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Biological evaluation of a protein isolate from cowpea (*Vigna unguiculata*) seeds

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Abstract

The present work was carried out to determine the nutritive quality of a protein isolate from cowpea (*Vigna unguiculata*) seeds. Previous data from our laboratory showed that cowpea protein isolate (CPI) presents good functional properties, including solubility, emulsifying and foaming activities. In this work, rats were divided into three groups that received, during 16 days, liquid diets containing casein or CPI as protein sources or a protein-free diet. The nutritional parameters measured for the diet containing CPI showed a positive nitrogen balance (NB = 0.5), a net protein retention (NPR) of 0.7 and digestibility of 87%. Furthermore, CPI did not present detectable hemagglutinating activity and very low trypsin inhibitory activity (18 TIU/mg protein) after heat treatment. In terms of its essential amino acid content, CPI presented a better composition than purified cowpea vicilin. No significant histological differences were found in several internal organs of the rats that were fed a diet containing CPI when compared to the group fed with a casein-containing diet. Taken together, these results suggest that CPI may provide a new, inexpensive source of protein for use as a potential functional and nutritional agent in the food industry.

Keywords: *Vigna unguiculata*; Protein quality; Cowpea seeds; Protein isolate

1. Introduction

Large segments of the population in developing countries suffer from protein malnutrition. Projections based on current trends indicate a widening gap between human population and protein supply. Hence, intense research efforts are currently directed toward identification and evaluation of underexploited sources, such as alternative protein crops for the world of tomorrow (Siddhuraju, Vijayakumari, & Janardhanan, 1996). In this regard, various studies are being carried out to assess the potential of legumes that are still not widely used as dietary sources of protein, as well as a genetic resource for the improvement of traditional legume crops (Siddhuraju et al., 1996).

Over the past 30 years, the use of concentrated proteins from plant seeds has increased tremendously because of greater knowledge of their functional properties, processing and nutritive value. While, historically, soybeans had a competitive advantage over other legume seeds, there is a need to develop other sources of concentrated plant proteins (Vose, 1980) which ideally should be crops that are widely grown in tropical countries.

The cowpea (*Vigna unguiculata*) is a widely grown legume food crop of the tropics. Like other legumes, cowpea seeds contribute to the level of dietary protein in starchy tuber-based diets through their relatively high protein content (25%), and their quality, by forming complementary mixtures with cereals (Phillips & Baker, 1987).

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Many of the antinutritional factors in legumes can be eliminated or inactivated, to a large extent, by appropriate heating and processing during food preparation. Wet-milling and processing techniques, employed during protein concentration and isolation, are known to be effective in the detoxification of seed materials (Mwanjala, Kharidah, Jamila, & Yaakob, 1999). Cowpeas are already widely used in diets for humans and other mammals, and this suggests that the seeds do not have major adverse nutritional effects. Phillips and Baker (1987) examined various processing techniques for their effects on cowpea protein quality, and found that the greatest improvement over the raw seed (expressed by an increase in protein efficiency ratio from 2.04 to 2.81) occurred when the meal was extruded. Previous data from our laboratory showed that cowpea protein isolate (CPI) presents good functional properties, including solubility, emulsifying and foaming activities. In the present study, diets containing CPI as sole protein source were tested in rats to establish the nutritive quality of the isolate and to investigate the presence of possible antinutritional and/or toxic factors. The CPI was prepared by isoelectric precipitation and PV was obtained by successive steps of precipitation using ammonium sulfate. Both PV and CPI show a major protein component of 150 kDa. The CPI also has a minor protein component of 30 kDa that coprecipitates with vicilin in the isoelectric precipitation step at pH 4.5. The use of CPI in the food industry is likely more advantageous than PV because CPI is considerably easier and cheaper to obtain and has a significantly higher protein recovery yield compared to PV.

2. Materials and methods

2.1. Preparation of proteins

2.1.1. Purification of vicilin

Vicilin was purified from pea and cowpea seeds as previously described (Pedrosa & Ferreira, 1994). Throughout the purification, the solutions contained a cocktail of protease inhibitors, including 0.5 μg/ml of leupeptin, 0.07 μg/ml of pepstatin, 2 μg/ml of PMSF and 0.05 μg/ml of soybean trypsin inhibitor. Vicilin stock solutions (~5 mg/ml) were stored at 4 °C in 50 mM ammonium bicarbonate, pH 8.0. Protein concentration was determined according to Lowry, Rosebrough, Farr, and Randall (1951).

2.1.2. Protein isolate

Cowpea protein isolate (CPI) was obtained by isoelectric precipitation from defatted cowpea meal as reported (Bender & Doell, 1957), with minor modifications. Briefly, the defatted flour was incubated for one and a half hours at 4 °C in buffer containing 50 mM Tris–HCl, 200 mM NaCl, pH 8.5, and centrifuged (10,000*g* for 30 min) to remove insoluble components such as fibres and pigments. The supernatant was then brought to pH 6.0 by addition of 1 M HCl and centrifuged at 10,000*g* for 30 min. The supernatant was then precipitated at pH 4.5 (corresponding to the isoeletric point of vicilin) and the insoluble fraction, representing the CPI, was collected.

2.2. Antinutritional and/or toxic factors

Trypsin inhibitory activity was measured according to the method of Kakade, Rackis, McGhee, and Puski (1974) using bovine trypsin (Merck, Darmstat, Germany), and *N*-benzoyl-DL-arginine-*p*-nitroanilide (Merck) as substrate. One trypsin unit (TU) was expressed as an increase of 0.01 absorbance units per 10 ml of reaction mixture at 410 nm. Trypsin inhibitor activity was defined in terms of trypsin units inhibited (TIU) per mg of protein. All experiments were done in triplicate. Hemagglutinating activity was assayed according to Udedibie and Carlini (1998) and reported as present or absent in the investigated samples.

2.3. Amino acid composition

Amino acid analyses were performed on a Biochrom 20 (Pharmacia Biotech, Uppsala, Sweden) automatic amino acid analyzer, based on the methodology described by Spackman, Stein, and Moore (1958). Protein samples were vapour-hydrolyzed in a Pico Tag workstation (Waters Corporation, USA) under vacuum, after saturation of the atmosphere with nitrogen, using 200 μl of 5.7 N HCl for 24 h at 108 °C. The amino acid composition was calculated considering the highest value obtained for each amino acid, except for tryptophan, which was determined by the ultraviolet molar absorption coefficient of each protein sample (Pace, Vajdos, Fee, Grimsley, & Gray, 1995).

Chemical scoring of amino acids was calculated using the FAO/WHO (1991) reference pattern. Essential Amino Acid Index was calculated according to Oser (1959), using the amino acid composition of the whole egg protein published by Steinke, Prescher, and Hopkins (1980) as standard. Nitrogen-to-protein conversion factors were calculated, based on the ratio of protein to amino acid N.

2.4. In vivo protein quality determination

Diets were prepared to contain the equivalent of 10% protein (Table 1) in the form of casein or CPI (Sgarbieri, 1996). Protein-free diets were also used as controls to estimate the endogenous nitrogen excretion of the rats. In addition to the protein sources, the diets contained vitamin and mineral mix (AIN-93G) according to Reeves et al. (1993).

2.5. Test animals

Male Wistar rats were weaned at 22 days of age and fed the casein diet ad libitum for 3 days as a period of adaptation to the experimental diets. The animals were then divided into 3 groups of seven rats each, housed individually in metabolic cages fitted with glass separators for urine and feces collection. The rats received diet containing casein, protein-free diet or experimental diet (containing CPI) for 16 days. Food and water were supplied ad libitum. The rats were kept in an air-conditioned room maintained at 22 °C with a 12 h light and dark cycle. Food intake was monitored daily and body masses were registered every other day. The concentration of nitrogen in feces and urine was estimated by the Kjeldahl method (AOAC, 1990) and conversion factors of 6.25 for casein and 5.70 for CPI were used. Feces were collected during the last 4 days of the experimental period, bulked, freeze-dried, weighed and ground. The data obtained from this experiment were used to calculate nitrogen balance (NB), net protein retention (NPR) and true digestibility (TD), as described by Bender and Doell (1957). All the parameters were calculated for each rat and means were calculated within each group. The following protein quality indices were calculated from the data collected:

$$\text{Nitrogen balance (NB)} = \text{Nitrogen intake} - (\text{fecal nitrogen output} + \text{urine nitrogen output})$$

$$\text{True digestibility (\%TD)} = \frac{\text{Nitrogen intake of test animals} - (F - Fm) \times 100}{\text{Nitrogen intake of test animals}},$$

where F = fecal nitrogen output by test animals, and Fm = fecal nitrogen output by "protein free" animals.

$$\text{Net protein retention (NPR)} = \frac{\text{weight gain of test animals-weight loss by "protein free" animals}}{\text{Weight protein consumed by test animals}}.$$

Table 1
Composition of the experimental diets (g/l)

Ingredients	Casein (g/l)	Protein-free (g/l)	CPI[a] (g/l)
Casein	26.5	–	–
CPI	–	–	28.5
Sucrose	26.5	26.5	26.5
Cellulose	13.3	13.3	13.3
Maltose dextrin	161.9	188.4	159.9
Soybean oil	18.6	18.6	18.6
t-Butylhydroquinone	0.004	0.004	0.004
Salt mix[b]	9.28	9.28	9.28
Vitamin mix[c]	2.65	2.65	2.65
L-cystine	0.8	0.8	0.8
Choline bitartrate	0.66	0.66	0.66
Xanthan gum	3.0	3.0	3.0
Total calories (kcal)	1039	1039	1039

[a] Cowpea protein isolate.
[b] Salt mix.
[c] Vitamin mix, according to Reeves, Nielsen, and Fahey (1993).

2.6. Histological processing

Rats fed with casein, CPI or protein-free diets were sacrificed on the 16th day of the experiment and internal organs (stomach, intestine, brain, pancreas, kidney, liver) of 3 rats from each group were dissected. Representative fragments of each organ were fixed in 10% formalin and embedded in paraffin, in order to obtain serial sections of approximately 5 μm. The sections were stained with hematoxylin-eosin (H&E) and inspected on an Axioshop 2 Routine microscope adapted with a MC80 DX camera (Zeiss) for morphological examination.

3. Results and discussion

3.1. General

Preparations of purified cowpea vicilin (PV) and CPI were routinely examined by SDS-PAGE under denaturing conditions and exhibited main protein bands of 50 and 52 kDa, respectively (data not shown). These are in the typical molecular mass range of 7S storage proteins (vicilins), in agreement with data previously reported by several research groups (Domoney & Casey, 1985; Gatehouse, Lycett, Delauney, Croy, & Boulter, 1983; Pedrosa, De Felice, Trisciuzzi, & Ferreira, 2000).

It is important to note that CPI (prepared by isoelectric precipitation) is considerably easier and cheaper to obtain than purified vicilin, which is obtained by successive steps of precipitation using ammonium sulfate (Pedrosa & Ferreira, 1994; Pedrosa, Trisciuzzi, & Ferreira, 1997, 2000). Furthermore, the protein yield of the CPI was significantly higher than the yield of purified vicilin: out of 1 g of the defatted seed flour, approximately 10 mg of purified vicilin and 120 mg of CPI could be routinely obtained.

3.2. Amino acid composition

The nutritive value of dietary proteins is determined by the pattern and quantity of essential amino acids

present. The presence of one or more of the essential amino acids in adequate amounts increases the nutritive value of the protein. Table 2 shows the amino acid compositions of PV and CPI. The amino acid analyses were quite similar to previously reported results for pea vicilin (Baniel, Caer, Colas, & Gueguen, 1992) and are also in line with a very recent report on the composition of seeds from *Vigna unguiculata* and *Phaseolus vulgaris* (Onwuliri & Obu, 2002). There were only slight variations in the amino acid compositions of the two preparations of protein. Both samples were rich in aspartic and glutamic acids. Tryptophan was the limiting amino acid in both preparations. The low methionine content can be complemented when legume proteins are consumed in conjunction with other food ingredients containing high or moderate amounts of sulphur-containing amino acids, such as cereal proteins. The quality of seed proteins as sources of amino acids can usually be assessed by comparison with the FAO/WHO recommended pattern of essential amino acids (FAO/WHO, 1991; Table 2). CPI and PV contained adequate levels of lysine, leucine, isoleucine, threonine, valine, phenylalanine, arginine and histidine, based on the FAO/WHO/UNU (1985) reference pattern for infants. The data also indicated that cowpea proteins contained adequate amounts of most essential amino acids for pre-school children and all essential amino acids for adults.

Table 2
Amino acid compositions of purified vicilin (PV) and protein isolate (CPI) from *Vigna unguiculata*

Amino acid	CPI (mg/g protein)	PV (mg/g protein)	FAO/WHO (1991) Requirement pattern (mg/g protein)
Aspartic acid	116	128	
Threonine	50	34	34
Serine	53	51	
Glutamic acid	162	192	
Glycine	38	26	
Alanine	39	32	
Valine	50	54	35
Cystine	18	3	25[a]
Methionine	14	10	
Isoleucine	47	50	28
Leucine	74	88	66
Tyrosine	39	32	
Phenylalanine	66	77	63[b]
Lysine	68	64	58
Histidine	37	33	19
Arginine	75	81	
Proline	45	41	
Tryptophan	9	4	11

[a] Cystine + Methionine.
[b] Phenylalanine + Tyrosine.

On the basis of chemical score, the CPI had a higher value than PV (Table 3). CPI and PV had chemical scores of 82 and 36, respectively, and both had tryptophan as the first limiting amino acid. PV presented sulfurated amino acids (Met + Cys) as secondary limiting amino acids. Similarly, Okezie and Bello (1988) demonstrated that protein isolate from winged bean (*P. tetragonolobus)* presented tryptophan and methionine as limiting amino acids. CPI had a higher essential amino acid index (EAAI) than PV. These results indicated that CPI presented better composition in terms of essential amino acids than PV.

Crude protein analysis is conventionally based on multiplying the total nitrogen (Kjeldahl N) by a factor of 6.25. The value of 6.25 is based on the assumption that proteins contain 16% N, which, though valid for animal proteins, may not be true of plant proteins. Several reports have indeed demonstrated that, for plant tissues, the protein conversion factor is less than 6.25 (Mossé, 1990; Yeoh & Wee, 1994). Conversion factors recommended for cereals and grain products range between 5.70 and 5.83, while factors of 5.18–5.46 are generally used for proteins from nuts and seeds (FAO, 1982). In this study, we have evaluated the true protein contents and established the nitrogen-to-protein conversion factors for PV and CPI (Table 4). The conversion factors for PV (5.92) and CPI (6.00) showed that these proteins present a good nitrogen content. Ezeagu, Petzke, Metges, Akinsoyinu, and Ologhobo (2002) recently studied the conversion factors for tropical plant seeds and suggest the use of the values of 5.0 and 5.9 for legumes and non-legumes, respectively. Therefore, both purified vicilin and CPI showed better proteic values than legumes in general.

3.3. Trypsin inhibitor activity

Protein quality is affected by antinutritional factors that interact with the intestinal tract, such as protease inhibitors, lectins and tannins that reduce protein digestibility and amino acid absorption. Unless destroyed or inactivated by heat or some other suitable treatment,

Table 3
Biological values of purified cowpea vicilin (PV) and CPI

Preparation	Chemical score (%)	Limiting amino acids		EAAI (%)
		First	Secondary	
Purified cowpea vicilin	36	Tryptophan	Met + cys	29
Cowpea protein isolate	82	Tryptophan	–	64

EAAI, Essential amino acids index.

Table 4
Conversion factors for purified cowpea vicilin (PV) and cowpea protein isolate (CPI)

Amino acid	M.W.	N	PV (mg/g ptn)	N (mg)	CPI (mg/g ptn)	N (mg)
Aspartic acid	132.12	0.2120	128	27.14	116	24.59
Threonine	119.12	0.1176	34	4.00	50	5.88
Serine	105.09	0.1333	51	6.80	53	7.06
Glutamic acid	146.15	0.1917	192	36.81	162	31.05
Glycine	75.07	0.1866	26	4.85	38	7.09
Alanine	89.09	0.1572	32	5.03	39	6.13
Valine	117.15	0.1196	54	6.46	50	5.98
Cysteine	121.15	0.1156	3	0.35	18	2.08
Methionine	149.21	0.0939	10	0.94	14	1.31
Isoleucine	131.18	0.1068	50	5.34	47	5.02
Leucine	131.18	0.1068	88	9.40	74	7.90
Tyrosine	181.19	0.0773	32	2.47	39	3.01
Phenylalanine	165.19	0.0848	77	6.53	66	5.60
Lysine	146.19	0.1916	64	12.26	68	13.03
Histidine	155.16	0.2708	33	8.94	37	10.02
Arginine	174.20	0.3216	81	26.05	75	24.12
Proline	115.13	0.1217	41	4.99	45	5.48
Tryptophan	204.23	0.1372	4	0.55	9	1.23
Total			1000	168.91	1000	166.58
			FACTOR = 5.92		*FACTOR* = 6.00	

these substances can exert adverse physiological effects when ingested by man and animals (Liener, 1994).

It is well established that the feeding of raw soybean and many other legume products, which contain high levels of proteinase inhibitors, to experimental animals such as rats, mice and chickens leads to growth depression, pancreatic hypertrophy and/or hyperplasia (Gallaher & Schneeman, 1984; Liener & Kakade, 1980) and the potentiation of pancreatic carcinogenesis (Gumbman, Spangler, Dugan, & Rackis, 1986; McGuiness, Morgan, & Wormley, 1984). Most of those compounds inhibit the digestive enzymes or react with essential amino acids, limiting the application of the whole seed in food products. This problem can be potentially overcome if legume seed proteins are isolated instead of being used in whole-seed preparations.

The results of trypsin inhibitory activities are summarized in Table 5. CPI exhibited the highest trypsin inhibition levels (52 TIU/mg), whereas purified pea vicilin (used as a negative control) produced the lowest inhibition levels (2 TIU/mg). Interestingly, a marked decrease in trypsin inhibitory activity (18 TIU/mg) was observed after the CPI was heated by 80 °C for 5 min. The trypsin inhibitory activity found for CPI was similar to that previously reported for a proteic fraction from *Vigna unguiculata* (Elkowicz & Sosulski, 1982). The trypsin inhibitory activity of CPI was also similar to that found in other legumes, such as *Acacia nilotica*, *C. arietinum* and *Lens esculenta* (Siddhuraju et al., 1996). Early investigators (Jaffé, 1950; Liener, 1976) considered soybean, lima and kidney beans to be high in trypsin inhibitory activity, while cowpea and lentil showed intermediate levels. Nti and Plahar (1996) showed that trypsin inhibitors from cowpea were totally inactivated after cooking the seeds and only partially inactivated after cooking the meal. Moreover, chickpea proteins are partially denatured during the preparation of protein isolates, becoming more accessible to digestive enzymes, and improving their hydrolysis (Lynch, Rha, & Catsimpoolas, 1977; Paredes-López, Ordorica-Falomir, & Olivares-Vásquez, 1991). Indeed, a marked (~70%) reduction in the concentration of trypsin inhibitors has been observed in the process of extraction and precipitation of isolated soy protein (Waggle, Steinke, & Shen, 1989).

Table 5
Trypsin inhibitor and hemagglutinating activities of purified cowpea vicilin, purified pea vicilin and cowpea protein isolate

Protein	TIU/mg sample[a]	Hemagglutination[b]
Vicilin from pea (*Pisum sativum*)	2	Absent
Vicilin from cowpea (*Vigna unguiculata*)	15	Absent
Cowpea protein isolate	52	Absent
Cowpea protein isolate after heating at 80 °C for 5 min	18	Absent

[a] TIU = Trypsin inhibitor units.
[b] Hemagglutination assays were performed using rabbit erythrocytes, as described in Section 2.

3.4. Hemagglutinating activity

Hemagglutinins (lectins) are a class of proteins or glycoproteins characterized by their ability to bind particular sugar residues that belong to polysaccharide moieties of glycoproteins, glycolipids, polysaccharides or simple glycosides (Murray, 1984). The lectins are capable of agglutinating animal and/or human

erythrocytes and stimulating mitosis in resting lymphocytes (Hankins & Shannon, 1978). Higuchi, Suga, and Iwai (1983) demonstrated that lectins bind to the intestinal mucosa impairing digestion and absorption of nutrients. The lectins reduce protein digestibility by inhibiting digestive enzymes (Thompson, Tenebaum, & Hui, 1986). In the present study, no hemagglutination was observed in any of the samples assayed, indicating low or non-detectable lectin levels in the samples (Table 5). These results are in line with those reported by Elkowicz and Sosulski (1982), who showed very low hemagglutinating activity for a protein concentrate from *Vigna unguiculata*. Sathe and Salunkhe (1981) showed absence of hemagglutinating activity in the albumins, globulins, protein concentrates, and protein isolates from *Phaseolus vulgaris*. These observations suggest that CPI and PV may be advantageous for nutritional application and/or functional improvements in foods.

3.5. In vivo protein quality

Food intake of the casein diet (positive control) was the highest (52.9 ml/rat/day) compared to an intake of 29.3 ml/rat/day for the CPI diet (Table 6). The reduced intake of CPI diet could be due to poor palatability, a result of the presence of residual salt in the preparation. Protein intake of the casein diets was 21.0 g per rat, higher than the protein intake of the group fed on CPI-containing diet, which was 12.5 g per rat (Table 6).

While the gain in body weight of the casein group was 63.13 g over 16 days, the gain in body weight of the test group (CPI diet) was 3.45 g per rat. This low body weight gain may be, in part, associated with the food intake, since the rats fed on the CPI diets ate much less than those on casein diets. Rubio, Grant, Bardocz, Dewey, and Puztai (1991) showed a reduced body weight gain and lower protein content after feeding *Vicia faba* meal to rats in comparison to lactalbumin. The growth depression and reduced food intake in the group consuming CPI may also be related to the excess of sulfur-containing amino acids, as shown in Table 2. This is unusual for naturally occurring legume proteins, which are reported to have a low content of sulfur-containing amino acids (Zarkadas, Voldeng, Yu, & Choi, 1997).

A significant reduction occured in nitrogen consumption in the CPI group (0.64g/rat/4 days) compared to the casein group (1.07g/rat/4 days) (Table 7). In the control group, nitrogen absorption was 1.01g/rat/4 days while, in the CPI group, it was 0.56g/rat/4 days. It is important to note that nitrogen intake in the CPI diets was lower than in the casein diets. Thus, the nitrogen absorbed in rats fed on the CPI diet was good. The nitrogen balance was positive in both groups, showing that nitrogen intake was larger than the fecal and urinary excretion of nitrogen.

Table 6 indicates the distinct superiority of the casein diet over the CPI diet, with respect to NPR. The NPR values of the casein and CPI diets were 3.2 and 0.7, respectively, probably owing to lower food intake of CPI diet, and also to an imbalance in essential amino acids.

The true digestibility (TD) for the casein group was higher than for the CPI group. TD was 95.6 % for the casein group and 86.9% for the CPI group. The digestibility of the CPI diet can be considered very good, since legumes in general show digestibilities ranging from 70 to 80% (Seabra et al., 2001). The TD values of the CPI diet was also good compared with TD for other seeds, such as *Adansonia digitata* (74%), *Enterolobium cyclocarpium* (51%) and *Sesbania pachycarpa* (83%) (Proll, Petzke, Ezeagu, & Metges, 1998).

Dietary trypsin inhibitors are often responsible for the poor digestibility of dietary protein by interference with the proper function of trypsin, leading to growth inhibition and pancreatic hypertrophy (Liener, 1994). Armour, Pereira, Bucham, and Grant (1998) reported that the overall contribution of protease inhibitors or lectins to the impaired nutritional performance of animals fed with soy-based diets was small and that, whilst these components alter pancreas and small intestine

Table 6
Protein intake, body weight gain of rats and NPR of CPI and casein diets[a]

Dietary group	Food intake (ml/day)	Protein intake (g)	Body weight gain (g)	Body weight loss[b] (g)	NPR
Casein	52.9 ± 10.3	21.0 ± 1.2	63.13 ± 11.0	4.7 + 0.3	3.2
CPI	29.3 ± 9.8	12.5 ± 0.2	3.45 ± 2.0	4.7 + 0.3	0.7

[a] The data refer to the total (16 days) period of treatment.
[b] Group the rats fed with protein-free diet.

Table 7
Nutritional parameters of rats fed on CPI compared with those of rats fed on casein and protein-free diets

Dietary group	Nitrogen consumed (g/rat/4 day)	Nitrogen absorbed (g/rat/4 days)	True digestibility (%)	Nitrogen balance (g/rat/4 days)
Casein	1.07 ± 0.11	1.01 ± 0.01	95.6	1.0 ± 0.09
CPI	0.64 ± 0.06	0.56 ± 0.06	86.9	0.50 ± 0.06

metabolism, other factors may be responsible for much of the growth impairment and poor utilization observed on soy feeding. Grant, Dorward, Buchan, Armour, and Pusztai (1995) showed that the presence of different legume meals in diets for young rats significantly impaired food conversion efficiency and growth. However, that study also showed that the growth-reducing effects of dietary legumes appeared to diminish with time and, after 200 days, the growth rates of rats on the test diets were close to those of controls given the same daily intake. It is likely that age-linked changes in metabolism decreased the susceptibility of rats to the effects of the legume-based diets.

3.6. Histology

The ability of soy bean agglutinin (SBA) to inhibit the growth of rats was first demonstrated by Liener (1953). About 60% of the lectin content of diets survives intestinal transit and becomes bound to the intestinal epithelium, where it causes disruption of the brush border (Pusztai et al., 1990), atrophy of the microvilli (Jindal, Soni, & Singh, 1984), and reduces the viability of the epithelial cells (Ishiguro, Nakashima, Tanabe, & Sakakibara, 1992). As a consequence of the interaction of lectins with the epithelial surface of the proximal small intestine, there is an increase in the weight of the small intestine because of hyperplasia of the crypt cells (Grant, Watt, Stewart, & Pusztai, 1987). This effect is believed to involve the accumulation of polyamines, mostly spermidine (Bardocz, Grant, Brown, Ewen, Nevison, & Pusztai, 1990), a known stimulant of cellular proliferation. Several studies showed that raw peas contain significant levels of protease inhibitors and lectins (Alonso, Orue, & Marzo, 1998, 2000) and these stimulate hypersecretion of digestive enzymes in rats, leading to pancreas enlargement due, mainly, to hyperplasia and hypertrophy of the acinar cells (Grant, 1999). Inactivation of protease inhibitors and lectins prevents these effects on pancreas metabolism (Armour et al., 1998; Grant, 1999). The small intestines and pancreas of rats fed with the CPI diet presented normal histological appearances (Fig. 1, panels C and D, respectively) when compared to internal organs of rats fed the casein diet (Fig. 1, panels A and B). In particular, no intestinal or pancreatic enlargement could be detected in rats fed

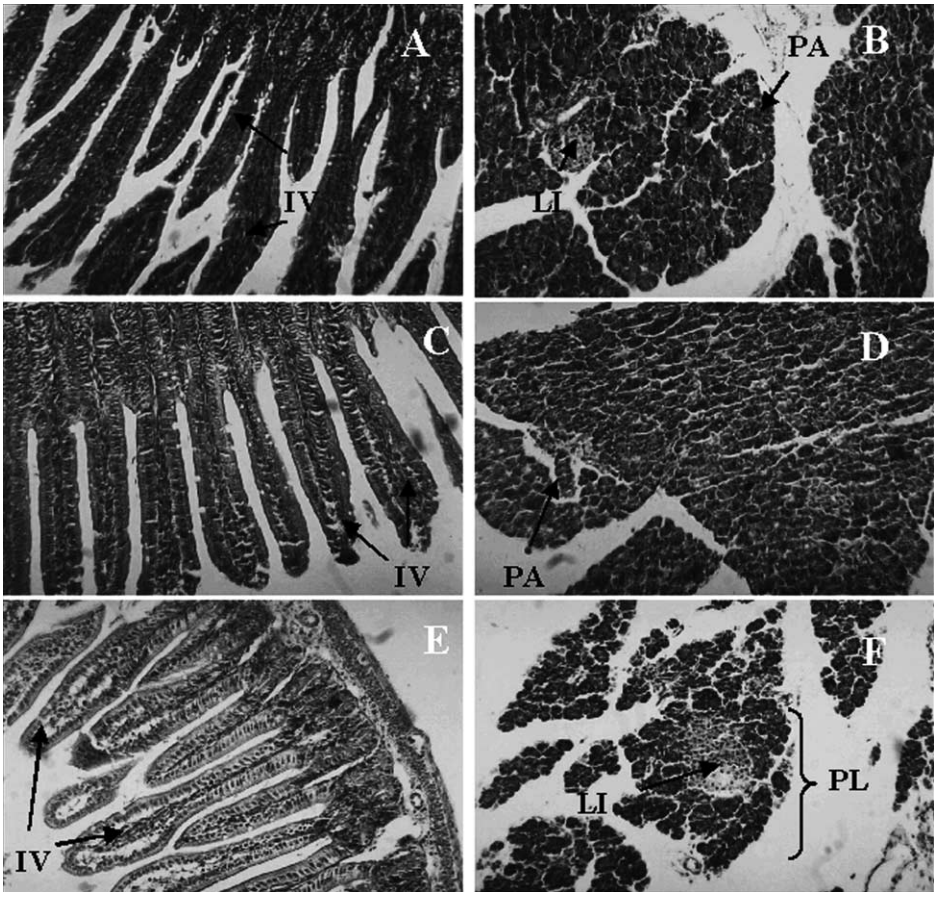

Fig. 1. Hystologic sections of the small intestine (panels A, C and E) and pancreas (panels B, D and F) of rats fed with casein (panels A and B), CPI (panels C and D) or protein-free (panels E and F) diets. IV, intestinal villi; PA, pancretic acini; LI, Langehans' Islet; PL, pancreatic lobule. The sections were stained with hematoxylin-eosin (H&E). Magnification is 50X.

with the CPI diet. As expected, rats fed the protein-free diet showed evidence of a little narrowing and irregular size of the villi and decreased size of the pancreatic lobules (Fig. 1, panels E and F).

4. Conclusions

This study shows that CPI contained adequate levels of most essential amino acids for pre-school children and all essential amino acid for adults and its in vivo protein digestibility is higher than that of other common legumes. The presence of anti-nutritional factor (i.e., trypsin inhibitor) identified in the current report, should not be a problem to human health if the CPI is properly processed (e.g. by heat treatment) prior to its incorporation in food products. Furthermore, no evidence of toxicity was found in experimental diets containing this isolate. CPI may thus be an economic and alternative protein source to alleviate protein malnutrition among the socio-economic lower classes of the population in developing countries.

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