

Anti-Nutrients-Nutrients Profiles of Bambara Groundnut as Feeding Stuff and Implications in Nutrition and Health of the Fed Broiler-Chicken

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Abstract

The chemical composition, anti-nutrients and nutrients of raw Bambara groundnut seeds (BGNS) were assessed and the raw and processed BGNS (water-washed and ensiled), were subjected to biological, nutritional and biochemical evaluation using DOC broiler chicks as test animal models. Native BGNS was found to contain some high levels of secondary metabolites, alkaloids, flavonoids, haemagglutinins/lectins, trypsin inhibitors, saponins. High concentration of the primary metabolites, carbohydrate, protein, fat/oil, minerals, vitamins was as well recorded including the nuts nutraceuticals, carotenoids, ascorbic acid (vitamin C), total phenolic content (TAC) and total antioxidant capacity (TAC). Most of the measured phytochemical anti-nutrients and/or toxic factors or secondary metabolites could elicit harmful biological effects when ingested at certain levels in diet. Dietary inclusion of untreated BGNS beyond 10% in this study reduced parameters of feed intake and growth rate, $SGR(P < 0.05)$. Feed consumption and growth rate on the conventional diet were superior to those on the dietary raw and processed BGNS ($P < 0.05$). Dietary intakes of raw and processed BGNS improved some biochemical indices while reducing others such as those of the detoxifying tissue soluble liver enzymes ($P < 0.05$). Further research works are recommended to obtain conclusive results on some biological and biochemical parameters investigated in this study.

Keywords: Anti-Nutrients, Bambara, Feeding, Groundnut, Stuff-Health

Introduction

Wide distribution of biologically-active constituents exists throughout plants particularly those used in human nutrition and food animals feeding. The plants feeding stuffs are known to contain natural sources (toxins) and artificial (man-made) sources of chemical compounds. The natural chemical compounds in plants are called toxins or anti-nutrient-phytochemicals while the artificial toxicants include fertilizers, pesticides and so on. These chemical compounds are known to elicit both toxic and beneficial biological responses. The chemicals in plants (phytochemicals) are grouped into “primary” and “secondary” metabolites. Primary metabolites include carbohydrates, proteins, fats/oils, minerals, vitamins while secondary metabolites as some examples are alkaloids, coumarins, estrogenic principles, flavonoids, glycosides (cardiac and cyanogenic glycosides), haemagglutinins, lectins, oxalic, phytic, tannic acids, phenolic compounds, saponins, trypsin or protease inhibitors, terpenoids, tryterpenes (Soetan, 2008). Most of these secondary metabolites exert very poisonous biological responses when fed in diet at certain amounts. However, some have

advantageous effects and are widely used in nutrition and as pharmacologically-active agents (Soetan, 2008). Apart from cyanogenic glycosides that have been properly researched upon in commonly used food/feedstuffs, food or feed poisoning from plants natural toxins has not been adequately received attention in developing countries, Nigeria inclusive. There is therefore need to do research works to obtain data and information on the chemical composition of the more commonly used plants food/feedstuffs locally. This is to ascertain the composition of the plants chemicals since origin, climate, soil, processing methods, pesticides, fertilizers influence the phytochemical composition of plants obtained from a particular region.

Bambara groundnut, scientifically called *Vigna subterranean* investigated in this project is a leguminous crop indigenous to Africa where it has been cultivated for centuries. It is known for its attributes like drought tolerance, ability to thrive in poor soils, a vital crop for food/feed security in semi and arid regions (Hillocks *et al.*, 2012; FAO, 2019). Bambara is a close relative of Peanuts popularly known as groundnut or *Arachis hypogaea* (Hillocks *et al.*, 2012). Bambara ground nuts are primarily grown in West African countries including Nigeria but also cultivated in Central and Eastern African countries (FAO, 2019). Over the centuries, Bambara nut has become a staple food and an important protein source particularly in areas with harsh climate (Azam-Ali *et al.*, 2001). Like all legumes, *Vigna subterranea* contains anti-nutritional and/or toxic factors that can negatively impact on the nutrition and health when fed in diet raw and at certain levels (Chibabada *et al.*, 2017). Thus, even though Bambara nuts possess valuable nutrients, the presence of anti-nutritional factors therein necessitate careful feeding following proper research consideration before use for optimum nutrition and good health of humans and food animals.

Justification of the Project

The knowledge and data obtained following research works on the nutritional and/or anti-nutritional (toxic) substances present in Bambara nut used as food and feed and ways of reducing the anti-nutrients in the nuts to safe levels of consumption is therefore needed.

Objectives of the Study

The main objectives of the project are to:

- (i) determine the nutrients and anti-nutrients composition of Bambara ground nut in question.
- (ii) obtain data on anti-nutritional factors in the Bambara nut in order to adopt proper processing methods for maximum dietary inclusion therefore enhancing nutrients availability and utilization.
- (iii) evaluate data on feeding raw and processed Bambara nut in diets compared with the conventional diet.
- (iv) investigate on the toxicological effects of feeding raw and processed Bambara nuts at graded levels in diets using detoxifying enzymes activities as parameters of study.

Review of Literature

Bambara ground nut (BGN) is receiving research attention due to its potential as a versatile crop for food/feed security and agricultural sustainability. Research works are therefore ongoing on BGN based on nutritional properties, anti-nutritional content and for its potential uses in both human and animal nutrition (Adebayo *et al.*, 2019). Numerous studies have shown that BGN is rich in essential nutrients making it valuable for human consumption and livestock feeding (Murevanhema *et al.* 2019; Olayemi *et al.*, 2020; Adesina *et al.*, 2021). Bambara nuts contain high proportions of nutrients proteins (18-24%) and carbohydrates (50-60%), making the nuts suitable as energy and protein sources (Murevanhema & Jideani, 2015). The protein of BGN has a balanced amino acids profile with lysine and methionine, thus making it comparable to other legumes such as soyabeans used as a conventional plant protein source (Ijarotimi & Esho, 2009). In addition, the nuts contain significant mineral nutrients like calcium, iron, potassium, magnesium which are vital for growth and health (Murevanhema & Jideani, 2015). The high protein content of BGN suggests that it can be used as a valuable source of food/feed particularly in supplementing energy-rich but protein-deficient diets (Oluoch *et al.*, 2018). For instance, experimental trial to evaluate the effect of using Bambara nuts as protein source in the diet for growth and nutrients utilization showed significant higher performance indicating that Bambara ground nut was accepted and digested by the experimental animal models. Conclusively, it could be inferred from the study that BGN has potential to be used as a protein source that could support growth (Murevanhema & Jideani, 2015).

On the other hand, the presence of anti-nutritional factors in BGNs and inclusion in diet at certain amount beyond the normal threshold could negatively impact the nutritional value of the diet. BGN is reported to contain phenolic compounds like tannins, oxalates, phytates. Tannins can bind proteins and potentially reduce the digestibility of proteins while oxalates do bind calcium and other divalent cations affecting their digestibility and absorption (Chibarabada *et al.* 2017). Thus, while BGN possesses valuable nutrients, its inherent anti-nutritional factors necessitates research investigation for use as a feeding material. Besides, BGN anti-nutrients content of tannins, phytates, oxalates may limit its use in diets if not properly processed (Temba *et al.*, 2016). Tannins are found in higher concentrations in BGN more than in legumes like soyabeans. Although tannins exhibit antioxidant properties and can help protect against pathogens, they can interfere with protein digestibility and other nutrients by binding to proteins (Murevanhema and Jideani, 2013). The content of phytates in BGNs which are known to bind minerals such as calcium, zinc, other minerals lead to reduction of the minerals bioavailability by preventing minerals digestion and absorption (Chibarabada *et al.*, 2017). This property of BGN may limit the use of the nuts as a primary feed source for single stomached-animals like poultry and swine including human beings (Aremu *et al.*, 2006). Anti-nutrients like phytic and oxalic acids bind to minerals such as calcium, zinc, magnesium reducing their bioavailability and affecting the overall digestibility of the nuts in diet. Appropriate processing methods like water-washing, toasting, anaerobic ensiling have been shown to reduce the levels of the anti-nutrients making Bambara nuts a safe and more efficient food/feedstuff (Adeparusi, 2001).

Phytates levels can be reduced in diet by anaerobic fermentation, soaking which enhance the food/feedstuff nutritional value and mineral availability (Fasoyiro et al., 2006). Another efficient method of treatment to improve the nutritive value and ensure BGN nutrients use, digestibility and absorption to improve growth performance is to remove or decorticate the nuts coats by dehulling. Past works (Adeparusi, 2001) reported that processed Bambara nuts improved protein digestibility and protein availability making the nuts a potential food/feed ingredient in monogastrics and multiple-stomached feeding.

Bambara ground nuts are also reported to contain phytochemicals like iso-flavones which make certain consumers (females) sensitive or vulnerable to hormonal alterations for negative performance (Murevanhema and Jideani, 2013). BGN is noted to be high in phenolic compounds such as flavonoids, tannins which offer beneficial effects as anti-oxidants but could be harmful when fed at certain levels beyond the normal threshold level (Aremu et al., 2006).

BGN as well contains saponins which possesses immune-modulating effects and promote gut health. However, high levels of dietary saponins can lead to digestive issues especially in monogastric animals including humans and hindering of nutrients digestibility and absorption (Yeboah, 2017). Yeboah (2017) reported the content of alkaloids in BGN. Alkaloids are observed to possess bioactive properties which can also elicit toxicity if ingested in excess in diet, High level of alkaloids in BGN may make the nuts unsuitable for use in species that are sensitive to these chemical compounds like monogastric animals and man (Murevanhema and Jideani, 2013).

Materials and Methodology

Procurement of the test feedstuff (Bambara nut): One hundred and fifty kilogram (150kg) Bambara nuts was procured from a local market in Ilorin, Kwara State for use for the feeding trial. Nuts were divided in two parts: one part was ground raw for inclusion in diets 2, 3 and 4 at 10, 20 and 30kg/100kg diet. The other part (90kg) was divided into two equal parts of 45kg each. 45kg raw nuts was subjected to water-washing (soaking) in tap water for 48h while the other 45kg was treated under carbon dioxide environment (anaerobic ensiling) for 168h as described by Annongu *et al.* (1996). Nuts treated by water-washing were incorporated in diet 5 while BGN treated by ensiling was included in diet 6. The control or reference diet contained zero percent BGNS. BGN processed by water-washing and ensiling was properly sun-dried prior to pulverizing with a blender to suitable particle size for chicks at hatch (NRC, 1994).

Diets Formulation, Test Birds and Feeding Trial

Six experimental diets were formulated made of a maize-soybeans basal diet 1 and five other diets containing milled raw BGN at graded inclusion levels of 10, 20 and 30kg/100kg diet corresponding to diets 2, 3 and 4 while BGN treated by water-washing and ensiling was included in diets 5 and 6 at the fixed level of 45kg/100kg diet respectively. One



hundred and twenty six (126) broiler chicks at hatch of a given breed from a commercial source were used for the experiment and housed in an eighteen – units metabolic brooding cage with supply of electricity for the brooding period. The experiment will be designed as a one-way classification. Each dietary treatment was replicated thrice and a replicate contained 7-chicks. The composition of the experimental diets on as fed basis is shown in Table 1.

Table 1: Composition of the Experimental Diets on as fed basis (kg/100kg diet).

Diets	1	2	3	4	5	6
Ingerdients& Treatments	0%BGN	10%RAW BGN	20%RAW BGN	30%RAW BGN	45%WW BGN	45%AF BGN
Maize	59.27	51.00	42.00	34.00	24.00	24.00
Soybeanmeal	37.52	36.00	35.00	33.00	28.00	28.00
BGN meal	0.00	10.00	20.00	30.00	45.00	45.00
Bone meal	1.44	1.23	1.23	1.23	1.23	1.23
Oyster shell	0.52	0.52	0.52	0.52	0.52	0.52
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Dl-methionin	0.24	0.24	0.24	0.24	0.24	0.24
Lysine	0.24	0.24	0.24	0.24	0.24	0.24
Premix	0.52	0.52	0.52	0.52	0.52	0.52

BGN, Bambara groundnut, WWBGN, Water-washed. AFBGN, Anaerobic fermented, RawBGN.

Blood Collection

At the end of the experiment, one broiler chick per replicate was randomly taken and blood collected for the analyses of serum biochemical analysis. Blood samples for the serum biochemical analysis was first collected in labeled test tubes, allowed to stand for 10-15 min to separate into serum and corpuscles then centrifuged at 3000rpm to obtain clear sera. The sera samples were stored at -20°C until thawed and used for biochemical analysis.

Chemical Analyses

Proximate analysis or nutritional constituents of the native BGNS was carried out on dry matter, crude protein, crude fat (EE), crude fibre, mineral matter by the standard methods of AOAC (2005). Crude protein (CP) and total soluble carbohydrate were calculated by NX6.25 and by difference respectively. Chemical determinations of the nutraceuticals, carotenoids, ascorbic acid, total phenolic content (TPC), total antioxidant capacity (TAC) were carried out using procedures described by past research workers (Prieto *et al.*, 1999; Tambe and Bhambar, 2014). Analyses of the anti-nutritional factors, phytic, oxalic, tannic

acids, total phenolic compounds, haemagglutinins/lectins, trypsin inhibitors, flavonoids, saponins, alkaloids were conducted according to the standard methods of early research workers (Obadoni and Ochuko, 2001; Ifemeje *et al.*, 2014; Tambe and Bhambar, 2014; AOAC, 2005).

Determination of the detoxifying enzymes activities was carried out on the enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP/AP), Glutathione -S-transferase (GST) and superoxide dismutase (SOD). The enzyme ratios, AST/ALT and ALT/ALP was calculated from data obtained on the respective enzymes determined.

Data Analysis/Statistical Analysis

All data obtained on nutritional composition, nutraceutical constituents, anti-nutritional and/or toxic factors were subjected to descriptive statistics (SAS, 2008). Data obtained on performance characteristics and biochemical parameters including the enzymes activities of ALT, AST, AP/ALP, GST and SOD were analyzed statistically by analysis of variance (ANOVA) following the single factor design (CRD) model at 5% protection level. Significant differences between treatment averages were separated by using Duncan Multiple Range Test after Steel and Torrie (1980).

Results and Discussion

Table 1(a): Antinutrients constituents of native Bambara ground nut seed.

Antinutrients	BGNS*	SBS*	Unit
Alkaloids	13.60	0.01-0.05	%
Flavonoids	33.92	10-50	Mg/100g
Haemagglutinins/lectins	1400	1000-3000	HU/g
Oxalates/Oxalic acid	8.60	0.01-0.10	%
Phytates/Phytic acid	0.40	1.0-2.50	%
Saponins	9.21	0.5-6.00	%
Tannins/Tannic acid	33.20	0.1-0.50	%
Trypsin inhibitors	25.00	15-40.00	TIU/mg

*BGNS, Bambara ground nut seeds, *SBS, Soyabean seeds.

Anti-nutritional and/or toxic factors of the native Bambara ground nut seeds (BGNS) are presented in table 1(a). The phytochemical toxins are related to those of the soyabean as the conventional plant protein source. Whole BGNS (from Middle Belt of Nigeria) used in this study contain high levels of the phytochemicals, trypsin inhibitors, tannins, alkaloids, flavonoids, oxalic and tannic acids compared with the same phytochemicals in soyabean. Phenolic compounds (tannins-condensed, complex, soluble, flavonoids, phenolic acid) as contained in BGNS are reported to bind dietary proteins and reduce their digestibility and availability as well as other nutrients (Murevanhema and Jideani, 2013). Oxalates including phytates in the BGNS used in this investigation bind divalent cations such as calcium and affect their digestibility and absorption (Chibarabada *et al.*, 2017). Phytates or phytic acid in BGN chelate minerals like Ca, Zn, Mg and similar other minerals leading to reduction in the

minerals bioavailability. For these reasons, the presence of oxalic and phytic acids chemicals of raw or unprocessed feeding materials like BGNS in question may limit the use of the seeds as a protein feedstuff for simple stomached animals (monogastrics) including human beings (Aremu *et al.*, 2006).

However, processing methods like water-washing/soaking, ensiling/anaerobic fermentation, thermal treatments/toasting, boiling, cooking, autoclaving have been reported to reduce the levels of the anti-nutritional factors in such plants food/feedstuffs to make them safe for feeding to humans and food animals (Adeparusi, 2001). Water-washing for example, has been shown to improve nutritional value and minerals availability (Fasoyiro *et al.*, 2006). Past research works (Adeparusi, 2001) reported that processed BGNS enhanced nutrients (proteins) digestibility and availability making the BGNS a potential feeding stuff in monogastrics and multiple chambered (ruminants) animals.

BGNS used in this investigation contain flavonoids. Part of flavonoids like iso-flavones are plants oestrogens and mimic the function of these chemicals in humans and animals. This being the case, dietary intake of such phytochemicals in excess of the safe level makes some consumers, especially females, sensitive or vulnerable to hormonal alterations eliciting sub-optimal growth performance, physiological and hormonal imbalance issues (Murevanhema and Jideani, 2013). BGNS used for this research study contain the chemicals, saponins as alkaloids. Yeboah (2017) reported similarly, appreciable levels of the alkaloids in BGN and observed their high bioactive properties which do cause binding of cholesterol, lysing of red blood cells and other toxicity if ingested in diet at certain levels.

Table 1(b): Nutritional/ Proximate composition of BGNS compared with the conventional soyabenseed protein (%).

Nutrient	GGNS	Conventional soyabean
Dry matter	89.80	88-92
Crude protei	17.80	35-40
Crude fat (Ether Extract)	5.30	18-22
Crude fibre	4.46	4-6.00
Mineral matter/total ash	3.24	4.50-6.50
Soluble carbohydrate/NFE	59.00	25-30

Table 1(c): Nutraceutical/medicinal value of BGNS in comparison with Soyabean seeds.

Nutraceutical	Bambara ground nut	Conventional SBS
Carotenoids (mg/100g)	0.067	1-10.00
Ascorbic acid/vitamin C	31.00	0.5-2.50

Total Phenolic Content(TPC),mg/GAE/100g	243.47	50-300
Total Antioxidant Capacity (TAC)mmol/Fe/100g	46.71	1.5-4.00

Analytical results on the nutritional and therapeutic/nutraceutical composition of BGNS in tables 1(b & c) showed the presence of high nutritive and medicinal values of the nuts. These proximate analyses results on BGNS in this study agreed with past findings (Unigwe et al., 2018; Melini and Melini, 2021; Chelangat et al., 2023) which reported that BGN seeds are rich in nutrients and have medicinal values. These authors also established that some of the BGNS phytochemicals of the secondary metabolites, if ingested in excess in diet, induce health hazards. However, some of the secondary metabolites have therapeutic/medicinal values and promote good health.

Table 2: Influence of raw and processed dietary BGNS at graded levels on growth Performance of broiler chicks.

Diets indices	1	2	3	4	5	6	SEM
Daily FI (g/b/d)	21.04 ^b	21.33 ^b	19.67 ^a	19.67 ^a	21.40 ^b	20.00 ^b	0.38
Daily wt, gain (g/b/d)	19.33	15.00	14.33	13.43	15.00	13.33NS	5.48
Feed/Gain (Ratio)	1,10	1.45	1.44	1.48	1.49	1,55NS	0.04
*SGR	39,70 ^d	21.70 ^b	33.30 ^c	33,90 ^c	15.00 ^a	22.43 ^b	*
Survival rate (%)	100 ^b	96 ^a	97 ^a	96 ^a	96 ^a	97 ^a	*

a-b-c-d means in rows not sharing common superscript letters differed significantly ($P < 0.05$). NS, not significant ($P > 0.05$).

Result of feeding dietary untreated and treated BGNS at graded levels is presented in table 2. Raw or processed BGNS in diets did not influence daily body weight gain or feed efficiency/FCR ($P > 0.05$). Survival rate on the conventional diet was (100%) however, the 3 or 4% mortality observed on diets containing raw or treated dietary BGNS was not connected with dietary effect but probably caused by natural death. Feed consumption was reduced on diets with 20 and 30% inclusion of raw BGNS compared with the control diet and diets 5 and 6 containing processed BGNS by water-washing and fermentation under carbon dioxide environment ($P < 0.05$). Reduction in feed intake at 20 and 30% raw BGNS is suggestive of the inherent anti-nutritional and/or toxic factors in the nuts operating in concert with cumulative effects of the BGNS phytotoxins.

**Table 3: Some biochemical indices in broiler-chicks fed dietary untreated and treated BGNS.**

Diets	1	2	3	4	5	6
Index						
BGL(mmol/L)	2.70 ^a	3.40 ^a	4.00 ^b	3.00 ^a	2.00 ^a	2.80 ^{a}
Total protein(g/L)	60 ^b	62 ^b	55 ^a	68 ^c	77 ^d	74 ^d
Albumin(g/L)	18.00 ^b	22.00 ^{bc}	31.00 ^d	17.00 ^b	13.00 ^a	20.00 ^b

Table 3 shows some biochemical indices in broiler-chicks fed dietary untreated and treated BGNS. Biochemical parameters are blood-based measurements that help evaluate internal health and metabolic functions of the visceral organs like the detoxifying organs, the liver and the kidneys including the body. The measured biochemical parameters indicated that there was no significant difference in blood glucose level (BGL) between the control diet and the diets containing processed BGNS except the raw BGNS ($P < 0.05$). Serum total protein of birds receiving dietary treated BGNS was higher comparable with the control connoting that processing methods used were feasible in detoxifying or improving the nutritional value of the dietary BGNS on these parameters. The measured biochemical indices (BGL and STP) also showed how well the experimental chicks metabolized the BGNS nutrients and maintained their immune function. Results on the measured biochemical parameters of BGL and STP in this study also showed that treating the Bambara nuts before dietary inclusion at high level of 45% is safe for consumption and not toxic to the body and its organs.

Table 4: Detoxifying enzymes activities as influenced by dietary raw and processed BGNS

Diets	1	2	3	4	5	6
Enzymw activity (IU/L)						
GST	164 ^f	111 ^e	87 ^c	55 ^b	99 ^d	22 ^a
SOD	0.56 ^b	0.59 ^b	0.60 ^c	1.10 ^d	1.10 ^d	0.34 ^a
ALT	69 ^e	14 ^a	19 ^b	41 ^d	28 ^c	29 ^c
ALP	103	100	77	74	76	93 ^{NS}
AST	47 ^c	22 ^b	14 ^a	12 ^a	12 ^a	25 ^b

Ingestion of dietary BGNS treated by water-washing or ensiling (table 4) reduced drastically the activities of the enzymes, GST, SOD, ALT and AST relative to the control diet activity ($P < 0.05$). Reduction in activity of the enzymes may hinder or affect their specific function and optimum operation.

This study therefore established that Bambara ground nut is a rich source of both primary and secondary metabolites and that most of the secondary metabolites could elicit very harmful biological responses when ingested in diet beyond the normal threshold level. However, some of the secondary metabolites are reported to be medicinal and find use in nutrition and good health. The experiment also showed that inclusion of raw BGNS in diet beyond 10% is attended with poor performance and inferior/reduced results on some biochemical parameters like the detoxifying enzyme activities. Processing BGNS before inclusion in diet improved the biological and some biochemical indices measured.

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