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Evaluation of flaxseed, sesame and pumpkin seeds as an alternative source of functional feed ingredients

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Target Audience: *Feedmillers, Farmers, Analysts*

Abstract

This research work presents the proximate, minerals and anti-nutritional factors of edible oil seeds; flaxseed, sesame and pumpkin seeds. The seeds were procured from an open market in Umuahia, Abia State and analyzed following standard procedure. Results from the proximate analysis showed that the seeds had appreciable amount of dry matter (93.13%; 95.78% and 93.92%), crude protein (19.84%; 26.23%; 29.83%), also with a high value for ether extract which are 43.87%; 52.88% and 48.77% respectively. The seeds also had lower value of crude fibre (9.66%; 5.17%, 6.16%). The mineral analysis showed that the seeds had sodium (0.03%; 0.60%; 0.43%), potassium (0.75%; 1.27%; 0.90%), calcium (0.24%; 1.18%; 0.68%) while the micro minerals showed that flaxseed, sesame and pumpkin seeds had appreciable concentration of zinc (43.90mg/kg; 35.41mg/kg; 31.97mgkg), copper (14.71mg/kg; 6.13mg/kg; 5.68mg/kg) and iron (55.78mg/kg; 94.58mg/kg; 78.58mg/kg). The anti-nutritional factor constituents of flaxseed, sesame and pumpkin seeds showed that phytate concentrations were 0.88%; 0.12% and 0.21% for flaxseed, sesame and pumpkin seeds respectively. The oxalate recorded a value of 0.39%; 0.09%and 0.11% for flaxseed, sesame and pumpkin seeds, while saponin concentration was 0.36% for flaxseed, 0.20%for sesame seed and 0.23% for pumpkin seed. Tannin had concentration of 0.01% for flaxseed, sesame and pumpkin seeds respectively while trypsin inhibitors were 26.77mg/g, 28.67mg/g and 31.05mg/g for flaxseed, sesame and pumpkin seeds respectively. This study showed that the seeds of flaxseed, sesame and pumpkin can be good sources of protein, ether extract, carbohydrate and minerals. Therefore, these seeds can be exploited as commercial source to supplement livestock feedstuffs.

Keywords*: Seeds, Proximate, Minerals, Anti Nutrients and Livestock.*

Description of Problem

The increase in human population had placed significant pressure on food production which is greatly affected by global warming, economy, skill development and insecurity especially in third world countries. Despite the global pandemic (Covid-19) which had greater effect on human mortality rate and economies of nations, the quest for food production to meet with demands had consistently increased. In Nigeria specifically, poultry industry growth index as at mid-2020 was estimated to be around 2 million birds with a total value of over ₦16 trillion (1). Thus, there is need for significant investment in livestock production in other to sustain the growing demands and population. Thus, livestock production, poverty, food security, people's health and nations economy are inextricably linked.

Meeting the nutritional requirements of

the animals with the objective of optimizing digestibility, absorption of nutrients and reduced cost of feed is certainly one of the most important elements (2). Cost of production of livestock which is associated with the cost of feed is also related to the cost of ingredients especially protein and fat sources which contributes about 30%-40% of the total composition of feeds, are expensive, examples include; soya bean meal, fish meal, groundnut cake, full fat soya, soya oil, poultry meat meal etc. With these trends, researchers have continued to search for cheap, sustainable alternatives on how to reduce cost of production of feed despite increase in cost of ingredients.

Edible seeds such as flaxseed, sesame and pumpkin seeds had been recognized as suitable alternatives. Flaxseeds, also known as Linseed is one of the most important oilseed crops for industrial as well as for food and feed purposes. The Latin name of flaxseed is *Linum usitatissimum*, which means “very useful”. Every part of the seed is utilized either directly or after processing. The seed provides oil rich in omega-3, digestible proteins and lignans. The seed is also unique among oilseeds because of its exceptionally high content of α-Linolenic acid (ALA, 18:3n-3). It is known that fatty acid profile of poultry meat and fat is directly affected by the source of fat in diet. Therefore, it is possible to change the fatty acid profile, especially the ratio of omega-3 fatty acids, by feeding flaxseed (3).

Sesame seed (*Sesamum indicum* L.), another widely consumed seed, is a good source of omega-6 fatty acid. The world largest producers of sesame are India and China, followed by Sudan, Mexico, Nigeria, Turkey, Uganda an Ethiopia (4). It is commonly known as Benniseed in Nigeria. Although sesame seed is primarily used as a source of edible oil for cooking, it is also used in other alternative ways important to human and livestock nutrition. Like every other edible seed, it can sustain livestock production by ensuring the availability of various sources of nutrients that are required for the formulation of animal feed (5). It had been reported that Sesame seeds has high protein content with high levels of methionine and tryptophan (6) which are essential for metabolism and optimum performance of livestock.

Pumpkin seed can be consumed either by boiling or frying (7). They serve as good source of lipids, proteins, carbohydrates and other nutrients that are important for maintaining good health (8). Pumpkin seeds have exhibited pharmacological properties such as anti-diabetic (9), antifungal, anti-bacterial, anti-inflammation and can serve as an antioxidant (10). Researches have shown that pumpkin seeds do not only contain nutritionally important bio-compounds but are also sources of other phyto-compounds which at certain levels have significant anti-nutritional effects (11).

It is evident that these seeds contained adequate amount of nutrients that can be made available for livestock production, it is imperative to document the nutritional value of these locally available seeds. It is therefore the aim of this study to provide proximate, minerals and anti-nutritional components of these selected seeds.

Materials and method

Procurement and processing of experimental material

The flaxseed, sesame and pumpkin seeds were purchased from the open market in Umuahia (Abia State, Nigeria) metropolis. Each seed was grinded to fine powder using an electric blender. Thereafter, they were packed separately into a zip lock bag and transferred to Institute of Agricultural Research and Training Laboratory in Ibadan for further analysis.

Proximate compositions and gross energy of Flaxseed, Sesame and Pumpkin seeds

Samples of test ingredients (flaxseed, sesame and pumpkin seeds) were analyzed for proximate constituents according to the methods of A.O.A.C (12). The gross energy was determined using adiabatic bomb calorimeter.

Mineral determination

Minerals were analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled, de-ionized water with a few drops of concentrated hydrochloric acid. Sodium, calcium and potassium were determined using a flame photometer (Model, 405, Corning, UK) using NaCl and KCl to prepare the standards. Mg, Cu, Zn, and Fe were determined by atomic absorption spectrophotometry using buck 600 AAS. Phosphorus, selenium and manganese were determined using spectric 21D digital spectrometer. All determinations were done in triplicate.

Determination of anti-nutritional factors

Determination of Phytate

Phytate was determined using the method of Maga (13) where 2g of sample was weighed into 250ml conical flask. 100mls of 2% Hydrochloric acid was added to soak each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50ml of each filtrate was placed in 0.50ml conical flask and 107mls distilled water was added in each case to give proper acidity. 10mls of 0.3% Ammonium Thiocyanate (NH_4SCM) solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195g Iron per ml. The end point was slightly brownish-yellow which persisted for 5 minutes. The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{Wt. of Sample}}$$

Determination of Oxalate

2g of sample was boiled in 40ml of water for 30 minutes in a reflux condenser. 10ml of 20% Na_2Co_3 was added and boiled for another 30 minutes. The liquid extract was filtered and washed with hot water until the wash water showed no alkaline reaction. The combined wash water and filtrate were concentrated to a small volume and cooled. With constant stirring, HCL (1:1) was added drop wise until the final acid concentration after neutralization is about 4% at which stage a heavy precipitate appeared (which was allowed to flocculate). The extract was carefully filtered into a 250ml flask and made up to mark. Kept overnight, then filtered supernatant liquid through a dry filter paper in a dry beaker. An aliquot of this filtrate was taken in a 400ml beaker, diluted with water to 200ml and made just ammoniacal, and reacidify with Lactic Acid. In the cold, medium, 10ml of a 10% calcium chloride solution was added and stirred well to include calcium oxalate precipitate to appear, and allow it settle overnight. The clean supernatant liquid was carefully decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. The precipitate in HCL (1:1) was thereafter dissolved. Oxalic acid is re-precipitated by adjusting the pH with ammonium hydroxide solution. The contents were boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution.

Calculation:

1ml of 0.05N $KMNO_4$ = 0.00225 anbydrous Oxalic Acid

= % Oxalic Acid

$$= \frac{\text{Titre value x 0.00225}}{2} \text{ x } \frac{100}{1}$$

$$= \text{T.V x 0.1125.}$$

Determination of Saponin

The Spectrophotometric method of (14) was used for Saponin Analysis, where 1g of finely ground sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a whatman No1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of maganesium carbonate was added. The mixture obtained with saturated MgCO3 was again filtered through a Whatman No1 filter paper to obtain a clear colorless solution. 1ml of the colorless solution, was pipetted into 50ml volumetric flask and 2ml of 5% FeCL3 solution was added and made up to mark with distilled water. It was allowed to stand for 30min for blood red color to develop. 0-10ppm standard Saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2ml of 5% FeCL3 solution as done for 1ml sample above. The absorbance of the sample as well as standard saponin solutions were read after color development in a Jenway V6300 Spectrophotometer at a wavelength of 380min

$$\%\text{Saponin} = \frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt.of sample X 10000}}$$

Determination of tannins

Tannin in the test feedstuff was determined according to the method of (13). 2g of sample was weighed into a beaker and soaked with solvent mixture (80mL of acetone and 20mL of glacial acetic acid) for 5 hours to extract tannin. Each filtrate was in the water-bath for 4 hours, after which the filtrates were removed. The samples were filtered through double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 10ppm to 50ppm. The absorbencies of the standard solution as well as that of the filtrates were read at 500nm on a spectronic20. The percentage tannin was calculated using the formula:

$$\%\text{ tannin} = \frac{\text{absorbance x average gradient x dilution factor}}{10{,}000}$$

Determination of trypsin inhibitor

Determination of trypsin inhibitor was carried out according to the procedure outlined by (15). This involved weighing of 0.2g of the sample into a screw cap centrifuge tube. Also, 10mL of 0.1M phosphate buffer was added and the contents shaken at room temperature for 1 hour on a UDY shaker. The suspension obtained was centrifuged at 5000rpm for 5 minutes and filtered through Whatman No. 42 filter paper. The volume of was adjusted to 2mL with phosphate buffer. The test tube was placed in water bath, maintained at 370C. Again, 6mL of 5% TCA solution was added to one of the tubes to serve as a blank. Then, 2mL of casein solution was added to all the tubes, which were previously kept at 370C. The content was incubated for 20 minutes. The reaction was stopped after 20minutes by adding 6mL TCA solution to the tube and was shaken using UDY shaker. The reaction was allowed to proceed for 1 hour at room temperature. The mixture was filtered through Whatman No. 42 filter paper. Absorbance of filtered sample and trypsin standard solutions was read at 280nm. The trypsin inhibitor in mg/g was calculated using the formula:

$$\text{T.I. mg/g} = \frac{\text{A standard - A sample x Dilution factor}}{\text{0.1g x sample wt in g 1000 x sample size}}$$

Data analysis

All data analyses were done using IBM® SPSS version 20.0. The data were then subjected to descriptive analysis where the separation of means and standard deviation was computed.

Results and Discussion

Table 1: Proximate composition of flaxseed, sesame and pumpkin Seeds

Parameters %	Flaxseed	Sesame seed	Pumpkin seed
Dry matter	93.13 ± 0.06	95.78 ± 0.02	93.92 ± 0.04
Crude protein	19.84 ± 0.08	26.23 ± 0.08	29.83 ± 0.07
Ether extract	43.87 ± 0.03	52.88 ± 0.02	48.77 ± 0.02
Crude fibre	9.66 ± 0.02	5.17 ± 0.02	6.16 ± 0.04
Ash	3.37 ± 0.03	6.57 ± 0.03	2.84 ± 0.03
Nitrogen free extract	23.27 ± 0.00	9.21 ± 0.01	12.44 ± 0.07
Gross energy (kcal/g)	5.36 ± 0.00	5.71 ± 0.00	5.82 ± 0.00

± Standard deviation

The result of the proximate composition of flaxseed, sesame and pumpkin seeds are presented in Table 1 where flaxseed was detected to have dry matter (DM) content of 93.13%, crude protein (CP) of 19.84%, ether extract (EE) of 43.87%, crude fibre (CF) of 9.66%, ash component of 3.37%, nitrogen free extract (NFE) content of 23.27% and gross energy of 5.36 kcal/kg. The DM reported is within the range reported by (16) while the CP was found to be 19.84% which is similar 20.30% as reported by (3). The ether extract (EE) content reported (43.87%) is relatively higher than 37.10% reported by (16), 33.60% by (17) but less than 45.20% reported by (15). The variation in EE may be due to environmental conditions and so as for other constituents. The average fibre content reported 9.66% is higher than 3.08% as presented by (18). The chemical composition of flaxseed depends upon growing environment, genetics and processing conditions. The above result showed that flaxseed is a good source of fat, protein and carbohydrates

Sesame seed recorded DM 95.78%; CP 26.23%; EE 52.88%; CF 5.17%; Ash 6.57%; NFE 9.21% and gross energy of 5.71 kcal/g. The chemical composition of sesame seed result showed above occurred in the following order; DM > EE > CP > NFE >Ash > Gross Energy > CF. DM content (95.78) is high which is an index of stability, quality, shelf life and also high yields (19). The crude protein 26.23 is higher than that reported by (20) (14.73%), this implies that the level of CP can qualify sesame seed as protein source of feed. The result further showed that sesame seeds are rich source of fat with the value reported as 52.88%, this suggest the seed is a potential source of edible vegetable oil and the extracted meal as a potentially vital source of carbohydrate, fibre and protein for livestock feed. The result of the CF at 5.17% which is lower than what was reported by (20). According to (21), values of ash and CF content are important in terms of the suitability of foo vegetables an digestibility. The presence of ash is a reflection of inorganic matter.

Pumpkin seeds had DM content of 93.92% which is similar to 95.00% as reported by (22). The result showed that the DM content was higher than other of vegetable spaghetti seeds (55%), and gardenia aqualla seed (51%) (23). The high dry matter content will give it a storage advantage. The crude protein an ether extract values of the see was found to be 29.83% and 48.77% respectively, which showed that the see can serve as a source of protein and also classified as oil seed for livestock feeds. The ash content of the seed is 2.84% which is lower than 5.50% as reported by (22). The percentage ash content gave an idea of on the inorganic content of the samples from where the mineral elements, which are expected to speed up metabolic processes, improve growth and development. The result of the CF is within the range of 6.16% which is lower than what was reported by (22). Although, fibre containing feedstuffs are known to expand the walls of the colon, easing the passage of waste, thus making it an effective anti-constipation, it also helps to lower cholesterol level in the blood and reduce risk of cancer.

Table 2: Macro mineral composition of flaxseed, sesame and pumpkin seeds

Parameters %	Flaxseed	Sesame seed	Pumpkin seed
Sodium	0.03 ± 0.00	0.60 ± 0.00	0.43 ± 0.00
Potassium	0.75 ± 0.00	1.27 ± 0.00	0.90 ± 0.00
Calcium	0.24 ± 0.00	1.18 ± 0.00	0.68 ± 0.00
Phosphorous	0.37 ± 0.00	0.49 ± 0.00	0.38 ± 0.00
Magnesium	0.36 ± 0.00	0.41 ± 0.00	0.39 ± 0.00

± Standard deviation

Table 3: Micro mineral composition of flaxseed, sesame and pumpkin seeds

Parameters mg/kg	Flaxseed	Sesame seed	Pumpkin seed
Zinc	43.90 ± 0.02	35.41 ± 0.02	31.97 ± 0.01
Copper	14.71 ± 0.02	6.13 ± 0.03	5.68 ± 0.01
Iron	55.78 ± 0.02	94.58 ± 0.02	78.58 ± 0.02
Manganese	32.86 ± 0.01	8.97 ± 0.02	7.37 ± 0.01
Selenium	0.08 ± 0.00	1.17 ± 0.01	0.10 ± 0.00

± Standard deviation

The result of the macro and micro minerals composition of flaxseed, sesame and pumpkin seeds is showed in Table 2 and Table 3 respectively. The sodium level for flaxseed is 0.03%; sesame seed 0.60% and pumpkin seed is 0.43%; this element is required by the body to regulate blood pressure and blood volume. It helps regulate the fluid balance in the body; it also helps in the proper functioning of the muscles and nerves (24).

Potassium is one of the most abundant elements found in flaxseed, sesame and pumpkin seeds (0.75%; 1.27% and 0.90%). High amount of potassium in the body was reported to increase iron utilization (25).

The calcium content of the samples was found to be 0.24%; 1.18% and 0.68% for flaxseed, sesame and pumpkin seeds respectively, calcium helps to ease insomnia and helps regulate the passage of nutrients through cell walls, without calcium the muscles in the body cannot contract correctly, the blood in the body will not clot and the nerves will not carry message. Calcium ions are also necessary for the normal functioning of nerves and muscles (26).

The concentration of Phosphorus in the samples were estimated as 0.37% for flaxseed, 0.49% for sesame seed and 0.38% for pumpkin seed, phosphorus is found bound in the blood and cells, while most of the non-skeletal phosphorus is inorganic in the form of nucleic acids, phosphor-lipids, ATP and sugar phosphate (24). Phosphates play important roles as buffers that prevent change in the acidity of body fluids because of their ability to combine with additional hydrogen ion. The combination with phosphorus makes it possible for nutrients to cross the cell membrane (27).

The concentration of Magnesium in the samples were 0.36% for flaxseed, 0.41% for sesame seed and 0.39% for pumpkin seed, magnesium is a constituent of bone and teeth and is closely associated with calcium and phosphorus. Magnesium is necessary for the release of parathyroid hormone and for its action in the backbone, kidney and intestine and for the reactions involve in converting vitamin D to its active form. Magnesium is important in tissue respiration, especially in oxidative phosphorylation leading to formation of Adenosine triphosphate (ATP). It is also involved in normal muscular contraction; calcium stimulates muscles while magnesium relaxes the muscles (27).

Flaxseed, sesame and pumpkinseed presented fairly high values for Zinc (43.90mg/kg; 35.41mg/kg; 31.97mgkg). Zinc plays a very important role in protein and carbohydrate metabolism and also help in mobilizing vitamin A from its storage site in the liver and facilitates the synthesis of DNA and RNA necessary for cell production (27).

The concentration of copper in the samples were found to be 14.71mg/kg for flaxseed; 6.13mg/kg for sesame seed and 5.68mgkg for pumpkin seeds, Copper is essential for cellular respiration, free radical defense, neurotransmitter functions and tissue biosynthesis (27).

The values for Iron in flaxseed are 55.78mg/kg while sesame seed had 94.58mg/kg and pumpkin with 78.58mg/kg, Iron performs several functions in the body; it helps in the formation of blood, it also helps in the transfer of oxygen and carbon dioxide from one tissue to another (27).

Manganese was 32.86mg/kg in flaxseed, 8.97mg/kg in sesame and 7.37mg/kg in pumpkin seed. Manganese plays an important role in all mental functions and aids in the transfer of oxygen from lungs to cells, it is important as an activator for enzyme reactions concerned with carbohydrate, fat and protein metabolism (24).

The concentration of Selenium in the samples were found to be 0.08mg/kg for flaxseed; 1.17mg/kg for sesame seed and 0.10mgkg for pumpkin seeds, Selenium is necessary for the normal functioning of the immune system and thyroid gland(28).

Table 4: Anti-Nutritional factors composition of Flaxseed, Sesame and Pumpkin Seeds

Parameters %	Flaxseed	Sesame seed	Pumpkin seed
Phytate	0.88 ± 0.00	0.12 ± 0.00	0.21 ± 0.00
Oxalate	0.39 ± 0.00	0.09 ± 0.00	0.11 ± 0.00
Saponin	0.36 ± 0.00	0.20 ± 0.00	0.23 ± 0.00
Tannin	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Trypsin Inhibitors (mg/g)	26.77 ± 0.01	28.67 ± 0.02	31.05 ± 0.01

± Standard deviation

The result of the anti-nutritional factors constituents of flaxseed, sesame and pumpkin seeds are presented in table 4, where the phytate concentration were 0.88%; 0.12% and 0.21% for flaxseed, sesame and pumpkin seeds respectively. The oxalate recorded a value of 0.39%; 0.09%and 0.11% for flaxseed, sesame and pumpkin seeds, while saponin concentration was 0.36% for flaxseed, 0.20%for sesame seed and

0.23%for pumpkin seed. Tannin had concentration of 0.01% for flaxseed, sesame and pumpkin seeds respectively while trypsin inhibitors were 26.77mg/g, 28.67mg/g and 31.05mg/g for flaxseed, sesame and pumpkin seeds respectively.

Generally, no feedstuff is free from toxic substances; it is the concentration of the toxic constituents that determine the edibility of the food. The concentration of anti-nutrients are highly variable; the levels that researchers find when they analyze a specific feedstuff probably depends on growing conditions, harvesting techniques, processing methods, testing methods and even the age of the feedstuff being tested (29). Phytic acid for example will be much higher in foods grown using modern

High phosphate fertilizers than those grown in natural compost (29). (30) reported that about 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds are in form of organically bound phytin phosphorus, respectively. Although the result obtained does not signify any deleterious effect as to when the materials are used as livestock feedstuff. Nevertheless, the concentration of this anti-nutrients can be further reduced into insignificant values by further processing such as boiling, roasting, fermentation, etc.

Conclusion and Applications

1. In conclusion the results obtained in this present study suggest that flaxseed, sesame and pumpkin seeds investigated can serve as an alternative source of protein, ether extract, carbohydrate, crude fibre and bioavailability of minerals.
2. Therefore, it can be exploited as an alternative feedstuff for livestock.

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