Dehulling reduces toxicity and improves in vivo biological value of proteins in vegetal milk derived from two mucuna (*Mucuna pruriens* L.) seeds varieties

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Abstract The present work was carried out to evaluate the nutritive quality (proximate and antinutrients composition) of vegetable milks prepared from whole and dehulled mucuna bean flours. Casein and mucuna milk diets were fed to rats (four weeks old; $n=8$ per group) for 28 days to determine protein efficiency ratio (PER), net protein efficiency ratio (NPER), true and apparent digestibility (TD and AD, respectively), organ-to-body weight ratios and hematological parameters. The experimental design was a factorial design with two variety of mucuna (cochinchinensis and veracruz) and two treatments (whole and dehulled beans). Protein, total sugar, dry matter and ash-content of mucuna milks ranged from 6.40 to 12.13 g/100 mL, 10.52 to 13.08 g/100 mL, 8.59 to 12.88 g/100 g and 0.31 to 0.92 g/100 g, respectively. Milks from dehulled flours had lower contents of tannins (80–87.08 %), phytates (76.67–78.16 %) and L-Dopamine (44.45–66.66 %) than that from whole flours. The PER of dehulled mucuna diets were 22.76–

21.74 %, but negative PER and low NPER was observed for whole mucuna milk diets. TD for dehulled mucuna milk (85.15–85.96 %) were higher and similar to casein when compared to that of whole mucuna milk (47.87–51.17 %). Rats fed with diets containing whole mucuna milk lost weight and had higher kidney weight. In addition, the rats fed with milk from whole mucuna flours showed significantly lower levels of lymphocytes, granulocytes, red blood cells, hemoglobin and hematocrit than that fed with dehulled mucuna milk.

Keywords *Mucuna pruriens* · Dehulling · Vegetable milk · Chemical composition · Protein quality · Hematological parameters

Introduction

Malnutrition remains a major problem nowadays with approximately 925 million people undernourished in the world and 98 % of them in developing countries (FAO 2012). Among the identified forms of malnutrition, protein energy malnutrition is the most prevalent (Siddhuraju et al. 2000; Mezajoug et al. 2011). Protein energy malnutrition believes vertiginously in Africa where the rate of people affected has increased from 100 million in 1970 to 200 million in 2012 (FAO 2012). In Cameroon, 33 % of children under 5 years old are affected by protein malnutrition, with the North and Far-North regions being the most affected (INS 2013). In order to fight against malnutrition, the use of legumes dry seeds has been suggested (Siddhuraju et al. 2000). In this respect the use of legume dry seeds such as soy and peanuts beans in the production of milk has long been used in several countries to enrich the diet of young children with protein (Sowonola et al. 2005). However, due to population growth and low agricultural yields,

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conventional legume seeds regarded as a major source of dietary protein for the population in developing countries have become insufficient (Janardhanan et al. 2003). In addition, there is a competition between people, animals and industries for these common leguminous seeds as these are used in animal nutrition and represent a source of raw material for industries. Faced to this situation, it becomes important to diversify protein food resources to improve people's needs and to meet industrial demand. *Mucuna* dry seeds have been recently the subject of scientific attention (Mang et al. 2015a, b, c).

Mucuna pruriens is an underutilized tropical leguminous seeds with nutritional qualities comparable to soya beans and other conventional leguminous seeds as it contains similar proportions of proteins, lipids, minerals, and other nutrients. They are traditionally used as a soup thickener by rural population in Far-North region of Cameroon. Outside Cameroon, the seeds are also eaten by Ibos in south-eastern Nigeria, Indian tribal sects, Mundari and Dravidian groups (Adebawale et al. 2005). In our previous studies (Mang et al. 2015a, b, c), we have determined the optimal conditions for milk production from either whole or dehulled *Mucuna* dry seeds. The results revealed that while the milk made from dehulled *mucuna* were higher in protein and carbohydrate; those made from whole *mucuna* were higher in total phenols. These results suggested that *mucuna* milk can be used either for its nutritional or antioxidant point of view, however the biological value of proteins in *mucuna* milk still not evaluated, at the best of our knowledge.

The ability of a protein source to be efficient for the supply depends not only on its chemical composition, but also on the digestibility of its protein (Dahouda et al. 2009). Ngatchic et al. (2013a) recently reported the amino acid score of *mucuna* protein isolate and found that sulfur amino acid were the limiting amino acids. In addition Ngatchic et al. (2013a, b) also demonstrated in rat systems that when ingested, *mucuna* protein may exhibit either toxic or positive biological activity dependent on the extraction method. In the method of production of *mucuna* milk recently reported (Mang et al. 2015a, b), we used the whole and dehulled seeds which undergone process of boiling, drying and milling to obtain flours which was used for milk production by extraction in water at 60 °C. The fundamental question to be answered in this study was then: what is the effect of the dehulling process on the biological value of the *mucuna* milk since these are the determinant parameters that conditioned its use as food for human.

Therefore, this work was done to 1) evaluate the effect of dehulling on nutrients and antinutrients composition and in vivo protein digestibility of *mucuna* milks and 2) to investigate the influence of *mucuna* milks on hematological parameters of rats.

Materials and methods

Sampling and production of *mucuna* bean flours

Two varieties of *M. pruriens* seeds (Cochinchinensis and Veracruz mottle) used for this study were obtained from the International Institute of Tropical Agriculture (IITA) of Yaounde, Cameroon. *Mucuna* bean flours were produced as previously described by Mang et al. (2015a). Dried beans were carefully cleaned, sorted to remove defective ones and graded according to size. Beans were soaked in distilled water (1:10, w/v) in a controlled temperature water bath at 35 °C for 48 h. Steep water was replaced every 12 h. At the end of soaking, seeds were divided into two sets: one part was dehulled manually while the another one was left whole. Both whole and dehulled beans were boiled in water for 1 h and dried in a ventilated electric turning dryer (brand Riviera & Bar) at 40 ± 2 °C for 48 h. After drying, the beans were separately ground to make fine flour using an electric grinder (Culatti, Polymix, France) equipped with a sieve of diameter 500 µm mesh. The obtained whole and dehulled bean flours were directly used for milk production.

Procedure of production of *mucuna* milk

The production of vegetable milk samples from *Mucuna* bean flours was performed at optimal conditions as described earlier (Mang et al. 2015c). Briefly, 8 g flour was blended with 100 mL distilled water. The slurry was stirred at 3500 rpm for 60 min at 60 °C using an electric stirrer (TECHNICON stirrer motor, England). After incubation, the sample was centrifuged at 1500 g for 15 min at 20 °C using refrigerated centrifuge. The supernatant was collected and the residue was re-extracted as mentioned above. The supernatants were combined and packaged in 100 mL volumetric glass vessels and stored at 4 °C in the refrigerator for analysis within a maximum of 4 h. The vegetable milks resulting from Veracruz flours were coded as WVM (whole veracruz milk) and DVM (dehulled veracruz milk), while those resulting from Cochinchinensis flours were coded as WCM (whole cochinchinensis milk) and DCM (dehulled cochinchinensis milk).

Chemical analysis

Proximate analysis

Dry matter content of milks was determined following the AOAC (2003) method. Total protein content (Nx6.25) was analyzed using approved method (AOAC 2003). Total sugar was determined after digestion in concentrated sulfuric acid followed by analysis with aqueous phenol as described by Dubois et al. (1956). Ash was determined as the residue of incinerated milk (10 g) in a crucible at 550 °C in a muffle furnace (AOAC 1990). pH and total titrable acidity (TTA)

were determined by the AOAC (2003) method. The pH of milk was determined at ambient temperature using a pH Meter (Eutech Instruments, Malaysia). Total acidity was then measured by titration with 0.1 N NaOH to equivalent point, using an automatic Titroline apparatus (Schott Schweiz AG, St. Gallen, Switzerland). Percent total titratable acidity was expressed as malic acid using a conversion factor of 0.067.

Analysis of anti-nutrient and toxicant in mucuna milk

The content of total phenolic in milk expressed as mg gallic acid per 100 mL of milk was determined by the Folin–Ciocalteu method, according to the method of Gao et al. (2000). Tannin level (equivalent of mg tannic acid per 100 mL) of milk was determined by the method of Bainbridge et al. (1996). The phytic acid content was determined following a method described by Vaintraub and Lapteva (1988), and results were expressed as mg phytic acid per 100 mL of milk. L-Dopa level in mucuna milk was determined by the spectrophotometric method using a standard curve made of L-dopamine concentration varying from 2 to 100 µg/mL (Ezeagu et al. 2003) (Table 1).

Animal experiments and biological assay

Diet formulation

Diets were formulated according to Ngatchic et al. (2013a) and fed to 25–30 days old male albino rats (strain wistar) weighing between 60 and 70 g. The composition of the diets is shown in Table 2. There were 6 experimental diets with 2 made of Cochinchinensis milk (dehulled and whole bean milk), 2 from Veracruz milk (dehulled and whole bean milk), a positive control diet consisting of casein (10 %) and a negative control diet without protein. The volume of milks incorporated into the respective experimental diets were determined so as to make up a final protein content of 10 %.

Animal experiments and determination of protein digestibility

A total of forty-eight rats obtained from the animal house of National School of Agro-Industrial Sciences were used for the experiment. The animals placed in individual metabolic cages were randomly grouped into the six groups of eight animals each. After an acclimatization period of 4 days during which the rats were fed standard diet made of casein as protein source, each group of rats was fed with one of the experimental diets. The laboratory temperature was 24 ± 3 °C while the experimental period was 28 days, with alternate periods of light and dark of 12 h. Rats received water and their experimental diets ad libitum. Individual rat body weight, feed intake and feed waste were measured and recorded per 2 days and used in calculating weight gain or loss, protein intake,

protein efficiency ratio (PER) and net protein efficiency ratio (NPER) following Adrian et al. (1998) procedure. The true (TD) and apparent (AD) digestibility were determined by measuring the amount of nitrogen ingested in the diet, the amount eliminated in the feces, and the metabolic loss in the feces, which corresponds to the fecal nitrogen in the protein free group. At the end of the experiment, the feces were dried at 105 °C for 24 h, cooled, weighed and ground in a food processor for the determination of nitrogen concentration by the Kjeldahl method (AOAC 1990). The samples were analyzed in six replicate determinations. The protein digestibility parameters were calculated as followed:

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein consumed (g)}}$$

$$\text{NPER} = \frac{\text{Gain in body weight (g)} + \text{Loss in body weight of protein free diet (g)}}{\text{Protein consumed (g)}}$$

$$\text{TD} = \frac{Ni - (NF1 - NF2)}{Ni} \times 100; \quad \text{AD} = \frac{Ni - NF1}{Ni} \times 100$$

Where: Ni = Nitrogen intake of animals fed the test diet; NF1 = Nitrogen excreted in feces of animals fed the test diet; NF2 = Nitrogen excreted in feces of animals fed the protein free diet.

Evaluation of organ-to-body weight ratios and hematological parameters

At the end of the 28 days experiment, rats were fasted for 14 h, anaesthetized by inhalation of diethyl ether impregnated on a cotton wool, and blood was collected via cardiac puncture on ethylene diamine tetracetic acid (EDTA) tubes for hematological analysis. The liver, kidneys, brain, testicles and heart were removed and weighed precisely to determine organ to body weight ratios. Hematological analyses were performed on blood samples using an automated hematology analyzer (Humacount; Human, Weisbaden, Germany). The recorded parameters were white blood cells, lymphocytes, granulocytes, red blood cells, hemoglobin, hematocrit and platelets.

Statistical analysis

The data reported in the tables and figures were carried out in triplicate or more replicate determinations. Analysis of variance was performed to determine the effect of variety and bean dehulling on the proximate composition and antinutrient in mucuna milk. In addition, the effect of these factors was determined on in vivo proteins digestibility of milk and hematological parameters of bloods samples. When statistical differences were found, the Duncan's

Table 1 Chemical composition of *Mucuna pruriens* bean flours (Mang et al. 2015a)

Components (g/ 100 g dry matter)	var. Cochinchinensis		var. Veracruz	
	Whole beans flour	Dehulled beans flour	Whole beans flour	Dehulled beans flour
Moisture	10.21 ± 0.71 ^b	9.62 ± 0.13 ^a	10.63 ± 0.15 ^b	9.91 ± 0.07 ^a
Total carbohydrate	46.52 ± 1.29 ^a	55.40 ± 1.01 ^b	47.11 ± 1.02 ^{ab}	54.44 ± 1.09 ^b
Crude proteins	26.63 ± 0.33 ^b	32.91 ± 0.65 ^d	25.62 ± 0.26 ^a	30.41 ± 0.52 ^c
Crude lipid	6.04 ± 0.50 ^{ab}	6.25 ± 0.30 ^{ab}	5.83 ± 0.57 ^a	6.73 ± 0.30 ^b
Ash	2.94 ± 0.13 ^b	2.25 ± 0.14 ^a	3.32 ± 0.11 ^b	1.94 ± 0.11 ^a
Total phenolics	2.33 ± 0.07 ^c	0.82 ± 0.04 ^a	3.91 ± 0.11 ^d	1.32 ± 0.11 ^b
Tannins	1.32 ± 0.02 ^c	0.52 ± 0.01 ^a	1.12 ± 0.01 ^b	0.52 ± 0.02 ^a
Phytates	1.24 ± 0.07 ^c	0.95 ± 0.05 ^b	1.15 ± 0.02 ^c	0.83 ± 0.02 ^a
L-Dopa	2.04 ± 0.06 ^c	0.95 ± 0.06 ^a	1.62 ± 0.07 ^b	0.94 ± 0.07 ^a

Means ± SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

Multiple Range Test was applied in order to classify samples at the significant level of 5 %. Statgraphics Program (Statically Graphics Educational, version 6.0 1992 Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis.

Results and discussion

Proximate composition

The proximate composition of dehulled and whole mucuna milk samples is presented in Table 3. The protein content did not vary significantly with variety; however, while the content in dehulled mucuna milks showed higher protein

content (12.0–12.1 %) than whole mucuna milks (6.40–6.80 %). The significant ($p < 0.05$) effect of variety was observed for total sugar content which was higher for Veracruz (11.65–13.08 %) than for Cochinchinensis milks (10.52–12.11 %). As for protein content, carbohydrates content of whole mucuna milks were significantly ($p < 0.05$) lower than that of dehulled mucuna milk, irrespective of the variety. The increase of protein (78.38–87.96 %) and carbohydrate content (10.93–15.11 %) after removal of the hull may be attributed to the complexation of proteins and carbohydrates by tannins and polyphenols present in the beans hull. Comparatively, mucuna milks had higher protein content (6.4–12.1 %) than common vegetable milk such as soya (2.1 %) and peanut milk (3.1 %) (Isanga and Zhang 2009).

Table 2 Composition of the diets (100 g) used in the experiments with rats

Ingredients	Diets					
	Protein free diet	Casein	DCM	WCM	DVM	WVM
Mineral mix (g)	1	1	1	1	1	1
Vitamin mix (g)	4	4	4	4	4	4
Cellulose (g)	5	5	5	5	5	5
Tournesol oil (g)	10	10	10	10	10	10
Casein (g)	–	10	–	–	–	–
DCM (mL)	–	–	85	–	–	–
WCM (mL)	–	–	–	160	–	–
DVM (mL)	–	–	–	–	85	–
WVM (mL)	–	–	–	–	–	160
Cassava starch (g)	80	70	70	70	70	70

Ingredients are given in g/100 g of the mixture

DCM dehulled Cochinchinensis milk, WCM whole Cochinchinensis milk, DVM Dehulled Veracruz milk, WVM whole Veracruz milk

Table 3 Effect of variety and dehulling on chemical composition and some chemical properties of *Mucuna pruriens* milk

Components	Var. Cochinchinensis		Var Veracruz	
	Dehulled beans milk	Whole beans milk	Dehulled beans milk	Whole beans milk
Proximate composition				
Dry matter (g/100 g)	12.14 ± 0.17 ^c	8.59 ± 0.67 ^a	12.88 ± 0.37 ^d	9.96 ± 0.27 ^b
Total sugar (g/100 mL)	12.11 ± 0.24 ^c	10.52 ± 0.13 ^a	13.08 ± 0.34 ^d	11.65 ± 0.93 ^b
Protein content (g/100 mL)	12.03 ± 0.22 ^b	6.40 ± 0.47 ^a	12.13 ± 0.43 ^b	6.80 ± 0.11 ^a
Ash (g/100 mL)	0.31 ± 0.04 ^a	0.92 ± 0.06 ^d	0.46 ± 0.02 ^b	0.87 ± 0.04 ^c
pH	5.97 ± 0.16 ^a	6.89 ± 0.20 ^b	5.95 ± 0.07 ^a	6.85 ± 0.09 ^b
TTA (%)	0.37 ± 0.03 ^c	0.20 ± 0.05 ^b	0.39 ± 0.05 ^d	0.19 ± 0.01 ^a
Antinutritional factors				
Total phenolics (mg/100 mL)	122.83 ± 2.84 ^a	135.33 ± 1.75 ^b	178.83 ± 2.08 ^c	199.51 ± 2.78 ^d
Tannins (mg/100 mL)	0.43 ± 0.02 ^c	2.15 ± 0.07 ^d	0.27 ± 0.05 ^b	2.09 ± 0.05 ^a
Phytates (mg/100 mL)	0.69 ± 0.12 ^a	3.16 ± 0.53 ^c	0.73 ± 0.03 ^b	3.13 ± 0.23 ^c
L-Dopa (g/100 mL)	0.05 ± 0.01 ^b	0.09 ± 0.01 ^c	0.03 ± 0.01 ^a	0.09 ± 0.01 ^c

Means ± SD ($n = 3$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

Vegetable milk is a combination of proteins, carbohydrates, lipids and other nutrients which formed the bulk and nutritive part of the product. In this respect the behavior of dry matter does reflect that of proteins and carbohydrates content. In this respect it may be observed from Table 3 that the dry matter which represents all the components other than water in the milk was mostly influenced by the variety with Veracruz having higher values. In addition dehulling induced a significant increase in dry matter. *Mucuna* milk was reported to represent a fairly good source of minerals represented globally as ash, in concordance with literature on vegetable milk source of potassium, copper, iron and zinc (Agunbiade et al. 2011). No significant effect of variety was observed while dehulling induced 47 to 66 % reduction in ash content. These observations highlighted the high level of mineral in the beans hull as recently demonstrated for *mucuna* (Odedeji and Oyeleke 2011). The ash content of *mucuna* milk produced in the present work was higher than corresponding values (0.09–0.15 %) reported for peanut milk (Kouane et al. 2005).

The pH and titratable acidity play an important role in the selection of a vegetable for use in food formulation. Angeles and Marth (1971) found that, the vegetable milk has a desirable characteristic as an ingredient when it has a titratable acidity of 0.23–0.25 % or a pH of 5.7. In the present study, we found that the pH of *mucuna* milk (5.95–6.89) was fairly high compared to the desired value, but fall in the range (6.50–6.70) reported for other vegetable milk such as soya milk and peanut milk (Wang et al. 2003; Manhal and Kamal 2010). In addition, the acidity of *mucuna* milk decreased with dehulling probably as a consequence of decrease in phenolic acid, confirmed by the strong negative correlation between pH and

phenolic content ($r = -0.94$, $p < 0.05$) or titratable acidity and phenolic content ($r = -0.91$, $p < 0.05$) of milk.

Anti-nutritional compounds

Mucuna bean have antinutritional factors such as phenolic compounds, tannins and L-Dopamine and studies have shown that consumption of untreated *mucuna* beans is related to several anti-physiological effects (Josephine and Janardhanan 1992; Ngatchic et al. 2013a). These antinutrients were present in significantly higher concentrations significantly ($p < 0.05$) higher in whole *Mucuna* milk than in dehulled *mucuna* milk (Table 3). Compared to chemical composition of *mucuna* flours (Table 1), antinutrients contents were reduced to about 95–99 % during the extraction process of *Mucuna* milk. Phenolic compounds, notably tannins are known to have ability to decrease digestibility by complexing with dietary proteins and to lower the activity of several digestive enzymes (Vadivel and Pugalenthil 2010). In this regards, the proteins of all the *mucuna* milk, and particular *mucuna* with high levels of polyphenol are potentially less digestible, but this needs to be investigated. Inversely the polyphenols may also be beneficial due to their strong antioxidant activity (Matthaus 2002). Total phenolic (122.83–199.5 mg) and tannins content (0.27–2.15 mg) of *mucuna* milk were most affected by the variety. Veracruz milk showed higher content of total phenolics as compared to cochinchinensis milks, while opposite trend was observed for tannins content. The elimination of *mucuna* beans hulls which contained a lot of phenolic compounds (Siddhuraju et al. 2000) induced 80 % (DCF) to 87 % (DVF) reduction in tannins contents of *mucuna* milk. The values of phytate content of *Mucuna* milk sample was

between 0.69 and 3.16 %. Phytic acid has been reported to lower the nutritional value of legume seeds by limiting the bioavailability of proteins, dietary minerals and essential trace elements (Reddy and Salunkhe 1981). Dehulling also reduced phytate content of mucuna milk. The major toxicant in Mucuna bean is a non-protein amino acid, 3, 4-dihydroxy-L-phenylalanine (L-Dopa) (Gurumoorthi et al. 2008). Dehulling also induced reduction of 44–67 % of L-Dopamine in milks. L-Dopa content in mucuna milk was between 0.03 and 0.09 %, values lower than 0.1 % recommended in food (Dahouda et al. 2009). The low content of L-Dopa in mucuna milk added positive attributes favoring the use of this vegetable milk.

Protein quality

The growth performance of the experimental animals fed with diets containing casein and mucuna milks are illustrated in Table 4. The food intake (FI) value of the animals fed with diet containing whole mucuna milk (337.86–357.48 g) was significant lower ($p < 0.05$) when compared to diet containing casein (532.14 g) and dehulled mucuna milk (501.46–530.73 g). As reported by Ngatchic et al. (2013b), the high amount of antinutritional factors in diet reduces the appetite of rats, and this seems to be the case in this study. In addition low quality of proteins in the diet, notably deficiency and imbalance of amino acids, may also reduce appetite (Tome 2004). It could also be due to poor palatability of the test protein (Marrineze-Villaluenga et al. 2007). Low food intake in the rats fed with whole mucuna milk corresponded to low nitrogen intake (6.38–7.94 g) as compared to the rats fed with casein (10.86 g) and dehulled mucuna milk (9.58–10.27 g). Low protein ingestion generally resulted in protein malnutrition symptoms such as delayed growth, negative nitrogen (protein) uptake or disturbances in protein synthesis (Suijak et al. 2006). Figure 1 shows the variation in rat weight fed in diet made of different protein regime. Rat fed in diet made of whole mucuna milk and those fed in diet free protein showed a gradual decrease in weight. Inversely rat fed in diet made of dehulled mucuna milk gradually increased in weight. The low daily weight gain may be associated to different factors including lower food intake, lower digestion and absorption through the intestine, the deficiency in some essential amino acids and toxicity of the product. Determination of the digestibility parameters revealed evidence of low absorption of protein and an eventual toxicity. Proteins are firstly consumed to support growth of body, and in this respect the ratio of growth weight to the ingested protein, known as PER, of casein and proteins in dehulled mucuna diets were comparable (range 2.47–2.17 g body weight/g protein ingested). PER reported in this work also fall within

the range 1.5–3.8 g body weight/g protein reported for many protein including casein, soy beans and wheat proteins. However the PER for whole mucuna milk and free protein diet rats group were negative. These results indicate that proteins of dehulled mucuna milk, irrespective of the variety, can promote growth of a living organism. Furthermore, PER of dehulled mucuna milks were superior to those of soybean (Schaafsma 2000). In reverse mucuna milk protein made from the whole seeds rather reduced weight than promoting growth.

The TD coefficient provides a more accurate method of ranking dietary protein quality (Darragh and Hodgkinson 2000). High TD is indicative of low fecal nitrogen and vice versa. TD differs AD in the way that the fecal nitrogen is adjusted by removal of the nitrogen from basal metabolism, which is determined in rat group fed protein free diet. Dehulled mucuna milk possessed a TD of 85.2–86.0 % which was not significantly different from 87.0 %, the TD observed for casein. In this respect dehulled mucuna milk can be considered a source of protein of good quality as compared to whole mucuna milk which had a TD of 47.87–51.17 %. The low TD of the whole mucuna milk is a result of low hydrolysis of protein in the intestine which consequently increases the fecal nitrogen. Indeed, we observed no significant difference in fecal nitrogen between groups fed with mucuna milk and casein as protein source, while significant higher fecal nitrogen was observed in rat groups fed with whole mucuna milk, irrespective of the variety. Higher fecal nitrogen might result either by inhibition of proteolytic enzyme in the intestine or unavailability of protein diet for digestion. In this respect, molecules such as polyphenols and phytate which were higher in whole mucuna milk might form complexes with either enzymes or protein (Liener 1994). The complexation of enzymes in the gut by antinutrients present in the hulls of mucuna seeds appeared as the more probable reason of reduction of TD. Polyphenols and phytates may also be responsible for reduction in TD as highly significant correlations ($r = -1.00$; $p < 0.001$) were found between them. Apart their complexations with endogenous enzymes, phytates and polyphenols have been shown to exert a barrier effect to absorption which increases the endogenous losses of the organism in the intestine (Laterme et al. 1990). Mugendi et al. (2010) reported very low TD (58 %) of mucuna protein as compared to casein (93 %) following dehulling and soaking at 60 °C pH 3.2. This result suggests that both treatments dehulling and boiling need to be combined to improve the biological value of the mucuna protein.

Organs to body weight ratio and hematological parameters

Organ-to-body weight ratios of rats fed with mucuna milk were comparable to those fed casein except that for kidney

Table 4 Effect of variety and dehulling on food intake and parameters of protein digestibility of rats fed with *Mucuna pruriens* milk for 28 days

Parameters	Casein	Protein free diet	var. Cochinchinensis		var. Veracruz	
			DCM	WCM	DVM	WVM
Food intake (g/day)	19.15 ± 1.93 ^e	11.16 ± 2.14 ^a	17.90 ± 2.13 ^d	12.06 ± 1.65 ^b	18.95 ± 2.31 ^e	12.76 ± 2.08 ^c
Nitrogen intake (g/day)	1.81 ± 0.01 ^f	0.00 ± 0.00 ^a	1.59 ± 0.02 ^d	1.06 ± 0.01 ^b	1.71 ± 0.01 ^e	1.32 ± 0.02 ^c
PER	3.39 ± 0.01 ^d	/	3.24 ± 0.03 ^e	0.35 ± 0.01 ^a	3.26 ± 0.02 ^c	0.47 ± 0.04 ^b
NPER	2.37 ± 0.01 ^c	/	2.28 ± 0.02 ^d	−1.40 ± 0.01 ^b	2.17 ± 0.01 ^c	−0.93 ± 0.01 ^a
TD (%)	86.74 ± 0.33 ^d	/	82.15 ± 0.83 ^c	44.35 ± 2.51 ^a	83.62 ± 1.32 ^c	47.37 ± 1.29 ^b
AD (%)	88.03 ± 1.11 ^{cd}	/	85.87 ± 1.76 ^c	48.15 ± 0.37 ^b	86.96 ± 1.93 ^c	50.21 ± 0.47 ^a
Faecal Nitrogen (g)	0.24 ± 0.01 ^b	0.04 ± 0.01 ^a	0.24 ± 0.01 ^b	0.55 ± 0.02 ^c	0.24 ± 0.02 ^b	0.65 ± 0.02 ^d

Means ± SD ($n = 6$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test
 PER protein efficiency ratio, NPER net protein efficiency ratio, TD true digestibility, AD apparent digestibility, DCM dehulled Cochinchinensis milk, WCM whole Cochinchinensis milk, DVM dehulled Veracruz milk, WVM whole Veracruz milk

(Table 5). Generally, naturally occurring toxic materials present in the food are absorbed, neutralized by the liver and excreted by the kidney in the urine. Treatments generally applied to beans are adequate to eliminate the toxic materials; these include soaking overnight and boiling. In the present work, the mucuna beans were soaked for 2 days and boiled 1 h. The observed toxicity after the treatment suggested that the toxic material have not been eliminated or destroyed. The rat body's protective mechanisms, was in the case of this study, overwhelmed by the intake of mucuna milk made from whole seeds. This observation is indicative of stress probably as a consequence of accumulation of the toxic material (Jenkins and Mitchell 1989). The level of antinutrients was significantly higher in whole mucuna milk compared to dehulled mucuna milk, but the values were all

either in the same range or lower than the recommended safe level. In particular the level of L Dopa, a molecule produced by triangular-shaped glands located on top of the kidney, is considerably higher in whole mucuna milk, but the value still under the recommended $<0.1\%$ value. These observations suggested that the toxicity observed could be due to other toxin not included in this work. Similar atrophy of kidney was reported by Mugendi et al. (2010) with mucuna protein processed by dehulling and soaking 60°C pH 3.2. They further observed on histological section the loss of kidney function characterized by interstitial infiltrates, fibrosis and inflammatory atrophy. The observed normal function of kidney following consumption of dehulled /boiled mucuna milk suggested an inhibition or destruction of the toxic molecule, not investigated in the present study.

Fig. 1 Variation of mean ± SD body weight of rats groups ($n = 8$) fed diets formulated with different proteins sources: Casein diet, Protein free diet, WVM (Whole Veracruz milk diet), DVM (Dehulled Veracruz milk diet), WCM (Whole Cochinchinensis milk diet) and DCM (Dehulled Cochinchinensis milk diet)

Table 5 Effect of variety and dehulling on organ-to-body weight ratios of rat fed with *Mucuna pruriens* milk for 28 days

Organs (%)	Casein diet	Protein free diet	Var. Cochinchinensis		Var. Veracruz	
			DCM	WCM	DVM	WVM
Liver	3.14 ± 0.28 ^a	3.23 ± 0.32 ^a	3.14 ± 0.13 ^a	3.15 ± 0.20 ^a	3.16 ± 0.23 ^a	3.11 ± 0.21 ^a
Kidney	0.60 ± 0.02 ^a	0.61 ± 0.02 ^a	0.61 ± 0.03 ^a	0.89 ± 0.07 ^b	0.61 ± 0.04 ^a	0.91 ± 0.09 ^b
Testis	1.38 ± 0.01 ^a	1.39 ± 0.02 ^a	1.38 ± 0.01 ^a	1.37 ± 0.04 ^a	1.38 ± 0.02 ^a	1.38 ± 0.03 ^a
Lung	1.81 ± 0.02 ^a	1.82 ± 0.05 ^a	1.81 ± 0.02 ^a	1.82 ± 0.01 ^a	1.83 ± 0.01 ^a	1.81 ± 0.02 ^a
Heart	0.33 ± 0.01 ^a	0.32 ± 0.01 ^a	0.32 ± 0.02 ^a	0.31 ± 0.01 ^a	0.31 ± 0.02 ^a	0.32 ± 0.02 ^a

Means ± SD ($n = 6$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

DCM dehulled Cochinchinensis milk, WCM whole Cochinchinensis milk, DVM dehulled Veracruz milk, WVM whole Veracruz milk

Hematological parameters

As shown in Table 6, the hematological parameters of rats varied significantly ($p < 0.05$) amongst diet groups. The rats groups fed with casein and dehulled mucuna milk exhibited higher levels of white blood cell count, lymphocytes, granulocytes, red blood cells (RBCs), hemoglobin, hematocrit and platelets compared to those fed whole mucuna milk. These results showed a direct link between all the cells level in blood, probably as a consequence of their dependence to protein synthesis. In the rat groups fed whole mucuna milk, the low digestibility of protein have probably reduced the level of amino acid available to support cells synthesis and differentiation, thus contributing to the low level of blood cells. In addition the linear relation between the levels of the hematological parameters is probably a consequence of their similarity in function. In fact RBCs contain hemoglobin, its main component, which transports oxygen from the lungs to the rest of the body,

such as to the muscles, where it releases the oxygen load. In addition WBC's also called leucocytes are classified as agranular (B, T lymphocytes and monocytes) and granular (neutrophils, eosinophyls and basophyls). These special cell help the body defend its self against invasion of pathogens. Not only do they help our immune system but they remove toxins. However the level of either granulocytes or lymphocytes was significantly high in blood of rat groups fed casein and dehulled mucuna milk compared to whole mucuna milk. This result suggest that the immune system is not activated by any pathogen of toxin, and in this respect, the level of WBC's is governed essentially by the level of quality of protein ingested.

Platelets are membrane bound cell fragments which accumulate at site of broken blood vessels to form clots. They have a big role in hemostasis, by preventing blood loss. Their level in the blood also followed the quality of ingested protein, lower for whole mucuna milk than for dehulled mucuna milk and casein as protein source.

Table 6 Effect of variety and dehulling on hematological parameters of rats fed with *Mucuna pruriens* milk for 28 days

Parameters	Casein diet	Protein free diet	Var. Cochinchinensis		Var. Veracruz	
			DCM	WCM	DVM	WVM
WBC ($\times 10^9/L$)	6.16 ± 1.01 ^c	2.55 ± 0.62 ^a	4.15 ± 0.34 ^b	2.47 ± 0.51 ^a	4.38 ± 0.89 ^b	2.03 ± 0.55 ^a
Lym (%)	79.62 ± 4.66 ^b	60.67 ± 5.35 ^a	76.55 ± 3.68 ^b	59.82 ± 6.05 ^a	74.65 ± 4.65 ^b	65.15 ± 3.72 ^a
Gra (%)	17.75 ± 2.48 ^b	12.89 ± 1.22 ^a	15.97 ± 0.33 ^{bc}	13.25 ± 0.79 ^a	16.65 ± 1.07 ^b	14.45 ± 0.23 ^{ab}
RBC ($\times 10^{12}/L$)	6.82 ± 0.36 ^b	1.82 ± 0.41 ^a	5.68 ± 1.09 ^b	1.45 ± 0.53 ^a	6.24 ± 1.21 ^b	1.43 ± 0.64 ^a
Hb (g/dL)	12.90 ± 2.03 ^b	4.90 ± 1.15 ^a	11.05 ± 1.20 ^b	6.28 ± 0.84 ^a	12.43 ± 1.72 ^b	5.08 ± 1.36 ^a
Ht (%)	37.27 ± 4.37 ^b	12.50 ± 2.18 ^a	33.77 ± 4.23 ^b	15.15 ± 1.96 ^a	33.43 ± 4.10 ^b	12.83 ± 2.43 ^a
Plt ($10^9/L$)	630.75 ± 38.11 ^b	306.50 ± 14.54 ^a	510.50 ± 62.87 ^b	308.25 ± 15.30 ^a	577.50 ± 77.67 ^b	342 ± 24.80 ^a

Means ± SD ($n = 6$) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test

WBC white blood cell count, Lym lymphocytes, Gra granulocytes, RBC red blood cells, Hb hemoglobin, Ht hematocrit, Plt platelets, DCM dehulled Cochinchinensis milk, WCM whole Cochinchinensis milk, DVM dehulled Veracruz milk, WVM whole Veracruz milk

Conclusion

Irrespective of the variety, mucuna milk is a good source of proteins, but it contains high amounts of tannins, polyphenols and phytate. The application of dehulling during the process of mucuna milk production yields a product with a protein twice that obtained from whole seeds. While the PER by dehulled mucuna milk is comparable to that of casein, that of whole mucuna milk is negative, leading to weight loss, impairment of proteins and blood cells synthesis. In addition consumption of a mucuna milk made from whole seeds induces a kidney hypertrophy which disappeared when dehulled seeds are used. The dehulled mucuna milk represents a good source of proteins for human nutrition, but a chronic toxicity study needs to be investigated.

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