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Insoluble dietary fiber of grain food legumes and protein digestibility

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SUMMARY. The present work aimed to verify the digestibility of cooked whole food grain legumes. Samples of beans (*Phaseolus vulgaris and Vigna sinensis*), chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*) were used in the experiment. The interrelationship between the insoluble dietary fiber presented in the food grain legumes and the low protein digestibility was studied. The insoluble dietary fiber and the proteic nitrogen presented in the neutral detergent fiber (NDF) were determined. «In vivo» digestibility was performed in rats fed with diets containing cooked grain legumes, casein and protein free diet. The experiments were perfomed on rats over a period of 21 days. High excretion of nitrogen was observed by rats fed with cooked food grain legumes compared to casein diet. «In vitro» digestibility was performed by enzymatic hydrolysis with pepsin and trypsin. No significant differences was found between «in vivo» and «in vitro» digestibility. The heat treatmet caused increased in the values of insoluble dietary fiber by the complexation of its components with protein and aminoacids. The results obtained showed the increased of the insoluble dietary fiber, in the cooked samples compared with raw samples. Significants values of protein nitrogen were found in the NDF, suggested that it was originated by complexation with proteins and aminoacids. This fact contributed to become proteic nitrogen nonavailability decreasing consequently the digestibility of the proteins.

RESUMEN. Fibra dietaria insoluble de leguminosas y su influencia en la digestibilidad. El objetivo del presente estudio fue verificar la digestibilidad de tres variedades de frijol común (*Phaseolus vulgaris*), *Vigna sinensis, Cicer arietinum y Lens culinaris*. La relación entre el nitrógeno proteico presente en la fibra insoluble y la baja digestibilidad de las proteínas de las leguminosas fue estudiadas. La fibra insoluble y el nitrógeno proteico presente en la NDF fueron determinadas. Se usaron ratas machos, alimentados con dietas a base de leguminosas, caseina y dieta libre de nitrógeno, para la determinación de la digestibilidad «in vivo». La duración del experimento fue de 21 días. Fue observada alta excreción del nitrógeno fecal en las ratas alimentadas con diets de leguminosas comparadas con la dieta de caseina. La digestibilidad «in vitro» fue determinada a través de la hidrólisis enzimática con pepsina y tripsina. No fueron encontradas diferencias significantes entre la digestibilidad «in vivo» e «in vitro». El tratamiento térmico causa el aumento de fracción fibra insoluble debido a la formación de complejos de los componentes de la fracción con proteínas y aminoácidos. Los resultados obtenidos muestran el aumento de la fracción fibra insoluble de las muestras cocidas en relación a las muestras crudas. Valores significantes de nitrógeno proteico fueron encontrados en los residuos de la NDF y sugieren la interacción de las proteínas y aminoácidos con los componentes de la fracción fibra insoluble. El nitrógeno proteico presente en la NDF contribuye en la fracción no utilizada del nitrógeno no proteico, disminuyendo, en consecuencia, la digestibilidad de las proteínas de las leguminosas.

INTRODUCTION

The food grain legumes constitute an important source of dietary protein for large segments of world's population. The grain legumes have a high percentage of protein, however, their nutritive value is limited by the low digestibility of protein. Dietary fiber causes decreased utilization of many nutrients, including proteins.

Many authors have show that heat treatment induces

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quantitatives modifications in the dietary fiber componentes (1, 2, 3, 4, 5).

The increased faecal nitrogen excretion observed in food grain legumes was correlated to decrease in the digestibility of dietary protein (6, 7, 8).

The objective of the present work was to verify the relationship between proteic nitrogen present in insoluble dietary fiber (NDF) and the low protein digestibility of grain legumes.

MATERIAL AND METHODS

Materials

Studies were performed with three varieties of cooked whole common beans (*Phaseolus vulgaris*, L): black beans (cultivar BR1—Xod6 and commercial); «carioca» beans (cultivar and commercial) and white beans (commercial); blackeye pea (*Vigna sinensis*) (commercial); chickpea (*Cicer arietinum*) (commercial) and lentil (*Lens culinaris*) (commercial).

Black bean -cultivar BR1-Xod6 and «carioca cultivar» were obtained from Estação Experimental de Campos-RJ. The commercial samples were purchased from a local supermarket.

For the biological assay wealing rats of the «Wistar» strain were used and casein diet, protein-free diet and diet based on grain legumes. The diets for all the biological experiments were prepared at a 12,50% protein. The composition of 100 g of diet based on grain legumes was as follow: test sample flour calculated weight to give 8% fat; 4% mineral mixture (The mineral mixture contained 17 mg $CuSO_4$, 517 mg Mn SO_4, 17 mg KI, 16g $CaCO_3$, 11,6 KCl, 417 mg Mg SO_4, 6,6 NaCl, 11,6 Na_2HPO_4, 47 g Ca_3 $(PO_4)_2$, 333 mg ferric citrate and 217 mg zinc carbonate); 1% vitamin mixture (The vitamin mixture used contained 500 mg thiamine, 500 mg riboflavin, 2 g Ca pantothenate, 3 mg vitamin B_{12}, 500 mg vitamin B_6, 200 g choline chloride, 150 mg retinol, 1,25 g ergosterol, 5 g vitamin E, 2 g vitamin K, 5 g niacin, 200 mg folic acid, 100 g ascorbic acid, 100 g inositol, 10g p-aminobenzoic acid and 30 mg biotin made up 1000 g with sacarose).

Methods

The grain legumes and the diets were analysed for moisture content, lipids, ash, proteic nitrogen, NDF, ADF, lignin, starch and pectic substances. Moisture contents, lipids and ash were determined by the procedures described in the methods of Analyses of Institute Adolfo Lutz (9).

Proteic nitrogen was determined by the microkjeldahl method (10), and the protein content was calculated by multiplying the proteic nitrogen by 6,25.

Insoluble dietary fiber was determined using the neutral detergent fiber (NDF) and the acid detergent fiber (ADF). The NDF was estimated by the method modified by Méndez at al (11), applying the following procedure:

Gelatinization of starch with 15 ml of 0.5 N NaOH for 15 min with constant stirring, at 37°C; incubation of 2 hours with 5 ml of 22,5 g% amyloglycosidase* solution in acetate buffer, pH 4,8, to remove starch and reflux in neutral detergent solution for 1 hour. The residue was filtered through a preweighed sintered glass filter crucible, was washed with hot water and acetone. It was dried in a 110°C oven, for 12 hours, cooled in a dessicator, and weighed.

Acid detergent fiber was determined applying the method described by Van Soest (12). Lignin was determined in the residue of ADF. The residue was added 72% H_2SO_4 at 0-4°C for 3 hr and was ocasionally stirred with a glass rod. After hydrolysis, the residue was filtered, and washed with hot water, acetone, dried in a 110°C oven, for 12 hours, cooled in a dessicator and weighed.

Pectic substance were extracted by the method described by Mc Cready and Mc Comb (13) and were determined by the carbazol method (14).

Starch was extracted by the technique purpose by Dekker and Richards (15) and was determined according to Areas and Lajolo (16).

For the biological experiment, 60 males weanling rats of the «WistaR» strain, weighing 35-40 g, were distributed in groups of six animals and fed with the following diets:

Group 1	- Casein diet
Group 2	- Protein-free diet
Group 3	- Diet based on common bean black, cultivar BR1-Xod6
Group 4	- Diet based on common bean black, commercial
Group 5	- Diet based on common bean «carioca», cultivar
Group 6	- Diet based on common bean «carioca», commercial
Group 7	- Diet based on common bean white, commercial
Group 8	- Diet based on blackeye pea, commercial
Group 9	- Diet based on chickpea, commercial
Group 10	- Diet based on lemtils, commercial

Composition of diets are given in Table 1

* Amyloglycosidase E.C. Nº 3.2.1.3 from Rhizopus, Sigma Chemical Co. A7255, activity of 5000-10.000 U/solid g.

TABLE 1
COMPOSITION OF DIETS*. g/100 g DRY MATTER

Determination \ Diets	Casein	Protein free	Common bean Black Cultivar BR1-Xod6	Common bean Black Commercial	Common bean Carioca Cultivar	Common bean Carioca Commecial	Common bean White Commercial	Blackeye pea Commercial	Chickpea Commercial	Lentil Commercial
Protein	13.27	——	14.50	14.84	16.33	14.84	16.70	14.15	13.47	14.34
Lipid	2.69	2.59	3.82	4.27	4.66	4.87	6.92	5.82	9.94	4.39
Ash	4.74	3.80	5.84	6.40	6.45	6.39	7.14	5.86	5.94	5.41
Total dietary fiber	3.64	3.94	10.24	12.33	10.01	11.33	10.12	10.30	7.51	9.97
Cellulose	3.61	3.93	5.22	5.67	6.01	6.34	5.85	5.29	4.15	4.78
Hemicelluloses	0.03	0.01	1.27	1.23	0.67	1.08	0.73	3.62	1.17	3.64
Lignin	—	—	1.43	1.95	1.01	1.04	0.75	0.22	0.56	0.45
Soluble pectin	—	—	1.46	1.48	1.44	1.20	2.45	1.13	1.06	0.97
Protopectin	—	—	0.86	2.00	0.87	1.67	0.34	0.03	0.57	0.13
Carbohydrate	75.93	89.54	65.79	60.57	63.39	61.74	61.31	60.86	58.11	66.70

*Diets based on grain legumes are supplemented with 0.03% DL-methionine

The experiment was peformed over a period of 21 days. Rats were housed in individual cages. Diets and water were fed ad libitum. Food intake was recorded daily and body weight was recorded each 2 days.

Values of nitrogen ingestion were calculated by the food intake. The faeces were collected daily and dried at 55°C. The fecal samples were pooled by dietary group at the end of test period; ground in a laboratory mill and the proteic nitrogen determined by the microKjeldahl method of AOAC (10).

The apparent digestibility coefficient was calculated according to the formula:

$$\text{Apparent digestibility} = \frac{(\text{ingested N} - \text{fecal N}) \times 100}{\text{ingested N}}$$

The true digestibility coefficient was calculated according to the formula:

$$\text{True digestibility} = \frac{\text{ingested N (fecal N-endogenous N)} \times 100}{\text{ingested N}}$$

«In vitro» digestibility was determined for casein and cooked grain legumes employing the method of Akeson and Sttahmann (17). Total aminic nitrogen was determined after acid hydrolysis of the samples with 10 ml of HCl and reflux for 22 hours at 110°C, according to the method described in USP XXI (18).

Student - Fisher test (19) was applied to verify the relationship between digestibility coefficients «in vivo» and «in vitro».

RESULTS AND DISCUSSION

The studies reported confirmed that the residual activity of the antinutritional (antitrypsin and phytohemaglutinin) after heat treatment is very low (20, 21, 22). Therefore, these factors have not contributed to the low protein digestibility of cooked grain legumes.

Other factors also contributed to low digestibility of grain legumes:

1. The heat treatment was responsible for interaction and complexation of proteins with other components, as lignin, cutins and polyssacharides and the formation of Maillard polimers (23).

All of these complexes were characterized by insolubility and indigestibility and therefore quantitatively recovered in the insoluble dietary fiber.

Table 2 shows the increased for the insoluble dietary fiber in the cooked samples against raw samples. Significants differences were observed in the value of lignin in the common beans samples.

Differences in the percentage of non hidrolysated protein present in the insoluble dietary fiber were observed (Table 6): lentils (32,59%) and blackeye pea (27,51%) having the higher value and common bean white (5,19%) the lowest value and the other samples having the average value of about 19%.

These results suggested interactions of aminoacids and proteins with components of the insoluble dietary fiber. The protein content of grain legumes are given in Table 2.

2. The proteins of beans presented greater resistance to action of digestive enzymes. Schneeman (24) suggested that the mechanism by wich fiber influence enzyme activity was that part of enzyme was absorved into the fibre matrix decreasing the availability of enzyme activity and that may be responsible for the decrease in protein digestibility.
3. Many authors (6) (7) (8) (22) (25) (26) have attributed the low digestibility of grain legumes proteins, verified in biological assays, to the increased in the fecal nitrogen.
   The increase in the fecal nitrogen could be originated.
   a) An intestinal fermentation resulting in a bacterial protein synthesis increase.
   b) An increase excretion of endogenous fecal nitrogen.
   c) In complexation of nitrogen with the components of insoluble dietary fiber.

The correlation coefficient between percentage of retained proteiñ in NDF residue and «in vitro» digestibility (y=7x +40.4628 and r=0.7901) in the common beans (*Phaseolus vulgaris*) and (y=0.84x + 71.74 and r= -0.7001) in the blackeye pea, chickpea and lentils shows the negative influence of the retained nitrogen in the NDF residue and the digestibility was significantly acentuated in the common beans samples than the other grain legumes investigated.

The results of «in vivo» and «in vitro» digestibility are given in Tables 4 and 5, respectively. No significant differences were found (Student-test: t=0.07 and n=10) betweeen «in vivo» and «in vitro» digestibility at 0.001, 0.01 and 0.05 levels.

The correlation coefficient between of «in vitro» and «in vitro» digestibility 9y = 93.3 x -5.67 and r = 0.9927), showed there was no difference between the enzymatic and biological methods, in the grain legumes samples.

The results sugest that increase in fecal nitrogen could be originated by the nitrogen present in protein and or aminoacid complexed with insoluble dietary fiber. The resulting complexes are compounds insoluble and indigerible.

The proteic nitrogen found in NDF contributed to the fraction of nonavailable proteic nitrogen decreasing consequently the digestibility of the grain legumes protein.

TABLE 2
VALUE OF PROTEIN AND INSOLUBLE DIETARY FIBER IN COOKED* DRY AND RAW LEGUMES, g/100g DRY MATTER

Grain Legumes \ Determination		Protein	Insoluble dietary fiber		
			Cellulose	Hemicellulose	Lignin
Common bean (Black) Cultivar BR1-Xod6	Raw	21.23	5.37	1.82	0.68
	Cooked	24.04	7.85	2.62	2.74
Common bean (Black) Commercial	Raw	19.48	5.47	0.57	0.89
	Cooked	20.09	7.20	0.47	3.93
Common bean (Carioca) Cultivar	Raw	18.36	5.10	0.94	0.26
	Cooked	20.61	8.25	0.44	1.72
Common bean (Carioca) Commercial	Raw	14.70	4.96	0.66	0.83
	Cooked	18.31	9.00	0.42	1.55
Common bean (White) Commercial	Raw	15.53	3.60	0.31	0.69
	Cooked	18.31	7.29	0.46	1.08
Blackeye pea commercial	Raw	21.52	7.17	6.25	1.68
	Cooked	25.42	8.20	3.74	1.65
Chickpea Commercial	Raw	18.24	4.32	0.22	0.42
	Cooked	19.88	5.66	4.33	0.49
Lentil Commercial	Raw	22.72	4.95	1.22	0.66
	Cooked	23.72	6.80	4.58	0.78

* Cooked for 30 min. under pressure.

Table 3 shows the nitrogen in the NDF residues and the amount of retained protein in the NDF residues.

TABLE 3
NITROGEN IN THE NDF RESIDUE AND RETAINED PROTEIN IN THE NDF RESIDUE (COOKED* DRY GRAIN LEGUMES)

Grain Legumes \ Determination	Nitrogen (g) in 100 g of NDF	Retained protein (g) in NDF residue in 100 g of grain legumes**	Retained protein (g) in NDF residue, in 100 g of protein***
Common bean (Black) Cultivar BR1-Xod6	2.26	1.87	7.78
Common bean (Black) Commercial	2.44	1.77	8.81
Common bean (Carioca) Cultivar	1.91	1.24	6.03
Common bean (Carioca) Commercial	1.98	1.36	7.42
Common bean (White) Commercial	0.57	0.31	1.72
Blackleye pea Commercial	1.93	1.64	6.45
Chickpea Commercial	1.24	0.81	4.09
Lentil Commercial	2.22	1.70	7.17

* Cooked for 30 min, under pressure

** $\frac{\text{\% Protein in NDF x NDF}}{100}$

*** $\frac{\text{\% Protein in NDF in grain legumes x 100}}{\text{Protein}}$

TABLE 4
APPARENT AND TRUE DIGESTIBILITY OF CASEIN DIET AND DIETS* BASED ON GRAIN LEGUMES

Diets	Nitrogen Intake (g)	Fecal Nitrogen (g)	Apparent Digestibility (%)	True Digestibility (%)
Casein	4.977±0.686	0.4933±0.081	90.12±0.49	92.30±0.65
Protein-free	—	0.107±0.018	—	—
Common bean (Black) Cultivar BR1-Xod6	3.866±0.696	11.536±0.287	60.1±4.70	62.21±3.23
Common bean (Black) Commercial	4.422±1.304	1.858±0.436	53.37±3.73	59.97±3.37
Common bean (Carioca) Cultivar	5.112±0.714	1.855±0.376	63.75±4.58	65.86±4.53
Common bean (Carioca) Commercial	5.517±0.496	2.316±0.076	57.75±3.85	59.71±3.68
Common bean (White) Commercial	5.631±1.119	1.958±0.285	64.79±2.78	66.78±2.32
Blackeye pea Commercial	4.189±0.787	1.028±0.069	75.02±3.67	77.64±3.16
Chickpea Commercial	4.724±0.546	1.074±0.194	77.38±1.68	79.67±1.94
Lentil Commercial	4.512±0.853	1.119±0.135	75.03±1.73	77.47±1.31

* Diets based on grain legumes are supplemented with 0,03% DL-methionine
Experiment was performed over a period of 21 days.

TABLE 5
«IN VITRO» DIGESTIBILITY OF CASEIN AND COOKED* DRY GRAIN LEGUMES

Grain legumes	Total Aminic Nitrogen (%)	Hydrolysated Nitrogen (%)	«In vitro» Digestibility (%)
Casein	12.76	12.28	96.24
Common Bean (Black) Cultivar BR1-Xodó	4.30	2.60	60.47
Common Bean (Black) Commercial	3.83	2.26	59.01
Common Bean (Carioca) Cultivar	3.90	2.64	67.69
Common Bean (Carioca) commercial	3.57	2.16	60.50
Common Bean (White) Commercial	3.56	2.38	66.85
Blackeye pea Commercial	4.65	3.56	76.56
Chickpea Commercial	3.66	2.90	79.23
Lentil Commercial	4.00	3.12	78.00

* Cooked for 30 min under pressure

TABLE 6
PERCENT OF NON HIDROLYSATED PROTEIN PRESENT IN THE INSOLUBLE DIETARY FIBER

Grain legumes	% Retained protein in NDF residue	% Non hidrolysated protein	% Non hidrolysated protein present the insoluble dietary fiber
Common Bean (Black) Cultivar BR1-Xodó	7.78	39.53	19.68
Common Bean (Black) Commercial	8.81	40.99	21.49
Common Bean (Carioca) Cultivar	6.03	32.31	18.66
Common Bean (Carioca) Commercial	7.42	39.50	18.78
Common Bean (White) Commercial	1.72	33.15	5.19
Blackeye pea Commercial	6.45	23.44	27.51
Chickpea Commercial	4.09	20.77	19.69
Lentil Commercial	7.17	22.00	32.59

* Cooked (for 30 min under pressure) and dry

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