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BIOCHEMICAL ASSAY OF SELECTED VARIETIES OF KIDNEY BEAN (*Phaseolus vulgaris* L.) SEEDS GROWN ON THE JOS PLATEAU, NIGERIA

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ABSTRACT

A study was conducted to determine the nutrient and anti-nutrient composition of five (5) selected varieties of kidney bean seeds grown on the Jos Plateau, Nigeria, for possible use as a feedstuff for non-ruminant animals. Four samples were taken from each of the five varieties and analyzed to determine their proximate composition and anti-nutritional factors. The varieties studied were white, brown, red, gray and black varieties. There were five treatments replicated four times in a completely randomized design. The anti-nutritional factors investigated were trypsin inhibitor, hydrocyanic acid, tannins, phytate, oxalate and haemagglutinins.From the results of the studies, the red and black varieties had a significantly ($P<0.05$) higher dry matter content than all other varieties. The white variety had the least dry matter content. There were no significant differences ($P>0.05$) in the crude protein and ether extract contents of the kidney bean varieties. The white variety had a significantly ($P<0.01$) higher crude fibre content than all other varieties, and the red variety had the least. The five varieties did not differ significantly ($P>0.05$) in their contents of hydrocyanic acid, phytic acid, oxalate and haemagglutinin. The red and black varieties had significantly ($P<0.01$) higher contents of trypsin inhibitor than other varieties, while the black variety had significantly ($P<0.01$) higher content of tannin than other varieties. The relatively high crude protein and low crude fibre contents of all five kidney bean varieties studied confer a high potential for use as protein feedstuffs in the diets of non-ruminant animals. However, due to the presence of anti-nutritional factors in the beans, there is a need to subject them to some form of processing to facilitate the efficient utilization of its nutrients by monogastric animals.

INTRODUCTION

The high cost of feed and feed ingredients, particularly protein concentrates such as groundnut cake and soyabeans, has been a major problem in monogastric animal production in developing countries today, apart from the incidence of disease (Adeniyi and Balogun, 2002; Amaefule *et al*., 2004). The ultimate goal of livestock production is the attainment of sustainable production with minimum cost and maximum returns. This has however been difficult to achieve due to the prohibitive cost of these feed ingredients. The urgent need to arrest the escalating cost of feed ingredients has prompted animal nutritionists, farmers and other players in the livestock industry to direct research focus on alternative feedstuffs which are locally available and affordable to farmers. Efforts have been made to use other vegetable protein sources such as pigeon pea (Amaefule and Obioha, 2001, Iorgyer*et al*, 2009, Iorgyer, 2010); Mucuna seeds (Emenalom and Udedibie, 1998; Tuleun *et al*., 2008a & b) and Jackbean (Esonu *et al*., 1998) in monogastric diets with encouraging results. There are, however, some other legumes whose seeds can be explored for their

nutritional value for monogastric animals. One of such legumes is the kidney beans (*Phaseolus vulgaris* L.).

Kidney bean is a leguminous crop grown in different parts of the world. Several varieties of this crop are currently cultivated in fairly large quantities in Mangu and Bokkos areas on the Jos Plateau, Nigeria. The varieties cultivated have crude protein contents ranging from 17 to 28% (Olomu, 2011), and are worth investigating for possible use as a source of protein in monogastric animal feeds. However, like other grain legumes, its usefulness as a feed ingredient for monogastric animals may be limited due to the presence of some anti-nutritional factors (Kingsley, 1995). The presence of these anti-nutritional substances has been associated with growth depression and pancreatic hypertrophy in many species (Birk, 1988; Olomu, 2011).

The objective of this study was to investigate the proximate composition and anti-nutritional factors of the commonly cultivated varieties of kidney beans on the Jos Plateau, Nigeria, for possible use in non-ruminant animal feed production.

MATERIALS AND METHIODS

Sources of kidney bean seeds

Five most widely cultivated kidney bean varieties on the Jos Plateau, Nigeria, were selected for the study. These were the white, brown, red, gray and black varieties. A sample (1kg) of each variety, obtained from two different locations each in Mangu and Bokkos areas, was taken to the laboratory to analyze for its proximate composition and anti-nutritional factors. The five varieties served as experimental treatments, and the four collection points of each variety, as replicates in a completely randomized design.

Proximate analyses of the kidney bean seeds

The dry matter (DM) content was determined based on the weight loss after 24 hours in an oven set at 100^0C. The nitrogen content was determined by Kjeldhal method of AOAC (1995) and crude protein calculated as N x 6.25. The ash content was determined as the residue remaining after incinerating the sample at 600^0C for 3 hours in a muffle furnace. The AOAC (1995) method was also employed for ether extract and crude fibre determinations. The nitrogen free extract (NFE) was obtained as the difference between the weight of the sample dry matter and the sum of the weights of the crude protein, ether extract, crude fibre and ash contents.

Determination of Anti-nutritional Factors

The anti-nutritional factors determined were trypsin inhibitor, hydrogen cyanide, tannin, phytic acid, oxalate and haemagglutinin.

Estimation of trypsin inhibitor

A ground sample of each kidney bean variety was extracted for 3 hours with petroleum ether (b.p. 60-80^0C) at room temperature. The defatted materials were sieved through a 40 mesh screen. One gramme of each sample was suspended in 20ml of 0.5N HCl and kept overnight at 4^0C in a refrigerator. The insoluble matter was removed by centrifugation at 800 rpm for 20 minutes in a Unipan Centrifuge Model No. 310. The supernatant was used as trypsin inhibitor (TI) extract and diluted at 1:1000 with water before use. The trypsin inhibitor activity (TIA) of sample extracts was determined according to the method of Kakade *et al*., (1974) with the modification described by Liu and Markakis (1989).

Estimation of hydrogen cyanide (HCN)

The hydrocyanic acid content of the different kidney bean varieties was determined using the procedure of Cooke and Maduagwu (1978) as modified by Ikediobi and Fashagba (1985). Two grammes of each sample was extracted with 15ml 0.1M of sodium phosphate buffer pH 6.8 for 30 minutes using a shaker. The mixture was centrifuged at 100 rpm and the supernatant obtained was decanted into a 50ml

volumetric flask. Exactly 0.2ml of the supernatant was pipetted into a test tube and the volume made up to 2ml with 0.1M sodium phosphate buffer pH 6.8. Four millilitres (ml) of alkaline picrate was added and mixed thoroughly. The mixture was incubated at 95^0C in a water bath for 10 minutes. It was allowed to cool and absorbance was read at 490nm. The total cyanic content was obtained by extrapolation from a standard curve of potassium cyanide.

Estimation of tannins

The kidney bean sample was defatted and extracted with 5% ethyl ether and ethanol, respectively. Tannic acid was estimated using the method of Earp *et al*., (1981). The method employed the vanillin-HCl procedure based on acid catalyzed addition of vanillin to flavonols and their polymers as well as other phenolic compounds such as dihydrocalcone and flavones. These reactions were determined colorimetrically at 500nm using a spectrophotometer (Spectronic 20).

Estimation of phytic acid

Eight grammes of defatted, finely ground kidney bean samples were soaked in 200ml of 2% HCl solution for 3 hours and then filtered through two layers of hardened filter paper. In a 400ml beaker, 50ml of the filtrate was placed and 107ml distilled water was added to give it correct acidity and thereafter titrated with standard ferric chloride solution containing 19.5mg iron per millilitre until a brownish yellow colour persisted for 5 minutes. Phytic acid was thereafter determined using the method of Sutardi and Buckle (1985).

Estimation of oxalate

One gramme of each kidney bean sample was placed in 250 cm^3 conical flask. One hundred and ninety centimetre cube (190cm^3) distilled water and 10ml 6M hydrochloric acid (HCl) were added. The mixture was warmed on a waterbath at 90^0C for four hours and the digested sample was centrifuged at a speed of 2000 rpm for 5 minutes. The supernatant was diluted to 250 cm^3. Three aliquots of 50 cm^3each of the supernatant was evaporated to 25 cm^3, the brown precipitate was filtered off and washed. The combined solution and washings was titrated with concentrated ammonia (NH_3) solution in drops until salmon pink colour of methyl orange changed to faint yellow. The solution was heated on a waterbath at 90^0C and the oxalate was precipitated with 10 cm^3 of 5% calcium chloride solution. The solution was allowed to stand overnight and then centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO_4 diluted to 125 $cm^3$with distilled water and warmed to 90^0C. It was then titrated against 0.05M $KMnO_4$ according to the procedure of AOAC (2006).

Estimation of haemagglutinin (lectins)

The haemagglutinin content of each kidney bean variety was determined using spectrophotometric method. A weighed sample (0.5g) of the ground kidney bean seeds was dispersed in a 10ml normal saline buffered at pH 6.4 with a 0.01M phosphate buffer solution. It was allowed to stand at room temperature for 30 minutes and then centrifuged to obtain the extract. A volume of 1ml trypsinized rabbit blood was added to 0.1ml of the extract diluent in a test tube. A control was mounted with the test tube containing only the blood cells, and both tubes were allowed to stand for 4 hours at room temperature. Normal saline (1 ml) was added to all the test tubes and allowed to stand for 10 minutes after which the absorbance was read at 620nm. The haemagglutinin was determined using the method of Lees (1971).

The data generated from these studies were subjected to analysis of variance using the SAS (1995) procedure, and treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS

Proximate composition

The results of the proximate analysis of the five selected varieties of kidney beans were as presented in Table 1. The results showed that the dry matter contents of the red and black varieties did not differ significantly, but were significantly ($P<0.05$) higher than the values for the brown and gray varieties (92.96% and 92.96% respectively), which also did not differ significantly ($P>0.05$) from each other. The white variety had the least dry matter content. There were no significant differences ($P>0.05$) in crude protein and ether extract contents among the varieties. The kidney bean varieties differed significantly in their contents of crude fibre, ash and nitrogen free extract. The white variety had a significantly ($P<0.01$) higher crude fibre content than all the other varieties. This was followed by the gray and black varieties which did not differ significantly ($P>0.05$). The red variety had the least crude fibre content. The ash content of the gray variety was significantly ($P<0.05$) higher than that of the white variety. The brown and red varieties did not differ significantly in their ash contents but had significantly ($P<0.05$) lower values than that of the white variety, and higher values than the black variety. The black variety had the least ash content of all the five varieties. The red variety had a significantly ($P<0.01$) higher content of nitrogen free extract than all the other varieties. It was followed by the brown and black varieties, which did not differ significantly ($P>0.05$) from each other. The red variety had a significantly ($P<0.01$) higher content of nitrogen free extract than all other varieties. The white variety had the least nitrogen free extract.

Anti-nutritional factors of selected varieties of kidney bean seeds

The contents of anti-nutritional factors of the five kidney bean varieties were as presented in Table 2. There were no significant differences ($P>0.05$) in the hydrogen cyanide, phytic acid, oxalate and haemagglutinin contents among the varieties. The varieties differed significantly ($P<0.01$) in their contents of trypsin inhibitor and tannin. The red and black varieties did not differ significantly ($P>0.05$) in trypsin inhibitor, but had significantly ($P<0.01$) higher values than the other varieties. The white, brown and gray varieties did not differ significantly ($P>0.05$) in trypsin inhibitor.

DISCUSSION

Proximate composition

The dry matter content, which ranged from 91.35 – 93.90%, was comparable to 89.0% given by Olomu (2011). There were no significant differences ($P>0.05$) in crude protein and ether extract contents among the varieties. The crude protein contents, which ranged from 24.93 – 27.38%, were comparable to 23.90% and 24.0% given by Olomu (2011) and Anon. (2012) respectively, for the crop. The crude protein content of kidney beans is also comparable to those of cowpea and pigeon pea seeds given as 24.67% and 23.77% respectively, by Aduku (2005). The ether extract contents of the varieties, which ranged from 12.18 – 13.73%, were much higher than 7.8% and 1.5% reported by Banerjee (2009) and Olomu (2011) respectively. These differences may be due to varietal differences. This agrees with an observation by Olomu (2011) that there are wide variations in the chemical compositions of kidney bean varieties. These ether extract values are lower than 19.0% and 46.5% for full-fat soyabeans and groundnut, respectively (Aduku, 2005; Olomu, 2011). This implies that kidney beans may not be regarded as an oilseed. The crude fibre content of the varieties ranged from 9.46 – 5.65%. The red variety had the least crude fibre content (5.65%), which is even lower than 6.50% for soyabean meal (Aduku, 2005). The generally low crude fibre content of kidney beans makes the crop suitable as a feedstuff for non-ruminant animals. The red variety had a significantly ($P<0.01$) higher content of

nitrogen free extract than all the other varieties. This connotes a significantly higher content of soluble carbohydrates in the red variety than all other varieties. It was followed by the brown and black varieties, which did not differ significantly (P>0.05) from each other.

Anti-nutritional factors of selected varieties of kidney bean seeds

The trypsin inhibitors, which ranged from 3.10 – 4.12mg/100g was much lower than 593.87mg/100g reported for raw Dolichos lablab seeds by Bawa (2003). Trypsin inhibitors interfere with the enzymic digestion of proteins into their basic amino acid components. These protease inhibitors are partly responsible for the growth retarding influence of raw legume seed meals (McDonald *et al*., 1995). When rats and chickens were fed diets supplemented with trypsin inhibitors from soyabeans, a significant growth retardation and enlargement of the pancreas was observed (Birk, 1988). This was attributed to the endogenous loss of essential amino acids in the faeces as a result of hyperactivity of the pancreas to enhance the secretion of enzymes of pancreatic origin. Pancreatic enzymes (trypsin, chymotrypsin and amylase) are very rich in cystine. Since much of the cystine required for the biosynthesis of these enzymes is presumably derived from methionine, an accelerated rate of synthesis of these enzymes in the pancreas as a result of pancreatic hypertrophy would, in effect, deplete the rest of the body tissues methionine that would have been channeled into body protein synthesis. Trypsin and chymotrypsin inhibitors are heat labile, and are inactivated by heat, especially, humid moist heat, and the rate of denaturation increases with rising temperature (Damang, 2011). Trypsin inhibitors are destroyed by rumen microorganisms in ruminant animals (McDonald *et al*, 1995).

The hydrogen cyanide content, which ranged from 18.83 – 23.96mg/100g, was much higher than 1.58mg/100g reported for Dolichos lablab (Bawa, 2003). According to Olomu (2011), hydrocyanic acid exists as two major aliphatic cyanogenicglucosides: linamarin and lotaustralin. The glucoside, especially linamarin exists with the enzyme, linamarase, along with some free hydrocyanic acid (also called prussic acid). During decomposition, linamarase hydrolyzes linamarin to produce B-glucose, acetone and more prussic acid. It is the free prussic acid that is poisonous by inhibiting the respiratory chain at the cytochrome oxidase level. Soaking for a while, and fermentation have been shown to reduce the concentration of hydrogen cyanide (Olomu, 2011).

The phytic acid content of the kidney bean varieties, which ranged from 25.40 – 29.30mg/100g, was higher than 1.56g/kg reported for *Mucunautilis* seeds (Tuleun and Patrick, 2007). Phytic acid forms stable chelates with divalent and trivalent metals such as calcium, phosphorus, iron, zinc, copper and magnesium (Cheeke and Shull, 1985; Osagie, 1988). The chelating phytic acid reduces their bioavailability and absorption in the intestinal tract. The endogenous phytase activity in the intestinal mucosa of monogastric animals is extremely low (Moore and Veum, 1983). Consequently, monogastric animals cannot hydrolyze phytate phosphorus effectively. On the other hand, ruminants have an exceptional ability to utilize phytin phosphorus and inorganic phosphorus with the aid of phytase produced by rumen micro-organisms (McDonald *et al*., 1990). Phytic acid is resistant to heat treatment. Heat treatment was found to be ineffective in detoxifying phytate in kidney beans (Emiola *et al*., 2007). Damang (2011) reported that only 37.10% and 44.28% phytate was destroyed in African locust bean seeds after cooking in water at 100^0C for 30 minutes and 60 minutes respectively.

The tannin content of the black variety was significantly (P<0.01) higher than the values for the other four varieties, which did not differ significantly (P>0.05) from one another. The

tannin content, which ranged from 143.95 – 263.96mg/100g, was lower than 141mg/g of raw *Mucunautilis* seeds (Tuleun and Patrick, 2007). Tannins have been found to form complexes with proteins, thus interfering with the digestion process, and also by inactivating the digestive enzymes, trypsin, chymotrypsin, amylase and lipase (Rao and Doesthale, 1982). Poultry diets high in tannin contents have been shown to decrease egg production and body weight gain (Rao and Prabhavathi, 1982; Tuleun and Patrick, 2007). Heat treatment was found to be ineffective in detoxifying tannin in kidney beans (Emiola *et al*., 2007).

The oxalate content of the kidney bean varieties ranged from 16.00 – 19.70mg/100g. Oxalates are naturally occurring anti-nutritional factors that are capable of interfering with the absorption of essential mineral elements such as calcium, iron and to some extent, zinc by forming complexes with them (Olomu, 2011). The reduction in the absorption of calcium and iron may have implications on bone formation and the formation of red blood cells, especially in monogastric animals (Oke, 1996). However, calcium oxalate is degradable in the rumen and may not impair the performance of the ruminant animal significantly (Onwuka, (1996). Diets of monogastric animals containing ingredients that are high in oxalates may require exogenous calcium. Oxalates, when present in a high concentration in diets of monogastric animals, may result in extensive scouring, distress, dullness, loss of appetite, progressive in-coordination and, in extreme cases, death (McDonald *et al*., 1988). Heat treatment has been found to be ineffective in destroying oxalate in legume seeds (Emiola *et al*., 2007).

The haemagglutinin contents of the kidney bean varieties ranged from 3.65 – 3.96mg/100g. Haemagglutinin is a glycoprotein which is capable of agglutinating red blood cells in rabbits, chickens and man, but not in sheep and cattle (McDonald *et al*., 1988). When ingested, haemagglutinins are resistant to digestion, and bind to the mucosal surface of the gut leading to altered epithelium morphology and renewal, impaired intestinal permeability and nutrient transport, resulting in adverse effects on immune function, protein metabolism, enzymatic activities and hormonal regulation (Pusztai, 1989). Unlike in monogastric animals, the rumen microorganisms in ruminants degrade these compounds. Haemagglutinins are destroyed by heat (Oke, 1996). Kidney beans heated at 80^0C for 20 minutes had increased haemagglutinating activity, while the same beans heated at 100^0C for 2 minutes lost all haemagglutinating activities (Bender, 1983).

Table 1: Proximate Composition of Five Selected Varieties of Kidney Bean Seeds

	Kidney Bean Varieties					
Proximate Composition (%)	**White**	**Brown**	**Red**	**Gray**	**Black**	**SEM**
Dry matter (DM)	91.35^{c}	92.96^{b}	93.79^{a}	92.96^{b}	93.90^{a}	0.22*
Crude protein (CP)	27.38	26.38	24.93	26.44	27.15	0.66^{NS}
Ether extract (EE)	13.73	13.30	12.28	14.74	12.18	0.29^{NS}
Crude fibre (CF)	9.46^{a}	7.23^{c}	5.65^{d}	8.44^{b}	8.12^{b}	0.20**
Total ash	4.72^{b}	4.30^{c}	4.40^{c}	5.15^{a}	4.17^{d}	0.06*
Nitrogen free extract (NFE)	35.06^{c}	41.76^{b}	46.53^{a}	30.17^{d}	42.28^{b}	0.72**

^{abcd} Means within the same row bearing different superscripts are significantly different
SEM = Standard error of mean
NS = Not significant
* = P<0.05
** = P<0.01

Table 2: Anti-nutritional Factors of Five Selected Varieties of Kidney Bean Seeds

	Kidney Bean Varieties					
Anti-nutritional Factors (mg/100g)	**White**	**Brown**	**Red**	**Gray**	**Black**	**SEM**
Trypsin inhibitor	3.40^{b}	3.12^{b}	3.83^{a}	3.10^{b}	4.12^{a}	0.06**
Hydrogen cyanide	21.96	19.39	23.96	18.83	19.60	1.66^{NS}
Phytic acid	29.00	25.40	29.30	27.80	27.10	0.08^{NS}
Tannin	143.95^{b}	149.15^{b}	176.66^{b}	166.27^{b}	263.96^{a}	8.35**
Oxalate	16.40	18.30	17.80	19.70	16.00	0.11^{NS}
Haemagglutinin	3.88	3.65	3.91	3.96	3.96	0.10^{NS}

^{a,b}Means within the same row bearing different superscripts are significantly different
SEM = Standard error of mean
NS = Not significant
** = P<0.01

CONCLUSION

From the result of the study on the proximate composition of the white, brown, red, gray and black varieties of kidney beans cultivated on the Jos Plateau, Nigeria, each variety had a potential for use as a protein feedstuff in the diets of monogastric animals due to the relatively high crude protein and low crude fibre contents. The five varieties had various levels of all the anti-nutritional factors investigated. It is recommended that for kidney beans to be used for non-ruminant animal feed production, it will need to be subjected to some form of processing in order to destroy the anti-nutritional factors for efficient utilization of its nutrients by such animals.

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