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Antinutritional Factors and Aminoacids Content Comparison with Different Processing Methods of *Balanites aegyptiaca* in Formulated Broiler Diets

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Authors' contributions

This work was carried out in collaboration among all authors. Author IA designed the study. Author IKA performed the statistical analysis. Author JO performed the nutritional analysis, author FOA wrote the protocol. Author OSK supervise the experiment, author AML managed the literature searches. Author OOR managed the data processing. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to evaluate the effect of processing methods on the antinutrients and amino acids composition of *Balanites aegyptiaca.* The durations for soaking, roasting and fermentation were 0, 24, 48, 72 and 96 hours to determine the optimal duration of inclusion of processed *Balanites aegyptica*. Raw and processed samples were analysed for amino acids composition and anti-nutritional factors.The trial showed a linear increase in processing duration resulted into a decrease in the concentration of antinutritional factors. Raw *Balanites aegyptica* had

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higher concentration of concentration of amino acid than the soaked and boiled *Balanites aegyptica*. Soaking, fermentation and boiling up to 96 hours reduced the antinutritional components of *Balanite aegyptica.* Percentage reduction of anti-nutrients in *Balanites aegyptica*. fruit meal was best at 96 hours of soaking and fermentation and 60 minutes of boiling and roasting. All anti-nutrients in *Balanites aegyptiaca* fruit meal showed remarkable reduction post processing. In all processing methods, results showed that anti-nutrients in the *Balanites aegyptiaca* reduced up to the maximum durations of processing (96 hours and 60 minutes). It was therefore advisable to include *Balanites aegyptiaca* upto 96 hours in the diets of broiler birds.

Keywords: Balanites aegyptiaca seeds; amino acids; antinutritional factors; processing methods.

1. INTRODUCTION

Worldwide, use of alternative feed ingredients in poultry production has increased consistently over the years, and this trend is expected to continue due to competition between animals and humans for conventional feed ingredients. It is extrapolated that most increases in cost of feed ingredients during the next two decades will occur in developing countries, where rapid economic growth, urbanization and higher household incomes will increase the demand for animal proteins. Anti-nutritional factors are compounds found in most food substances which are poisonous to humans or in some ways limit the nutrient availability to the body thus preventing optimal exploitation of the nutrients present in food and decreasing its nutritive value. Anti-nutritional factors are present in different food substances in varying amounts depending on the kind of food [1].

The high cost of conventional feed ingredients used in poultry feed formulation has necessitated the search for alternative feedstuffs in most developing countries. This is because soybean and groundnut which are consumed by man as plant protein are also conventionally the major protein sources used in poultry feed formulation [2]. It is important that Agricultural researchers explore and promote Nigerian's vast novel feed resources such as *Balanites aegyptiaca* and inculcate it in the poultry industry. This will go a long way to increase the feedstuff base especially for 'conventional plant protein sources' and reduce the human-animal competition for the 'conventional' plant proteins as it is presently.

One of such legume is *Balanites aegyptiaca,* also known as Desert Date in English [3]. In Nigeria, it grows abundantly in States such as Kaduna, Kano, Jigawa, Kebbi, Adamawa and Katsina. [4] reported that the raw fruit contains high level of anti-nutritional factors such as tannin, cyanide, oxalate, phytate and saponin. According to [5], the reduction in trypsin inhibitor at 72 hours of fermentation was greater than tannins and phytic acid with a reduction level of 89% in an experiment with African locust bean. Cooking of Africa oil bean seed (*Pentaclethra macrophylla* Benth) resulted to 51.6% reduction in the tannin content but fermentation further reduced it to 56% [6]. Roasting (toasting) and autoclaving have been reported to decrease phytic acid in dry bean, chickpea and black gram, cowpea and black bean [7]. Trypsin inhibitor, phytic acid, haemagglutinin and oxalate were significantly reduced by roasting of Bambara nut. Roasting greatly reduced the level of trypsin inhibitor compared to boiling [8]. It is against this background that this study was designed to evaluate the potential of *Balanites aegyptiaca* fruit meal as an alternative protein source in feeding farm animals.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment were carried out at the Teaching and Research farm, in the poultry unit, of the Department of Animal Science, Ahmadu Bello University, Zaria. The Departmental farm is located on latitude 11°9'45" N and longitude 7°38'8" E at an altitude of 610 m above sea level. The temperature of the area ranges between 26-40°C depending on the season while the relative humidity during the dry and wet seasons are 21 and 72% respectively.

2.2 Collection of *Balanites aegyptiaca* Fruits

Raw samples of fruits were collected from various open market in Jigawa state.

2.2.1 Processing of fruits

The fruits were processed by soaking in water, boiling, roasting and fermentation.

2.2.2 Soaking of *Balanite aegyptica* Fruit Meal

Samples of the raw fruit were processed by soaking in water for 96 hours at the rate of 200 g per four litter of water in a container. The water was changed at regular intervals of 8 hours. At the end of the soaking duration, the water was drained and the fruits were sun dried after which they were taken to laboratory for proximate analysis.

2.2.3 Boiling of *Balanite aegyptica* fruit meal

Four liter of water was poured into 8 liter aluminium capacity pot and allowed to boil over an open stove flame. Two hundred gram (200) grams of raw the fruit was poured into the already boiling water and it was covered with a lid. At each stipulated time of 60 minutes, an aluminium spoon of about 50 g capacity was used to remove the processed samples. The water was drain and the fruits were allowed to cool and sundry. The samples were later be taken to the laboratory for proximate analysis.

2.2.4 Roasting of *Balanite aegyptica* fruit meal

About 250 g of raw fruit samples was processed by roasting in silver pot using kerosene stove which maintained steady blue flame. While roasting, the samples were stirred to avoid charring until a consistent dark brown colour of the fruit samples is attained. The fruit was roasted for duration of 60 minutes. An aluminum spoon of about 50 g capacity was used to remove the processed samples. The fruit was then allowed to cool and later taken to laboratory for proximate analysis.

2.2.5 Fermentation of fruit

A batch of 250 g of raw fruits was soaked in four liter of water for a period of 24 hours before the water was drained. The fruit was then bagged in air tight polythene bags to promote anaerobic fermentation in four different bags at 50 g weight each. The samples were allowed to ferment for 96 hours after which the fruits were removed and sundried before they were later taken to laboratory proximate analysis.

2.3 Laboratory Analyses

Samples of raw and differently processed *Balanite aegyptiaca* fruit were analysed for their antinutritional factors and amino acids composition at the Biochemical laboratory of the National Research Institute for Chemical Technology (NARICT). Bassawa, Zaria. Kaduna State, Nigeria according to procedures described by [9].

2.3.1 The effect of processing methods on the concentration of Anti nutritional factors of *Balanites aegyptiaca* fruits

Samples of differently processed *Balanites aegyptiaca* fruits were further subjected to quantitatively phytochemical analysis for Anti-nutrients by the standard methods described by [9] at the Biochemical Laboratory of the National Research Institute for Chemical Technology (NARICT). Bassawa, Zaria. Kaduna State, Nigeria.

2.3.2 Determination of Tannin in raw and differently processed *Balanites aegyptiaca* fruits

Tannin was determined using the standard method described by [9]. Two grams of the sample of *Balanites aegyptiaca* fruit was boiled with 300 ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non- absorbent cotton wool. Twenty five mls of the distilled water was measured into a 2 liter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution turned green, then few drops of 0.1N potassium permanganate was added. The titre was multiplied by 0.0066235 to obtain the amount of tannin in the sample.

2.3.3 Determination of phytate in raw and differently processed *Balanites aegyptiaca* fruits

Phytate was determined using the standard method described by [9]. A known weight (5 g) of ground sample of *Balanite aegyptica* fruit was soaked into 100 ml of 2% Hcl for 5 hours and filtered through a filter paper and 25ml of the filtrate was taken and 50 $cm^3$of 0.3% potassium thiocynate solution was added in a conical flask. The mixture was titrated using a standard solution of ferric chloride ($FeCl_{3)}$ until a brownish – yellow color persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v

Calculation:

Mole ratio of $FeCl_3$ to Phytate = 1:1
Concentration of Phytate phosphorus

$$= \frac{\text{Titre value} \times 0.064}{1000} \text{ weight of sample}$$

2.3.4 Determination of oxalate in raw and differently processed *Balanitess aegyptiaca* fruits

The method of [10] was used to determine the oxalate content in the *Balanite aegyptica* fruit. The total oxalic acid of the powdered samples was determined by weighing 2 g into a 250 ml flask. Then 190 ml distilled water and 10 ml of 6 M hydrochloric acid were added. The mixture was heated for 1 hour in boiling water bath, cooled, then transferred into a 250 ml volumetric flask, and diluted to volume and filtered. Four drops of methyl red indicator was added followed by concentrated ammonia till the solution turned faint yellow. It was then heated to 100°C and allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled and 10 ml of 5% calcium chloride was added with constant stirring. It was then allowed to stand overnight. The mixture was filtered through Whatman No 40 filter paper. Then the precipitate was washed several times with distilled water and transferred to a beaker and 5 ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample from the amount of potassium permanganate used, the oxalate content of the *Balanite aegyptica* fruit. sample was calculated using the formula below:

1 ml of potassium permanganate = 2.24 mg oxalate.

2.3.5 Determination of Saponin in raw and differently processed *Balanites aegyptiaca* fruits

The method of [10] was used to determine saponin content of *Balanites aegyptiaca* fruits. A gravimetric method employing the use of a soxhlet extractor and two different organic solvents was used. The first solvent extracted lipids and interfering pigments while the second solvent extracted the sapon in proper. A known weight (2 g) of the sample was weighed into a thimble and transferred into the soxhlet extraction chamber fitted with a condenser in a round bottomed flask. Acetone was poured into the flask, and the sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hotplate and the solvent was distilled off.

For the second extraction, a pre-weighed round bottom flask was fitted into the Soxhlet apparatus which had the thimble containing the sample. Then 5% methanol was poured into the flask. The saponin was then exhaustively extracted for 3 hours by heating the flask on a hotplate after which the solvent was distilled off. The flask was reweighed, and the difference between the final and initial weights of the flask represented the weight of the saponin extracted.

$$\% \text{ saponin} = \frac{\text{weight of saponins}}{\text{weight of sample}} \times 100$$

2.3.6 Estimation of amino acids content of raw, soaked and boiled *Balanites Aegyptiaca* fruit

The amino acid profile in the known sample was determined using methods described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyser (TSM).

2.4 Defatting Sample

The sample was defatted using chloroform/ methanol mixture of ratio 2:1. About 4 g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus [9,10].

2.5 Nitrogen Determination

A small amount (200 mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4

drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured

$$Nitrogen \quad (\%) \quad = \frac{(a-b)x0.01x14\ xVx\ 100}{WxC}$$

Where:

a. = Titre value of the digested sample
b. = Titre value of blank sample
v. = Volume after dilution (100 ml)
W. = Weight of dried sample (mg)
C. = Aliquot of the sample used (10 ml)
14. = Nitrogen constant in mg.

2.6 Hydrolysis of the Sample

A known weight of the defatted sample was weighed into glass ampoule. 7 ml of 6 NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis.

The filtrate was then evaporated to dryness in hot air oven. The residue was dissolved with 5 ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

2.7 Loading of the Hydrolysate into TSM Analyser

The amount loaded was between 5 to 10 microliters. This was dispended into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. The period of an Analysis lasted for 76 minutes.

2.8 Method of Calculating Amino Acid Values from the Chromatogram Peaks

An integrator attached to the analyser calculates the peak area proportional to the concentration of each of the amino acids. Approximately area of each peak was then obtained by multiplying the height with the width at half-height.

The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{Area\ of\ Norceucine\ Peak}{Area\ of\ each\ amino\ acid}$$

A constant S was calculated for each amino acid in the standard mixture:

Where S_{std} = NE_{std} x Molecular weight x μMAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g/16 gN or g/100 g protein using the following formula:

Concentration (g/100 g protein) = NH x W@NH/2 x S_{std} x C

where,

$$C = \frac{Dilution x\ 16}{SampleW_t(g)xN\%X10\ XVol.loaded} \div \text{NHxW(nleu)}$$

where,

NH = Net height
W = Width @ half height
nleu = Norleucine

2.9 Data Analysis

Data recorded for antinutrients and amino acid profile of Balanite aegyptica fruit meal obtained from this study were subjected to analysis of variance (ANOVA) using general linear model procedure of [11]. Significant differences between treatments were separated using the Tukey test.

3. RESULTS AND DISCUSSION

The percentage reduction in anti-nutritional factors of raw, fermented, soaked and roasted *Balanites aegyptiaca* fruit are shown in Tables 1, 2 and 3. There was a percentage reduction in anti-nutritional factors as duration of processing increases. There was a linear decrease in the concentration of antinutritional factors in *Balanites aegyptiaca* fruit as the processing duration increases. All the processed *Balanites aegyptiaca* fruit had lower values for anti-nutritional factors as compared to the raw. This

agrees with the report of [12] studied the effect of ethanol extraction on reduction of anti-nutritional factors in seeds and their reports showed that processing methods cause a significant reduction in antinutrient and recommended for inclusion in animal diets. Raw *Balanites aegyptiaca* fruit meal was found to contain 0.91% tannin, 7.00% phytate, 8.70% saponin and 129.8 mg/100ml oxalate. All anti-nutrients in *Balanites aegyptiaca* fruit meal showed remarkable reduction post processing. It has been established that cooking and other processing methods exert beneficial effect by destroying the anti nutritional factors inherent in legume grains [13].

Table 4 shows the amino acid composition of raw, soaked and boiled *Balanites aegyptiaca* fruit meal. There were no significant differences ($p>0.05$) between the raw and differently processed amino acids composition. The raw *Balanites aegyptiaca* fruit meal had higher numerical values for all the amino acids composition in this study than the processing methods, with the exception of soaking methods which had higher composition of threonine than the raw and boiling method. The raw, boiled and soaked *Balanites aegyptiaca* fruit meal were rich sources of essential amino acids which make it a useful supplement for cereal grains which are generally low in these amino acids [13]. The lower level of lysine (4.19- 5.17 g/100 g cp) as compared to report of severa [13, 14] researchers may be due to reaction with oxidized lipids. Highest digestibility of amino acids observed in soaking as a processing method in this study could be linked to break down of the proteinaceous toxins such as typsin inhibitors and haemaglutinins and down regulation of sulphur containing compounds which enhance the high digestibility of threonine [15]. The range of proline contents (3.05-3.55/100g cp) in the analyzed samples of *Balanites aegyptiaca* seed meal are notably lower than the values reported in literature (4.02 g/g cp). The decrease in amino acid content in the processed *Balanites aegyptiaca* fruit meal as compared to the raw *Balanites aegyptiaca* could be linked to the lower ability of hydrolyzing the antinutritional components during processing which will then allow less release of amino acids.

Table 1. Percentage reduction of anti- nutritional factor in fermented balanite fruit

Description	Duration (minutes)				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hr
Tannin (%)	0.91	0.35(61.54)	0.30(67.02)	0.28(69.23)	0.27 (70.33%)
Phytate(%)	7.00	5.47(21.86)	4.33(38.14)	3.04(56.57)	2.66 (62%)
Saponin(%)	8.70	7.10(18.39)	6.40(26.44)	5.30(39.02)	4.80 (44.83%)
Oxalate mg/100	129.8	30.80(76.92)	28.60(84.69)	24.20(84.69)	17.60 (92.31%)

Table 2. Percentage reduction of anti- nutritional factor in soaked balanite fruit

Description	Duration (minutes)				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hr
Tannin (%)	0.91	0.29(68.31)	0.28(69.23)	0.27(70.33)	0.25 (72.52%)
Phytate(%)	7.00	4.34(38.0)	2.97(29.0)	2.21(68.43)	1.45 (79.29%)
Saponin(%)	8.70	6.90(22.99)	6.10(29.89)	5.70(34.48)	2.97 (65.86%)
Oxalate mg/100	129.8	33.00(76.92)	26.1(84.61)	19.80(92.31)	8.80 (92.31%)

Table 3. Percentage reduction of anti- nutritional factor in roasted balanite fruit

Description	Duration (minutes)				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hr
Tannin (%)	0.91	0.72(20.88)	0.70(23.03)	0.52(42.86)	0.38 (58.24%)
Phytate(%)	7.00	3.43(51.0)	2.74(60.86)	2.21(68.43)	1.24 (82.28%)
Saponin(%)	8.70	7.20(17.24)	69.0(20.69)	5.80(33.33)	3.80 (56.32%)
Oxalate mg/100	129.8	121.00(7.69)	105.60(15.38)	83.60(38.46)	70.40 (46.15%)

Table 4. Amino acids composition of raw, soaked and boiled *Balanites aegyptiaca*

Amino acids	Raw	Soaked	Boiled	SEM	p-value
Leucine	5.00	4.47	4.65	1.19	0.58
Lysine	1.55	1.24	1.29	0.47	0.71
Isoleucine	2.39	1.99	1.19	1.01	0.89
Phenylalanine	2.97	2.61	2.61	1.24	0.19
Trytophan	1.37	1.21	1.26	0.89	0.80
Valine	2.33	1.86	1.92	1.19	0.34
Methionine	0.50	0.34	0.44	0.24	0.53
Proline	3.55	3.05	3.05	1.21	0.19
Arginine	6.19	4.99	5.33	2.22	0.09
Tyrosine	3.61	3.35	3.44	1.37	0.52
Histidine	3.32	3.19	3.19	1.24	0.61
Cystine	1.45	1.33	1.39	0.83	0.55
Alanine	3.49	2.81	3.03	1.22	0.18
Glutamic acid	11.65	10.30	10.75	1.35	0.39
Glycine	5.03	4.42	4.47	1.66	0.83
Threonine	1.11	1.94	1.00	0.98	0.46
Threonine	3.94	3.51	3.67	1.27	0.18
Serine	10.98	9.93	10.17	1.36	0.06

Sem-Standard error of mean

4. CONCLUSION

- It was concluded that processing by 96 hours of soaking or fermentation showed remarkable reduction of intrinsic anti-nutrients in raw *Balanite aegyptiaca* fruit meal. The amino acid composition of processed *Balanite aegyptica* was comparable with other fruit meal which revealed the overall suitability as an alternative feed ingredient in poultry nutrition and may serve as a potential source of functional ingredients in Nigeria especially in areas where insurgency is highly predominant. The amino acids composition of *Balanite aegyptica* revealed the overall suitability as an alternative feed ingredient in poultry nutrition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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