

Effects of *Balanites Aegyptiaca* (del) Seed Cake on Haematological and Serum Biochemical Indices of Growing RabbitsOgori AF^{1*}, Makinde OJ² and Ogori Joeguluba³¹Department of Home Science, Federal University, Nigeria²Department of Animal Science, Federal University, Nigeria³Department of Agricultural education, Federal College of Education Kontagora, Nigeria

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*Corresponding author

Ogori AF, Department of Home Science, Faculty of Agriculture, Federal University, Gashua, Nigeri, Tel: +234 0803-6458-262; Email: ogorifaraday@gmail.com

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Abstract

A 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 rabbit. Five experimental diets were formulated representing the following treatments: T1, T2, T3, T4 and T5 respectively. 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 the 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 vitalyte 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 diet. Each treatment contained 20 rabbits and these treatments' (T₁-T₅) each was replicated in four portions and each portion had five rabbits each. Results of BASM on hematology and serum chemistry showed 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. More so, there was no significant difference in the parameters measured for hematological and biochemical characteristics of rabbits studied. Rabbitfed BASM diet performed significantly like (P>0.05) those fed with the control diet. Inclusion of 25% to 100% roasted BASM contain bio nutrient that can be used in rabbit diet to substitute for groundnut cake without any adverse effect especially in the area of study and related ecology.

Introduction

Balanites aegyptiaca being a browse plant has been reported to improve the feeding potential of ruminant animals in semi-arid [1]. *Balanites aegyptiaca* is widely grown tree in Nigeria. Early studies [2] showed that *Balanites* offers the most rapid and lowest means of providing adequate supplies of bioactive nutrients to the tropical people and their animals. Ogori et al., [3] posit that *Balanites* fruit pulp could be used for making juice, drink and yoghurts at optimal boiling of fruit. Works on the chemical and nutritional composition of *Balanites* however, showed that *Balanites* tree contains chemical compounds namely saponins, tannins and nitrites, which could elicit deleterious effects in animals when consumed in large quantities [4].

Balanites aegyptiaca have been reported to have anti-inflammatory and analgesic, anthelmintic, antioxidant, antidiabetic, hepatoprotective, antibacterial and larvicidal activities in animals. *Balanites aegyptiaca* roots, barks, fruits, pulps and seeds of *Balanites* are lethal to aquatic animals. Thus, the presence of phytotoxins in *Balanites* may limit its intensive utilization in diets for man or livestock [5]. According to Ahamfulen et al., [6] raw or processed pigeon pea did not support changes in haematological and biochemical blood profile for weaner rabbits at 20% dietary level of inclusion. Similarly Saleh et al., [7] inferred that inclusion of *Balanites aegyptiaca* leaves up to 20% in the formulated diets for rabbit in arid region does not have any negative effect on haematological and biochemical indices of growing rabbits. Pretreatments by boiling, roasting, five minutes heating, soaking of *Balanites* have been reported to reduce alkaloid, saponin and phenol contents but also a biochemical turn on flavonoid, and tannin [3]. The seed cake from *Balanites* seed after boiling, roasting and soaking treatments have been reported safe for animal feed formulation and an excellent anti-oxidant substance to keep additives in feed. The quality of meat from meat slab depends largely on feed ration and feed formulations. Haematological parameter is an important and reliable medium used to monitor and alleviate health and bio nutrition status of animals [8]. 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 [9]. Protein from animal sources are widely choice. Harmful and or importance bioactive could also be passed to man in biological cycle. The hematological and biochemical indices are indexes 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 [4] and also the level of anti-nutritional factors present in the feed also influence the hematological and

biochemical bioavailability values [10]. Blood indices are considered to be critical indicators of the physiological stages of farmanimal, thus reflecting the relationship between their nutrition and health [11]. They are useful for clinical evaluation of various animal diseases and feed quality [12]. Packed cell volume and red blood cells have been reported by Jiwuba et al., [12] to determine the feed toxicity and anemia in farm animals. Blood parameters change in relation to the physiological status of an animal [11,12].

This present study is therefore carried out to determine the effects of percentage levels of inclusion of *Balanites aegyptiaca* pretreated seed cake in the diets of weaner rabbits haematological and serum biochemistry which may help the feed and meat industries.

Materials and Methods

Study area

This study was conducted in the North eastern parts of Nigeria, specifically in Gashua, Bade local Government area of Yobe state, Nigeria during 2016/2017 dry session at the Department of Home Science and Management, Federal University Gahua, Yobe state. The feed trial was carried out at the Rabbitry research Unit of the Department of Animal Science, Faculty of Agriculture, Federal University, Gashua, Yobe State Nigeria. The town 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.

Analysis of diets

Proximate analysis of the *Balanites* cake 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) was calculated according to the formula of Ponzenga (1985): $ME = 37 \times \%CP + 81 \times \%EE + 35.5 \times \%NFE$.

Management of experimental rabbits

The rabbits were raised in cages. The entire rabbit houses were thoroughly disinfected. The Rabbits, Feed and water were supplied *ad-libitum* (healthy state). Management practices of cleaning the cages and the replacement of feed as well as water were done three days interval due to the number of rabbits per cage throughout. Vaccination programme against stress using vitalyte and against wormed using ivermectin respectively before the one week of feeding trials or adjustment and for subsequent feeding trials. This was followed strictly.

Experimental design

The rabbits were allocated to four lots with five rabbits each. Each treatment contained 20 rabbits and these treatments' (T_1 - T_5) each were replicated in four portions and each portion had five rabbit each treatment (T_1 - T_5) were replicated four times in a Complete Randomized Design (CRD).

Experimental diets

Five experimental diets were formulated and designated as T_1 , T_2 , T_3 , T_4 and T_5 respectively. T_1 (control) contain groundnut cake as its protein source while T_2 , T_3 , T_4 and T_5 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.

Data collection

Blood collection and analysis: At the end of the trial study, about 5 mL of blood sample was collected from the jugular vein of each rabbit before slaughtered was done. The slaughtering was done at the animal science laboratory after cleaning the animal of external debris' and bowl well cleaned with alcohol before final rinsing with water. The blood was 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 then finally stored at freezing temperature (-21°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. The blood serum was used to determine serum total protein (STP) following the Kjeldahl method as described by Kohn and Allen. Albumin was determined using the BCG (bromocresol green) method as described by Peters. Globulin (Gb) concentration was computed as the difference between

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
*Balanites	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
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.

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

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	108.5
A Cake	4.20	6.80	13.40	8.80	10.40

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 ($P > 0.05$).

Result and Discussion

The proximate compositions of raw, roasted cake from *balanites aegyptica* before the diet experimentation diets are presented in Table 2. Proximate analysis of raw, roasted *balanites aegyptica* seed cake revealed the presence of dry matter (93%), crude protein (17.7%) crude fiber (5.95%), ash (9.10%), nitrogen free extract (49.71%) and energy (4.31%) for raw balanites seed cake flour while roasted cake had dry matter (92.83%), crude protein (19.26%) crude fiber (5.20%), ash (10.25%), nitrogen free extract (49.57%) and energy (4.12%). The crude protein and ash values of the roasted cakes were more higher than the control (raw) seed cake flour.

Results are mean from duplicate samples.

Table 4: 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	P-value
Packed cell volume (%)	30.60 ^b	29.17 ^a	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	85.8 ^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.

Key:

A1=Raw Balanites seed powder sample,

A= Roasted balanites seed cake sample.

Table 3 showed phytochemical in cake from raw (A1) and roasted *balanites* seed cakes (A). Cake alkaloid from roasted sample (4.20 %) and raw cake (29 %) samples shows reduced value implying reduced antimicrobial potency for feed use. The saponin content in the A1 and A samples showed a drastic reduction in saponin content. This revealed that in feed formulation, bitter associated compound from *balanites aegyptica* may be reduced especially when roasted cake seeds are used to formulate meals. Hence Cake from roasted *balanites aegyptica* seed cake may not cause haemolytic problem, precipitating and coagulation of red blood cells in animal when use as feed ration.

The flavonoid values were high for roasted cake (12.40%) compare to raw or control sample. This confers that cake from *balanites aegyptica* seed oil may serve as natural anti-oxidants and also keep feed products longer. Phenolic is a conjugate bioactive that varies depending on expose condition in food, either in a *vivo* or *in vitro* systems. Raw balanites seed flour and A1 have high estimated phenolic material lower than the roasted (A) sample (108.05) %. Phenolic values were low on roasted *balanites aegyptica* sample before use as feed mix, having the value (10.40%) compared to raw sample of (108.5%) value which was not used as feed mix but to ascertain the level of phenolic materials after roast processing. Hence roasting drastically reduced phenolic materials of balanites aegyptica seed cake hence reducing its anti- nutritional efficacy. The ability of these cakes to inhibits microbial growths or activities may be reduced however, may have traceable microbial and toxicological inhibition on feed or animals.

The results on the haematological indices are presented in Table 4. There is 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 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. Roasted BASM Nutritional adequacy did not indicate under nutrition [13]. However the RBC values were within the range 3.07 to 7.50 x10⁶/mm³ as reported by Fudge. The WBC ranges of 6.40 - 12.90 x10³/mm³ were reported by Hillyer for healthy young rabbits. This showed that rabbits (animals) fed with these percentage BASM cake were healthy. 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 [14]. However

Table 5: 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	P-value
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.

this was not the case here rather BASM feed also served *in vivo* as anti parasitic, probably because of the alkaloid and saponin contents of the feed cake. Saleh et al., [7] and Hackbath et al., [15] asserts that there is a strong influence of diet on haematological traits with PCV and Haemoglobin, indicating nutritional status of animals when fed with balnites leaves. The observed balance between PCV and haemoglobin of BASM feed implies that this experimented rabbits were at good and health condition. Also these values are within recommended values.

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 43-31g/dl. These values were above the range (2.50 - 5.80 mmol/l) similar with the one reported by Njidda and Isidahomen [11]. This value were higher compare to that obtained in temperate regions (4.6 to 10.4) as reported by Duncan and Prasse [16]. Implying high protein content of the cake feed trial. The Globulin values for treatment T₂ (3.36g/dl) and T₃ (2.41g/dl) were much lower than the values reported by Duncan and Prasse [16] while that of T₁ (1.7g/dl) falls within the range (1.94 to 2.26 g/dl) reported by Onifade and Tewe [17], 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 [17]. The normal values for albumin, total protein and globulin obtained in this study indicates nutritional adequacy of the dietary proteins for the rabbits. This also agreed with the report of Saleh et al., [7] on adequacy of 20% inclusion of balanites leaves in rabbit feed and optimal metabolism. The biochemical indexes increased at increased percentage cake inclusion with T₅ trial having the best inclusion ration to maintain normal biochemical profile and metabolism in the rabbits.

Conclusion

The results obtained in this study suggest that inclusion of *Balanites aegyptiaca* roasted seed cake in the diets of rabbit at 25%-100% 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 roasted seed cake enhanced the nutritional value and has therapeutic advantages on the biomeal- feed.

Recommendation

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

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