AJFAND ONLINE
Volume 10 No. 4
April 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

EFFECTS OF PROCESSING METHODS ON THE PROTEIN QUALITY OF MUCUNA BEAN *(MUCUNA PRURIENS L.)*

Mugendi JB[1*], Njagi ENM[2], Kuria EN[3], Mwasaru MA[4], Mureithi JG[5] and Z Apostolides[6]

Beatrice Mugendi

[*] Corresponding author email: beatricemugendi@yahoo.com

[1] Department of Foods, Nutrition and Dietetics, Kenyatta University, P.O. Box 43844-00100, Nairobi. Kenya

[2] Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi. Kenya

[3] Department of Foods, Nutrition and Dietetics, Kenyatta University, P.O. Box 43844-00100, Nairobi. Kenya

[4] Department of Foods Science and Post Harvest Technology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi. Kenya

[5] Kenya Agricultural Research Institute (HQ), P.O. Box 57811-00100, Nairobi. Kenya

[6] Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa

AJFAND ONLINE
Volume 10 No. 4
April 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

ABSTRACT

Mucuna bean (*Mucuna pruriens* L.) is grown in many parts of Kenya as a green manure/cover crop. The bean contains a high content of crude protein. However, it remains a minor food crop due to the presence of anti-nutritional compounds such as 3,4-dihydroxy-L-phenylalanine (L-Dopa). The potential for utilization of mucuna bean as an alternative source of protein was evaluated by assessing the effect of various processing methods on its protein quality. Mucuna bean was processed to remove L-Dopa and other anti-nutritional compounds by different methods such as soaking, autoclaving, roasting, germination, and alkaline fermentation. Protein quality was determined by amino acid composition, *in vitro* and *in vivo* rat balance methodologies. All processing methods except roasting improved *in vitro* protein digestibility (IVPD). Soaking in acidic medium (pH 3.2) at 60˚C for 48 hrs significantly improved IVPD (80.5%) and biological value (80.8) of mucuna bean protein. The content of essential amino acids met the recommended FAO/WHO reference requirements for 2-5 yr old except for tryptophan. However, true digestibility for processed bean diet was poor (58%) and protein digestibility-corrected amino acid score (PDCAAS) low (0.4) compared to that of reference casein (1.0). This was attributed to both low sulphur amino acids content and possible presence of factors that affect protein hydrolysis such as phenolic compounds. Mucuna protein diet did not support growth of weanling rats indicating amino acids pattern incompatible with the needs of weanling rats. Histological examination of liver and kidney tissues revealed that consumption of processed mucuna bean as the only source of protein caused inflammation of the organs. This suggests possible presence of other antitoxins in processed bean even though mucuna bean diet contained the recommended safe level of residual L-Dopa (<0.1%). Processing mucuna bean by soaking in acidic medium (pH 3.2) at 60˚C for 48 hrs improved protein quality. However, mucuna bean is not recommended as a sole protein in human diet.

Key words: bean, protein quality, anti-nutrients, processing

INTRODUCTION

Legume seeds are an important source of plant proteins constituting more than 80% of available protein in human diets in developing countries [1]. The primary nutritional importance of proteins is that they are a source of amino acids. High quality proteins contain essential amino acids in quantities corresponding to human requirements and are readily digestible. Humans require certain minimal quantities of essential amino acids from a biologically available source as part of a larger protein/nitrogen intake. It is important to determine the relative efficiency with which individual protein sources meet these requirements. Evaluation of dietary protein quality has consisted of monitoring the metabolic responses of an animal model to changes in amino acid composition of the same [2]. Protein quality has been evaluated by different methods and has been expressed in various parameters such as protein efficiency ratio, net protein utilization and biological value. However, introduction of the protein digestibility-corrected amino acid score (PDCAAS) is a significant change in the assessment of dietary protein quality. It is the recommended protein quality index by FAO/WHO [3]. The PDCAAS method measures the amino acid score (AAS) with an added protein digestibility component and uses amino acid requirements of a 2 to 5-year old child as the standard. It reflects food protein's amino acid content, true digestibility, and ability to supply essential amino acids in amounts adequate to meet requirements. True digestibility of a food protein is determined based on the nitrogen balance obtained from rat feeding trials.
PDCAAS = AAS x True digestibility [3].

Mucuna bean, like other legume seeds, contains anti-nutritional compounds such as phytate, polyphenols, protease inhibitors and aromatic amino acids that cause physiological and biochemical effects including decreased protein digestibility, growth inhibition in animals [4]. A variety of processing techniques such as soaking, heat treatment (boiling and autoclaving), roasting, fermentation and germination have been used to remove anti-nutritional compounds and hence improve bean nutritional value [5]. Mucuna bean contains a major anti-nutritional compound, a non-protein amino acid, 3,4-dihydroxy-L-phenylalanine (L-Dopa) [6]. The objective of this study was to evaluate the effects of processing mucuna bean (to remove L-Dopa and other antinutrients) on protein quality in terms of amino acid composition, *in vitro* and *in vivo* digestibility.

MATERIALS AND METHODS

Materials

Mature and dry seeds of mucuna bean were obtained from Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. Cleaned seeds were stored in plastic containers at room temperature before analysis. Beans were dehulled with a hammer mill and ground using a Waring commercial blender (Smart Grind, Black and Decker, Towson, MA, USA). A set of standard sieves, American Society for Testing and Materials (ASTM E11, 8 inch) were used to segregate the sample into four particle size categories with the following particle diameter size range: 0-0.36mm, 0.36 –

0.50mm, 0.50 – 1.00 mm and 1.00 –1.70 mm for processing. Samples of processed bean were ground with a pulverizer (Fritsch Pulverizer, 02.102, Germany) for analysis.

Processing Methods

Soaking at different temperature, pH and particle size diameter levels

A 40 g bean sample (particle size 1.00 – 1.70 mm) was placed and stirred for 1 minute in a 1 litre capacity glass jar containing distilled, deionized water (800 ml).The ratio of sample to water was 1:20 (w/v). Samples were placed in an automated temperature control water bath (Scientific engineering, Grant Instruments Ltd, Cambridge, England) set at 20, 40 or 60°C. The pH was adjusted from 6.4 to 7 ± 0.2 using 1 M NaOH solution. To evaluate pH effect, samples were placed in the water bath set at 20°C. The pH was adjusted from 6.4 to three levels (3, 7 and 9 ± 0.2) using 18 N acetic acid and 1M NaOH solutions accordingly. To study effect of particle size on extraction, samples were placed in a water bath set at 20°C and pH was adjusted from 6.4 to 7 ± 0.2 using 1M NaOH. Three different particle size diameters were used (0.36– 0.50, 0.50– 1.00 and 1.00–1.70 mm). Samples of 10g each were taken at the following time intervals: 6, 12, 24, 36 and 48 hrs. They were frozen overnight at ⁻21°C then freeze dried at -(40-50)°C, (Freeze Mobile Twin 6, United Scientific, Alcatel vacuum pump M 2008A) and (United Scientific, Virtis Bench Top freeze dryer, Gerdiner, NY).

Autoclaving

A 20 g bean sample (particle size 1.0– 1.7 mm) was placed in a 1 litre capacity glass jar. Distilled, deionized water (400 ml) was added and the mixture stirred for 1 min. The ratio of sample to water was 1:20 (w/v). The pH was adjusted from 6.4 to 7 ± 0.2 using 1M NaOH. Samples were placed in an autoclave and heat treated at 121°C at 1 Kgf /cm^2 pressure for 30 min. They were cooled, frozen at ⁻21°C then freeze dried.

Alkaline fermentation

A 40 g bean sample (particle size 1.0– 1.7 mm) was placed in a 1 litre capacity glass jar. Distilled, deionized water (800 ml) was added and the mixture was stirred for 1 min. The ratio of sample to water was 1:20 (w/v). Samples were autoclaved at 121°C at 1 Kgf /cm^2 pressure for 30 min, cooled, and inoculated with activated *Bacillus subtilis* (Microbiology and Plant Pathology Culture Bank, University of Pretoria) at 5% v/v. Starter culture averaged approximately 10^6 cfu/ml. Fermentation was carried out at 32°C for 72 hr. Samples were frozen at ⁻21°C then freeze dried.

Germination

A 100 g bean sample was sterilized by soaking in ethanol for 1 min. Seeds were soaked in distilled water (1:10, w/v) for 12 hr at room temperature (25°C). Water was drained and the seeds were spread between thick layers of wet cotton wool on a tray and allowed to germinate in the dark for three days. Seeds that had not germinated were discarded. Germinated seeds were removed from the cotton wool, seed coats

removed manually and samples placed in plastic bags and frozen at -21°C for 12 hrs to stop germination. Seeds were thawed and dried in an oven at 50°C for 24 hrs. Dried germinated seeds were ground into powder, passed through a 500 µm sieve, frozen at ⁻21°C then freeze dried.

Roasting

A 10 g bean sample (particle size 1.0– 1.7 mm) was mixed with approximately 50 g of sand. The sand had been preheated to 80 °C for 1 hr. Samples were heated in oven set at 100°C for 15, 30, 45 and 60 min. Mixtures were cooled in desiccators for 2 hrs, and samples separated from sand by sieving. Samples were then ground into powder, freeze dried and stored at ⁻21°C until analysis.

ANALYTICAL METHODS

Crude protein

Mucuna bean was ground into fine flour (particle size diameter <0.5mm) and analyzed for crude protein according to AOAC method [7]. Samples were analyzed in triplicate.

Trypsin inhibitor activity

Trypsin inhibitor activity (TIA) was determined using the method reported by Kakade *et al*. [8]. Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) was used as substrate. Absorbance (A) was read at 410 nm wavelength. Trypsin inhibitory activity was defined as the number of trypsin inhibitor units (TIU). One TIU was defined as an increase of 0.01 in absorbance units under conditions of assay.

Amino acid profile

Amino acid profile of mucuna bean protein was determined according to the Pico-Tag Amino Acid Analysis System (Waters Chromatography Div., Millipore Co., Milford, MA, USA) as reported by Bidlingmeyer *et al*. [9]. Samples were acid hydrolyzed, derivatized and subjected to HPLC analysis. Calibration was done using standard amino acid kit (Stock No. AA-S-18) from Sigma –Aldrich, Inc., Germany. Detection was at 254 nm wavelength using Detector Model 440, auto sampling by WISP 712, while identification and quantification was done using the software Millenium 32 Chromatograph (Waters Corp., Milford, MA, USA).

L-Dopa content

L-Dopa was determined after acidic extraction of sample by the method reported by Siddhuraju and Becker [10]. The standard solution of L-Dopa concentration was 200 mg/ml. L-Dopa analysis was on a Pico-Tag C_{18}, 3.9 x 150mm column under the following conditions: injection volume 20 µl, flow rate: 1.0 ml/min, and column temperature of 37°C.

***In vitro* protein digestibility (IVPD)**

This was determined by the pH drop multi-enzyme method reported by McDonough *et al.* [11]. The multi-enzyme (Sigma-Aldrich Inc, Germany) solution was composed of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per ml of distilled water. *In vitro* protein digestibility of sample was calculated using the following equation:

$$\% \text{ digestibility} = 210.464 - 18.103x$$

where x is the pH after the 10-minute incubation.

Biological evaluation of protein quality

This was based on the nitrogen balance method reported by McDonough *et al.* [12]. Mucuna bean was processed under the following conditions: pH 3.2, 60°C, particle size range 1.0 -1.7 mm for 48 h. A sample of 30 clinically healthy weanling albino rats of wistar strain at approximately 4-5 weeks of age were obtained from Teaching and Research rat colony of the Department of Biochemistry and Biotechnology, Kenyatta University. They were divided into four groups of ten rats each on the basis of initial weight, sex and litter origin. Weights of the rats were adjusted in such a way that the mean initial group weights were similar (70 ±10 g). Rats were individually housed in stainless steel screen cages to facilitate separate fecal and urinary collection. They were maintained between 24-25°C and 40-60 % relative humidity with alternating 12 hr periods of light and darkness throughout the study. One group of ten rats was given the N-free basal diet, and remaining two groups were randomly allocated to the test (processed mucuna bean) and reference diets. The composition of basal diet was as described previously [13]. Processed mucuna bean protein to be evaluated was added at the expense of maize starch to give approximately 10% crude protein on a dry matter basis. Casein was used as the reference protein. Proximate composition of the diets used in the study was in the following ranges: protein, 9.8 - 10.2%; fat, 8.9 –9.4%; ash, 5.0 -5.6%; fibre, 2.5 –2.8% and carbohydrate 72.0% for casein and mucuna diets and 82.0% for protein-free diet. Rats were offered water and diets ad libitum for 14 days. Records were kept of weight changes and total food intake. A 10-day (days 5 –14) fecal and urine collection was made from rats during the trial. Urine from each rat was collected and placed in tubes containing 1 ml of 1.0 M sulphuric acid as preservative, with each day's collection being stored separately at $^{-}18$ °C. Fecal samples were collected daily, bulked for each rat, weighed, and stored. Duplicate samples of urine, feces and diets were analyzed for nitrogen. Based upon nitrogen content of the feed, feces and urine, the following definitions of methods of protein assessment were used:

(i) Biological Value (BV) = $\{I – (F – M) – (U – E)\} / \{I – (F – M)\}$. [14]

(ii) True Digestibility of Nitrogen (TD) = $\{I – (F – M) \times 100\} / I$ [15];

where I is the nitrogen intake (mg), F the nitrogen excreted in feces (mg), M the metabolic fecal nitrogen (the amount of nitrogen in the feces of rats fed the protein-free diet was used as the estimate metabolic fecal nitrogen from basal diet) (mg), U the nitrogen excreted in urine (mg), and E the endogenous urinary nitrogen (from basal diet) (mg).

PDCAAS = AAS x True digestibility. [3]

$$AAS = \frac{\text{(\% amino acid in test protein)}}{\text{(\% corresponding amino acid requirement)}}$$

At the end of the study period, rats were anesthetized using diethyl ether and sacrificed. The brain, heart, liver, kidney and lungs were harvested, weighed and preserved in 40% formalin for histological examination.

Histology of tissue specimens

Tissues were trimmed and washed in running water overnight to remove excess formalin. The tissues were then processed using an automatic tissue processor and dehydrated sequentially in increasing concentrations of alcohol at 50, 80, 90 and 96% at hourly stepped intervals. Tissues were cleared of alcohol twice in two changes of xylene. Infiltration with paraffin wax was then done for 3 hrs in paraffin wax oven set at 2˚C below the melting point of wax. Tissues were then embedded in fresh molten wax and allowed to dry. Embedded tissues were sectioned at 0.5 mm thickness with a microtome and floated in warm water to spread out before attaching them onto clean microscope slides. Tissue sections were placed in hot oven for 15 min, dewaxed in xylene and then stained with haematoxylin and eosin dyes using standard histological protocols. Stained tissues were cover slipped with DPX mountant, dried and examined microscopically for any pathological changes.

STATISTICAL ANALYSIS

A complete randomized design was used where mucuna bean was randomized to the treatments (processing methods). Data was exported from excel and analyzed using the Statistical Package for Social Sciences (SPSS) Version 11.5 (SPSS Inc., Chicago, IL USA). Differences between means were compared using paired T-test. Amino acid data was subjected to one way analysis of variance (ANOVA) and post hoc Tukey B test. Differences in means were considered statistically significant at $p < 0.05$. Values expressed as means ± standard deviation (SD).

RESULTS

Amino acid composition of raw and mucuna bean processed by soaking in acidic media is shown in Table 1. Concentration of essential amino acids increased after processing mucuna beans. Effects of various processing methods on crude protein and *in vitro* protein digestibility (IVPD) content of mucuna bean are shown in Table 2. Total crude protein decreased significantly ($P<0.05$) during processing of mucuna

bean by soaking at various temperature, pH and particle size levels. However, processing by germination and fermentation methods significantly (P<0.05) increased the crude protein to 32.9% and 37.6%, respectively. The IVPD for raw mucuna bean was 67.21%. All processing methods except roasting at 100˚C for 60 min significantly (P<0.05) improved IVPD.

Mucuna bean processed at pH of 3.2, 60˚C and particle size diameter of 1.0-1.7mm had high *in vitro* digestibility value of 80.54%. As shown in Table 3, crude protein content was 27.0%, residual L-dopa content was within the recommended level of ≤0.1% [16]. Other anti-nutrients such as phytates and total phenolics content was low (0.39% and 0.06%, respectively) while no tannins or trypsin inhibition were detected. Based on the composition of processed mucuna bean, this processing method was selected and used for *in vivo* digestibility study.

Mean food intake, gain/loss in body weight and nitrogen loss by rats fed on different diets is presented in Table 4. Rats fed on casein, mucuna bean and protein-free (basal) diets consumed feed at an average rate of 8.64, 7.57 and 5.95 g/day, respectively. In addition, rats fed casein diet gained an average weight of 3.77g while those fed on mucuna bean and basal diets lost an average of 5.22 and 9.51g, respectively. Total nitrogen losses in feaces for rats fed casein, mucuna bean and basal diets ere. Total nitrogen loss for rats consuming casein diet significantly differed with the highest loss observed in rats consuming mucuna beans and least in basal diet. Rats fed on mucuna diet significantly (P<0.05) consumed less feed, lost weight and lost more nitrogen in feces compared to those fed on casein diet. Protein quality measurement parameters are presented in Table 5. The PDCAAS for mucuna diet was 0.37 while that for casein diet was 1.00. True digestibility (TD) for processed mucuna and casein diets were 58% and 93.6% while biological values (BV) for the same were 80.8% and 94.2%, respectively. The PDCAAS, TD and BV for mucuna diet were significantly (P<0.05) lower than for casein diet.

Average weights of internal organs of rats fed on the casein, processed mucuna bean and basal diets at the end of the study period are shown in Table 6. Average organ weights for rats consuming mucuna bean diet were significantly higher (P<0.05) than those for rats fed on casein diet. In addition, organ weights for rats consuming basal diet were significantly higher (P<0.05) except for the pancrease whose weight was significantly (P<0.05) lower than that for rats fed on casein diet.

Histological examination of liver and kidney specimens from rats fed on the three different diets are shown in Figures 1 to 6. Liver specimens from rats fed the casein diet demonstrated normal liver histology. However, liver specimens obtained from rats fed on processed mucuna bean diet revealed liver infiltrates, vacuolar degeneration, venous congestion and necrosis of liver cells while specimens from rats fed on basal diet exhibited liver fatty degeneration. Examination of kidney tissue specimens from rats fed on processed mucuna bean diet revealed infiltrates and tubular atrophy while those obtained from the casein and basal diet fed rats exhibited

normal histology. Histological examination of the liver specimens from rats fed the basal diet showed fatty liver degeneration that is associated with lack of protein.

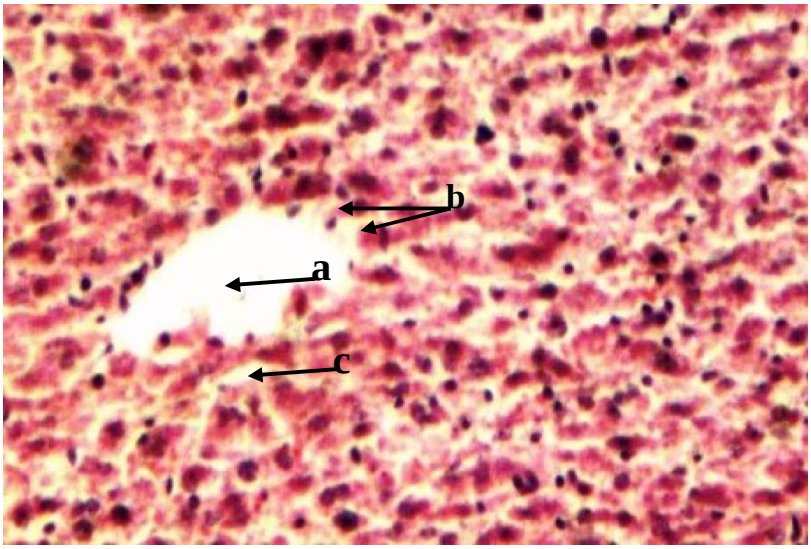

Figure 1: Histological section of liver of a rat fed the casein diet showing normal liver histology: a) central vein, b) hepatic cords and c) liver sinusoid. Magnification: X 100

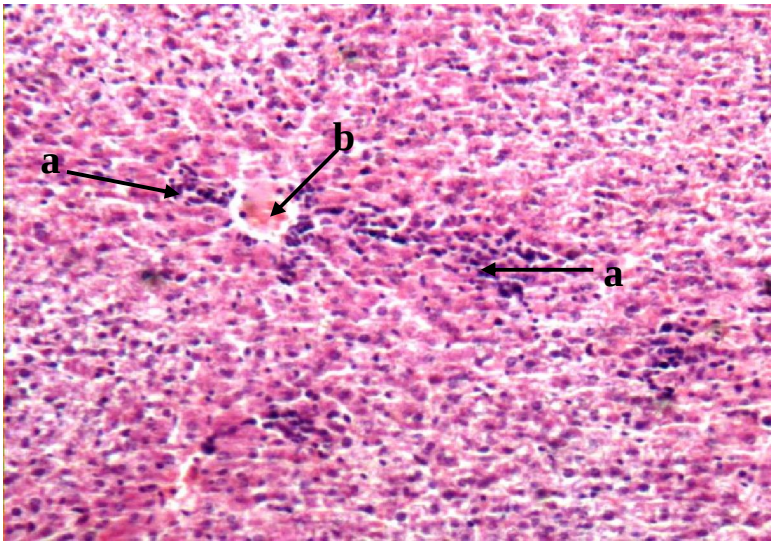

Figure 2: Histological section of liver of a rat fed mucuna bean diet showing a) infiltration and b) venous congestion. Magnification: X 100

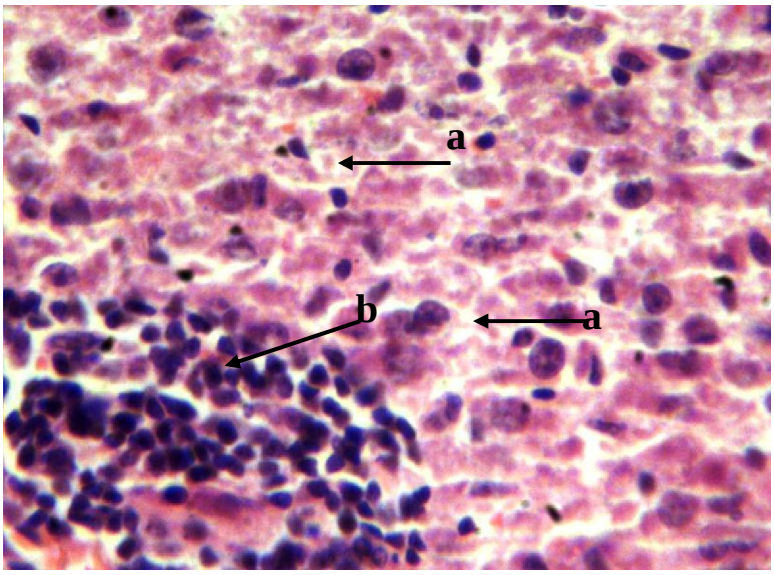

Figure 3: Histological section of liver of a rat fed mucuna bean diet showing a) necrosis and b) infiltration. Magnification: X 400

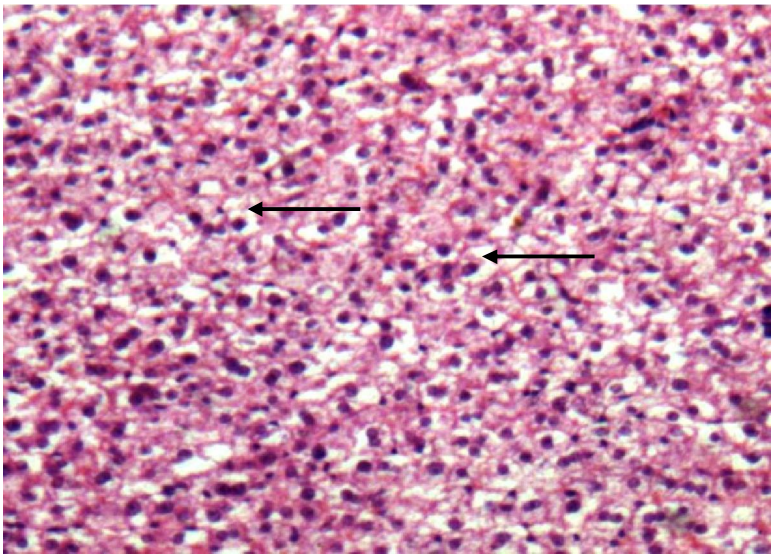

Figure 4: Histological section of liver of a rat fed protein-free diet showing fatty degeneration of hepatocytes. Magnification: X 100

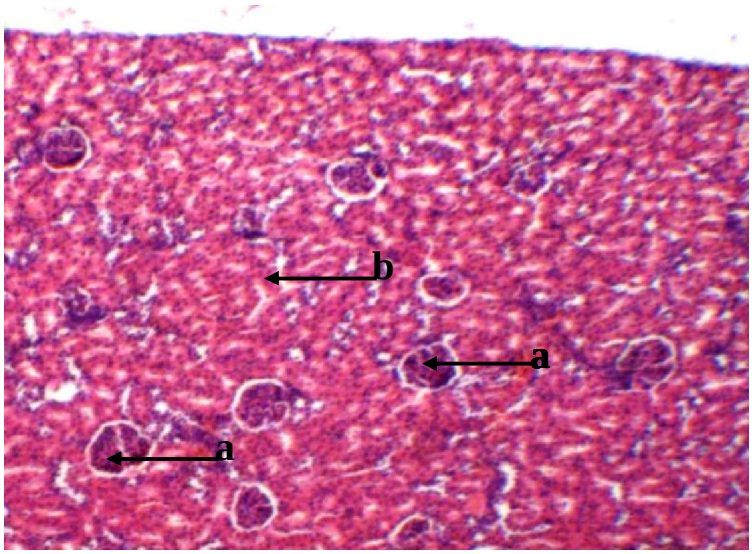

Figure 5: Histological section of kidney of a rat fed casein diet showing normal kidney histology: a) glomerulus and b) tubules. Magnification: X 100

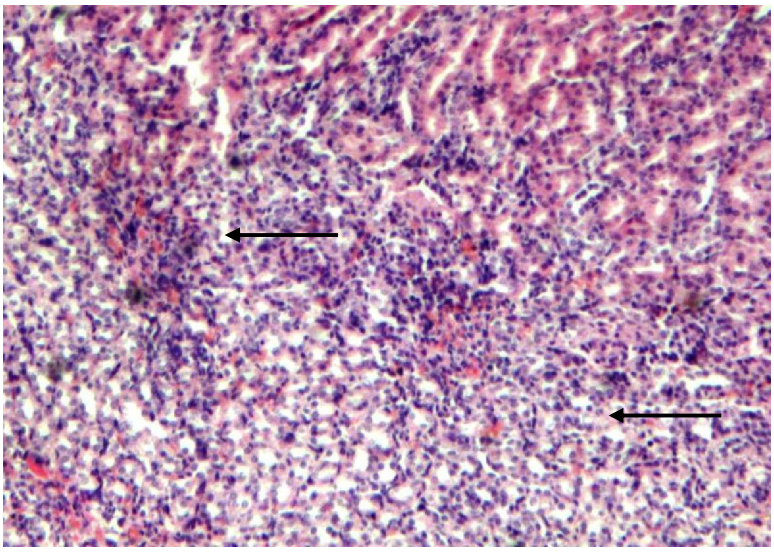

Figure 6: Histological section of kidney of a rat fed mucuna bean diet showing medulla tubular atrophy. Magnification: X 100

DISCUSSION

The protein quality of food depends on content and physiological utilization of its amino acids. Processing mucuna bean (by extraction at pH 3.2, 60˚C) increased the content of all essential amino acids. Content of essential amino acids observed in mucuna bean are consistent with those reported for three mucuna species [17]. Except for methionine and tryptophan, the content of other essential amino acids in raw and processed mucuna bean protein met the recommended reference requirements for 2-5 yr old [3]. Increase in crude protein of mucuna bean during germination may be attributed to extensive breakdown of seed-storage compounds and synthesis of structural proteins and other cell components occurring during germination. High amino acid biosynthetic activity in seedlings resulted in increased contents of free amino acids to support protein synthesis [18].

The IVPD for processed mucuna bean (80.54%) was higher than the range (68.0-76.9%) reported in other studies [19]. However, it was in the range of IVPD values reported for cowpea (75.5-78.3%), pigeon pea (76.8-80.1) and white beans (77.9-79.2) [20, 21]. Legumes have low protein digestibility partly due to their structural characteristics. The major protein fraction of legume seeds, the globulin, is fairly resistant to enzymic hydrolysis making denaturation by cooking important in protein digestion. Improvement in IVPD of soaked legumes is attributed to changes in activities of endogenous enzymes or alteration of storage protein structures including structural disintegration of enzyme inhibitors [22].

Protein-free diet was used to demonstrate endogenous nitrogen. The BV of processed mucuna bean was high indicating significant utilization of protein. However, inspite of high BV, PDCAAS was low and proportion of total nitrogen intake lost in feces by rats fed on mucuna protein was significantly higher (6.7%) compared to that for rats fed on the casein diet that lost 1.8%. This was attributed to low sulphur amino (methionine and cysteine) acids and poor (58%) true digestibility of mucuna bean protein that resulted to weight loss. Poor digestibility indicates presence of antinutrients such as phytates, protease inhibitors, condensed tannins and polyphenols that interact with protein to form complexes. This interaction leads to increased cross-linking, reduce protein solubility rendering them less susceptible to hydrolysis and consequently lower nutritional value of protein [23]. In addition, oxidation products of L-Dopa may conjugate with sulphur amino acid residues (cysteine) of proteins to form a protein bound 5-Scysteinyldopa cross-link resulting to polymerization of proteins and may contribute to reduction in protein digestibility of mucuna bean [24]. Anti-nutritional compounds have also been associated with increased losses of endogenous proteins at the terminal ileum of pigs [25].

Increase in organ weights may be attributed to fluid retention and relative proportion of organ in the reduced weight rat. A significant ($p<0.05$) increase in the weight of liver, kidney and pancrease was observed with mucuna diet fed rats. Increase in liver weight has been reported on broiler chicks fed partly on mucuna protein [26]. Liver specimens obtained from rats fed on processed mucuna bean diet revealed liver

infiltrates, vacuolar degeneration, venous congestion and necrosis of liver cells while specimens from rats fed on protein-free diet exhibited fatty liver degeneration that is associated with lack of protein. Fatty liver results from impairment of the normal secretion of fat-containing proteins (lipoproteins) by the liver [27]. Liver infiltrates, perivascular cuffing with lymphocytes, vacuolar degeneration and necrosis from mucuna fed rats indicated liver function abnormality leading to toxic injury [28]. Inflammatory response by cells close to the central veins of the liver suggested that toxins may have been carried in the blood. Mucuna bean has been associated with reduced growth or loss in weight and acute toxic hepatitis in pigs and reduction of growth rate and feed utilization in common carp [19, 29]. Examination of kidney specimens showed loss of kidney function characterized by interstitial infiltrates, fibrosis and inflammatory atrophy associated with the mucuna diet. However, report on studies on raw and roasted mucuna bean do not indicate presence of mutagenic or substances that can be converted into mutagens by metabolism in the liver [30]. This implies that though the mucuna diet contained the recommended safe level of residual L-Dopa (<0.1%) there could be other toxins present in the processed mucuna bean that were toxic to the rats such as steroids [16, 29].

CONCLUSION

All processing methods (except roasting) improved protein quality (IVPD) of mucuna bean. In addition processing in acidic (pH 3.2, 60˚C) medium at particle size diameter range of 1.0 – 1.7mm improved BV of protein. However, the processed bean did not support growth of weanling rats when fed as sole protein. Low PDCAAS for mucuna bean protein could be attributed to very low content of sulphur amino acids and possible presence of factors that hinder protein hydrolysis thereby reducing nutritional value. Consumption of processed mucuna bean by weanling rats caused inflammation of liver and kidney suggesting presence of toxins other than L-Dopa. Hence, mucuna bean may not be used as the sole protein in the human diet.

ACKNOWLEDGEMENTS

The authors are grateful to Legume Research Network Project, Kenya Agricultural Research Institute, Nairobi, Kenya, for providing mucuna beans and partly funding this research; University of Pretoria, Department of Biochemistry where processing of mucuna bean, protein and L-Dopa analysis was done and Department of Science and Technology, South Africa; Tropical Soils Biology and Fertility Institute, and Kenyatta University for funding this study.

Table 1: Amino acid composition of raw and processed (soaked in acidic medium at pH 3.2 and at 60˚C for 48 hrs) mucuna bean (mg/g)

Amino acid	Raw mucuna bean	Processed mucuna bean	Essential amino acid requirement for ildren[1] (2-5 yrs)
Non essential amino acids			
Alanine	32.6	41.5	
Arginine	60.2	57.7	
Aspartic acid	84.3	92.3	
Cysteine	15.7	16.2	
Glutamic acid	110.3	135.7	
Glycine	35.7	40.4	
Proline	48.6	52.6	
Serine	40.1	49.3	
Essential amino acids			
Histidine	20.1	25.0	19.0
Isoleucine	39.2	49.3	28.0
Leucine	66.8	82.7	66.0
Lysine	58.0	69.5	58.0
Methionine	8.2	11.8	25.0
Phenylalanine and tryrosine	87.2	101.1	63.0
Tryptophan	7.2	8.0	11.0
Threonine	39.2	41.5	34.0
Valine	44.8	51.8	35.0

Adapted from: [1]FAO/WHO (1991);Give no, Not year Values are means of duplicate determinations

Table 2: Crude protein (%) and *in vitro* digestibility (%) of processed[1] mucuna bean

Treatment	Crude protein (%)	*In vitro* digestibility (%)
Raw mucuna bean	31.9 [c] ±0.30	67.21 [g] ±0.05
Roasting at 100˚ C	31.8 [c] ±0.14	67.15 [g] ±0.18
Soaking (pH 3.2, 20˚ C)	26.9 [g] ±0.00	80.73 [a] ±0.02
Soaking (pH 9.0, 20˚ C)	23.1 [h] ±0.42	75.48 [c] ±0.01
	22.0 [i] ±0.14	73.91 [d] ±0.02
Extraction at 20˚ C, pH 7.0	28.9 [e] ±0.14	75.54 [c] ±0.06
Extraction at 60˚ C, pH 7.0	27.8 [f] ±0.14	79.28 [b] ±0.01
Soaking (pH 3.2 and at 60˚C)	27.2 [fg] ±0.0	80.54 [a] ±0.01
Autoclaving	29.7 [d] ±0.28	78.68 [b] ±0.05
Fermentation	37.6 [a] ±0.14	70.83 [e] ±0.08
Germination	32.9 [b] ±0.14	69.14 [f] ±0.10

Values are means ± SD of triplicate determinations; Values followed by different superscripts in the same column are significantly *(P<0.05)* different; [1]Processing conditions (pH 3.2, 60˚C, particle size diameter (1.0 – 1.7mm)

Table 3: Proximate composition and anti-nutritional compounds content of processed[1] mucuna bean

Component	Processed dehulled mucuna bean[1] g/100g (dwb)
Crude protein	27.0 ± 1.1
Crude fat	5.5 ± 0.1
Crude fibre	1.8 ± 0.2
Ash	0.3 ± 0.01
L-Dopa	0.1 ± 0.01
Trypsin inhibitor activity[A]	No inhibition
Phytic acid[B]	0.4 ± 0.04
Total phenolics[C]	0.1 ± 0.01
Tannins[D]	0

Values are means ± SD of triplicate determinations; Values followed by different superscripts in the same row are significantly *(p < 0.05)* different; [A]As TUI / mg sample; [B]As phytic acid; [CD]As tannic acid equivalents. [1]Processing conditions (pH 3.2, 60˚C, particle size diameter (1.0 – 1.7mm)

Table 4: Responses of rats fed on different feeds

Assay	Casein	Mucuna bean diet	Protein-free diet (Basal)
Feed intake (g)	$8.64^{a} \pm 0.75$	$7.57^{b} \pm 0.83$	$5.95^{b} \pm 0.66$
Gain in body weight (g)	$3.77^{c} \pm 0.41$	$-5.22^{b} \pm 0.65$	$-9.51^{a} \pm 0.89$
Total nitrogen loss in feaces (%)	$12.12^{b} \pm 0.04$	$15.60^{a} \pm 0.05$	$9.35^{c} \pm 0.03$

Values are means ± SD of ten replicate determinations; Values followed by different superscripts in the same row are significantly *(P<0.05)* different

Table 5: Protein quality of casein and mucuna bean fed to rats

Assay	Casein	Mucuna bean diet
TD (%)	$93.6^{a} \pm 3.14$	$58.0^{b} \pm 4.40$
BV (%)	$94.2^{a} \pm 2.35$	$80.8^{b} \pm 2.62$
AAS[1]	1.24^{a}	0.63^{b}
PDCAAS	$1.16^{a} \pm 0.05$	$0.37^{b} \pm 0.05$

Values are means ± SD of ten replicate determinations; Values followed by different superscripts in the same row are significantly *(P<0.05)* different, [1]Values are means of duplicate determinations

Table 6: Weight (g) of internal organs of rats fed casein, mucuna bean and protein-free diet

Diet	Brain	Liver	Heart	Pancrease	Kidney	Lungs
Casein	$2.09^{c} \pm 0.08$	$6.24^{b} \pm 0.49$	$0.44^{b} \pm 0.03$	$0.66^{b} \pm 0.09$	$0.94^{b} \pm 0.08$	$1.02^{c} \pm 0.08$
Mucuna bean	$2.67^{b} \pm 0.09$	$6.83^{a} \pm 0.54$	$0.51^{a} \pm 0.04$	$0.74^{a} \pm 0.13$	$1.20^{a} \pm 0.09$	$1.19^{b} \pm 0.11$
Basal	$2.80^{a} \pm 0.06$	$6.85^{a} \pm 0.53$	$0.55^{a} \pm 0.04$	$0.59^{c} \pm 0.08$	$1.16^{a} \pm 0.08$	$1.29^{a} \pm 0.09$

Values are means ± SD of ten replicate determinations; Values followed by different superscripts in the same column are significantly *(P<0.05)* different

REFERENCES

1. **Young VR** Soy protein in relation to human protein and amino acid nutrition. *J. Ame. Diet. Asso.* 1991; **91**: 828-835.

2. **Darragh AJ and SM Hodgkinson** Quantifying the Digestibility of Dietary Protein. *J. Nutrition,* 2000; **130**: 1850S-1856S.

3. **FAO/WHO.** Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation, Bethesda, MD, USA, 4–8 December 1989. FAO Food and Nutrition Paper No. 51. FAO, United Nations, Rome. 1997.

4. **Liener IE** Implications of antinutritional components in soybean foods. *Critical Reviews in Food Sci. Nutri*. 1994; **34**: 31-67.

5. **Alonso R, Aguire A and F Marzo** Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *J. Food Chem.* 2000; **68**: 159-165.

6. **Duke JA** Handbook of Legumes of World Economic Importance Duke, J.A. (Ed); Plenum Press: New York. 1981; 170-173.

7. **AOAC.** Official methods of Analysis, 15th Edition. *Association of Official Analytical Chemists,* Washington, DC. 1990.

8. **Kakade ML, Rackis JJ, McGhee JE and G Puski** Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chem.* 1974; **51**: 376– 382.

9. **Bidlingmeyer BA, Cohen SA, Tarvin TL and BA Frost** New, rapid, high sensitivity analysis of amino acids in food samples. *J. Assoc Off. Anal. Chem.* 1987; **70(2):** 241-247.

10. **Siddhuraju P and K Becker** Rapid reversed-phase high performance liquid chromatographic method for the quantification of L-Dopa (L-3,4-dihydroxyphenylalanine): non-methylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. *J. Food Chem*. 2005; **91**: 275–286.

11. **Hsu HW, Sutton NE, Banjo MO, Satterlee LD and JG Kendrick** The C-PER and T-PER Assays for Protein Quality, *Food Technol.* 1977; 32 (**12**): 69-73.

12. **McDonough FE, Steinke FH, Sarwar G, Eggum BO, Bressani R, Huth PJ, Barbeau WE, Mitchell GV and JG Phillips** *In vivo* rat assay for true protein digestibility: collaborative study. *J. of Asso. Off. Analy. Chem.* 1990; **73**: 801-805.

13. **Apata DF and AD Ologhobo** Some aspect of the biochemistry and nutritive value of African yam bean seed (*Sphenositylis stenocarpa)*. *J. Food Chemistry.* 1990; **36**: 271 –280.

14. **Phillips DE, Eyre MD, Thompson A and D Boulter** Protein quality in seed meals of *Phaseolus vulgaris* and heat-stable factors affecting the utilization of protein. *J. Sci. Food Agric.* 1981; **32**: 423 –432.

15. **Dreyer JJ** Biological assessment of protein quality: digestibility of the proteins in certain foodstuffs. *South African Medical J.* 1968; **42**: 1304–1313.

16. **Teixeira AA, Rich EC and NJ Szabo** Water extraction of L-Dopa from *Mucuna* bean. *J. Trop. Subtropical Agroecosystems.* 2003; **1**: 159-172.

17. **Prakash D, Niranjan A and SK Tewari** Some nutritional properties of the seeds of three *Mucuna* species. *Inter. J. Food Sci. Nutri.* 2001; **52**: 79-82.

18. **Kuo Y-H, Rozan P, Lambein F, Frias J and C Vidal-Valverde** Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *J. Food Chem*. 2004; **86**: 537–545.

19. **Siddhuraju P and K Becker** Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian tribal Pulse, *Mucuna pruriens var. utilis. J. Agric. Food Chem.* 2001:**49**: 3058-3067.

20. **Preet K and D Punia** Proximate composition, phytic acid, polyphenols and digestibility (*in vitro*) of four brown cowpea varieties. *International J. Food Sci. and Nutr*. 2005; **51**: 189–193.

21. **Sangronis E and CJ Machado** Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan. LWT Food Sci. Technol.* 2005; **40**: 116-120.

22. **Pugalenthi M, Vadivel V and P Siddhuraju** Alternative Food/Feed Perspectives of an underutilized Legume Mucuna pruriens var *Utilis* – A Review. *Plant Foods Human Nutri*. 2005; **60**: 201-218.

23. **Reddy NR, Pierson MD, Sathe SK and DK Salunkhe** Dry bean tannins: a review of nutritional implications. *J. Ame. Oil Chem. Society.* 1985; **62**: 541 - 549.

24. **Takasaki S and S Kawakishi** Formation of protein-bound 3,4-dihydroxyphenylalanine and 5-S-cysteinyl-3,4-dihydroxyphenylalanine as new cross-linkers in gluten. *J. Agric. Food Chem*. 1997; **45**: 3472-3475.

25. **Salgado P, Montagne L, Freire JPB, Ferreira RB, Teixeria A, Bento O, Abreu MC, Toullec R and JP Lalles** Legume grain enhances ileal losses of specific endogenous serine-protease proteins in weaned pigs. *J. Nutri*. 2002; **132**: 1913-1920.

26. **Ukachukwu SN, Ezeagu IE, Tarawali G and JEG Ikeorgu** Utilization of *Mucuna* as a food and feed in West Africa. **In** Flores M, Eilittä M, Myhrman R, Carew L and R Carsky. (Eds.), *Mucuna as a Food and Feed: Current Uses and the Way Forward.* Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. 2002; 189-217.

27. **Broody T** Nutritional Biochemistry, 2nd edition. New York: Academic press. 1999; 421-489.

28. **Patrick RS and JO'D McGee** *Biopsy Pathology of the Liver*. Chapman and Hall Ltd. London. 1988; 19-48.

29. **Flores L, Esnaola MA and R Myhrman** Growth of pigs fed diets with Mucuna bean flour (*Mucuna pruriens*) compared to soybean meal. In *Mucuna as a Food and Feed: Current Uses and the Way Forward.* M. Flores, M. Eilittä, R. Myhrman, L. Carew and R. Carsky. (Eds.), Workshop held April 26-29, 2000 in Tegucigalpa, Honduras. 2002; 288-305.

30. **Burgess S, Hemmer A and R Myhrman** Examination of raw and roasted *Mucuna pruriens* for tumerogenic substances. *J. Trop. Subtropical Agroecosystems.* 2003; **1**: 287–293.