Nutritional and Biochemical Evaluation of Canavalia Ensiformis seeds from Ghana

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ABSTRACT

Canavalia ensiformis seed was analysed for moisture, 9.10%, fat 3.30%, protein 30.91%, crude fibre 8.94%, ash 3.50% and amino acids. The seed was found to contain anti-nutritional factors, tannins 0.52%, phytate 0.08%, oxalate 0.43%, hydrogen cyanide 0.02% and saponins 1.11%. Toxicological evaluation of the raw and autoclaved seed flours carried out on albino male rats fed at 10% protein level for thirty (30) days showed several biochemical and histopathological abnormalities with the raw flour while such abnormalities were absent in the animals fed with the autoclaved seed flour diet. The transaminases (AST/ALT), lactate dehydrogenase and amylase in the liver, kidney and heart increased tremendously in rats fed with the raw seed flour. The alkaline dehydrogenase in the liver of the rats on the autoclaved was the same as the control. The red and white blood cell populations of rats on raw seed flour decreased drastically. Nutritional evaluation of the flour by rat assay gave PER of 0.76 for the autoclaved and 0.14 PER for the raw flour. The apparent protein digestibility was high 75.73 for the autoclaved flour and 59.14 for the raw flour but the net protein utilization was low 57.96 for autoclaved flour and 39.04 for the raw flour.

KEYWORDS: *Canavalia ensiformis, digestibility, amino transaminases, anti-nutritional factors, histopathological changes.*

INTRODUCTION

Protein is an essential nutrient required by both man and animals for healthy development. There is no question that protein malnutrition is prevalent in most developing countries. Over half the population dies before the age of fifteen and half or more of the infants die before the age of five (1); a direct result of malnutrition and wide spread infestious diseases in the developing countries (2). The mortality of the children is the best indication of the seriousness of protein malnutrition in the developing countries (3). Protein deficiencies can be either of total dietary protein or of one or more of the essential amino acids. Plant proteins are generally inferior to animal proteins in quality as the former often do not contain one or more of the essential amino acids or may contain anti-nutritional factor such as tannins, phytates, etc. A mixture of plant proteins, however, improves the quality of the plant protein. Animal proteins are scarce and expensive in the developing countries. The plant protein may therefore be the solution to the problem of protein malnutrition in the developing countries particularly in Africa where seed-bearing plants abound.

To be able to improve the nutritional quality of plant proteins various seeds and vegetables need to be blended. But little is known of the chemical composition of indigenous seeds. To contribute to the search for good plant proteins to blend with other proteins, this work was undertaken to determine the nutritional value of Canavalia ensiformis seeds. The plant grows well in all soils in Ghana and bears many pods which contain a large number of seeds. Even though the yield per hectare is high, and the seeds are reported to be rich in protein (4), the plant is used as a cover crop and as green manure by farmers. Only few people venture to use a little in their diet since little or nothing is known about its nutritional value in Ghana. Various researchers have however reported on the presence of L. Canavalia, trypsin inhibitors, the true protein content, the soluble sugars and minerals content of Canavalia ensiformis from Brazil (5). This will try to fill that gap.

MATERIALS AND METHODS

Canavalia ensiformis seeds were collected from the Department of Horticulture, University of Science

and Technology (UST), Kumasi. For gross composition forty seeds were selected randomly and weighed. Their seed coats were manually removed from he cotyledon. Both the cotyledon and the seed coat were collected separately and weighed. The weights of the cotyledon and the seed coat were each expressed as a percentage of the weight of the whole seed.

ANALYSIS

500g of Canavalia ensiformis seeds were milled to pass through a 40-mesh sieve. The seed flour was analyzed for moisture by oven method. Protein by Kjeldahl method, fat by soxhlet continuous extraction using petroleum either Bp 80°C, crude fibre and ash by methods in AOAC (6). Tannin was determined by Folin-Denis Collorimetric method as described by Joslyn et al (7). Hydrogen cyanide (HCN) and oxalate were determined as described by Oke (8). Saponin level was estimated following the method of Gestetner (9) and phytate by the method of Haug and Hans-Joachim (10). The amino acid content was determined by the method of Moore and Stein (11) after oxidizing cystein to cysteic acid by formic acid method.

Animal Assay

One kilogram of Canavalia ensiformis seeds flour was mixed with calculated amount of water (~200ml) into a paste and autoclaved at 110°C for ten minutes at 15 psi. The sample was used to prepare diet 1. One kilogram of raw flour was also used to prepare diet [11]. A control diet containing casein was prepared. Each diet containing protein at 10% level was kept at 10°C and was fed to a group of six male albino rats of local strain obtained from the animal house, Department of Biological Sciences, UST, Kumasi. The animals weighing 45-48g were caged and fed for thirty days. Water and diet were given ad libitum. Animals were weighed every other day, but the initial and final weights were used for quality indices and other parameter calculations. Feaces and urine were collected for five days after twenty-one days feeding and analyzed for crude protein and used for calculating Net Protein Utilization (NPU), True Digestibility (TD) and Biological Value (BV) and the Protein Efficiency Ratio (PER).

Biochemical Analysis

The rats were killed after thirty days feeding and the blood was collected for biochemical studies. The red blood cells (RBC) and the white blood cells (WBC) were counted using haematocrit. Blood smear was prepared, stained and examined. The liver, kidney and heart were separately homogenized in 9ml buffer per gram of tissue to give 10% homogenate. The homogenates were centrifuged at 0°C at 10^4 rpm for thirty minutes and the supernatants were used for determining the enzyme activities. The method of Reitman and Frankel (12) was used to estimate aminotransferases (AST/-ALT) and lactate dehydrogenase (LDH). Incubation was done at 37°C for 60, 30 and 15 minutes respectively and absorbance was read at 510nm. ALP was estimated by the methods of Varley et al (13) and amylase by the method of Henry and Chlamori (14). Incubation was done at 37°C and absorbance read at 710nm and 700nm respectively.

Histopathological Studies

The kidney, liver, heart, pancreas and spleen were separately weighed and examined for histopathological lessions and slides of the organs were prepared and examined for abnormalities with the help of a pathologist at Okomfo Anokye Teaching Hospital, Kumasi.

RESULTS

A physical description of Canavalia ensiformis seed and proximate composition and the toxic substance in Canavalia ensiformis seed flour are shown in Table 1. The seed coat formed 13.44% and the cotyledon 86.56% of the whole seed. The seed flour contained 3.30% fat, 30.91% crude protein, 3.50% ash, 8.94% fibre, 0.52% tannins, 0.02% hydrogen cyanide (HCN), 0.43% oxalate, 0.08% phytate and 1.11% saponins.

TABLE 1: THE GROSS COMPOSITION, PROXIMATE COMPOSITION AND SOME ANTI NUTRIENTS IN CANAVALIA ENSIFORMIS SEEDS

Cross component	% On Dry WT.Basis
Seed Coat	13.44
Cotyledon	86.56
Proximate Composition	
Moisture	9.10
Fat	3.30
Protein (Nx 6.25)	30.91
Fibre	8.94
Ash	3.54
Carbohydrate (by difference)	44.21

Anti-Nutrients	
Tannins	0.52
Phytates	0.08
Oxalates	0.43
HCN	0.02
Saponin	1.11

TABLE 2: AMINO ACID CONTENT OF CANAVALIA ENSIFORMIS SEED MEAL

	g/16gN	
Arginine	9.25	
Aspartic Acid	13.12	
Alanine	4.60	
Histidine	2.53	
Lysine	5.77	
Threonine	3.58	
Serine	4.65	
Glutamic Acid	11.01	
Proline	2.58	
Glycine	3.50	
Valine	3.45	
Cysteine	0.40	1.59
Methionine	1.19	
Isoleucine	3.49	
Leucine	9.75	
Phenylalanine	4.51	
Tyrosine	3.35	

The amino acid spectrum showed low level of S-amino acids (1.50g/16gN) and a good level of lysine (5.77g/16gN) (Table 2). The rat assay of the quality of the protein showed that the autoclaved protein was digestible (TD 75.73). The other protein quality indices obtained for the autoclaved flour were per 0.76, BV 47.65 and NPU 57.14 and for the raw flour were BV.37.00, PER 0.14, NPU 39.04 and digestibility 59.14 (Table 3). The mean weights of the animals and the organs are also given in Table 3. With the exception of the weight of the heart, the weights of the organs of rats on raw meal diet II were significantly lower than the control but not very much lower than those on diet I containing autoclaved flour. The results obtained from the animal assay showed that it is not good to feed animals with raw Canavalia. Canavalia protein alone in a diet provided poor results. All the animals on the control diet and the autoclaved flour, diet I were normal in appearance at outopsy but rats on diet II looked emaciated. There were inflammation and degenerative changes in the organs of rats on diet II especially, the liver showed inflammation, sclerosis, degerative and fatty changes, while the spleen looked darker than that from animals on the control diet and the kidney look inflamed and had no perirenal fat. There was very little body fat on the animals fed diet II. Organs of rats on the control and diet I, did not show any pathological changes.

Homogenate of the liver, kidney and heart were used for determination of enzyme activity instead of blood because very little blood could be ob-

TABLE 3:
EFFECT OF CANAVALIA ENSIFORMIS SEED ON CONSUMPTION QUALITY INDICES, GAIN IN BODY AND ORGAN WEIGHTS

PARAMETER	CONTROL DIET (CASEIN)	DIET I AUTO-CLAVED MEAL	DIET II (MEAL)
PER	2.21[a]	0.76[b]	0.14[c]
TD	81.71[a]	75.73[b]	59.14[c]
NPU	72.18[a]	57.96[b]	39.04[c]
BV	69.15[a]	47.65[b]	37.00[c]
Gain in body weight	53.87[a]	16.34[b]	2.93[c]
Heart g/100 weight	0.45[a]	0.46[b]	0.42[a]
Kidney g/100 weight	0.91[a]	0.55[b]	0.49[a]
Liver g/100 weight	5.48[a]	2.49[a]	2.43[a]
Pancreas g/100 weight	0.47[a]	0.25[b]	0.22[a]

tained from rats on diet II. The enzyme activities in the homogenates of the organs of animals on diet I, were comparable to those of animals on the control diet but the activities of those on diet II were higher (Table 4).

The haematological studies revealed abnormalities in the cells of rats on diet II but the cells of the rats on diet I and the control were normal. The RBC counts were 9,600,000/mm^3 for control, 8,320,000/-mm^3 for diet I and 1,280,000/mm^3 for diet II. The blood smear showed that the erythrocytes of rats on diet II were microcytic and hypochromic.

DISCUSSION

The 13.44% seed coat contributed immensely to the fibre content of the diet when whole seed flour was added to the diet. The results of the analysis of the flour showed that the seed contained a high level of

TABLE 4:
THE EFFECT OF CANAVALIA ENSIFORMIS MEAL AND ACTIVITIES OF ENZYMES IN ORGANS AND BLOOD CELLS OF RATS

Enzyme/organ	control	Diet I	Diet II
Alanine Transaminase (ALT)	(Casein)	(Autoclaved)	(Raw Meal)
Liver	410.0	405.0	564.0
Kidney	76.0	73.0	240.0
Heart	80.0	90.0	134.0
Aspartate Transaminase (AST)			
Liver	142.0	160.0	422.0
Kidney	142.0	147.0	264.0
Heart	260.0	280.0	312.0
Alkaline Phosphates (ASP)			
Liver	1750.0	1750.0	3625.0
Kidney	95.0	98.0	147.0
Heart	-	-	-
Lactate Dehydrogenase (LDH)			
Liver	206.0	210.0	386.0
Kidney	45.2	50.3	160.8
Heart	87.0	90.5	160.8
-Amylase			
Liver	42.0	42.0	134.7
Kidney	9.8	10.5	27.6
Heart	159.0	148.0	1506.0
Blood Cell Count			
RBC	6,000/mm^3	4,000/mm^3	2,800/mm^3
WBC	9,600,000/mm^3	8,320,000/mm^3	1,280,000/mm^3
Not determined			

crude protein, 30.91%. The protein was low in sulphur amino acids 1.50/16 gN. The 30.91% crude protein was below 33.90% reported by Gohl (15) but far above 26.90% reported by Bressani et al (16). The difference in the crude protein may be due to location of growth and variety of seeds. The low level of sulphur-containing amino acids is a big disadvantage if the seed is used without blending with another protein rich in sulpho-amino acids. The fibre content of 8.94% was higher than the 4.9-8.0% reported by Daisy (17). The high fibre level is advantageous as **Canavalia ensiformis** seed flour contained a low amount of tannins and phytates and therefore could serve as a good source of fibre in the diet. The HCN content of 0.02% was higher than 0.01% reported by Daisy (17). The level of HCN in the flour is far below the lethal dose of about 0.07% reported by Honing et al (18) but is on the uppermost limit of the safe limit range of 0.01-0.02% suggested by Montgomery (19). The low level of tannins in the Canavalia seed flour will save consumers from tannin intoxication. The saponin level of 1.11% was more than twice the value 0.50% found in the soyabean by Gestetner et al (9). Canavalia saponins could cause nausea and vomiting but could be eliminated by soaking the seed prior to cooking.

Oxalate and phytate contents of the seed were found to be 0.43% and 0.08% respectively and were comparable to levels usually found in many legumes.

ANIMAL ASSAY

Protein digestibility of 75.73 for the autoclaved seed flour diet i fell within the range of 75.85 reported for the legumes by Jaffe (20) but the 59% digestibility recorded for the raw flour fell outside the range, but not unexpected because of the presence of antitrypsin factors usually present in legumes. The antitrypsin was not determined in this work. The PER of 0.76 was lower than 1.21 reported by Bressani (16) but higher than 0.64 reported by D'Mello et all (21).

The low PER of 0.14 and 0.76 for diet II and I respectively might be due to the presence of concanavaline A and B (21) which impaired absorptions and utilization of nutrients from the gastro-intestinal tract and the low sulphur amino acids levels which were the limiting factor in the utilization of the protein. Oke (8) using a leaf protein and cassava recorded a negative PER 0.73 and attributed the negative PER to the presence of Cyanogenic glycoside. D'Mello (21) indicated that toxic amino acids appear to play a major role in determining nutritional value of a number of tropical legumes. Liener (22) proposed that the toxic amino acids act antagonistically towards certain nutritionally important amino acids. Canavaline, a structural analogue of arginine may act antagonistically to arginine and therefore canavalia in the diet may require arginine and s-amino supplementations. But D'Mello et al (21) reported that Jack bean diet with supplementation of arginine alone did not improve growth of chicks significantly. The BV 47.65 obtained for diet I reflected on the nature of the protein. The low PER, BV and NPU values obtained for diet II may be due to the presence of trypsin inhibitors, saponins, tannins, lectins and canavaline (lectins and canavaline were not determined), since all these retard digestion, absorption and utilization of protein. This therefore might account for the high faecal protein level (3.80%) which was observed.

Histopathological studies of the liver of the rats fed with diet II showed severe inflammation, fatty and degenerative changes and sclerosis.

The spleen looked darker in colour than normal and kidney looked inflamed and had no perirenal fat. There was very little body fat on the animals fed on diet II. The organs of the rats which were fed on diet I and the control diet looked normal in appearance and did not show any pathological changes. The autoclaved Canavalia flour (diet I) fed to rats at 10% protein level caused serious abnormalities in the way of growth retardation and in evaluation of blood and organ biochemical and histopathological parameters. Examination of the liver and the kidney of the rat on diet II revealed acute inflammation. The liver appeared as if it was cooked. Anderson (23) attributed such a situation to the presence of harmful substances in the organ, the increase of blood flow into the organ and the resultant increase in heat production in the cells. These changes result in inflammation. The fatty changes of the liver might have resulted from impaired lipoprotein synthesis due to the body's inability to use the protein due to the presence of anti-nutritional factors thus causing accumulation of neutral fat in the cytoplasm (25). Mandel (28) reported a similar result when rats were fed on akashoni diet.

The haematological studies revealed abnormalities in the blood cells of rats on diet II containing raw flour. The RBC count was extremely low, 1,280.-000/mm^3 as against 8,320,000/mm^3 for diet I and 9,660,000/mm^3 for control diet. Raticliffe (25) reported on RBC count of 9,000,000/mm^3 for rats.

The low RBC count and the low blood volume of rats on diet II may be due to the presence of lectins and saponins in the Canavalia flour. These substances can cause agglutination and haemolysis of RBC. The autoclaving of the flour possibly destroyed the lectins and saponins and therefore their effect was not felt in diet I. Again the WBC count of 2,800/mm^3 for diet II rats was lower when compared with 4,000/mm^3 reported for diet I and 6,000/mm^3 for the control diet. Ratcliffe (25) reported 6,000 to 18,000/mm^3 for rats. Lesson et al (26) reported that WBC migrate readily through blood vessel walls and develop into phagocytic cells at site of inflammation and injury. This may account probably for the low WBC count observed in rats on diet II which caused inflammation to all the organs.

Donald et al (27) reported that the response of liver to any form of biliary obstruction is to synthesize more ALP-enzymes so that some of the newly formed enzymes can enter into circulation to raise the enzyme levels in the serum. The high enzyme levels found in the organs of rats fed with diet II may possibly be due to several degenerative changes which occurred in the organs. Mandel et al (28) used evaluation of aminotransferasses and alkaline phosphates activities as an indication of tissues damaged particularly in the liver and kidney.

CONCLUSIONS

The overall assessment of the results have shown that the raw canavalia seed flour was highly toxic but autoclaving of the flour improved its nutritional value as the autoclaving seemed to have destroyed the antitryptic factor, lectins, saponins and hydrolyzed partially or completely the low levels of tannins and phytates. When used as the main source of protein, activities of ALT/ALP in the rats showed that rats fed on diet I indicated the absence of any hepatotoxic agent but those on diet II indicated the presence of hepatotoxic agents in the diet. The low PER (0.76) is an indication that the protein needs supplementation of s-amino acids and arginine when used as the main source of protein in a diet. The increased levels of ALT and ALP indicate histological changes in the liver and the kidney.

From the results obtained Canavalia seed if well cooked could be a good source of plant protein especially when blended with proteins rich in s-amino acids and arginine. The eating of uncooked Canavalia seed should be avoided completely as its consumption could be detrimental to the consumer.

REFERENCES

1. **Berg A. and Miscat R.J.** *The Nutrition Factor, its role in National Development.* The Brookling Institution, Washington DC 20066, 1973.
2. **Schaefer, A.E.** *Nutritional deficiencies in developing countries, J.* Am. Dietet, Association. 42 295. 1963.
3. **Scrimshaw, N.S. and Behar, M.** *Protein malnutrition in young children.* Science 133 2039. 1961.
4. **Rodrigues, B.F., Torne S.G.** *Chemical study of seeds in three Canavalia Species.* Tropical Science 31 (1) 101-103. 1991.
5. **Carlini, C.R., Barcellos, G.S.B and Baeta-Neves D. V.** *Immunoreactivity for Canatoxin and Concanavalin Among proteins of leguminous seeds.* Phytochemistry 27 25-30. 1988.
6. **AOAC: Association of Official Analytical Chemists.** *Official methods of Analysis. 12th Edition.* Association of Official Analytical Chemists, Washington D. C. 1975
7. **Joslyn, M.A.; Morris, M. and Hengenberg, G.** *Die bestiming de Genbsaure and Verwandter Phenoltubstanton Mittels de Phosphormolybdat-phosphowol-framt-Reagenz Mitt* (Klosterneuberg) 18 17-34. 1968
8. **Oke, O.L.** *Chemical studies on the more commonly used leaf vegetables in Nigeria.* Journal of West African Science Association Vol. II Nos. 1 and 2. 1966
9. **Gestetner, B.; Birk, Y. Bondi, A and Tencer, Y.** *A method for the determination of Sapogenin and Saponin contents in Soybeans.* In Phytochemistry, Vol. 5 Petgamon Press Limited, England pp 803-806. 1965
10. **Haug, W and Hans-Joachim, L.** *Sensitive for the rapid determination of phytate in cereals and cereals product: J.* Sci. Food Agric. 34 1423-1426. 1983
11. **More, S. and Stein, W.M.** *Chromatographic determination of amino acids by the use of the automatic recording equipment.* In methods in enzymology Vol. 6 (Colowish, S. P. and Kplan, N. D. (eds) Academic Press New York. 1963
12. **Reitman, S. and Frankel, S.** *A colorimetric Method for the Determination of Serum AST/ALT and LDH.* Am J. Clin. Pathology 28 56-62. 1975
13. **Varley, H.; Gowenlock, A. K. and Bell M.** *Practical Clinical Biochemistry.* Vol. 1 5th Edition pp 897-900. Whitefraires Press London. 1980
14. **Henry, R.J. and Chiamori, N.** *Clinical Chemistry Principles and Techniques 471 Harpen and Row.* New York. 1964
15. **Gohl, B.** *Tropical Feeds FAO - Animal Production and Health Series* No. 12 FAO of the UN Rome pp 143. 1981

16. **Bressani, R., Brenes, R.C. Garcia, A. and Alias L.G.** *Chemical composition amino acid content and protein quality of Canavalia Spp. seeds* J. Sci. Food Agric. 40 17-23. 1987
17. **Daisy, E.K.** *Food Legumes. Tropical crops product digest* No. 3. 1979
18. **Honing, D.H., Dogkridge, M.E., Gould, R.M. and Rackis, J.J.** *Determination of Cyanide in Soya bean products.* J. Agric. Food Sci 31 272-275. 1983
19. **Montgomery, R.D., Cyanogene.** *In Liener, L. E. ed Toxic Constituents of Plant Foodstuffs* 2nd Edition. Academic Preas New York pp 143-156. 1969
20. **Jaffe, W.C.** *Protein digestibility and Trypsin inhibition activity of legume seeds* Proc. Soc. Expt. Biol. Med. 75 219. 1950
21. **D'Mello, J.P.F., Acanovic T. and Walker, A.G.** *Nutritive value of Jack bean (Canavalia ensiformis) for young chicks,* Tropical Agric. (Trinidad) 62 145-149 Publ. Butterworths. 1984
22. **Liener, I.R.** *Toxic constituents of plant foodstuff* pp. 582 New York Academic Press. 1980
23. **Anderson, J.R.** *Muir's Book of Pathology* pp. 43-45 Publ. Arnold E. London. HD. Anderson. 1980
24. **MacSween, R.N.** *Muir's Text Book of Pathology* pp 43-45 and 67 Publ. Arnold R. London Edition Anderson J. R. 1980
25. **Rateliffe, N.A.** *Practical Illustrated Histology* Publ. The Machillah Press Limited, London pp. 69. 1982
26. **Lesson, C.R. and Lesson, T.S.** *Histology* pp. 135 Publ. W. E. Saunders Co. London. 1968
27. **Donald, W.K., Henderson, A.R. and Kachmar, F.J.** *Fundamental of Clinical Chemistry* pp. 346-398. Publ. DW. R. Saunders CO. London. 1987
28. **Mandel, B., Gosh, S. and Maity, C.R.** *Processing and Toxicological Evaluation of Akaslunosi and Encalyptus of seed meal. Human Nutrition.* Food Sciences and Nutrition 41F 135-143. 1987