E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(5): 380-385

www.fisheriesjournal.com
Received: 21-07-2020
Accepted: 23-08-2020

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Nutritive and phytochemical assessment of cotton (*Gossypium spp.*) seed meal for fish feed

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Abstract
The Proximate, Phytochemical and Mineral analysis of mechanically extracted cotton seed meal was carried out in a laboratory. The samples were collected from three different retailers of cotton seed cake in Abuja 'Yan Dusa, a local market along Club Road in Kano state. The proximate analysis shows that cotton seed cake has 3.2 moisture content, 6.9% ash, 3.2% lipid concentrations, 14.16% crude fiber content, 26.02% crude protein and NFE 46.2%. The phytochemical analysis shows that cottonseed cake has 0.8% flavonoid, 2.4% concentration of alkaloid, 0.4% concentration of Saponin. The glycoside concentration was 0.3% and Tannin finally recorded 0.9%. In the other hand, the mineral content of Cotton Seed meal (CSM) shows that the cake has 85.05mg/kg concentration of sodium, 8694.2mg/kg concentration of potassium. 0.8578mg/kg was recorded for calcium. 0.3055mg/kg was recorded for magnesium and finally 3.71mg/kg was recorded for phosphorous. The result shows that Cotton Seed meal (CSM) contained nutritive values and phytochemical constituents. It could be used as a protein supplement in fish feed formulation, more research should carry out on the processing of cotton seed cake to remove the anti-nutritional factors.

Keywords: Nutrient contents, phytochemical, Mineral, cotton seed cake

Introduction
Animal's feedstuffs have been in the decline in recent years, because of the diminishing output of certain traditional crops. Statistics show that the country relies on imports to meet the needs of an expanding livestock and aquaculture industry [1]. This has reaches in the increase in prices of feed resources which intensify the already high cost of fish feed which have been a major problem to fish farmers in Nigeria [2]. The feed cost constitute 40 to 60 percent of the recurrent cost of most intensive fish farm ventures which affect the economic viability of the farm when cheaper alternatives are not available [3]. Fish meal is the most commonly used in fish feed which is considered to be the best ingredients due to its protein requirement of fish [4]. The anti-nutrients component of feedstuff has to be inactivated by thermal or non-thermal processing before being used to feed fish. This may be done to increase the palatability of food, increase its quality for fish feed, minimizing risk of food spoilage and produce good product from the basics ingredients. According to [5] there are several anti-nutriti
onal factors that are very significant in plants used for human foods and animal feed. Trypsin present in soybean is factors that can interfere with the utilization of its protein [6]. reported that Moringa leaves contain negligible amount of tannins, but high level of crude saponins (5%) which may have anti nutritional effects on animals.
Research on the nutritive value of some fish feed ingredients of both plant and animals sources such as plantain [7], poultry offal [8], maggot meal [9, 10], Calabash Seed [11], calabash Seed Meal [12] and water hyacinth [13] were reported. There was dearth of information on the nutritive value of CSM. Despite all those studies, the
re indispensible to study the nutritive value of CSM. The aim of this research is to evaluate the nutritive value and the phytochemicals content of cotton seed meal

Materials and methods
Experimental location
The research was carried out in Agricultural chemical laboratory and Biochemistry laboratory of Usmanu Danfodiyo University Sokoto. Sokoto is located in the extreme North West of Nigeria; near the confluence of Sokoto River and River Rima. The state is within the longitude

11° 30-130° 50E and latitude 4°-6° 40N [14]. Sokoto state is located in the dry Sahel surrounded by sandy savannah and isolated hills with an annual average temperature of 28.3°C (82.9°) there are two seasons; the Dry and Wet season. Dry season begins in from October to
April and extends to May/June in some parts. Wet season starts in May and ends September to October. Harmatan is experienced between November and February. Heat is more severe around March and April.

Samples Collection
Cotton Seed cake was obtained from three different retailers of cottonseed cake in Abuja Yan Dusa, local market along Club Road in Kano state.

Chemical Composition of Sample Materials
The moisture content was determined by drying in an oven at 100-105º C to constant weight [15]. The crude protein content was evaluated by digestion of the sample. Nitrogen determination was done using a spectrophotometer as described by [16]. The crude protein is obtained by multiplying the quantity of nitrogen by the coefficient 6.25. Total lipids were determined by continuous extraction in a Sox let apparatus for 8 hours using hexane as solvent, ash by incinerating in a furnace at 550^0 C, crude fiber by sequential hot digestion of the defatted sample with dilute acid and alkaline and carbohydrate was calculated by difference of 100 with the sum of all contents obtained [15].

Minerals analysis
A number of methods, ranging from simple manual to automatic complex procedures, have been developed for the determination of various cations in plant extract. The methods make use or the simple forms of the common instruments generally available in analytical laboratories examples: colorimeters, flame photometers and atomic absorption spectrophotometers [17].

Determination of Potassium (K) and Sodium (Na)
Apparatus
Flame photometer
Reagents
K Standard, 100mg/l in aqueous solution: KCI 0.1907g/1.
Na Standard, 10Omg/l in aqueous solution: NaCl 0.2542g/l
Range of standard solutions
(a) K: 0, 2, 4, 6, 8, 10mg/l K OR 0, 5, 10, 15, 20 mg/l K OR 0, 10, 20, 30, 40 mg/l K
b) Na: 0, 2, 4, 6, 8, 10 mg/l Na

Procedure
Set up the flame photometer according to the instruction in the instrument manual.
Calibrate the instrument readout using the standard solution. Set the meter at zero while aspirating distilled water or blank solution. Set meter reading at 100% E while aspirating the top concentration of your standards. Record your reading of all the intermediate standard solutions. Plot the standard curve on a linear graph paper.
Aspirate the sample solution and record the reading (% B). (Check 0 and 100% E reading with 0 and top standard after every 10 to 20 sample determinations).
Read the concentration of the element in sample solution and calculate K and Na contents in plant tissue.
Note: Standard solutions should contain the same amount of reagents as in plant tissue digests.
Calculation

$$\%K \text{ or } \%Na = \frac{TV \times 0.01 \times 100}{20}$$

TV=Titre value

Determination of Calcium (Ca)
1ml of sample solution into titration flask add 19ml of distill water and 1ml of 10% NaOH add a tip of Murexide indicator and titrate against 0.01M EDTA from pink to purple color end point. Record the titer value.
Calculation

$$Ca = \frac{TV \times 0.01 \times 100}{20}$$

TV=Titer value

Determination of Phosphorus (P)
Pipette 2ml of sample into 50ml Volumetric flask add 2ml of phosphorus extraction and add Ammonium molybdate, add distill water to half of bottle and 1ml of dilute Stannous chloride and make to volume 50ml and take Absorbance reading using Spectrophotometer at 660 wave length.
Calculation

$$P = \frac{0.61x \ 25 \times 25}{\text{atomic weight of p (50.9)}}$$

Abs= Absorption reading

Determination of Magnesium (Mg)
1ml of sample solution into titration flask add 19ml of distill water and 5ml of buffer solution and 3 drops of Eriochrome black T indicator and titrate against 0.01M EDTA from purple to blue end point. Record the titre value.
Calculation

$$Mg + Ca = \frac{TV \times 0.01 \times 100}{20}$$

TV = Titre value

The titration is a measure of the total Ca and Mg' present in the sample, therefore the Magnesium can be calculated using the below formula:

%Mg = 2Mg + % Ca - % C

Phytochemicals Analysis
Determination of Flavonoids
Procedure
A weight sample five (5) gram of water lily was hydrolysed by boiling in 100mls of hydrochloric acid solution for about 35 minutes. The hydrolyte was filtered to recover the extract (filtrate). The filtrate was treated with ethyl acetate drop. The precipitated flavonoid was recovered by filtration using a weight filter paper after drying in the oven at 100°c for 30 minutes. It was cooled in a desiccator and reweighed. The difference in weighted gave the weighed of flavonoid which was expressed as a percentage of the weight of sample analysed [18].

Calculation

$$\% \text{ flavonoid} = \frac{W^2 - W_1}{5g} \times 100$$

Where
w1= weight of empty filter paper
W2= weight of paper + flavonoid precipitate
W3= weight of sample

Determination of Total Alkaloids

Principle

As with most alkaloids, the alkaloid Salt is soluble in water, while the alkaloid free bases are soluble in organic solvent. This fact is made use of in extraction of the free alkaloids with organic solvent [19].

Procedure

Fifty (50) gram powdered plant sample (Bark) was extracted with a liter of methanol mixture and solvent evaporated. The resultant residue was mixed with 200ml of 0.0025M H2SO4 and partitioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and was then extracted with excess chloroform to obtain the alkaloid fraction or separated by filtration. The chloroform extraction was repeated several times and the bulk extract was concentrated to dryness. The Alkaloid was weighed and the percentage was calculated with reference to the initial weight of the powder.

Calculation

$$\% \text{ Alkaloid} = \frac{W^2 - W_1}{5g} \times 100$$

Where
W1= weight of empty filter paper
W2= weight of filter paper +Alkaloid
W3= weight of sample

Determination of Saponins

Saponins were determined using method of [20].

Principle

Saponins are soluble in water or boiling dilute alcohol and are precipitated on the addition of acetone.

Procedure

From powdered plant extract, five (5) gram was place in a 250ml flask containing 30ml of 50% alcohol. The mixture was boiled under reflux for 30 minutes and was immediately filtered while hot through a coarse filter paper. Two (2) grams of charcoal was added; the content was boiled and filtered while hot. The extract was cooled (some Saponins may be separated) and an equal volume of acetone was added to complete the precipitation of Saponins. The separated Saponins were collected by decantation and dissolved in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter. The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of Saponins. The separated Saponins were collected by decantation and suspended in about 2 ml of alcohol and filtered. The filter paper was immediately transferred to a dissector containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference of extract used.

Calculation

$$\% \text{ Saponins} = \frac{W_2 - W_1}{5g} \times 100$$

Where
5g = weight sample
W1= weight of filter paper
W2= weight of filter paper+ sample.

Determination of Tannins

Tannins were determined by the method of [19].

Principle

The method is based on quantitative consumption of tannins and pseudotannins to iodine in alkaline medium, a character which is attributed to their phenolic nature. True tannins, in contrast to pseudotannins can be removed from the extract by precipitation with gelatin. This can permit the determination of each group of constituents alone. Excess iodine is determined by titration rendering acidic with sodium thiosulphate standard solution.

Procedure

Powdered sample (0.1) was put into a 100cm^3 conical flask and 50cm^3 volumetric flasks. The residue was washed several times and the combined solution made up with distilled water to 0,1,2,4, and 5cm^3 of the standard tannic acid and 10 cm^3 of the sample solution in a 50 cm^3 volumetric flask, 2.5cm^3 Folin-Denis reagent and 10cm_3 of Na_2CO_3 solution were added and made to volume with distilled water. The flask was allowed to stand for 20 minutes after which optical density was measured at 760nm. The calibration curve was plotted from which the concentration of tannic acid in the sample was extrapolated.

Calculation

% tannins = Abs of sample over Abs of standard × conc. of standard (100mg %)

Where
Abs= Absorbance of the color at 760nm

Determination of Glycosides

Glycoside is determined using spectrometric method [20].

Procedure

One (1) gram of the extract was extracted in 10ml of 70% alcohol and mixture was filtered. From the filtrate, 8ml of the mixture was added to 8ml of 12.5% lead acetate (to precipitate resins, tannin and pigments). The mixture was shaken well, completed to volume (100ml) with distilled water and filtered. The filtrate (50ml) was pipetted into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution (to precipitate excess lead) was added. The mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice through a whatman No. filter paper. Baijet reagent (10ml) was added to 10ml of purified filtrate. A blank sample of 10 ml of distilled water was also added to 10ml Baijet reagent. The two were allowed to stand for one hour (time maximum for color development). The intensity of the color was read at 495 nm using spectrometer against a blank (20ml distilled water). The color was stable for several hours.

Calculation

$$\% \text{ glycosides} = \frac{abs\ of\ sample}{17} \times 100$$

Where
Abs= Absorbance of the color at 495nm

Statistical analysis
Data obtained were subjected to analysis of variance (ANOVA) and, the treatment means were separated for significant differences following the procedure of Duncan's Multiple Range Test [21]. All the analyses were carried out using the computer software Statistical Package for the Social Sciences Version 9.0 for windows [22].

Results and discussion
Proximate analysis of Cotton Seed Cake
The result of proximate analysis of cotton seed cake is showed in Table 1. The moisture content of cotton seed cake was found to be 3.23±0.35% which was lower than the moisture content uncovered by [23, 24] which were 8.02 and 9.87% respectively. This variation was however as a result of the oil extraction method employed, storage and seed variety of the cake [25]. The moisture content found in all three samples was quite low in comparison with commercial standard which was 9.18% [26].
The Ash the content of cotton seed cake was 6.93±0.64% which was almost similar to 5.93% that was discovered by [27, 23] also analysed the Ash content of cotton seed cake and concluded that cotton seed cake contain 7.1% ash. Also reported by [24] cotton seed cake contains 4.55% ash. The slight variation in the ash content of the cotton seed cake was as a result of the oil extraction method, storage and seed variety [25] and can also be as a result of temperature [27].
The lipid/oil content of the cotton seed cake was less 3.22±0.56% compared to the lipid content reported by [28] which was 28.35%. In addition, the result differed from the value discovered by [24] who recorded 27.83 and 20.90% as reported by [23]. This variation is because the various methods used for oil extraction also explain the large range of residual oil present in cottonseed meal. Some solvent-extracted meals contain less than 2% oil, like the other major oilseed meals, but many cottonseed meals contain higher oil values, often in the 5-10% range, and over 20% is possible [29, 25]. Furthermore Cottonseed oil and protein contents can vary from 17 to 27% and 12 to 32%, respectively, among genetic variations [30, [31, 32].
The crude fiber of the cotton seed cake was found to be 14.16±0.61% which slightly differs from [27] which was 17.1%. [23] also found the crude fiber content of cottonseed cake to be 12.5 and 13.3% respectively, which were slightly differ from the result obtained in this study. The variation was however because the crude fiber content ranges from 2-2.7% in glandless and 7.9-16.0% in decorticated to 26.9% in un-decorticated form. Ether extract content varies from 4.2-11.3% in expeller and 0.9-2.9% in solvent extracted meals [33]. The fiber content may also vary accordingly, from 25% (non-dehulled) to 5% (fully dehulled) crude fiber [29, 25].
The crude protein of the cake analysed was 26.02±0.09% which was significantly higher than 21.1% obtained by [34]. In addition [27] also reported 22.06%. Furthermore, [35] reported a higher value of 31.23%, [24] also reported 27.27% crude protein. However these variations were because the protein content was highly variable as it depends on the amount of de-hulling and on the efficiency of oil extraction. The range of protein content was from 30% DM for non-de-hulled cottonseed meal up to 50% DM for fully dehulled meals [36]. Lower and higher values than these extreme have also been recorded [29, 25] respectively. In addition Cottonseed oil and protein contents can vary from 17 to 27% and 12 to 32%, respectively, among genetic variations [30, 31, 32, 37] found that N fertilization increased N levels in cottonseed with altered amino acid concentration.
The protein of cottonseed meal was found to be low in cystine, methionine and lysine [38]. It is also a good, though, variable source of thiamine but a poor source of carotene [39]. [40] had stated that the nutrient composition of the cotton seed cake varies according to climate, soil and moisture. Other factors that could be responsible for the results obtained in this trial could be due to processing techniques, length of storage, and variety among others [41].
The CHO of the cottonseed cake analysed was 46.21±1.114 that differed from the value obtained by [24] who reported 47.5% and [42] who uncovered 56.89%. The CHO variation was also affected by the temperature, seed variety, storage and method of oil extraction [25, 43] reported that varying planting dates or irrigation regimes altered cottonseed composition in terms of carbohydrate contents.

Mineral Analysis of Cotton Seed Cake
The result of mineral content of cotton seed cake is described in table 1 above and it shows that the cotton seed cake contain 85.05±0.39 mg/liter of Sodium and 8694.44±36.78 mg/liter of Potassium. The cotton seed cake is low in calcium with a value 0.85±0.02 mg/liter as well as magnesium 0.30±0.02mg/liter. 3.71±0.20mg/liter was the recorded value of phosphorous. The above result however presents a slight deviation from the result uncovered by [44] who analysed three different varieties of cotton seed cake which were obtained through a process involving de-hulling and solvent extraction which has 2.0g/kg, 12.4g/kg, 16.6g/kg, 0.3g/kg, 6.3g/kg concentration of Calcium, Phosphorus, Potassium, Sodium, Magnesium respectively and another cottonseed meal obtained by a process involving no or limited de-hulling and mechanical extraction (no solvent) which has 2.2g/kg, 11.9g/kg, 17.0g/kg, 0.2g/kg, 6.7g/kg concentration of Calcium, Phosphorus, Potassium, Sodium, Magnesium respectively. These variations discovered clearly shows that method of oil extraction in cotton seed cake significantly affects the mineral content. Other factors that may affect the mineral content of cotton seed cake may be as a result of long time storage and storage temperature [45].

Table 1: Proximate and minerals analysis of Cotton Seed Cake

S/N	Composition	Mean percentage%
1.	Moisture	3.23±0.23
2.	Ash	6.93±0.71
3.	Lipid	3.22±0.77
4.	Crude fiber	14.16±0.58
5.	Crude protein	26.02±0.10
6.	NFE	46.21±01.14
	Minerals	**Concentration (mg/kg)**
1.	Sodium (Na)	85.06±0.39
2.	Potassium(K)	8694.44±36.780
3.	Calcium(Ca)	0.85±0.02
4.	Magnesium(Mg)	0.30±0.02
5.	Phosphorous(P)	3.71±0.28

Phytochemicals analysis of Cotton Seed Cake

Table 2, describes the mean concentration of five important phytochemicals found in cotton Seed Cake.

The flavonoid concentration found in the cotton seed cake 0.80±0.40% which slightly differs with 1.2 which [45] uncovered. This variation was though because of long term storage.

In the other hand the alkaloid concentration of cotton seed cake was 2.4±0.40% which was the same range with 9h in [27] and [45] who turn up with 2.1 and 2.4 respectively. This little variation was as a result of long time storage and storage temperature [45].

The Saponins concentration as shown in table 2 was 0.40±0.20% which was differs from [27, 26] who discovered 1.38 and 0.97% respectively. The variation was also as a result of the storage facility temperature, as well as Seed variety [26].

The Glycoside value obtained was 0.39±0.01% which was in line with [45] who discovered 0.31% and [27] who reported 0.46%. This variation may perhaps be as a result of the method of oil extraction deployed and/or seed variety.

Finally, tannin concentration of cotton seed cake was found to be 0.96±0.01% which was in the same range with [27] who reported 0.48± 0.66% and [45] who uncovered 0.87%. This little variation was as a result of long time storage and storage temperature.

Table 2: Phytochemicals analysis of cotton seed cake

S/N	Phytochemical	Mean content%
1.	Flavonoid	0.80±0.40
2.	Alkaloid	2.40±0.40
3.	Saponins	0.40±0.20
4.	Glycoside	0.39±0.01
5.	Tannin	0.96±0.01

Conclusion and recommendation

The result shows that Cotton Seed meal (CSM) contained nutritive values and phytochemical constituents. It could be used as a protein supplement in fish feed formulation, more research should carry out on the processing of cotton seed cake to remove the anti-nutritional factors.

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