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INFLUENCE OF DIETARY PROCESSED NIGERIAN *Balanites aegyptiaca* FRUITS ON SOME BIOCHEMICAL, HAEMATOLOGICAL AND PHYSIOLOGICAL INDICES IN SWINE.

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ABSTRACT

Dietary effects of Nigerian processed *Balanites aegyptiaca* fruits on biochemical, haematological and physiological parameters in swine were investigated. Twenty weaner pigs were randomly allotted to five dietary treatments consisted of conventional diet, 20% Balanites fruits treated by boiling, roasting, boiling and roasting, boiling and fermenting, respectively, in a 4-week feeding trial. Results showed that the treated test feedstuff did not affect serum creatinine, blood glucose, serum protein albumin, respiratory rate and pulse rate, or the activities of alkaline phosphatase (ALP) (p 0.05) but affected (p <0.05) those of blood urea nitrogen, uric acid, urine urea or urine creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT) and acid phosphatase (ACP). The packed cell volume (PCV), haemoglobin (Hb) and the white blood cells (WBC) differential counts such as neutrophils, monocytes were similar (p>0.05) while the red blood cells (RBC),WBC, mean corpuscular haemoglobin concentration (MCHC), platelets and the lymphocytes were significantly influenced by the test feedstuff (p<0.05). It was concluded that *Balanites aegyptiaca*, roasted or boiled followed by fermentation and included at 20% level could serve a useful alternative feedstuff for monogastric animals considering most of the positive results obtained.

Keywords: *Balanites aegyptiaca*. Blood indices. Physiological indices. Swine

INTRODUCTION

In nutritional assessments, blood indices serve as useful criteria for evaluating animals' nutritional status, because feed components affect body constituents. Blood is very vital to life and before any meaningful work can be done on the biology of an animal, the blood must be studied in details (Harper *et al*., 1979; Church *et al*., 1984). The prohibitive cost of conventional feed ingredients has necessitated the use of novel feedstuffs in animal feeds in most of the developing countries, Nigeria inclusive. This has resulted to competition for the available orthodox feedstuffs between man and animal. This calls for alternatives hence current research is directed towards the application of non-conventional feedstuffs which are not usually consumed by man as staple food to provide for the nutritional needs of farm animals.

Balanites aegyptiaca is widely grown in Nigeria. The tree belongs to the family of *Balanitaceae* and is a savanna tree (Sulaiman and Jackson, 1959). Early studies (El-Khidir *et al*., 1983; Lars and Joker, 2000; Lockett *et al*., 2002) showed that Balanites offers the most rapid and lowest means of providing adequate supplies of 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 (Archibald, 1933; Kon and Weller, 1939; Hardman and Sofowora, 1972). 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. However, with emphasis on the search for alternative feedstuffs to substitute for the conventional ones, it becomes imperative to assess the utilization of this feedstuff as possible alternative to the conventional feedstuff. The work reported here attempted to detoxify Balanites fruits by traditional methods for use in nutrition of swine. Some biochemical, haematological and physiological parameters are used to measure the nutritional adequacy of the processed Balanites fruits in diets.

MATERIALS AND METHODS

Source and processing of test feedstuff: *Balanites aegyptiaca* fruits used for this study were obtained from Kebbi and Adamawa states of Nigeria The fruits were sun-dried to reduce the moisture content to less than 12% before grinding into flour using an attrition miller. The 200 kg flour was divided into four equal parts. One part was subjected to boiling (100^{o}C) while the other parts to roasting (100^{o}C), boiling and roasting, and boiling and fermentation (Annongu *et al.* 1996), respectively.

Diets formulation, experimental animals and management: Five iso-nitrogenous and iso-caloric diets were formulated with crude protein and energy values of 19.58% and 2884kcal/kg, respectively, corresponding to the nutrient requirements of weanling pigs (N. R. C., 1994). The control diet contained corn-soybeans basic ingredients while diets 2, 3, 4 and 5 had 20% inclusion of boiled, roasted, boiled and roasted, boiled-fermented, respectively.

Table 1(a) shows the percent composition of the experimental diets on as fed basis. Twenty weanling pigs, 10 males and 10 females weighing on average, 10 kg were used for the experiment. The pigs were randomly allotted to the five dietary treatments, each with four piglets in a completely randomized design. The experimental pigs were placed on a commercial diet for one week to acclimatize them to the experimental condition before the commencement of the trial. The piglets were individually housed and fed to appetite twice daily with free access to drinking water. The experimental period lasted for 4-weeks. In the course of the experiment, data were recorded on daily basis for feed consumption, body weight gain, and feed utilization efficiency and survival rate.

Data collected during and after the experimental trial included ambient and pen temperatures and some physiological parameters including the respiratory and pulse rates using a stethoscope, and the rectal temperature using a digital thermometer and these were taken in the mornings, afternoons and evening daily.

At the end of the feeding trial, whole blood samples were collected via ear-vein puncture into test tubes containing EDTA for haematological studies. Blood samples were analyzed for platelets, haematocrit (PCV), red blood cells (RBC), haemoglobin (Hb), white blood cells (WBC) and its differential counts of lymphocytes, monocytes, neutrophils, eosinophils and mean corpuscular haemoglobin concentration (MCHC). Blood samples for the analysis of blood glucose level, total protein, cholesterol, lipid, protein albumin, blood urea nitrogen (BUN) and creatinine were collected in test tubes and allowed to stand for some time before centrifuging at 2500 rpm to obtain clear sera (Singh, 1990). Activities of enzymes determined (Bassey *et al.,* 1946) were aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP).

Urine samples collection was carried out by placing the pig from each treatment in individual metabolic cages designed for the purpose. A bowl containing 5ml HCl to preserve the urine was placed under each metabolic cage to collect the urine passed by the pig. Urine samples collected were filtered to remove contaminants, bulked for each treatment before a part was taken and stored in the freezer at -4^{o}C for subsequent analysis of urine urea concentration, uric acid, urine creatinine.

Chemical analyses

The proximate analysis of the nutrients content of the experimental diets, Balanites fruit meal samples, both raw and treated (Tables 1b, 1c and 1d, respectively) were carried out using the standard procedures of AOAC (1990). Determination of biochemical parameters was carried out as described by Singh (1990). Total erythrocytic counts and total leukocytic counts were determined with the aid of Neubaur counting chamber (Haemocytometer) and Hb concentration was determined by Sahl's (acid haematin) method (Benjamin, 1978). MCHC values were calculated from PCV, Hb and RBC values (Jain, 1986). Physiological indices were conducted as outlined by Davis (1964)**.**

Statistical analysis

Data collected were analyzed by one-way ANOVA in a completely randomized design while differences between treatment means were further subjected to Duncan's multiple range test (Duncan, 1955).

RESULTS

Table 2 presents the response of weaner pigs to diets containing processed Balanites fruits using some biochemical indices. There were no significant differences in blood glucose and serum protein albumin levels ($p > 0.05$), however, significant differences existed on total protein and cholesterol levels ($p<0.05$). Serum total protein values were highest on dietary Balanites treated double (boiled and roasted or boiled and fermented). Blood total cholesterol level on the control diet was comparable with values obtained on the diet with Balanites treated by boiling and fermentation ($p>0.05$).

Table 2 shows the effect of dietary processed Balanites fruits on pigs using certain biochemical metabolites. Blood urea nitrogen, urine urea, uric acid and urine creatinine increased in values relative to the values obtained on the reference diet ($p<0.05$).

Table 1(a): Composition of the experimental diets (%)

Ingredients	Diets 1	2	3	4	5
Maize	59.93	46.93	47.93	45.93	46.93
Soybean meal	37.00	30.00	29.00	30.00	30.00
Balanites fruits meal	0.00	20.00	20.00	20.00	20.00
Lysine	1.01	1.01	1.01	1.01	1.01
Dicalcium phosphate	0.71	0.71	0.71	0.71	0.71
Limestone	0.90	0.90	0.90	0.90	0.90
Sodium Chloride	0.25	0.25	0.25	0.25	0.25
Mineral-vitamin. Premix	0.1	0.1	0.1	0.1	0.1
Antimicrobial premix	0.1	0.1	0.1	0.1	0.1
Total	100.00	100.00	100.00	100.00	100.00

Table 1(b): Analyzed nutrients content of the experimental diets (%)

Diets	Dry matter	Crude protein	Ether extract	Mineral matter	Crude fiber	GE Kcal/Kg
1	89.46	20.49	5.41	5.22	3.22	2833
2	86.83	19.01	6.14	6.01	5.11	2903
3	87.11	19.89	7.82	5.03	4.09	2913
4	86.43	19.21	7.91	3.71	5.78	2869
5	88.21	19.42	4.21	6.24	4.27	2903

Table 1(c): Proximate chemical composition of raw Balanites fruit meal

Nutrient	Concentration (%)
Dry organic matter	93.00
Crude protein	17.70
Ether extract	11.02
Crude fiber	5.95
Total ash	9.10
Soluble carbohydrate	49.71
Gross energy Cal/g	4.31

Table 1(d): Proximate composition of processed *Balanites aegyptiaca* fruit meal (%DM)

Diets	Dry matter	Crude protein	Crude fiber	Crude fat	Total ash	Nitrogen free extract
Boiled Balanites fruits.	86.83	18.91	5.11	6.14	6.01	50.66
Roasted Balanites fruits.	87.11	19.89	4.09	7.82	5.83	49.88
Boiled and roasted fruits.	86.43	18.21	5.78	7.91	6.24	52.52
Boiled and fermented fruits	88.21	19.42	4.27	4.21	6.24	50.37

Table 2: Response of pigs to dietary processed Nigerian Balanites fruits using some biochemical indices

	Diets					
Parameters	1	2	3	4	5	SEM
Blood glucose level, (mmol/l)	4.60	4.80	4.40	5.30	5.15	0.17
Serum total protein (g/l)	54.13^{a}	49.00^{a}	48.00^{a}	59.50^{b}	60.00^{b}	1.60
Serum albumin level (g/l)	27.50	36.00	38.00	36.50	30.00	1.16
Blood total cholesterol, (mmol/l)	3.80^{a}	4.30^{b}	6.10^{c}	5.10^{b}	2.70^{a}	0.34
Blood urea nitrogen (mmol/l)	7.55^{a}	8.80^{b}	8.50^{b}	7.00^{a}	5.40^{a}	0.13
Serum creatinine (mmol/l)	80.00	103.00	97.00	99.00	94.00	51.70
Uric acid (mmol/l)	9.67^{a}	13.38^{c}	11.13^{b}	13.76^{c}	12.10^{b}	0.001
Urine urea (mmol/l)	0.20^{a}	0.45^{c}	0.35^{b}	0.50^{c}	0.38^{b}	0.10
Urine creatinine	84.56^{a}	100.63^{b}	168.87^{b}	165.10^{c}	187.55^{b}	1.10

^{a,b,c} Means in the same row not sharing common superscripts differ significantly ($p<0.05$)

Enzyme activities influenced by diets containing processed Balanites are shown in Table 3. There were variations ($p<0.05$) in the activities of the enzymes except ALP ($p> 0.05$). There was decrease in the activity of both AST and ALT for the diet containing roasted and boiled Balanites fruits respectively compared with the control and other diets. However, the activity of ACP was higher in the processed diets compared with the control group.

Data on some haematological and physiological parameters in pigs fed processed Balanites in diets compared with the conventional diet are presented in Tables 4 and 5, respectively. The haematocrit (PCV), haemoglobin count and the white blood cell differential counts on the neutrophils and the monocytes showed no statistical significant difference between the values on the control group and the groups of pigs fed diets with treated Balanites fruits ($p>0.05$). There were, however, significant differences in red blood cells (RBC), white blood cells (WBC), mean corpuscular haemoglobin concentration (MCHC), platelets and WBC differential count of lymphocytes relative to the control group of animals ($p<0.05$) as presented in Table 4.

Table 5 shows the physiological response of the experimental pigs consuming treated Balanites fruits in diets compared with the control diet. Dietary Balanites fruits did not have effects on the respiratory or the pulse rate in relation to the control diet ($p> 0.05$). Consumption of treated Balanites in diets however, influenced the rectal temperature compared with the control diet ($p<0.05$). There was difference in pen temperature as shown in the pen with animals on diet 3 compared with the control and pens other than 3 ($p<0.05$).

Table 6 presents data on some performance characteristics of pigs received dietary treated Nigerian Balanites fruit meal. Feed intake and weight gain of piglets on all the diets containing the test feedstuff were comparable with those of the control diet ($p>0.05$). Some variation was observed on efficiency of feed utilization as the conversion of feed on the conventional diet was superior to that of Balanites based diets ($p<0.05$).

DISCUSSION

Results on the biochemical determinants (including that on performance) in pigs fed Balanites based diets in this study showed that serum total protein level increased following the various treatments of Balanites. Higher levels of the total protein were obtained on diets with double treatment of Balanites suggesting that the processing methods were effective in improving the nutritional value of *Balanites aegyptiaca* through its detoxification, hence, past works (Kon and Weller, 1939; Makkar, 2000; Onyango *et al*., 2003; Tijani, 2007) found by quantification, high levels of saponins, tannins, nitrites in unprocessed *Balanites aegyptiaca* fruits. Treatments adopted might have reduced the toxic levels of the Balanites toxins. Increment in total protein observed following nutritional improvement of dietary Balanites agreed with the past findings (Eggum *et al*., 1982; Onifade and Abu, 1998) which reported that serum total protein and albumin are responsive to the quality of dietary protein intake. Similarly, blood total cholesterol level on dietary Balanites processed by boiling and fermentation were comparable with results on the conventional diet which further confirmed the improvement in the nutritive value of the test feedstuff since normal body cholesterol level is useful as a

Table 3: Effects of dietary treated Balanites fruits on the activities of certain enzymes in the experimental pigs

	Diets					
Enzymes	1	2	3	4	5	SEM
AST, IU/L	68.50[b]	94.00[d]	59.00[a]	79.00[c]	62.00[b]	4.43
ALT, IU/L	92.50[b]	57.00[a]	91.50[b]	87.00[b]	90.50[b]	0.42
ALP, IU/L	38.50	40.00	43.00	49.00	54.00	0.36
ACP, IU/L	6.85[a]	15.65[b]	18.00[c]	14.45[b]	17.00[c]	0.02

[a,b,c] Means in the same row not sharing common superscripts differ significantly ($p<0.05$)

Table 4: Influence of dietary processed *Balanites aegyptiaca* fruits on some haematological indices in pigs

	Diets					
Parameters	1	2	3	4	5	SEM
PCV (%)	29.00	25.00	35.00	32.00	36.00	3.5
RBC (x 1012/L	3.54[b]	3.17[a]	7.43[c]	3.99[b]	5.49b	0.34
WBC (x109/L	12.20[b]	14.25[c]	11.85[b]	11.25[b]	10.95[a]	0.17
Hb (g/dl)	9.05	8.40	19.30	11.40	12.00	0.6
MCHC (g/dl)	31.00[b]	25.00[a]	32.00[b]	34.00[b]	30.00[b]	0.72
Platelets (109/l)	88b	95.00[d]	89.00[c]	90.00[b]	80.00[a]	1.22
Lymphocytes (%)	5.84[a]	8.28[b]	9.40[c]	8.12[b]	8.36[b]	3.24
Neutrophiles (%)	3.88	3.76	3.32	3.36	3.28	2.69
Monocytes (%)	1.52	1.72	1.68	1.60	1.20	0.83

[a,b,c] Means in the same row not sharing common superscripts differ significantly ($P< 0.05$)

Table 5: Effects of feeding treated Balanites fruits in diets on certain physiological characteristics in weanling pigs

Parameters	Diets 1	2	3	4	5	SEM
Respiratory rate (b/m)	24.00	20.00	26.00	20.00	25.00	0.82
Pulse rate (hb/m)	75.00	74.00	81.00	89.00	90.00	0.37
Rectal temp. (0C)	38.10[a]	38.10[a]	38.40[a]	39.70[b]	39.20[b]	0.01
Pen temp. (0C)	31.90[a]	31.70[a]	32.10[b]	31.70[a]	31.70[a]	0.001

[a,b,c] Means in the same row not sharing common superscripts differ significantly ($p<0.05$)
NB: Mean values were taken over a 4-week feeding trial and three times daily (morning, afternoon & evening).

Table 6: Performance data of piglets fed dietary treated Nigerian *Balanites aegyptiaca*

Parameters	Diets 1	2	3	4	5		SEM
Average daily feed intake, g/p/d	543	540	524	531	538	NS	0.35
Average daily weight gain, g/p/d	335	275	255	250	270	NS	7.90
Feed conversion ratio (feed/gain)	1.62[a]	1.96[a]	2.10[b]	2.12[b]	1.99[a]		0.0002
Mortality rate (%)	0.00	0.00	0.00	0.00	0.00		-

[a, b] Means significantly different ($p<0.05$); NS, no significant difference ($p>0.05$).

component of cellular membranes, precursor of steroid hormones and bile acids while excess or abnormal level in the blood is injurious to the body as it causes cardiovascular diseases (Nelson and Cox, 2005). Thus, significant levels of serum cholesterol obtained on dietary Balanites processed by boiling, roasting, boiling and roasting might induce cardiovascular disease in the young pigs fed these diets since high blood cholesterol level promotes deposition of hard fatty materials in the arteries causing them to clog and interrupting the activity of the heart to supply blood to the body.

Blood enzyme analysis showed that the activity of ALT was low on the diet with boiled Balanites relative to the reference diet or the other diets with Balanites treated by methods other than boiling. Acid phosphatase activity was high on Balanites based diets compared to the control diet. High activities of these enzymes might indicate tissue or organ damage or malfunction hence these enzymes, especially the transaminases, are used as indicators of myocardiac infection and liver damage (Bassey *et al*., 1946) and are needed in small or normal quantities for the metabolic processes of the body. However, the variations observed on the activities of these enzymes might probably relate to different susceptibility of the animals to the chemical compounds namely saponins, tannins, nitrites, coumarines which could elicit deleterious effects in the animals (Archibald, 1933; Kon and Weller, 1939; Hardman and Sofowora, 1972).

Experimental pigs fed the control diet had normal values of the biochemical metabolites, blood urea nitrogen, urine urea, uric acid and creatinine while values of these parameters in the groups of pigs offered the test feedstuff in some of the diets were relatively high. The high values could be attributable to the inability of the experimental animals to use the absorbed protein of the test feedstuff for protein synthesis or failure of the organs (kidneys) to fully utilize the nutrient. The insignificant difference recorded on the serum creatinine secretion also lend support that dietary treated Balanites had little or no adverse effect on muscle wastage (Eggum *et al*., 1982).

Data on haematocrit, haemoglobin and WBC differential counts on neutrophils and monocytes did not show significant difference between the control group and those maintained on treated Balanites fruits diets suggesting that treatments applied to detoxify Balanites fruits were effective in eliminating or reducing the anti-nutritional factors associated with *Balanites aegyptiaca*, making it to favorably compare with the standard diet. The differences noticed in mean RBC, WBC, MCHC, platelets and WBC differential count on lymphocytes might be due to the residual effects of the Balanites phytotoxins, nitrites, phytic acids, coumarines or saponins that persisted in the treated Balanites to elicit the differences. The differences might also be due to differences in the treatment methods adopted, some methods being more effective than others in detoxification. The differences observed in the blood parameters could also be as a result of the homeostatic response of the body system to the dietary treatments.

Ingesting processed Balanites in diets did influence neither the respiratory nor the pulse rate of the animals indicating that the Balanites fruits diets are comparable with the conventional diet with regards to physiological reactions on these indices. The significant difference recorded on the rectal temperature values in animals receiving diets 4 and 5, that is 1^{o}C higher than the control is though

remarkable, did not differ widely from normal rectal or body temperature ranges reported to be between 38.33 – 40.5°C (Davis, 1964) which is 2°C greater than the one degree difference obtained in this study.

In summary, this work shows by the results on daily feed intake and body weight gain (Table 6) and those on haematological, physiological and some of the biochemical data that Nigerian *Balanites aegyptiaca* fruits treated by dry heating or boiling followed by facultative fermentation could offer a good alternative source of feedstuff to monogastric animals. Further research to improve the nutritive value of the fruits to enable inclusion at levels higher than 20% will be given attention.

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