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Research Article
Evaluation of the Chemical Composition of Immature and Mature Ball Nut Seed (*Calophyllum inophyllum*) Meals

[1]Okokon Okon Effiong and [2]Adiah Inyang Ofem

[1]Department of Animal Science, University of Calabar, Calabar, Nigeria
[2]Department of Livestock, Ministry of Agriculture, Calabar, Cross River State, Nigeria

Abstract
Background and Objective: High cost of maize, sorghum and millet coupled with the inability of existing non-conventional energy feed resources to sustain the poultry and livestock industry in Nigeria has made it imperative for the animal nutritionist to further research into more sustainable and less competitive feed materials. This research was therefore conducted to determine the chemical composition of immature and mature ballnut seed meals. **Materials and Methods:** The immature and mature ballnut seeds were harvested, decorticated and the kernels oven dried at 50°C for 24 h before milling and storage at room temperature in labeled containers. The proximate, mineral, amino acids and the phytochemical composition of the ball nut seed meals were determined. Data generated were subjected to analysis of variance procedures using SPSS 17.0 windows computer software package with t-test as a statistical tool. **Results:** The results showed that immature ball nut seed was superior in metabolizable energy (3707.60$\pm$2.64 kcal kg^{-1}), sodium (0.25%), potassium (0.88%), magnesium (0.29%), calcium (0.19%) and iron (107.45%) to mature ball nut seed with metabolizable energy, sodium, potassium, magnesium, calcium and iron values corresponding to 3661.38 kcal kg^{-1}, 0.22, 0.69, 0.24, 0.35, 0.17 and 93.05%, respectively. The phytic acids, oxalic acid, alkaloids, saponins, tannins and hydrogen cyanides were the phytochemical present in the two samples. The variations in the composition of these compounds between the two samples were not significant ($p>0.05$). Tryptophan, glutamic acid, cysteine, phenylalanine, lysine and leucine with values corresponding to 18.23, 6.11, 5.06, 3.79, 3.77, 3.67% for the mature ball nut and18.47, 6. 38, 5.15, 3.88, 3.83, 3.78%, respectively for the immature ball nut were the most abundant amino acids. The concentration of these amino acids between the two samples were statistically ($p>0.05$) similar. **Conclusion:** From this study, it was concluded that immature and mature ball nut seed meal were similar in chemical composition and could therefore serve as potential animal's feed resource owing to high energy level, amino acids profile as well as low fibre and other anti-nutritional factors present in the meals.

Key words: Mature ballnut, immature ballnut, crude protein, anti-nutrients, minerals

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Corresponding Author: Okokon Okon Effiong, Department of Animal Science, University of Calabar, Calabar, Nigeria Tel: +2348036602048

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feeding of animals by livestock farmers in Nigeria has become very difficult recently, due to the high cost of raw materials meant for feed compounding. The prices of conventional energy concentrates (maize, millet and guinea corn) and plant protein concentrates (soybean meal, cotton seed meal and groundnut cake) which constitute about 50-65 and 30-35%, respectively in compounded feeds have tremendously risen recently due to drought, poor yield and massive export out of the country by grain farmers. This condition has created a big gap between the demand and supply of these conventional feed resources for feed mill industries in the country.

Utilizing non-conventional feed resources has been one of the approaches used in solving this problem but the traditional non-conventional feed resources (crop residues, agro-industrial by-products, slaughter house by-products, leaf and seed meals) are no longer sustainable[1]. Therefore, it imperative for animal nutritionist to search for more alternative feed resources to support the livestock industry. It is on this regards that the ball nut (*Calophyllum inophyllum*) seed has been considered.

Ball nut (*Calophyllum inophyllum* Linn) tree is valued for its hardiness and beauty as an ornamental tree that provides shade and shelter from the wind on streets, in parks and in coastal areas. The wood is a prized timber for carving, cabinet making and boat building[2]. The tree grows to a height of 8-20 m, sometimes reaching up to 35 cm, the canopy width which is often greater than the tree's height when the tree is grown in open location. It has a broad spreading crown often with large, gnarled, horizontal branches. According to Friday and Okano[2], ball nut tree has elliptical, shiny and tough leaves and flowers which were arranged in axillaries cymes with a sweet, lime-like fragrance. The tree flowers twice a year and bears numerous fruits (spherical drupes), which were arranged in clusters. Once ripe, their smooth, yellow epidermis discloses a thin layer of pulp, which tastes somewhat of apple. The grey, ligneous and rather soft nut contains a pale yellow kernel, which is odorless when fresh but once chewed, it coats the mouth and emulsifies saliva and its insipid taste becomes bitter.

Different parts of the plant, such as the leaves, bark and seeds possess medicinal values and can be used as astringent, antiseptic, diuretic, purgative and expectorant. Ball nut seed contains diverse compounds like coumarins, xanthones, flavonoids, steroids and triterpenoids. It was also rich in oil, having about 40-75% of it on dry weight basis and the mature tree may produce 1-10 kg of oil/year depending on the productivity of the tree and the efficiency of extraction process[3]. The oil obtained from ball nut seeds is greenish-yellow in color and contains benzoic and oxy-benzoic acids, small amount of vitamin E, phosphoamino lipids, free fatty acids, glycerides and steroids (canophyllal, canophyllol, canophyllic acids). The seed oil has also found to exhibit good physico-chemical properties and was useful as a biodiesel feedstock[4,3]. According to Chavan *et al.*[3] the oil demonstrates anti-inflammatory activity with 4-Phenylcoumarin calaphylloidea and a group of xanthones, including dehydrocycloguandin, calophyllin-B, Jacareubin, mesuaxanthone-A, mesuaxanthone-B and euxanthone. The presence of xanthones explains reduction of rashes, sores, swelling and abrasions with topical application of the oil. In addition, the presence of xanthones and coumarins in ball nut seed oil demonstrated anti-oxidant properties, specifically inhibiting lipid peroxidation and to protect skin cells from damage by reactive oxygen species. Praveena *et al.*[5] observed that oil of ball nut is rich on bioactive activities, such as anti-HIV, anti-cancer, anticoagulant, anti-inflammatory, anti-platelet aggregation and as well used traditionally for medicine, cosmetics and as lamp oil[6].

Significance of study: There were no reports in the literature highlighting the nutrient composition of ball nut seed meal as well as comparing the nutrient composition of the mature and immature ball nuts.

Conflict of interest: Authors have declared that no conflicting interests exist.

Objectives of study: Therefore, this study was designed to compare:

- Proximate composition of mature and immature ball nut seed (*Calophyllum inophyllum* Linn) meals
- Phytochemical composition of mature and immature ball nut seed meals
- Minerals and amino acids composition of mature and immature ball nut seed meals

MATERIALS AND METHODS

Sample collection: The experiment was conducted between March and June, 2016. The matured ball nut seeds were gathered from the floor at the premises of the Cross River State Library Complex, Calabar, whereas, the immature seeds were plucked in their greenish stage from the ball nut tree at the same premises (Fig. 1, 2).

Fig. 1: Immature ball nut

Fig. 2: Mature ball nut

Sample preparation: The hard layers covering the seeds were manually decorticated to expose the kernels and the kernels oven dried at 50°C for 12 h prior to milling. Oven-dried mature and immature ball nut seeds were milled using industrial blender to a fine flour. The milled samples were stored at room temperature using air tight plastic containers until needed for chemical analysis.

Chemical analysis: The chemical analyses were carried out at the Department of Animal Science Laboratory, University of Calabar and Animal Care Laboratories at Ibadan and Lagos, respectively.

Proximate composition: The proximate composition of the ball nut seed meals were carried out using the methods of AOAC[7]. The components analysed for were crude protein, ether extract (EE), ash, crude fibre and nitrogen-free extract (NFE). The percentage crude protein content (N×6.25) was determined by the micro-kjeldahl method and the metabolizable energy estimated using the equation:

$$\text{ME (kcal kg}^{-1}\text{)} = 37\ (\%\text{CP}) + 81.8\ (\%\text{E.E}) + 35.5\ (\text{NFE})$$

Mineral elements determination: Atomic Absorption Spectrometer (model703 Perkin Elmer, Norwalk, CT, USA) was used in the determination of calcium, manganese, magnesium, sodium, potassium and iron. Sodium and potassium were determined using flame photometer (Sherwood flame photometer 410, Sherwood Scientific Ltd., Cambridge, UK), while Phosphorus was determined using Vanadomolybdate method AOAC[7].

Amino acids determinations: Amino acids were determined by Ion Exchange Chromatography[7] using the Technicon Sequential Multi-sampling (TSM) amino acid analyzer. The period of analyses was 76 min for each sample. The gas flow rate was 0.50 mL min^{-1} at 60°C with reproducibility consistent within ±3 min. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of three determinations. Nor-leucine was the internal standard.

Phyto-chemical screening: The phyto-chemical analysis was carried out to determine the composition of cyanogenic glycosides, tannins, oxalate and alkaloids. Phytate was determined by the anion-exchange method as described by AOAC[7], using phosphate as the standard, while Saponins composition was determined using spectrometric method as described by Brunner[8]. The total flavonoids and alkaloids were determined according to the methods outlined by Harborne[9].

Statistical analysis: Data from the chemical analysis obtained in triplicate values were tested for significance using SPSS 17.0 windows computer software package with t-test as a tool for comparison between the two samples.

RESULTS AND DISCUSSION

Proximate composition: Result of the proximate composition of mature and immature ball nut seed meal showed that the percentage dry matter composition of the mature (97.02%) and immature (98.50%) ball nut seed meals were statistically ($p>0.05$) similar. The crude protein content of mature ball nut seed meal (8.16%) and immature seed (8.52%) were statistically ($p>0.05$), similar, having close resemblance with those of maize (8-10%) sorghum (10-11%) and guinea corn[10]. The total fat contents of mature ball nut seed (10.10 %) and immature (9.30%) did not differ ($p>0.05$) significantly from each other.

The crude fibre content was 6.00% for the mature ball nut seed meal and 4.50% for the immature seed meal. Variation in the crude fibre content between the two meals was significant ($p<0.05$).

The ash content of the ball nut was 2.20% for the mature seed and 2.05% for the immature seed meal. The results showed no significant difference between the two samples.

Nitrogen free extract (NFE) was 71.36% for the mature ball nut seed meal and 74.13% for the immature sample. Variation in the NFE composition between the two samples was not statistically ($p>0.05$) significant.

The metabolizable energy values differed ($p<0.05$) significantly, with the immature ball nut seed meal having the highest value corresponding to 3707.60 kcal kg^{-1} ME relative to 3661.38 kcal kg^{-1} ME observed for the mature seed meal. Variation in the free fatty acids composition between mature and immature seeds showed no significance, implying that at mature or immature stage, ball nut seeds have similar composition of free fatty acids.

Phytochemical composition: The results of the phytochemical composition of mature and immature ball nut seed meals were shown in Table 1.

The phytic acid, oxalic acid, alkaloids, saponins, tannins and hydrogen cyanide were present in the two samples. Variations in the composition of these compounds between the two samples were not significant ($p>0.05$).

Minerals composition of mature and immature ball nut seed meal: The results of the mineral constituents of the ball nut seed meals were shown in Table 2. The presence of sodium, potassium, magnesium, phosphorus, calcium and iron were noted in mature and immature ball nut seed meals. It was observed that immature ball nut seed meal was statistically superior in sodium, potassium, magnesium and calcium composition relative to mature ball nut seed meal, while the composition of the rest of the minerals were statistically similar between the two samples. The result indicated that immature ball nut seed meal is richer in some minerals and it becomes so important when the usefulness of such minerals like sodium, potassium, magnesium phosphorus, calcium in the body were considered.

Amino acids composition of the mature and immature bull nut seed meal: The result of the amino acids composition presented in Table 3 showed that mature and immature ball nut seed meals were rich sources of amino acids. The essential amino acids found in the mature and immature ball nut seed meals were histidine, threonine, methionine, phenylalanine, isoleucine, leucine, tryptophan, arginine and lysine, with the tryptophan, glutamic acid, cysteine, phenylalanine, lysine, leucine and alanine being the most abundant. The concentration of amino acids between the two samples were statistically ($p>0.05$) similar.

Proximate composition: The high dry matter content observed in the two samples implied that they contain less moisture with higher proportion of other nutrient components and could have longer shelf life, if well packaged and stored properly. The knowledge of the dry matter content of the feed stuff was essential as it helps to determine the feeding standards of the animal. Increase or decrease in feed dry matter content results in over or under feeding of nutrients[11].

The percentage crude protein content of ball nut seed meal was low compared with some nuts like walnut, containing 28.85% crude protein[12], Jatropha seed with 29.4% crude protein[13].

Fats are organic compounds and an important constituent of plants and animal tissues, supplying energy to the body. A diet is said to provide 1-2% of fat as its calories of energy as excess fat consumption is implicated in certain cardiovascular disorder[14]. Fat is important in diets because it promotes fat soluble vitamin absorption. It is a high energy nutrient and does not add to the bulk of the diet[14].

Table 1: Phytochemical composition of ball nut seeds (mg/100 g)

Composition	Mature	Immature
Phytic acids	1.86	1.83
Oxalate	6.30	6.12
Alkaloids	3.06	3.64
Saponins	1.70	1.70
Flavonoids	-	-
Tannins	0.02	0.02
Cyanide	10.26	10.58

Values are means of triplicate determinations

Table 2: Mineral composition of ball nut seed meal (%)

Minerals	Sodium	Potassium	Magnesium	Phosphorus	Calcium	Iron
Mature	0.22±0.02	0.69±0.01	0.24±0.01	0.35±0.090	0.17±0.01	93.05±0.20
Immature	0.25±0.01*	0.88±0.02*	0.29±0.01*	0.40±0.019	0.19±0.01*	107.45±0.09*

Means are value of triplicates determination, *Significant means

Table 3: Amino acids profile composition of mature and immature ballnut seed meal

Amino acid	Mature	Immature
Aspartic acid	1.07	1.18
Glutamic acid	6.11	6.38
Asparagine	0.65	0.77
Serine	0.78	0.89
Glutamine	5.23	5.46
Histidine*	1.08	1.17
Glycine	1.58	1.76
Threonine*	0.97	1.13
Arginine*	1.18	1.34
Alanine	3.45	3.53
Tyrosine	2.28	2.37
Cystine	5.06	5.15
Valine*	1.59	1.78
Methionine*	1.08	1.35
Norvaline	2.15	2.28
Tryptophan*	18.23	18.47
Phenylalanine*	3.79	3.88
Isoleucine*	2.35	2.52
Leucine*	3.67	3.78
Lysine*	3.77	3.93
Hydroxyproline	1.07	1.16
Sarcosine	2.17	2.33
Proline	2.36	2.57

Values are means of triplicate determination, *Essential amino acids

The results compared favourably with the earlier report of 9.99-11.89% for the de-oiled ball nut cake but higher than 8.40 and 3.77% reported by Ibironke *et al.*[15] and Chijioke *et al.*[12] for areca nut and walnut seeds, respectively.

The percentage crude fibre values obtained in this experiment were higher, compared with2.0% reported for maize and 2.57% for Jatropha[13] but similar to 6.01% for walnut seed[12,15] reported 34.25% as crude fibre content of areca nut, a value quite higher than results of this study. Crude fibre is a significant component of a diet, playing useful role in providing roughage that aids digestion and reduces the accumulation of carcinogen in the body[16]. It has been reported that the presence of fairly high crude fibre in food material decreases the dry matter digestibility in animals. The crude fibre content of the ball nut seed reported in this study is within the safety limit of monogastric animals.

The values for ash recorded in this study were lower than the range (5.44 -6.07%) earlier reported for the de-oiled seed cake of ball nut but comparable to 1.76% reported for areca nut by Ibironke *et al.*[15]. The ash content of food is a reflective of its minerals content and is an index for assessing the quality of feeds and feed stuffs. It is recommended that the ash content of nuts, seeds and tubers should be within the range of 1.5-2.5% in order to be suitable as animal feeds. The ash contents of the test samples were within this range, therefore, can be recommended for use in animals' feeds production.

Ibironke *et al.*[15] reported 79.18% as NFE value of areca nut. Nitrogen free extract content is an index of potentials of such feed material in supplying energy for animals.

The metabolizable energy recorded in the two samples were higher than 3430, 3300 and 3000 kcal kg^{-1} of metabolizable energy reported for maize, guinea corn and cassava meal, respectively. The above results indicated that ball nut seed meal has the potential of replacing the conventional feed resources as energy source in poultry and livestock feeds.

A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Boden[17] demonstrated that free fatty acids (FFA) cause both insulin resistance and inflammation in the major insulin target issues (skeletal muscle, liver and endothelial cells) and thus was an important link between obesity, insulin resistance, inflammation and the development of T2DM, hypertension, dyslipidemia, disorders of coagulation and Atherosclerotic Vascular Disease.

Phytochemical composition: The phytochemicals were synthesized for the defense and other biological functions in plants, with multiple health benefits at lower concentrations[18]. Phytic acids have been reported to possess anti-oxidant, anti-cancer, hypocholesterolemic and hypolipidemic effects, thereby preventing oxidative browning in fruits and vegetables by inhibiting polyphenol oxidase.

On the other hand, saponins reduce the absorption of certain nutrients like glucose and cholesterol at the gut, thereby preventing diabetes and cardiovascular diseases[18]. Alkaloids were also known to show medicinal as well as physiological activities[19]. Tannins and flavonoids have been shown to have biological activities that were beneficial in the prevention and treatment of many ailments[20,21]. Despite the beneficial effects of phytochemicals[22], observed that at high dosage, they are detrimental to human and animals' health. Tannins is known to depress growth by decreasing proteins quality and digestibility, cause liver damage and inhibit absorption of minerals such as iron which leads to anaemia. Studies have shown that areca nut, walnut and jatropha contain higher concentrations of phytic acid, oxalic acid, alkaloids, saponins and tannins when compared with those of mature and immature ball nut seed meals. The hydrogen cyanide concentration in the ball nut meals were lower compared to that of jatropha meal which was reported as 60.70 mg kg^{-1} [13]. Lower concentrations of the phytochemicals/anti-nutrients in the mature and immature ball nut seed meals imply that the seeds can be used in compounding feeds for animals without detrimental effects on performance.

Minerals composition of mature and immature ball nut seed meal: Minerals play important roles in health and nutrition, calcium and phosphorus are very essential for bone metabolism and assists in teeth development. Calcium is a cofactor of the three important enzymes, pyruvate dehydrogenase complex, isocitrate and a ketoglutarate dehydrogenase complex in the citric acid cycle. Iron plays important roles in many proteins and enzymes, notably in haemoglobin to prevent anaemia[14], while magnesium is also a cofactor for many enzymes. Sodium and potassium are very important for nerve transmission and osmolality of body fluid. The presence of minerals in the diet are generally required for normal growth, activities of muscle and skeletal development, cellular activity and oxygen transport, chemical reaction in the body and intestinal absorption, fluid balance and nerves transmission as well as the regulation of acid-base balance[23]. Low minerals consumption could lead to in proper proportion, reduction in production, infertility problems and weakness of the bone and increased incidences of non-infectious diseases. The minerals composition of ball nut seed meals observed in this experiment were lower than 2.89% for iron, 265.95% for phosphorus, 59.77% for magnesium, 44.99% for calcium, 24.08% for potassium and 9.59% for sodium reported by Ojobor[12] for walnut seed.

Amino acids composition of the mature and immature bull nut seed meal: From the recommended FAO and WHO[24] provisional pattern for some nuts and seeds, the ball nut seeds have been found to be lower with respect to aspartic acid, arginine, asparagine, glycine valine, threonine, methionine, serine, histidine and hydroxyproline but were adequate in tyrosine, norvaline, isoleucine, sarcosine and proline. Like in other oil seed cakes and cereal grains, it is essential to supplement the protein of the ball nut seed meal with the synthetic amino acids to take care of the deficiency for effective utilization of the other amino acids present in the meal. Failure to meet the requirement of any of the essential amino acids has serious health implications and can result in degradation of the body's proteins. The concentration of amino acids reported by Monforte-Braga *et al.*[25] showed that maize contained 3.7 g kg^{-1} of cysteine, 4.2 g kg^{-1} of arginine, 4.3 g kg^{-1} of phenylalanine, 3.1 g kg^{-1} of histidine, 2.5 g kg^{-1} of isoleucine, 8.8 g kg^{-1} of leucine, 4.8 g kg^{-1} of lysine, 2.1 g kg^{-1} of methionine, 1.8 g kg^{-1} of threonine and 3.5 g kg^{-1} of valine as essential amino acids. Similarly, the authors also reported 1.9 g kg^{-1} of cystine, 5.6 g kg^{-1} of arginine, 5.9 g kg^{-1} of phenylalanine, 4.2 g kg^{-1} of histidine, 4.2 g kg^{-1} of isoleucine, 13.6 g kg^{-1} of leucine, 4.7 g kg^{-1} of lysine, 1.4 g kg^{-1} of methionine, 2.1 g kg^{-1} of threonine and 5.3 g kg^{-1} of valine as essential amino acid composition in sorghum.

CONCLUSION AND RECOMMENDATION

From the results of this study, it was concluded that there was no significant difference in the nutrient composition between the immature and mature ball nut seed meals and therefore, could be utilized as alternative energy source in animal diets, owing to their high energy level, amino acids profile as well as low anti-nutritional factors.

Preliminary feeding trial using animals to determine the feeding value of this promising feed resource is hereby recommended.

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