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Evaluation of *Annona Senegalensis* Seed as an Alternative Source of Functional Feed Ingredient in Livestock Feed Production

Abdulwaheed Adeyemi BELLO^{1*}, Idris Mohammed ALIYU², Mohammed Shuaibu DOKO³, Bashir Adamu MU'AZU⁴, Musa MA'AJI⁵, Wosilat ABDULSALAM^{6,} Muftau BELLO⁷

^{1,3,4,5,6} Department of Animal Health and Production Technology, College of Agriculture, Mokwa, P.M.B. 109, Niger State, NIGERIA

² Department of Basic Science, College of Agriculture, Mokwa, P. M. B. 109, Niger State, NIGERIA ⁷ Department of Home Economics, College of Education, Minna, P.M.B. 39, Niger State, NIGERIA

¹https://orcid.org/0000-0002-5355-1039, ²https://orcid.org/0009-0000-3480-9121, ³https://orcid.org/0009-0000-6464-0224 ⁴https://orcid.org/0009-0007-0919-6221, ⁵https://orcid.org/0009-0007-8029-860x, ⁶https://orcid.org/0009-0005-1277-8906 ⁷https://orcid.org/0009-0001-1790-4562

* Corresponding author: aabello2003@yahoo.co.uk

Research Article	ABSTRACT
Article History:Article History:Received: 07 May 2023Accepted: 26 January 2024Published online: 01 June 2024Keywords:Annona senegalensis seedFeed ingredientsPhyto-chemical contentProximate compositionMineral contentVitamin content	Evaluation of <i>Annona senegalensis</i> seed as an alternative source of feed ingredients in livestock feed production was conducted. <i>Annona senegalensis</i> seed was evaluated for its, proximate constituent; mineral content; vitamin content, and Phyto-chemical content using standardized methods. The result of the chemical constituent indicates that the representative test material consist of 12.20% moisture, 12.08% Ash, crude fiber 17.80%, crude protein 8.86%, fat and oil 24.00%, carbohydrates 0.002506% = 25.06 mg/kg respectively, mineral's constituent from the current result showed that calcium, magnesium, potassium, sodium, phosphorus iron and lead values are 1.32, 0.23, 0.49, 0.11, 23.66, 1.81, and 1.10 in (%) respectively. The phytochemical analysis showed the sample contains Saponin, tannin, steroid, Flavonoid, and glycoside. The result further revealed 2.31, 0.88, 0.56, 0.42, 0.78, 2.64, 6.84, and 9.68 in (mg/100g) for vitamins A, B1, B2, B3, B6, D, E, and C content in the sample. Based on the findings of this investigation, <i>Annona senegalensis</i> seed is a good source of carbohydrates; crude fiber; crude protein; fat and oil; some minerals,
	and vitamins; Therefore, <i>Annona senegalensis</i> seed can be utilized as an alternative source of practicable feed ingredients in feed production for livestock production enterprise.
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INTRODUCTION

The livestock industry faces constant challenges in meeting the growing demand for high-quality feed ingredients. The rising costs of traditional feed sources and the need to address sustainability concerns have sparked the search for alternative feed resources (Akinmutimi, 2004). One such potential alternative is Annona senegalensis seeds. These seeds are known for their rich nutritional profile, including high protein content, essential amino acids, and beneficial fatty acids. (Ujowundu et al., 2008; Yisa et al., 2010; Ugochi et al., 2019; Villacís-Chiriboga et al., 2023). These seeds, after oil extraction, can be processed into cake meal, which may serve as a valuable ingredient in livestock diets. Moreover, Annona senegalensis trees are widely distributed in various regions in Africa, making the seeds easily accessible for potential utilization (Abdullahi et al., 2003). Several studies have investigated the nutritional composition and potential benefits of Annona senegalensis seed in various applications. According to Ugochi et al. (2019) Annona senegalensis seeds contain significant amounts of crude protein, crude fat, fiber, and carbohydrates. Additionally, Ugochi et al. (2019) reported that the nutrients composition of Annona muricata seed revealed that the seed can be used in feed formulation purposes. These nutrients make it a potentially valuable feed ingredient for livestock feed production. Similarly, Villacís-Chiriboga et al. (2023) reported in their study that the non-exploited soursop seeds could be a valuable source of oil and protein. In another study, Muhammad and Oluwaniyi (2022) reported that Ficus thonningii seed is a very good source of dietary nutrients. The authors reported high proportions of carbohydrate and protein hence its good nutritional value. Additionally, according to Odukwe et al. (2018), Jackfruit seed meal is a potential feed resources and could be included in diet of broiler chicks up to 10% level of inclusion without any adverse effect on its performance. While Eyoh and Udo (2020) reported that processed Jackfruit seed meal can be included in feeding West African dwarf goat as it enhanced their nutrient intake, digestibility and nitrogen utilization. Supplementation of Roselle seed meal in the diet of grazing red Sokoto bucks can successfully improve the performance of the goats without any detrimental effect on their body system (Abdurrahaman et al., 2021). Similarly, Nathaniel et al. (2022) reported that sesame seed meal can replace soya bean meal at 75%-100% inclusion level without any detrimental effect on the performance of the broiler starter chicks, Haematological parameters and serum biochemical indices. Additionally, according to Jimoh et al. (2023), sand box seed (Hura crepitans) has good nutritional values as feedstuff for poultry nutrition. The authors reported that the inclusion of sand box seed (Hura crepitans) in maize-soya based diets for starter broiler chickens had no negative effect on performance parameters. Additionally, the presence of bioactive compounds, such as antioxidants and antimicrobial agents, in Annona senegalensis seeds has been reported by Ugochi et al. (2019) These compounds could potentially enhance animal health and productivity when incorporated into their diet. This study

aims to evaluate the nutritional composition and potential benefits of incorporating *Annona senegalensis* seed into animal feed as an alternative source of practicable feed ingredients in feed production for livestock production.

MATERIALS and METHODS

Location of the Study

The experiment was carried out at of Niger State College of Agriculture Mokwa, Niger State, Nigeria (latitudes 9°N and 180°E and longitudes 5°N and 3°E). The area has an annual rainfall of between 4 mm and 14 mm and a temperature of about 24 °C to 32 °C during the day and 20 °C to 24 °C at night. The soil is sandy and loamy, and the vegetation is tropical Guinea savannah (CAM) (2021).

Source and Sample Preparation

Annona senegalensis seeds were sourced at the College Backyard along Boy's Hostel Road, Niger State College of Agriculture Mokwa, Niger State, Nigeria. The sample (*Annona senegalensis* seeds) was collected manually in April 2022 using a knife, washed with clean water, and then dried by air for a week. The electric grinder was used to mill the dried test material into a fine powder which was kept in a clearly marked airtight plastic bag for immediate laboratory evaluation at Central Service Research Lab - National Cereal Research Institute (CSL-NCRI) Badeggi-Bida Niger State, Nigeria.

Proximate Composition of Annona Senegalensis Seeds

The values obtained for proximate analysis were all estimated in percentages. Ash and Moisture components were ascertained using weight differences. The crucible's weight loss and its content upon lighting were used to derive the fiber content Carbohydrate component was derived when the total of the percentage of crude protein, fats, moisture, as well as ash were deducted from 100, and this shows presence of protein in the test material as was ascertained by micro Kjeldahl (AOAC, 1990), which comprises digestion, distillation, and titration processes. Protein was obtained by multiplying the value of nitrogen by a constant factor of 6.25. The ascertainment of the fat therein the sample was accomplished making use of the Soxhlet type of direct solvent extraction method of petroleum ether boiled at 40-600C for eight hours. The nitrogen free extract was derived obliquely by subtracting the total of Fibre, fats, crude protein, as well as ash from 100 as described in the 15th and 17th editions of (A.O.A.C, 2000)

Mineral Analysis of Annona Senegalensis Seeds

Two grams of the sample were digested using a 10 cm³ acid mixture consisting of HClO4, H2SO4, and HNO3 in a 1:4:3 ratio on a Kjeldahl digestion block till the solution became quite evident. The digested solution was filtered and filled 100 cm³ volumetric

flasks with deionized water to the mark. Metal concentrations of Ca, Mg, Fe, and Pb were analyzed using Atomic Absorption Spectrophotometry (A.A.S) (Perkin Elmer Analyst 200, USA). Potassium and Na concentrations were determined using flame photometers (SA-500f, China), as though phosphorus was ascertained colorimetrically using the Vanudo-Molybodate colorimetric method (Kf1700, Sweden).

The Vitamin Content of Annona Senegalensis Seeds in Mg Per 100 G

Vitamins analysis was executed using the procedure reported by (AOAC, 2006). **Determination of Vitamin A**

Vitamin A was ascertained by the colorimetric methodology. A quantity of between 1 or 2 grams of the test material and standard were measured and combined with 30 ml absolute alcohol. Afterward, 3 ml 50% KOH solution was added, and 30 minutes of gentle boiling were spent under reflux. Following a wash with purified water, vitamin A was extracted three times with 50 ml of diethyl ether. The extract was dissolved in 10 milliliters of isopropyl alcohol after being dried out at a low temperature. One milliliter of prepared the dissolved extract's solution and the standard vitamin A solution were moved to different cuvettes, and their respective absorbance values were read in a spectrophotometer at 325 nm with a reagent blank set at zero.

 $Conc. of vitamin A in sample = \frac{Absorbance of sample \times Conc. standard}{Absorbance of standard}$

Determination of Vitamin B1

1 g of the test material was placed and dissolved with 100 milliliters of deionized water in a conical flask by shaking vigorously. Later after five minutes of heating, it was cooled and filtered. Thereafter the filtrate was emptied into a cuvette and the respective wavelength (261 nm) for the vitamin was set to read the absorbances with the use of a spectrophotometer.

$$Concentration (mg \%) = \frac{A \times D.F \times volume of cuvette}{E}$$

Where A = absorbances, E = extinction coefficient = 25 for B1, and DF is dilution factor = 5.

Determination of Vitamin B2

1 g of the test material was placed and dissolved with 100 milliliters of deionized water in a conical flask by shaking thoroughly. Later after five minutes of heating, it was cooled and filtered. Thereafter the filtrate was emptied into a cuvette and the wavelength was set at 242nm for the vitamin B2 to read the absorbances using a spectrophotometer. $Concentration (mg \%) = \frac{A \times D.F \times volume of cuvette}{E}$

Where A = absorbance, E = extinction coefficient = 25 for B2, and DF is dilution factor = 5.

Determination of Vitamin B3 (Niacin)

Five grams of test material was mixed and 100 milliliters of purified water was added in order to dissolve all of the Nicotinic acid or Niacin that is present. The volume of 5 ml of this solution was filled into a 100 ml volumetric flask and made up to mark with distilled water. The amount of 10 to 50 ml of Niacin stock solution was also prepared and added. The absorbances of the diluted stock solutions and sample extract were measured at a wavelength of 385nm on a Spectrophotometer.

Determination of Vitamin B6

1 g of the test material was placed and dissolved with 100 milliliters of deionized water in a conical flask by shaking thoroughly. Later after five minutes of heating, it was cooled and filtered. Thereafter the filtrate was emptied into a cuvette and the wavelength was set at 242nm for the vitamin B6 to read the absorbances using a spectrophotometer.

 $Concentration (mg \%) = \frac{A \times D.F \times volume of \ cuvette}{E}$

Where A = absorbance, E = extinction coefficient = 25 for B6, and DF is dilution factor = 5.

Determination of Vitamin C

It was accomplished by the titrimetric methodology (Kirk and Sawyer 1991). To analyze the test material, One gram was homogenized in 6% EDTA/TCA solution and filtered. Then, 20 ml of 30% KI solution was added and titrated against 0.1M CuSO4 solution. Black coloring served as a marker for the endpoint. Additionally, a reagent blank was titrated. Vitamin C content was determined using the formula below. One ml of 0.1 moles CuSO₄=.88mg vit. C.

$$Vitamin C mg/100 = \frac{1 x .88 x titre - blank}{W}$$

Determination of Vitamin E

This was ascertained by the Futter – Mayer colorimetric methodology of the Association of Vitamin Chemists. 1 gram of the test material was blended with 10 ml of methanolic sulphuric acid and boiled gently under reflux for 30 minutes. The mixture was then moved to a separating funnel and treated three times with 30ml

diethyl ether, recovering the ether layer each time. The ether extract was then moved to a desiccator and allowed to dry for 30 minutes before being evaporated to dryness at room temperature. The resulting dried extract was dissolved in 10 milliliters of pure ethanol. One milliliter of the dissolved extract and an equal volume of standard vitamin E were moved into separate tubes. After adding continuously 5 milliliters of absolute alcohol and 1 milliliter of the concentrated nitric acid solution, the mixtures were left to stand for five minutes. And the respective absorbances were determined using the spectrophotometer at 410 nm with blank reagent set at zero.

 $Conc. of \ vitamimE \ in \ sample = \frac{Absorbance \ of \ sample \times \ Conc. of \ standard}{Absorbance \ of \ sample}$

Qualitative Analysis of Phyto-Chemical Content of Annona Senegalensis Seeds

The test sample was exposed to phytochemical examination for the existence of Saponin, glycosides, flavonoids, steroid, tannins, and alkaloids, using standard methods recommended by (AOAC, 2016).

Saponin

In a test tube, two grams of the sample were combined with roughly 5 milliliters of distilled water. The mixture was then agitated briskly and submerged for a few minutes in a water bath. The presence of saponin is confirmed by the production of a frothy liquid at the top of the mixture.

Alkaloids

Maeyers' reagent, which is a solution of 1.3 g HgCl2 and 5 g KI in 100 ml of purified water, was added dropwise to 1 ml of blended test material and roughly 2 ml of concentrated HCl in this test. The presence of alkaloids is confirmed by the production of a creamy-looking precipitate.

Quinone

Two milliliters of the sample solution were combined with one milliliter of concentrated H2SO4. The presence of quinones is indicated by the product's red color.

Glycosides

In a test tube, around 4 milliliters of the sample solution were combined with 2 milliliters of glacial acetic acid. The mixture was then exposed to a few drops of concentrated H2SO4. A deoxy sugar characteristic of cardiac glycosides was confirmed by the creation of a ring at the contact.

Phenol

In this test, one gram of sample was mixed with two milliliters of purified water, and then drops of 10% ferric chloride were added. The development of green or blue color shows the presence of phenols.

Tannins

One gram of the material was heated in a test tube with twenty milliliters of purified water, and after filtering, a few drops of 0.1% ferric chloride were added. The presence of tannins was determined by observing the development of a blue-black color.

RESULTS and DISCUSSION

The chemical constituent of *Annona senegalensis* seed is depicted in Table 1. The result shows higher composition of moisture 12.20% when compared to the 5.98% reported for *Hibiscus Sabdariffa* seeds by (Anhwange et al., 2006). The observed value is lower relative to the value observed for Afang seed (31.16%) and fluted pumpkin seed 45.8% (Ekop, 2007). The lower water level composition of the experimental test sample is an indication that when stored for a reasonable period, it will not go bad after drying (Daramola, 2022)

Parameters	Concentration (%)
Moisture	12.20
Ash	12.08
Crude fiber	17.80
Crude Protein	8.86
Fat and oil	24.00
Carbohydrate	0.002506% = 25.06 mg/kg

Table 1. Chemical constituent of Annona senegalensis seed

Ash content shows the mineral therein in the seed of plant material. The composition of ash in the representative part of test material was noted to be higher (12.08%) when compared to the trio of *Gnetum africanum* (1.20%), *H. Sabdariffa* seed (5.55%), and *Mucuna ureans* (6.00%). From the result of the current study, the fat content value was reported to be 24.00%, the current value is close to the 28.10% fat observed in *H. Sabdariffa* seed (Anhwange et al., 2006), the figure reported is higher than 11.99% reported in *Corchorus olitorius*, 13.15% in *G. africanum* and 4.30% in *M. Ureans* (Ekop, 2007, Idris et al., 2009).

Crude fiber composition of the test material were observed to be lower (17.80%) when compared to the figure reported in a handful of vegetables used up in Nigeria for instance fluted pumpkin at 41.60% (Ekop, 2007). The protein composition of the representative part of the test material (8.86%) is comparable to (the 8.80%) reported by Yisa et al. (2010) and was a little higher as compared to the (7.70%) figure reported for fluted pumpkin and the figure is lower relative to the 17.50% observed for *G*.

africanum and (24.3%) reported for *M. ureans* (Ekop, 2007). Additionally, the protein composition of *Annona senegalensis* seed may play an all-important role in boosting nutrient intake. Carbohydrate figure as reported in the current study were higher (25.06%) when compared to (19.56%) reported for *C. Olitorius*, and 10.56% in *B. diffuse* (Ujowundu et al., 2008; Idris et al., 2009). Carbohydrate supplies the animal's bodily system with energy that is needed for their daily metabolic activities.

Parameters	Concentration (%)	
Calcium	1.32	
Magnesium	0.23	
Potassium	0.49	
Sodium	0.11	
Phosphorus	0.0024% = 23.66ppm	
Iron	1.81	
Lead	1.10	

 Table 2. Mineral content of Annona senegalensis seed

The mineral composition of *Annona senegalensis* seed is depicted in Table 2. From the Table 2, the results of the current study shows the values of calcium 1.32%, magnesium 0.23%, potassium 0.49%, sodium 0.11%, phosphorus 0.0024% (23.66 ppm), iron 1.81%, and lead 1.10% are lower than the valves 7.05, 11.30, 160.29, 126.13, 105.60 and 15.30% reported by Mathew et al. (2018) and 6.94. 1.32, 24.15, 0.43, 0.05% reported by Adan et al. (2020). However, the values are comparable to that reported by (Yisa et al., 2010) for *Annona senegalensis* seed 1.35%, 0.24%, 0.47%, 1.80%, and 1.10% respectively.

Parameters	Inference
Saponin	+ve
Tannin	+ve
Alkaloid	+ve
Flavoniod	+ve
Steroid	+ve
Glycoside	+ve

Table 3. Phytochemical Content of Annona senegalensis seed

Key: +ve = present

The result of the phytochemical screening of *Annona senegalensis* seed presented in Table 3 above confirms the presence of all the phytochemical tested parameters quantitatively. Yisa et al. (2010) also reported the existence of these phytochemicals in *Annona senegalensis* seed except for tannins and alkaloids. A similar result by Tope et al. (2017) shows the existence of glycosides, flavonoids, steroid, alkaloids, and, tannins, and Terpenoids on qualitative phytochemical screening of *Moringa oleifera* seed. Furthermore, reports on *Hibiscus Sabdariffa* seeds (Anhwange et al., 2006) identify the presence of Saponin and tannins. (Muhammad and Oluwaniyi, 2022) also reported the presence of Saponin, tannins, Alkaloid, phenol, and Flavonoid in *Ficus thonningii* seeds. Additionally, Ugochi et al. (2019) also reported the presence of Saponin, tannins, alkaloid, Flavonoids, and Terpenoids in *Annona muricata* seeds.

	Parameters (Vitamins)	Concentration (mg/100g)
А		2.31
B1		0.88
B2		0.56
B3		0.42
B6		0.78
С		9.68
Е		6.84

Table 4. Vitamin Content of Annona senegalensis seeds (mg/100g)

The results shown above depict the presence of vitamin A- 2.31, B1- 0.88, B2 -0.56, B3, 0.43, B6 -0.78, D – 2.64, E – 6.84, and C-9.68 in (mg/100g) respectively. The vitamin therein in the seed can have a supplemental resultant effect on the routine vitamin of the livestock body system needs. The presence of important vitamin A, C, and E in the *Annona senegalensis* suggests that the seed is suitable for use as a dietary and healthy building block to enhance animal well-being and growth performance.

CONCLUSION and RECOMMENDATIONS

The findings from the present study indicate that *Annona senegalensis* seed is an excellent alternative source of crude protein, fat and oil, carbohydrate, and crude fiber, and they have the potential of being used as feed ingredient in livestock nutrition. They contain vital minerals like Ca, Mg, K, Na, P, and Fe, which are essential in livestock nutrition and also showed the presence of some important phytochemicals like Saponin, Tannin, Alkaloid, Flavonoid, steroids, and glycoside. The seed contains vital vitamins like vitamins, B1, A, B2, B3, B6, E, C, and K which are essential in livestock nutrition. The phytochemical content of *Annona senegalensis* seeds can be

reduced when dried before incorporating into livestock feed to minimize phytochemical effects.

Recommendations

i. *Annona senegalensis* seed is recommended as an alternative source of Crude protein, ether extract, carbohydrate, crude fiber, and minerals feed ingredients in livestock feed production.

ii. *Annona senegalensis* seed can be exploited as commercial source to supplement livestock feedstuffs.

iii.More research needed to be conducted to determine the complete potential of *Annona senegalensis* seed as a future feed ingredient to be used in formulating livestock feed.

Conflict of Interest

The authors have declared that there are no competing interests.

Authors Contribution

AAB contributed to the project idea, design and execution of the study. IMA, SMD, BAM, MB conducted the laboratory analyses. MM and WA supervised the experiment and AAB wrote the manuscript.

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