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Influence of the *Phaseolus vulgaris* phaseolin level of incorporation, type and thermal treatment on gut characteristics in rats

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Phaseolus vulgaris phaseolin has been shown to stimulate faecal losses of endogenous N in rats. Experiments with purified phaseolin were carried out in rats to test the hypothesis that these losses reflect intestinal disorders. Phaseolin composition varies depending on its constitutive subunits. Therefore, three phaseolin types (S, T, I) were tested. Phaseolin T was incorporated in varying levels (0, 33, 67 or 100 % of the dietary protein) as raw material in experiment 1. In experiment 2, the three phaseolin types were incorporated at 50 %, with or without previous thermic treatment. Raw casein was the basal protein source and was also heated in experiment 2. Faecal digestibility of phaseolin and gut integrity were evaluated in both experiments. The incorporation level or type of phaseolin had little effect on gross anatomy of gut segments but these factors influenced the weight and pH of fresh contents of the stomach and caecum ($P<0{\cdot}05$). Raw phaseolin T incorporated at various levels led to an enlargement of duodenal villi together with a tendency for increased crypt depth in the jejunum ($P=0{\cdot}06$). Activities of both alkaline phosphatase in the duodenum and aminopeptidase N in the ileum decreased ($P<0{\cdot}05$) after thermal treatment of casein while they increased ($P<0{\cdot}05$) for heat-treated phaseolin S and T, respectively. In conclusion, raw phaseolin had no effect on the tissue weight of gut segments and induced limited alterations in the small intestine. Differences due to phaseolin level or type were limited too.

Rats: Intestine: Phaseolin: Enzymes

Legume grains are important sources of dietary protein in various parts of the world. Common beans (*Phaseolus vulgaris* L.) account for 87 % of pulse consumption by humans in Latin America (Leterme & Muñoz, 2002). The nutritional value of common bean protein is lower than that of animal protein owing to low levels of sulfur amino acids and tryptophan. This limitation has also been ascribed to the presence of two types of proteins in the bean, lectin and phaseolin. *Phaseolus* lectin is highly resistant to proteolysis, reduces rat body growth, alters intestinal tissues (Pusztai *et al.* 1990) and also stimulates intestinal maturation in suckling animals (Radberg *et al.* 2000; Linderoth *et al.* 2000). By contrast, little is known of the effects of phaseolin, the bean storage globulin, on the gut, despite dramatic increases in faecal losses of endogenous N in rats (Santoro *et al.* 1997, 1999). Native phaseolin is highly resistant to hydrolysis *in vitro* (Nielsen, 1991) and digestion *in vivo* (Levi-Benshimol & Garcia, 1986; Coelho & Sgarbieri, 1995; Santoro *et al.* 1997, 1999). Its digestibility is largely increased by thermal treatment (Liener & Thompson, 1980; Marquez & Lajolo, 1981, 1990).

Another important point is the biochemical type of phaseolin. Data obtained in pigs fed diets with common beans belonging to the S and T types suggest that phaseolin S is digested better than phaseolin T (Begbie & Ross, 1993). Phaseolin subunits display a molecular heterogeneity that is reflected in the classification of *Phaseolus* beans. Three major patterns predominate over the numerous phaseolin types described so far: the T (Tendergreen), C (Contender) and S (Sanilac) types (Hall *et al.* 1999). In total, they comprise nine distinct polypeptides (Brown *et al.* 1981), the T and S types being essentially different one from each other and the C type being intermediate. More recently, the I (Inca) type, lacking the largest polypeptide, has been described as an ancestral line (Koening *et al.* 1990; Kami *et al.* 1995). Since small differences in protein structures can affect digestion and thermal stability (Rezaei *et al.* 2000; Fukuda *et al.* 2005), the present work was conducted to test the hypotheses that (a) there are differences between phaseolin incorporation levels and types for their digestibility and impact on gut characteristics and (b) thermal treatment reverses these effects.

Abbreviation: MW, molecular weight.

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Materials and methods

Isolation and purification of phaseolin

Seeds of *Phaseolus* bean breeds with S, T and I phaseolin patterns were provided by the Bean Breeding Department of the International Centre for Tropical Agriculture (CIAT, Cali, Colombia). The common bean meal was prepared as follows: whole seed beans were soaked in water (1:4, w/v) at 4°C for 24 h. Water was changed every 2 h for the first 10 h in order to extract as much as possible of soluble tannins and to soften the hull. The next day, the seeds were removed from the water, frozen and freeze-dried. The hull and embryo were removed by means of a scalpel. The cotyledon was then ground through a 0·5 mm mesh screen and the meal kept at 4°C until use. The purification of phaseolin was based on the methods of Hall *et al.* (1977). The flour was suspended in an acidic solution (1 g flour/20 ml; 0·5 M-NaCl and 0·025 M-HCl; pH 2·0) for 1 h and then centrifuged at 20 000 ***g*** for 20 min. The supernatant fraction was mixed with five volumes of distilled water (1:5) at 4°C. This caused immediate precipitation of the phaseolin. The solution was centrifuged for 20 min at 4°C and 20 000 ***g***. The precipitate was washed with distilled water and centrifuged again. The final precipitate was dialysed against distilled water (4°C) for 24 h, with water changed after 12 h, then frozen and freeze-dried. These modifications in the extraction protocol allowed a better yield than with the initial method.

Animals

The experiments were conducted in agreement with the guidelines of the National University of Colombia for care and use of laboratory animals. A total of twenty (experiment 1) and forty-five (experiment 2) Wistar rats (donated by the Zoo of Cali, Colombia) with initial body weights of 190 (SD 20) and 250 (SD 44) g were used. Female rats were used in order to conserve purified phaseolin because they eat less than male rats (Radcliffe & Webster, 1979). They were kept in individual metabolism cages (Tecniplast 150–300; Buguggiate, Italy) for the whole of the experimental periods.

Diets

Experiment 1. Four diets were formulated in order to contain 100 g protein/kg DM. The control diet (C) contained 118 g casein supplemented with DL-methionine/kg diet as the sole source. In the other diets, casein was replaced by 33, 67 and 100 % native phaseolin T, respectively (Table 1).

Experiment 2. Casein and the three types of purified phaseolin (S, T and I) were incorporated either raw or after thermal treatment into eight experimental diets. The proteins were dissolved in 1 M-NaOH (1:10, w/v) and pH adjusted at 7·5 using 1 M-HCl, then autoclaved at 121°C and 15 pounds per square inch for 15 min, frozen and freeze-dried. Eight diets were formulated in order to contain 100 g protein/kg DM provided by either casein alone (control) or a mixture of casein and phaseolin (1:1) (Table 1). The control diet based on casein was the same as in experiment 1. The diets were provided as dry flours to the rats. An additional protein-free diet was formulated and fed in the same conditions as below to five rats in order to determine the faecal true digestibility of protein and phaseolin.

Table 1. Ingredient and analytical composition of the experimental diets

		Diets of Expt 1*			Diets of Expt 2*	
	Casein control (Expts 1 and 2)	P33	P67	P100	S, T or I	PF
Ingredients (g/kg DM)						
Casein†	118	80	40	0	54	0
Phaseolin‡	0	35	70	106	52	0
Starch	582	585	590	594	594	700
Sucrose	100	100	100	100	100	100
Ground rice hulls	80	80	80	80	80	80
Vegetable oil	60	60	60	60	60	60
Vitamin–trace element mix§	10	10	10	10	10	10
NaCl	10	10	10	10	10	10
Calcium carbonate	15	15	15	15	15	15
Calcium phosphate	25	25	25	25	25	25
Analysis (g/kg DM)						
DM	91	91	91	91	90	90
Protein (N × 6·25)	109	103	106	102	111	9
Ether extract	65	65	64	66	65	62
Ash	71	67	67	64	66	64
NDF	63	70	70	60	68	68
Energy (MJ/kg DM)	16·31	16·33	16·36	16·33	16·92	16·58

NDF, neutral-detergent fibre.

* P33, P67 and P100 are diets with phaseolin contributing to 330, 670 and 1000 g/kg of the total dietary protein (experiment 1); S, T, and I are diets with phaseolin S (Sanilac), phaseolin T (Tendergreen) and phaseolin I (Inca) providing 500 g/kg of the total dietary protein (experiment 2); PF is a protein-free diet (experiment 2).

† Casein was either native (both experiments) or thermally treated (experiment 2). Casein was supplemented with 30 g DL-methionine/kg DM casein.

‡ Phaseolin T (raw; experiment 1) or S, T or I (raw or thermally treated; experiment 2).

§ Mineral and vitamin mixture supplied (per kg diet): 7·5 mg vitamin A; 0·2 mg vitamin D_3; 15 mg vitamin E; 6 mg vitamin K; 10 mg vitamin B_2; 35 mg calcium pantothenate; 75 mg niacin; 2·5 mg vitamin B_6; 0·05 mg vitamin B_{12}; 0·05 mg biotin; 200 mg choline; 150 mg Mn; 500 mg Zn; 40 mg Cu, 200 mg Fe; 2 mg I; 0·5 mg Se, 1 mg Co.

Experimental conditions

Experiment 1 was a randomised complete block design with twenty rats randomly allocated to one of the five blocks, each comprising four treatments (n 5 per treatment). Experiment 2 was a factorial design with protein type (four levels) and treatment (two levels) as the main factors with five replications of eight rats fed one diet (n 5 per treatment). For both experiments, food intake was limited to 10 g/d in order to limit food refusals in the diet with the highest phaseolin level, as observed in a pre-trial. After an adaptation period of 5 d to the diets, the faeces were quantitatively collected for 5 d and kept at −15°C until analysis for digestibility determination. At the end of the digestibility period, the rats were fasted for the rest of the day. They received a single meal the following day, 3 h before being killed by asphyxia with chloroform, after an intramuscular injection of Rompun© (Bayer HealthCare, Monheim, Germany). They were weighed and their abdomen immediately opened. The digestive tract was removed, weighed and positioned on an ice-containing tray covered with a glass square. Segments including the stomach, caecum and colon were isolated and weighed filled and empty. The pH of the contents of the stomach and the caecum was also measured. After being unrolled and length measured, the small intestine was divided into two halves by length, emptied and tissues and contents weighed. This was also done with the whole colon. Samples (1·5 cm in length) of the small intestine were taken at 10 cm distal from the pylorus (duodenum), in the middle of the small intestine (jejunum) and 10 cm before the ileo-caecal junction (ileum). Each fragment was cut longitudinally and washed with distilled water before being fixed in buffered formalin and kept at 4°C until morphology analysis (Salgado *et al.* 2001). Other samples of 3 cm in length were collected at the same sites of the small intestine and immediately frozen in liquid N_2 before enzyme activity determination.

Analysis

Phaseolin purity. The purity of phaseolin S, T and I preparations was checked using SDS–PAGE electrophoresis as described earlier (Lallès *et al.* 1999).

Diets and faeces. The diets were analysed for ash (furnace at 550°C for 8 h), N (Kjeldahl method), ether extract (Soxhlet method using petroleum ether as solvent), and for neutral-detergent fibre using the ANKOM fibre analyser (Ankom Technology, Madecon, NY, USA). The faeces were analysed for N (Kjeldahl method) only.

Histomorphometry. Methods for assessing small-intestinal morphology were based on microdissection as described by Goodlad *et al.* (1991). Villus and crypt length, width and surface area were measured using image analysis as previously described (Salgado *et al.* 2001). Mean values of these parameters were determined for fifteen individual villi and ten crypts from each specimen.

Intestinal enzyme activities. All the reagents used were from Sigma (St Louis, MO, USA). The frozen small-intestinal samples were thawed on ice and homogenised in ice-cold saline (NaCl 0·9 %) and refrozen at −40°C until analysis as previously described (Salgado *et al.* 2001). The specific activity of aminopeptidase N (*EC* 3.4.11.2) was determined according to Sangild *et al.* (1995). The specific activity of alkaline phosphatase (*EC* 3.1.3.1) was determined according to Martins *et al.* (2000) Alkaline phosphatase and aminopeptidase N were retained because the former is considered to be a biomarker of intestinal maturation and the latter, which contributes to peptide digestion, was found to vary similarly to other peptidases (aminopeptidase A and dipeptidylpeptidase IV) in piglets and calves fed various legume protein sources (Salgado *et al.* 2001; Montagne *et al.* 2002). Protein was measured by the method of Lowry *et al.* (1951) and specific enzyme activities were calculated.

Calculations and statistical analyses

The coefficients for apparent and true digestibility of protein (N × 6·25) were calculated as follows:

$$\text{Protein apparent digestibility} = (\text{Pi} - \text{Pf}) \times 100/\text{Pi},$$

$$\text{Protein true digestibility} = (\text{Pi} - \text{Pf} - \text{Pe}) \times 100/\text{Pi} \quad \text{(in experiment 2)},$$

with Pi as protein intake, Pf as faecal protein output, and Pe as endogenous faecal protein output measured with the protein-free diet.

In experiment 1, an ANOVA was conducted on the data in order to test the effect of the diet. Diet effects were also partitioned into single degrees of freedom orthogonal comparisons, i.e. into linear, quadratic and cubic effects of the dietary phaseolin incorporation level. In experiment 2, an ANOVA was conducted on the data in order to test the effect of the protein type, thermal treatment and protein type by thermal treatment interaction. In each experiment, when the F value of the ANOVA was significant ($P<0·05$) the means were compared using Duncan's multiple range test (Duncan, 1955). All statistical analyses were performed using the general linear model procedure of SAS (SAS Institute, Cary, NC, USA; SAS, 1999).

Results

Phaseolin quality

As expected, SDS–PAGE electrophoresis revealed two bands for phaseolin S and I, and three bands for phaseolin T, at molecular weight (MW) comprised between 40 and 50 kDa (Fig. 1). No bands were detected at MW inferior to 40 kDa.

Experiment 1

Voluntary food intake and protein digestibility. The voluntary food intake was not significantly different between diets with 0, 33 and 67 % phaseolin T but was lower with 100 % phaseolin T ($P<0·05$) (Table 2). Also, the apparent faecal digestibility of protein decreased with increasing phaseolin T incorporation rate ($P<0·001$). Food intake taken as a covariate tended to be significant for protein digestibility ($P=0·062$) but was not significant ($P>0·10$) for the rest of the measured parameters. The faecal apparent digestibility of phaseolin T was found to be 25, 25 and 38 % for the levels of incorporation of 33, 67 and 100 % of phaseolin T, respectively.

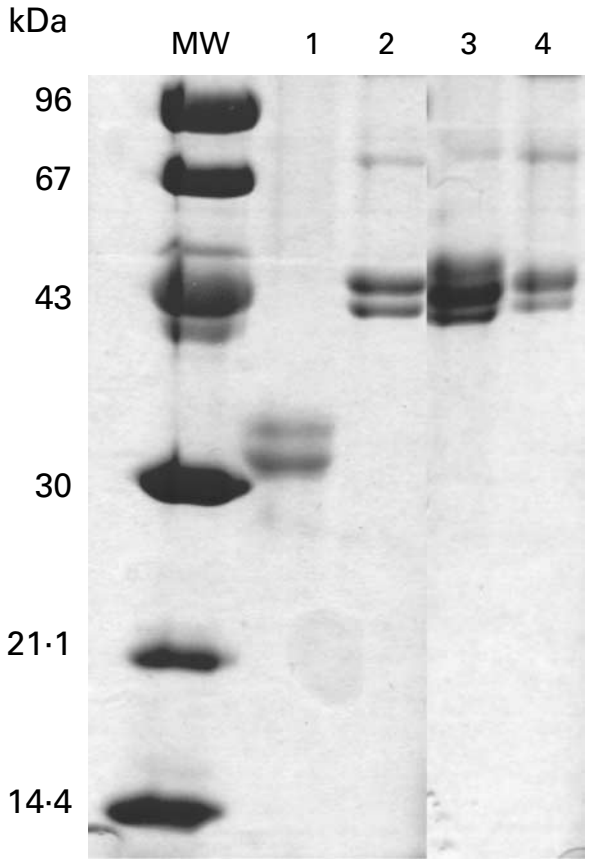

Fig. 1. SDS–PAGE electrophoresis of casein and phaseolins. MW, molecular weight standards; lane 1, casein; lane 2, phaseolin I; lane 3, phaseolin T; lane 4, phaseolin S.

Characteristics of the gastrointestinal tract. The only significant differences ($P<0·05$) due to treatments were observed for the weight of caecal contents and the pH of contents in the stomach and caecum (Table 3).

Morphology of the small intestine. The diet treatments impacted significantly on villus height in the duodenum ($P=0·045$), villus width in the duodenum and jejunum ($P=0·002$ and $P=0·01$, respectively), crypt depth in the jejunum ($P=0·06$) and crypt width ($P=0·004$) and on the villus height:crypt depth ratio in the duodenum ($P=0·02$) with a tendency in the jejunum ($P=0·08$) (Table 4).

Enzyme activities of the intestinal mucosa. There was no significant effect of the diet on the specific activities of alkaline phosphatase and aminopeptidase N at any of the three sites of the small intestine ($0·12 < P<0·63$) (data not shown).

Experiment 2

Protein digestibility. A significant protein type × thermal treatment interaction ($P<0·001$) was observed for both apparent and true digestibility of dietary protein (Table 5). The apparent and true digestibilities of phaseolin were found to be lower in the unheated as compared with the heated state ($P<0·001$); this without any difference between phaseolin types (apparent, 21·3 SE 1·5 and 79 SE 1·0 %; true, 33·3 SE 1·5 and 91·7 SE 1·3 %).

Characteristics of the gastrointestinal tract. The mucosa weight:muscularis weight ratio was influenced by thermal treatment in the second half of the small intestine ($P<0·05$) (Table 6). The length of the small intestine tended to be influenced by the type of protein ($P=0·078$) but not by heat treatment ($P>0·05$). The length of the colon was shorter with the thermally treated proteins ($P<0·05$).

A tendency ($P=0·057$) for an interaction between the type of protein and thermal treatment was noted for small-intestinal fresh content. The protein type × treatment interaction was significant ($P=0·030$) for the contents of the caecum but no significant differences between treatment means were detected ($P>0·05$).

The protein type × treatment interaction was significant for the pH of fresh contents in the stomach ($P=0·048$) but no significant differences between treatment means were detected ($P>0·05$).

Morphology of the small intestine. The protein type × thermal treatment interaction tended to be significant for villus height and width in the ileum (Table 7). Thermal treatment had a significant effect on various morphology variables along the small intestine ($P<0·05$).

Enzyme activities of the small-intestinal mucosa. The protein type × thermal treatment interaction was significant for the specific activity of alkaline phosphatase in the duodenum ($P<0·001$) and aminopeptidase N in the ileum ($P<0·05$) (Table 8).

Discussion

Quality of the prepared phaseolins

Two (phaseolins S and I) or three (phaseolin T) bands at MW between 40 and 55 kDa were obtained for phaseolin using SDS–PAGE electrophoresis, as expected (Hall *et al.* 1999). *P. vulgaris* lectin has a subunit MW at about 32 kDa (Felsted *et al.* 1981), protease inhibitor at 10 kDa (Pusztai, 1968) and α-amylase inhibitor subunits at 12·4, 15·2, 33·6 and 45 kDa (Lee *et al.* 2002). Therefore, it is unlikely that our phaseolin preparations were contaminated with these anti-nutritional

Table 2. Voluntary food intake and faecal apparent digestibility of protein and phaseolin T (experiment 1)
(Mean values and residual standard deviations)

	Diet						
	C	P33	P67	P100	RSD	Contrast	*P*
Voluntary food intake (g/d)	9·7[a]	9·6[a]	8·7[a]	6·0[b]	1·1	Quadratic	0·002
Protein digestibility (%)	83[a]	64[b]	44[c]	38[c]	6·1	Linear	0·001

C, casein control diet; P33, phaseolin T 33 % diet; P67, phaseolin T 67 % diet; P100, phaseolin T 100 % diet.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

Table 3. Weight of fresh contents in the caecum and pH in the stomach and caecum of rats fed diets with increasing levels of phaseolin T (experiment 1)

(Mean values and residual standard deviations)

	Diet						
	C	P33	P67	P100	RSD	Contrast	*P*
Fresh contents in the caecum (g/100 g BW)	0·65[b]	0·78[b]	0·74[b]	1·3[a]	0·24	Quadratic	0·02
Stomach pH	5·4[a]	4·6[b]	4·4[b]	3·5[c]	0·4	Linear	0·001
Caecum pH	7·4[b]	7·3[b]	7·6[a,b]	7·8[a]	0·2	Quadratic	0·020

C, casein control diet; P33, phaseolin T 33 % diet; P67, phaseolin T 67 % diet; P100, phaseolin T 100 % diet; BW, body weight.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

Table 4. Morphology of the small intestine of rats fed diets with increasing levels of phaseolin T (experiment 1)

(Mean values and residual standard deviations)

	Diet						
	C	P33	P67	P100	RSD	Contrast	*P*
Villus height (μm)							
Duodenum	556[a]	541[a]	503[a,b]	457[b]	52	Quadratic	0·045
Jejunum	414	466	407	420	34	NS	0·073
Ileum	243	270	284	271	56	NS	0·709
Villus width (μm)							
Duodenum	216[b]	342[a]	358[a]	326[a]	45	Quadratic	0·002
Jejunum	351	356	306	277	35	Quadratic	0·011
Ileum	253	274	267	219	50	Quadratic	0·341
Crypt depth (μm)							
Duodenum	211	205	253	223	31	NS	0·111
Jejunum	153	170	188	191	22	NS	0·057
Ileum	143	170	140	157	22	NS	0·164
Crypt width (μm)							
Duodenum	47[a,b]	53[a]	40[b,c]	35[c]	69	Quadratic	0·007
Jejunum	42	33	39	35	7	NS	0·282
Ileum	37	36	33	30	4	NS	0·057
Villus height:crypt depth ratio							
Duodenum	2·6[a]	2·7[a]	2·1[b]	2·1[b]	0·3	Quadratic	0·022
Jejunum	2·8	2·7	2·2	1·7	0·4	NS	0·083
Ileum	1·7	1·6	1·7	2·0	0·4	NS	0·377

C, casein control diet; P33, phaseolin T 33 % diet; P67, phaseolin T 67 % diet; P100, phaseolin T 100 % diet.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

Table 5. Faecal apparent and true digestibilities of protein in rats fed diets containing different types of proteins either unheated or heated (experiment 2)

(Mean values and residual standard deviations)

	Diet, protein unheated				Diet, protein heated*					*P*		
	C	S	T	I	C	S	T	I	RSD	Protein	Heat	Protein × heat
Apparent digestibility (%)	85[a]	52[b]	54[b]	53[b]	85[a]	81[a]	83[a]	82[a]	4	0·001	0·001	0·001
True digestibility (%)	97[a]	64[b]	66[b]	65[b]	97[a]	93[a]	95[a]	95[a]	4	0·001	0·001	0·001

C, casein control diet; S, T, I, diets with phaseolin S (Sanilac), phaseolin T (Tendergreen) and phaseolin I (Inca), respectively, providing 500 g/kg of the total dietary protein.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Thermal treatment was at 121°C and 15 pounds per square inch for 15 min.

factors because bands of MW below 40 kDa were not observed in the present work.

Impact of phaseolin on the gut

We used female rats restricted for food intake because in a pre-trial the diet with a high level of phaseolin incorporation (100 %) was poorly consumed. This was confirmed here. Such a negative effect of a low-digestible protein on food intake was documented earlier with zein, as compared with casein or gluten (Radcliffe & Webster, 1979). Part of the effects observed with the P100 diet can be ascribed to the limited feed intake of the rats fed this diet.

The first major observation of the present study is that raw phaseolin, irrespective of the biochemical type, impacted little on the gastrointestinal tract of our rats despite its poor

Table 6. Gastrointestinal tract characteristics of rats fed diets containing different types of proteins either unheated or heated (experiment 2)
(Mean values and residual standard deviations)

	Diet, protein unheated				Diet, protein heated*					*P*		
	C	S	T	I	C	S	T	I	RSD	Protein	Heat	Protein × heat
Small-intestinal mucosa:muscularis ratio (fresh weight, g/g)												
Second half	0·6	0·8	0·9	1·0	1·3	0·8	1·5	1·2	0·4	0·612	0·011	0·297
Length of intestines (cm)												
Small intestine	100	102	100	102	97	99	96	105	6	0·078	0·385	0·606
Colon	13·2	14·2	14·7	13·6	12·0	12·4	13·5	11·8	2·0	0·209	0·032	0·973
Weight of fresh contents (g/100 g BW)												
Small intestine	1·6	1·5	1·6	1·5	1·3	1·6	1·7	1·5	0·2	0·709	0·967	0·057
Caecum	0·6	0·6	0·4	0·6	0·5	0·6	0·7	0·5	0·2	0·820	0·604	0·030
Stomach contents pH	5·3	4·4	4·8	4·4	4·3	4·3	4·6	5·2	0·6	0·572	0·661	0·048

C, casein control diet; S, T, I, diets with phaseolin S (Sanilac), phaseolin T (Tendergreen) and phaseolin I (Inca), respectively, providing 500 g/kg of the total dietary protein; BW, body weight.
* Thermal treatment was at 121°C and 15 pounds per square inch for 15 min.

Table 7. Morphology of the small intestine of rats fed diets containing different types of proteins either unheated or heated (experiment 2)
(Mean values and residual standard deviations)

	Diet, protein unheated				Diet, protein heated*					*P*		
	C	S	T	I	C	S	T	I	RSD	Protein	Heat	Protein × heat
Duodenum												
Villus height (μm)	492	427	454	441	477	491	494	488	50	0·508	0·045	0·342
Crypt depth (μm)	164	185	198	186	192	205	209	196	21	0·152	0·016	0·729
Jejunum												
Crypt depth (μm)	154	172	171	157	150	144	148	149	22	0·667	0·040	0·601
Crypt width (μm)	30	31	30	25	37	31	33	35	5·8	0·413	0·012	0·232
Villus height:crypt depth ratio	2·6	2·0	2·4	2·5	2·5	2·8	2·8	2·7	0·5	0·306	0·065	0·217
Ileum												
Villus height (μm)	244	217	236	226	198	233	246	228	27	0·388	0·599	0·070
Villus width (μm)	292	271	244	302	286	256	258	215	40	0·088	0·080	0·062
Crypt depth (μm)	150	148	144	146	152	167	169	171	19	0·873	0·009	0·499
Crypt width (μm)	35	33	30	33	40	38	38	41	5	0·635	0·001	0·825
Villus height:crypt depth ratio	1·6	1·5	1·7	1·6	1·3	1·4	1·5	1·3	0·2	0·735	0·011	0·603

C, casein control diet; S, T, I, diets with phaseolin S (Sanilac), phaseolin T (Tendergreen) and phaseolin I (Inca), respectively, providing 500 g/kg of the total dietary protein.
* Thermal treatment was at 121°C and 15 pounds per square inch for 15 min.

Table 8. Specific activity of alkaline phosphatase and aminopeptidase N in the small intestine of rats fed diets containing different types of proteins either unheated or heated (experiment 2)
(Mean values and residual standard deviations)

	Diet, protein unheated				Diet, protein heated‡					*P*		
	C	S	T	I	C	S	T	I	RSD	Protein	Heat	Protein × heat
Alkaline phosphatase (nmol substrate degraded/mg tissue protein per h)												
Duodenum	411^{a}	190^{c}	$253^{b,c}$	$264^{b,c}$	199^{c}	512^{a}	210^{c}	225^{c}	146	0·006*	0·873	0·001
Jejunum	68	58	53	97	48	128	46	62	47	0·029†	0·089	0·094
Aminopeptidase N (× 10^{3}; nmol substrate degraded/mg tissue protein per h)												
Duodenum	36	30	31	27	33	43	40	35	9	0·367	0·082	0·098
Ileum	55^{a}	$35^{b,c}$	$35^{b,c}$	33^{c}	$46^{b,c}$	$53^{a,b}$	54^{a}	$39^{b,c}$	13	0·075	0·022	0·039

C, casein control diet; S, T, I, diets with phaseolin S (Sanilac), phaseolin T (Tendergreen) and phaseolin I (Inca), respectively, providing 500 g/kg of the total dietary protein.
^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Protein type effect: $305^{a,b}$, 351^{a}, 234^{c} and $244^{b,c}$ for C, S, T and I, respectively.
† Protein type effect: 58^{b}, 93^{a}, 50^{b} and $79^{a,b}$ for C, S, T and I, respectively.
‡ Thermal treatment was at 121°C and 15 pounds per square inch for 15 min.

digestibility. This is in contrast with observations by Santoro *et al.* (1997) who noted that raw phaseolin, as compared with lactalbumin, increased the dry weight of the small intestine. In addition, an abnormally high excretion of endogenous compounds in the faeces was observed in these acute feeding experiments. Here, there was no major detrimental effect of raw phaseolin on the gut. By contrast, common bean lectin is known to have various effects on the gut. This includes

decreased villus height and increased crypt depth all along the small intestine, and increased intestinal sucrase and maltase activities in suckling piglets (Radberg *et al.* 2001), stimulation of the fresh weight of the proximal and distal small intestine and increased length of this segment in suckling rats (Linderoth *et al.* 2000). In weaned rats, Pusztai *et al.* (1990) noted a cell hyperplasia and increased dry weight of the small intestine. Here, the fresh weight of gastrointestinal tract segments and intestinal villus height remained essentially unaffected by raw phaseolin consumption. Crypt deepening was limited (and only tended to be significant) with increasing proportions of phaseolin to casein in the diet, while phaseolin type had no effect on this parameter.

The second interesting point of the present study is that no consistent differences between phaseolin types (experiment 2) were observed for gastrointestinal tract characteristics and protein digestibility when phaseolin was incorporated in the unheated form. Our findings do not confirm the observation on phaseolin digestibility made in pigs by Begbie & Ross (1993). Apart from animal species differences, they used whole *P. vulgaris* beans of the S and T types and their data suggested that phaseolin S was digested better than phaseolin T. Here, the phaseolin type used in raw form did not influence phaseolin digestibility. This is in agreement with an *in vitro* study carried out by Deshpande & Nielsen (1987) on seventeen common bean varieties, some of them differing in their subunit composition. Besides, our data showed that increasing the incorporation level of raw phaseolin (T) between 33 and 67 % of the dietary protein (if one excludes the 100 % phaseolin T treatment group with a lower food intake) did not cause increased gastrointestinal tract disturbances or alterations in our experimental conditions. Therefore, phaseolin type and level of incorporation (of phaseolin T) are unlikely to explain variations between published digestibility data (Liener & Thompson, 1980; Coelho & Sgarbieri, 1995; Santoro *et al.* 1997, 1999).

Thermal treatment increases substantially the digestibility of dietary protein and phaseolin, with one exception (Carbonaro *et al.* 2005), and reduces the variability in digestibility observed with raw phaseolin (Liener & Thompson, 1980; Levy-Benshimol & Garcia, 1986; Marquez & Lajolo, 1990). Our data for thermally treated phaseolin are in agreement with the published values. The three phaseolin types studied here responded equally well to this treatment (+58 %). It also led to a reduction in the length of the small intestine and to an increase in the mucosa weight:muscular weight ratio in the ileum. These changes could have been the consequence of increased phaseolin digestibility, and therefore, increased availability of nutrients for the distal small intestine, upon thermal treatment.

Stomach pH dropped with increasing levels of phaseolin T. There was also an increased pH and an accumulation of undigested material in the caecum in the rats fed the diet with the highest level of phaseolin T incorporation. Stomach pH decrease associated with phaseolin consumption might be related to higher gastric emptying rate and a quicker return to preprandial pH values with diets with lower levels of casein that might, therefore, not clot. However, in preruminant calves fed various milk replacers, duodenal pH was found to be higher with a 1:1 mixture of skimmed milk powder and soyabean protein, as compared with skimmed milk powder alone, 2 to 4 h after the meal (Lallès *et al.* 1999). Other possibilities would be that the food containing phaseolin was consumed according to a pattern different from that of the control diet or that phaseolin stimulated acid secretion or was fermented already in the stomach of rats.

In conclusion, raw phaseolin incorporated to diets chronically fed to rats had little effect on the gross anatomy of the gut and on the architecture and enzyme activities of the small intestinal mucosa and was poorly digested, regardless of the level of incorporation and type of phaseolin. Thermal treatment completely normalised gut variables and improved the digestibility of the three phaseolin types similarly. The common bean is an important feature of human nutrition in particular areas of the world (Leterme & Muñoz, 2002). However, it is difficult to speculate on the relevance to man of the present study in rats because species differences do exist at the intestinal level (Rittler *et al.* 2000).

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