; the rabbits were housed in different hutches and fed their respective experimental diets. Each treatment contained 20 rabbits and there were four replicates for each treatment with 5 rabbits each. Results of BASM on haematology and serum chemistry shows that *Balanites aegyptiaca* seed meal supplementation levels had no adverse effect on red blood cell counts, white blood cell counts, and packed cell volume and biochemical indices. Moreover, there was no significant difference in the parameters measured for hematological performance and biochemical characteristics of rabbits studied. Rabbits fed BASM diets performed like ($P > 0.05$) those fed with the control diet. It was concluded therefore that 100% roasted BASM contain bio nutrients that can be used in rabbits diet to replace groundnut cake without adverse effect especially in the area of study and related ecology.

Keywords: Biochemical, Biomeal, *Balanites Seed*, cake, Haematology, Serum

1. INTRODUCTION

Balanites aegyptiaca being a browse plant have been reported to improve the feeding potential of ruminant animals in the semi-arid (Njidda and Ikhimioya, 2010). *Balanites aegyptiaca* is widely grown in Nigeria. Early studies (Lars and Joker, 2000; Lockett *et al.*, 2002) showed that *Balanites* offers the most rapid and lowest means of providing adequate supplies of bioactive nutrients to the tropical people and their animals. Works on the chemical and nutritional composition of *Balanites* however, showed that *Balanites* tree contains chemical compounds namely saponins, tannins, nitrites, coumarines which could elicit deleterious effects in animals when consumed in large quantities (Hardman and Sofowora, 1972).

Balanites aegyptiaca have been reported to have anti-inflammatory and analgesic, anthelmintic, antioxidant, antidiabetic, antinoceptive, hepatoprotective, antibacterial and larvicidal activities in animals (Dubey *et al.*, 2011). *Balanites aegyptiaca* like *Acacia Senegal*, (Diallo, 1997) and *Acacianilotica* (Tybirk, 1989) shows a synchronization between male (internal stamina cycle) and female phase (Ndoye *et al.*, 2004). These authors showed that the roots, barks, fruits, pulps and seeds of *Balanites* are lethal to aquatic animals. Thus, the presence of the phytotoxins in *Balanites* may limit its intensive utilization in diets for man or livestock. Haematological parameter is an important and reliable medium used to monitor and elevate health and bio nutrition status of animals (Gupta *et al.*, 2007). Blood composition of animal might be influenced by certain factors such as nutrition, management, sex, age diseases and stress factors which might affect blood values (Schalm *et al.*,

1975). The hematological and biochemical indices are an index and reflection of the effects of dietary treatment on the animals in terms of the type and amount of bio-feed ingested and which were available for the animals to meet its physiological biochemical and metabolically necessities (Ewuola *et al.*, 2004) and also the level of anti-nutritional factors present in the feed also influence the hematological and biochemical bioavailability values (Akinmutimi, 2004). This present study is therefore carried out to determine the effects of graded levels of inclusion of *Balanites aegyptiaca* in the diets of weaner rabbits on haematological and serum biochemical in Gashua, Nigeria

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in the North eastern parts of Nigeria, specifically in the Gashua, Bade local Government area of yobe state during 2016/2017 dry session at the Department of Home Science and Management Federal University Gahua, Yobe state. The feed trial research was carried out at the Rabbitry research Unit of the Department of Animal Science, Faculty of Agriculture, Federal University, Gashua, Yobe State Nigeria. Gashua lies between 12°52' 5"N and 11°2'47"E. the average elevation is about 299 m. The hottest months are March and April with temperature ranges of 38-40° Celsius. In the rainy season, June-September, temperatures fall to 23-28° Celsius, with rainfall of 500 to 1000mm. This region is located within the country's dry belt and *B. aegyptiaca* has nutritional significance. People from Gashua consume leaves and fruits..

2.2. Analysis of Diets

Proximate analysis of the *Balanites* wastes to be used for this study and that of the experimental diets were determined at the Teaching and Research Laboratory of the National Animal Production Research Institute (NAPRI), according to the procedure of AOAC (2000). The parameters determined include dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and Nitrogen free extract (NFE). Metabolisable energy (ME) will be calculated according to the formula of Ponzenga (1985): $ME = 37 \times \%CP + 81 \times \%EE + 35.5 \times \%NFE$

2.3. Management of Experimental Rabbits

The rabbits were raised in cages. The entire rabbit houses were thoroughly disinfected. The Rabbits, Feed and water were be supplied *ad-libitum*(healthy state). Management practices and vaccination programmes was followed strictly.

2.4. Experimental Design

The rabbits were allocated to four experimental dietary treatments of 20rabbits per treatment, while each treatment was replicated 5 times in a completely randomized design (CRD). Each replicate were contained of 10 rabbits.

2.5. Experimental Diets

Five experimental diets were formulated and designated as T₁, T₂, T₃, T₄ and T₅ respectively. T₁ (control) contain groundnut cake as its protein source while T₂, T₃, T₄ and T₅ contain 0, 25, 50, 75 and 100% *Balanites* cake replacing GNC in the diets. The gross composition of the experimental diets is shown in Table 1 below.

Table 1. Gross composition of formulated experimental diets

INGREDIENT	T1 (control)	T2 (25)	T3 (50)	T4 (75)	T5 (100)
Maize	50.00	50.00	50.00	50.00	50.00
Wheat offal	16.00	16.00	16.00	16.00	16.00
Fish meal	3.40	3.40	3.40	3.40	3.40
GNC	25.00	18.75	15.00	6.25	0.00
* <i>Balanites</i>	0.00	6.25	12.50	18.75	25.00
Palm oil	2.00	2.00	2.00	2.00	2.00
*Premix	0.40	0.40	0.40	0.40	0.40
Limestone	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25

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Methionine	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10
TOTAL	100	100	100	100	100
Calculated nutrients (%)					
Energy (Kcal/kg ME)	2990.56	2898.02	2767.89	2706.99	2706.99
Crude protein (%)	16.45	16.09	16.45	16.17	16.17

*Premix in diets provided per kg: Vit. A 10000 IU, Vit. B 2000 IU, Vit. E 13000 IU, Vit. K 1500mg, Vit. B12 10mg, Riboflavin 5000mg, Pyridoxine 1300mg, Thiamine 1300mg, Panthothenic acid 8000mg, Nicotinic acid 28000mg, Folicacid 500mg, Biotin 40mg, Copper 7000mg, Manganese 48000mg, Iron 58000mg, Zinc 58000mg, Selenium 120mg, Iodine 60mg, Cobalt 300mg, Choline 27500mg

2.6. Data Collection

Blood Collection and Analysis

At the end of the feeding trial, about 5 mL of blood sample was collected from the jugular vein of each rabbit slaughtered and put into sterilized glass tubes/bottles containing Ethylene Diaminetetra-acetic Acid (EDTA) for the haematological study. Blood samples meant for serum biochemical studies was collected into plain bottles (without anticoagulant) to enhance serum separation. Serum was obtained by centrifugation at 3,000 revolutions per minute (rpm) for 10 minutes at ambient temperature of 28°C and store at -20°C in a deep freezer until required for the serum tests. The haematological parameters which includes packed cell volume (PCV), red blood cells (RBC), haemoglobin (Hb), and white blood cells (WBC) were analyzed according to standard procedures described by Davise and Lewis (1991). The blood serum was used to determine serum total protein (STP) following the Kjeldahl method as described by Kohn and Allen (1995). Albumin was determined using the BCG (bromocresol green) method as described by Peters *et al.* (1982). Globulin (Gb) concentration was computed as the difference between total protein and albumin concentrations. Creatinine concentration was determined using a commercial kit (Creatinine Liquicolor, Germany). Serum glucose, nitrogen urea and cholesterol constituents were determined spectrophotometrically (Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) using commercial reagent kits (United Diagnostic Industry, Dammam, Saudi Arabia) as described by Coles (1986).

Data Analysis

Data generated from the study were subjected to analysis of variance (ANOVA) using statistical package (SAS, 2015) Version 9.3. The variation in means was separated using the Duncan Multiple Range Test (P=0.05).

3. RESULT AND DISCUSSION

Table 2. proximate composition of raw and roasted *balanites aegyptica* seed cake

Nutrients,%	*Raw	Roasted
Dry matter	93.00	92.83
Crude Protein	17.70	19.26
Crude fibre	5.95	5.20
Ether extract	11.02	10.55
Ash	9.10	10.25
Nitrogen free extract	49.71	49.57
Gross energy (Cal/100 g)	4.31	4.12

*Annongu *et al.* (2009)

The proximate compositions of raw and roasted cake before meal experimentation diets are presented in Table 2. Proximate analysis of raw and roasted *balanites aegyptica* seed cakes revealed the presence of dry matter, crude protein, crude fiber, ash, nitrogen free extract and energy. The crude protein and ash values of the roasted cakes values were more favorably than the control (raw) seed cake ,

Table 3. Phytochemical (quantitative) Analysis of *Balanites Seed Cake*

Sample	Alkaloid (%)	Saponin (%)	Flavonoid (%)	Tannin (%)	Phenol (%)
A1 (RBP)	29.0	30.0	2.03	0.069	108.05
A Cake	4.20	6.80	13.40	8.80	10.40

Results are mean from duplicate samples.

Key: A1== Raw *Balanites* seed powder sample, A= Roasted *balanites* seed cake sample

Table 3 showed bio-actives in cake from raw and roasted *balanites* seed cakes. Cake alkaloid from roasted (4.20 %) , raw cake (29 %) samples shows reduce value content revealing reduce anti – microbial potency for feed use . The saponin content in the two cakes samples showed a drastic reduction in saponin values. This revealed that in feed formulation , bitter associated compound from *balanites* may be reduce especially in treated cake seeds formulated meals. Cake from roasted *balanites* seed cake may not cause haemolytic problem, precipitating and coagulation of red blood cells in animal when use as feed ration.

The flavonoid value were high for roasted (12.40%) samples compare with the raw or control sample . This confers that cake from *balanites* seed oil will be natural anti-oxidants and also keep feed products longer. Phenolic values were low for roasted (10.40%) than the raw The ability of these cakes to inhibits microbial growths or activities may be reduce however, may have traceable microbial and toxicological inhibition on feed or animals

Table4. Haematological parameters of growing rabbits fed diets containing *BASM* as substitute for groundnut cake

Parameters	T1(0%)	T2(25%)	T3 (50%)	T4 (75%)	T5(100%)	SEM	Pvalue
Packed cell volume (%)	30.60 ^b	29.17 ^b	38.26 ^a	28.05 ^b	37.45 ^a	2.55	0.001
Haemoglobin (g/dl)	10.20	9.70	12.68	8.96	11.19	1.87	0.071
White blood cell X10 ⁶	75.10 ^c	85.81 ^b	97.90 ^a	82.29 ^b	95.35 ^a	5.71	0.003
Red blood cell X 10 ⁹	16.63 ^a	12.77 ^b	14.99 ^{ab}	13.05 ^b	15.43 ^a	1.93	0.012

abc = mean with different superscripts within the same row are significantly (P=0.05) different. SEM=standard error of mean

The results on the haematological indices are presented in Table 4. There was significant difference (P=0.05) among treated trials for all the haematological parameters except for haemoglobin. The PCV values (29.1%, 38.26%, 28.05% and 37.45 % for T₂,T₃,T₄ and T₅) respectively were within the range of 33% as reported by Hillyer (1994) for growing rabbits using moringa leaves. The packed cell volume (PCV), Red and white blood cell (WBC) were significantly (p=0.05) at this percentage composition. Roated *BASM* Nutritional adequacy did not indicate under nutrition (Church et al. 1984). However the RBC values were within the range 3.07 to7.50 x10⁶/mm³ as reported by Fudge (1999). The WBC ranges of 6.40 - 12.90 x10³mm³ were reported by Hillyer (1994) for healthy young rabbits.This show that the animals were healthy because decrease in number of WBC below the normal range is an indication of allergic conditions and certain parasitism, while elevated values (leucocytosis) indicate the existence of a recent infection, usually with bacteria (Ahamefule et al.,2008). Hackbath et al. (1983) asserts that there is a strong influence of diet on haematological traits with PCV and Haemoglobin being strong, indicating nutritional status of animals. There was observed balance between pcv and haemoglobin.

Table5. Serum Biochemical indices of growing rabbits fed diets containing *BASM* as substitute for groundnut cake

Parameters	T1 (0%)	T2 (25%)	T3(50%)	T4(75%)	T5(100%)	SEM	Pvalue
Albumin, g/dl	3.22	3.07	3.24	3.15	3.28	0.09	0.062
Glucose, g/dl	98.05 ^a	102.29 ^a	99.24 ^a	90.81 ^b	101.23 ^a	2.88	0.001
Total protein, g/dl	5.01	6.43	5.65	5.98	6.27	0.72	0.062
Globulin, g/dl	1.79	3.36	2.41	2.83	3.12	0.79	0.051
Urea, g/dl	31.28 ^b	43.16 ^a	35.94 ^b	38.94 ^a	42.23 ^a	2.97	0.001

abc = mean with different superscripts within the same row are significantly (P=0.05) different. SEM=standard error of mean.

The results on biochemistry are presented in Table 5. The Globulin values, Albumin, Glucose, Blood urea and Total protein showed significant difference (P=0.05) among treatments. The blood urea ranged from 43.– 31gl/dl. This values were above the range (2.50 - 5.80 mmol/l) similar with the one reported by Njidda and Isidahomen (2011) .This value were higher compare to that obtained in temperate regions (4.6 to 10.4) as reported by Duncan and Prasse (1986). The Globulin values for

treatment T₂(3.36g/dl) and T₃(2.41 g/dl) were much lower than the values reported by Duncan and Prasse (1986) while that of T₁(1.7.g/dl) falls within the range (1.94 to 2.26 g/dl) reported by Onifade and Tewe (1993), who fed various tropical energy feed resources to growing rabbits. The total protein values (6.43 - 5.01 g/dl) were within the range reported by Anon (1980) and of the range (5.81- 6.75 g/dl) reported by Onifade and Tewe (1993). The normal values for albumin, total protein and globulin obtained in this study indicates nutritional adequacy of the dietary proteins for the rabbits.

4. CONCLUSION

The results obtained in this study suggest that inclusion of *Balanites aegyptiaca* seed cake in the diets of rabbit does not have any negative effect on haematological and biochemical indices of growing rabbits. Roasted BASM could be used more as feed ingredient in animal feed, judging from the high carbohydrate and adequate crude protein content. This study showed that the anti-nutrients in the seed did not affect the health status of the rabbits. The reduction of these anti-nutrients through processing enhanced the nutritional value and adequacy of the biofeed.

RECOMMENDATION

Balanites seed cake could substitute ground nut cake in animal feed making therefore feed mills and animal feed makers should tap into this resource.

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