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Commercial *Phaseolus vulgaris* Extract (Starch Stopper) Increases Ileal Endogenous Amino Acid and Crude Protein Losses in the Growing Rat

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The effect of a commercial *Phaseolus vulgaris* extract (PVE, starch stopper) on ileal and fecal endogenous protein losses was studied. Growing rats were fed for 14 days a protein-free diet containing PVE at a nutritional concentration of 0% (PF1), 0.4% (PF2), or 1.1% PVE (PF3) or 1.1% autoclaved PVE (PF4). An indigestible marker (TiO_2) was included in each diet. Ileal endogenous amino acid (AA) losses were significantly higher ($P < 0.05$) in PF3 (20% higher than in PF1), except for Pro, Gly, Ala, and His. Endogenous ileal N losses were 22% higher in PF3 than in PF1. Endogenous fecal AA and N losses were all significantly higher ($P < 0.05$) in PF3. Starch digestibility (∼100%), food intake (single daily meal, d10−23), and body weight loss were not significantly different among the groups. PVE, at 1.1% of the diet, not only was ineffective in reducing starch digestibility but also led to increased ileal endogenous N losses, possibly due to the antinutritional factors (trypsin inhibitor, lectin) present in the PVE.

KEYWORDS: Gut endogenous losses; amino acid; nitrogen; *Phaseolus vulgaris*; rat; antinutritional factors

INTRODUCTION

Beans contain several antinutritional factors (ANF), such as enzyme (α-amylase, trypsin, chymotrypsin) inhibitors, lectins, phytic acid, flatulence factors, saponins, and toxic factors (*1*). The α-amylase inhibitor (αAI), *phaseolamin,* has been isolated from kidney beans (*Phaseolus vulgaris*) and characterized by Marshall and Lauda (*2*). It specifically inhibits animal α-amylases, especially human saliva amylase and human and porcine pancreatic amylases, but has no activity toward plant, bacterial, or fungal enzymes (*2*).

The αAI activity of kidney bean extracts has been exploited to produce products (commonly referred to as "starch blocker" or "starch stopper" products) that claim to reduce starch digestion and absorption in humans and thus assist in body weight loss. As a crude extract of kidney beans, starch stopper products contain not only αAI but also other antinutritional factors, found in variable concentrations (*3*). The major ANF in beans that can cause adverse physiological responses are trypsin−chymotrypsin inhibitors and lectins (*4*). Lectins are sugar-binding proteins that bind to the epithelial cell lining of the intestinal mucosa, causing damage to the microvilli and intestinal malabsorption. Trypsin−chymotrypsin inhibitors, part of the Bowman−Birk type inhibitors, not only inhibit intestinal protein digestion but can also lead to hypertrophy and hyperplasia of the pancreas and hypersecretion of digestive enzymes in rodents (*5*). Lectins and trypsin inhibitors, moreover, may increase overall gut endogenous protein losses in simple-stomached animals (*6*, *7*). Costa de Oliveira et al. (*8*) reported that endogenous protein losses in rat feces were higher when rats were fed a diet containing 10% of raw *P. vulgaris* than when they were fed a casein-based diet. There is, however, little information on this aspect of the effects of *P. vulgaris* when ingested as a commercial extract, known as a "starch stopper". The present study thus aimed to determine the effect of a commercial *P. vulgaris* extract (PVE), at nutritional concentrations, on ileal and fecal endogenous losses of amino acids in the growing rat. Protein-free diets were used to determine endogenous protein and amino acid flows at the terminal ileum and in the feces of the growing rat. PVE was added to a protein-free diet at concentrations equivalent to what a human subject would consume, according to the manufacturer's recommendations. Any undigested protein originating from the PVE was assumed to be a negligible proportion of the protein losses at the terminal ileum and in the feces. Two controls were included: a standard protein-free diet and a protein-free diet to which autoclaved PVE was added.

MATERIALS AND METHODS

***P. vulgaris* Extract and Analysis of ANF.** The commercial *P. vulgaris* extract (PVE, starch stopper) was purchased from a pharmacy (Palmerston North, New Zealand) as capsules containing dry powdered (500 mg/capsule) white kidney bean extract. According to the informa-

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tion supplied by the company, 1 g of PVE was extracted with water from 12 g of dry beans. PVE was removed from the capsules prior to use and was added to the diets either "as is" or after inactivation of the ANF (autoclaved PVE, aPVE).

To inactivate the ANF, PVE was soaked for 15 h in deionized water, as advocated by Carvalho and Sgarbieri (*9*). The wet flour was autoclaved at 121 °C for 15 min, as described in previous studies (*10*), and freeze-dried. The major ANF known to be present in beans (αAI, lectin, and trypsin inhibitor) were determined in both PVE and aPVE. Amylase and αAI activities were determined by an adaptation of Bernfeld's method (*11*), whereby αAI was extracted from PVE in a 0.02 M sodium phosphate buffer (pH 6.9) containing NaCl (9 g L^{-1}). After 1 h of vigorous shaking and centrifugation (15 min, 14000*g*, 4 °C), the supernatant was collected (adapted from refs *3* and *12*), mixed with 1 μg of porcine α-amylase (1:1, v/v, Sigma Chemical Co., St. Louis, MO), and preincubated (1 h, 37 °C, pH 6.9). When 50% of porcine α-amylase was inhibited, the αAI units were determined and expressed as the number of amylase units inhibited per gram of starch stopper dry matter (DM) (*3*). One amylase unit was defined as the amount of enzyme that released 1 μmol of maltose per minute (*11*). Appropriate blanks were included.

Lectin concentration was determined according to the method of Burbano et al. (*13*). A competitive indirect ELISA for quantification of the *Phaseolus vulgaris* lectin (PHA) using anti-PHA IgG antibody (Sigma Chemical Co.) was performed.

Quantitative trypsin inhibitor measurements were performed and trypsin inhibitor units (TIU) defined as previously described (*14*).

Total dietary fiber was determined on PVE and aPVE using the kit Megazyme Total Dietary Fibre (Megazyme International Ireland Ltd., Wicklow, Ireland), which follows the method of Prosky (*15*).

The protein profiles of PVE and aPVE were assessed by electrophoresis. Soluble protein was extracted by stirring the dried sample in borate buffer (100 mmol/L H_3BO_3, 150 mmol/L NaCl, pH 8.0) for 1.5 h and centrifuging at 12000*g* for 10 min at room temperature. The supernatants were collected and stored at −20 °C until electrophoresis analysis. Soluble protein was measured using the bicinchoninic acid protein (BCA) assay (Pierce Chemical Co., Rockford, IL), based on a colorimetric reaction. Electrophoresis was carried out using a mini-gel apparatus (Mini-Protean system, Bio-Rad, Richmond, CA) in a 125 g/L acrylamide separating gel and a 45 g/L acrylamide stacking gel, according to the method of Laemmli (*16*). The samples were dissolved in 1 mol/L Tris-HCl buffer (pH 6.8) containing 2.7 mol/L glycerol, 0.139 mol/L sodium dodecyl sulfate (SDS), and 0.4 mmol/L mercaptoethanol and heated to 100 °C for 3 min, for reduction of disulfide bonds. The samples were loaded onto the gel, with equivalent amounts of soluble proteins (200 μg) deposited in each well. Molecular mass standards obtained from Bio-Rad Laboratories (Hercules, CA) were also loaded in a separate well. Electrophoresis was performed in 62.5 mmol/L Tris-HCl buffer with 3.4 mmol/L SDS for 1.15 h. The electric field conditions were 170 V and 40 mA and were set in a Bio-Rad power supply unit (model 1000/500, Bio-Rad, Richmond, CA). The protein bands were fixed and stained using a solution of Coomassie Brilliant blue R-250.

Animals and Housing. Sixty-four Sprague−Dawley male rats (170−175 g body weight, 44 days of age) were obtained from the Small Animal Production Unit, Massey University (Palmerston North, New Zealand). Ethics approval was received from the Massey University Animal Ethics Committee (protocol 03/135). The animals were housed individually in raised stainless steel cages with wire mesh floors above individual trays collecting feces and urine. The room was maintained at 20 ± 2 °C with a 12 h light/dark cycle. Food was given during the light cycle. Water was continuously available.

Diets. Five diets were prepared including a preliminary diet, given to all of the rats, and four experimental diets, each given to 16 rats. The experimental diets, based on an essentially protein-free diet, consisted of a control (no PVE, diet PF1), two diets to which PVE was added at physiologically meaningful concentrations (0.4% PVE, diet PF2; 1.1% PVE, diet PF3), and a second control that contained 1.1% of aPVE (diet PF4). The experimental diets, PF1, PF2, and PF3, contained 0.06, 0.07, and 0.09 g of N/100 g of diet, which correspond to 0.36, 0.45, and 0.59 g of crude protein/100 g of diet, respectively (conversion factor = 6.25). PF4 contained the same concentration of crude protein as PF3.

Table 1. Ingredient Compositions of the Preliminary and Experimental Diets

	diet[a] (g/kg of air-dry wt)				
ingredient	preliminary	PF1	PF2	PF3	PF4
corn flour[b]	690	747	743	736	736
cellulose[c]	10	50	50	50	50
vitamin mix[d]	50	50	50	50	50
salt mix[e]	50	50	50	50	50
soya oil	80	100	100	100	100
lactic casein[f]	120				
titanium dioxide		3	3	3	3
PVE[g]			4	11	
aPVE[i]					11

[a] The preliminary diet was fed to the rats during the first 10 days. The other diets were based on an essentially protein-free diet with no commercial *P. vulgaris* extract (PVE) (PF1) or 0.4% PVE (PF2), 1.1% PVE (PF3), and 1.1% inactivated PVE (PF4). [b] Goodman Fielder Industries Limited, Summerhill, NSW, Australia. [c] Avicel PH101. Commercial Minerals Limited, Auckland, New Zealand. [d] Crop & Food Research, Palmerston North, New Zealand. The mixture supplied (mg kg^{-1} of diet) retinol acetate, 5.0; DL-α-tocopheryl acetate, 200; menadione, 3.0; thiamin hydrochloride, 5.0; riboflavin, 7.0; pyridoxine hydrochloride, 8.0; D-pantothenic acid, 20; folic acid, 2.0; nicotinic acid, 20; D-biotin. 1.0; *myo*-inositol, 200; and choline chloride, 1500; (μg kg^{-1} of diet) ergocalciferol, 25; cyanocobalamin, 50. [e] Crop & Food Research, Palmerston North, New Zealand. The mixture supplied (g kg^{-1} of diet) Ca, 6.29; Cl, 7.79; Mg, 1.06; P, 4.86; K, 5.24; Na, 1.97; (mg kg^{-1} of diet) Cr, 1.97; Cu, 10.7; Fe, 424; Mn, 78.0; Zn, 48.2; (μg kg^{-1} of diet) Co, 29.0; I, 151; Mo, 152; Se, 151. [d,e]The mixtures were formulated to meet the vitamin and mineral requirements of the growing rat as described by the Agricultural Research Council (*19*). [f] Lactic casein 30 mesh. Alacid, New Zealand Milk Products, Wellington, New Zealand. [g] *P. vulgaris* extract as dry powder taken from capsules of proprietary starch stopper product (50 caps/packet). Each capsule contained 500 mg of a water extract of *P. vulgaris*, equivalent to 6 g of raw *P. vulgaris*. [i] Autoclaved PVE. PVE was soaked, autoclaved, and freeze-dried.

Table 2. Determined Amino Acid and Total Nitrogen Contents of the Essentially Protein-free Diet (PF1) and of the Crude *P. vulgaris* Extract and Autoclaved *P. vulgaris* Extract (aPVE)

	diet (%)				diet (%)		
	PF1	PVE	aPVE		PF1	PVE	aPVE
Asp	0.008	1.9	1.9	Ile	0.003	0.5	0.6
Thr	0.003	0.6	0.6	Leu	0.006	1.0	1.1
Ser	0.005	0.7	0.7	Tyr	0.001	0.6	0.5
Glu	0.012	2.7	2.3	Phe	0.001	0.5	0.8
Pro	0.000	0.8	0.6	His	0.010	0.5	0.4
Gly	0.005	0.6	0.6	Lys	0.003	0.9	0.9
Ala	0.004	0.6	0.6	Arg	0.000	0.9	0.8
Val	0.007	0.6	0.7	N	0.058	3.1	2.7

PVE was included in the diet of the growing rat so as to provide an amount of PVE, on a food intake basis, equivalent to that ingested by humans taking the commercial starch stopper product. On the basis of the manufacturer's recommendations, a human would consume some 3 g of PVE/day. Assuming a daily food intake for the adult human of 436 g of DM (*17*) and a daily food intake for the growing rat of 11 g of DM (*18*) (same breed, similar body weight and diet), the equivalent dose of PVE for the rat was calculated to be 75 mg/day. Lower and higher test doses (45 and 120 mg/day/rat) were chosen, equivalent to 0.4 and 1.1% PVE added to the air-dry diet. Titanium dioxide was added to each diet as an indigestible marker. Dietary ingredient compositions are given in **Table 1** and determined amino acid (AA) and nitrogen (N) compositions of PF1, PVE, and aPVE in **Table 2**.

Experimental Design. The rats were acclimatized to the cages and to the feeding regimen over a 10-day period (days 0−9) and for the entire study received a single meal daily (9:00 a.m. to 12:00 p.m.). During the acclimatization period the rats were fed the preliminary

diet. The rats were then randomly and equally allocated to the four experimental diets for a 14-day experimental period (days 10−23).

The rats were weighed on days 9 and 22. Food intake was recorded daily. Feces were collected on absorbant paper. Collection was made three times a day on days 20, 21, and 22. Feces were cleaned of any spilled food and were immediately frozen at −20 °C.

On day 23, 3 h ± 15 min after the start of feeding, the rats were asphyxiated with carbon dioxide gas and decapitated (immediately ceasing all neural stimulation to the gut). The abdomen was opened by an incision along the midventral line, and the skin and musculature were folded back to expose the viscera. The final 20 cm of the ileum was dissected from the body, rinsed with deionized water to remove any traces of blood and hair, and gently dried with absorbent paper. Care was taken not to apply pressure to the intestine. The digesta were slowly flushed out with 10 mL of deionized water from a plastic syringe, and the pH was adjusted to 3.0 by the addition of 6 M HCl to prevent bacterial activity. The samples were immediately frozen at −20 °C.

Chemical Analysis. Feces, digesta and diet samples were freeze-dried and ground. Feces were pooled for each rat over the 3 days of collection and then pooled from two randomly selected rats within the same diet. Ileal digesta were pooled using the same pair of rats as for the feces. Furthermore, composite digesta samples were obtained by pooling equal quantities of ileal digesta across rats receiving the same diet. Samples were stored in a desiccator.

The starch content was measured in the feces, in the composite digesta samples, and in the diets, using the kit Megazyme Total Starch (Megazyme International Ireland Ltd.).

Total N was determined in duplicate in the feces, in the composite digesta samples, and in the diets following the Dumas method. The samples were combusted at 1050 °C in O_2 gas. The N was then reduced to N_2 by a catalyst, and this was measured using a Leco FP-2000 thermal conductivity cell (Leco Corp., St. Joseph, MI).

The AA composition was determined on individual ileal digesta and feces samples as follows: duplicate dried samples (5 mg) were hydrolyzed in 1 mL of 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110 ± 2 °C in glass tubes sealed under vacuum. AA concentrations were then measured using a Waters ion exchange high-performance liquid chromatography (HPLC) system calibrated against a reference AA mixture with known concentrations. The peaks of the chromatograms were integrated using the dedicated software Millenium (Waters Millipore), which identifies the AA by retention time against a reference AA mixture. Cysteine, methionine, and tryptophan, being destroyed during acid hydrolysis, were not determined.

Titanium dioxide was determined by a colometric assay following the method of Short et al. (*20*). The sample was ashed and dissolved in sulfuric acid. Hydrogen peroxide was subsequently added and absorbance measured at 410 nm using an automatic spectrophotometer (COBAS FARA 2, Roche Diagnostic, Basel, Switzerland).

Data Analysis. Ileal and fecal starch flows were determined using the following equation:

$$\text{starch flow [mg/100 mg of dry matter intake (DMI)]} = [\text{starch in digesta or feces (mg/100 mg of digesta or feces)} \times \text{TiO}_2\text{ in diet (mg/100 mg of diet)}] / \text{TiO}_2\text{ in digesta or feces (mg/100 mg of digesta or feces)} \quad (1)$$

Ileal and fecal starch digestibility was determined using the following equation:

$$\text{ileal or fecal starch digestibility (\%)} = 100 \times [\text{dietary starch intake (mg/100 mg of DMI)} - \text{ileal or fecal starch flow (mg/100 mg of DMI)}] / \text{dietary starch intake (mg/100 mg DMI)} \quad (2)$$

Endogenous N or AA flows were determined using the following equation:

$$\text{endogenous flow (mg/100 mg DMI)} = [\text{N or AA in digesta or feces (mg/100 mg of digesta or feces)} \times \text{TiO}_2\text{ in diet (mg/100 mg of diet)}] / \text{TiO}_2\text{ in digesta or feces (mg/100 mg of digesta or feces)} \quad (3)$$

The data were tested for homogeneity of variance using Bartlett's test using Minitab (release 14, Minitab Inc., State College, PA). The results were subjected to a one-way ANOVA using the computer program SAS (version 8.2, 1999; Statistical Analyses Systems Institute Inc., Cary, NC). The food intake data were subjected to a one-way ANOVA for repeated measures. For $P < 0.05$, the significance of difference between means was determined using Tukey's test.

RESULTS

The ANF activities in PVE and aPVE are given in **Table 3**. The lectin was completely destroyed in aPVE, whereas αAI and trypsin inhibitor activities were reduced by 96 and 62%, respectively. Determined total dietary fiber contents were 34.9 and 36 g/100 g of DM in PVE and aPVE, respectively. The protein profile of PVE (**Figure 1**) showed strong signals between 43 and 50 kDa and close to 30 kDa and lower signals at 20 and 25 kDa. These signals were lower in aPVE. A band at 10 kDa was visible for both PVE and aPVE.

The rats consumed the diets readily and remained healthy, although they lost body weight over the study. Body weight losses over the 14 day experimental period were (mean ± SE) 14.8 ± 2.0, 13.7 ± 1.3, 12.5 ± 1.5, and 12.4 ± 2.0 g for PF1, PF2, PF3, and PF4, respectively, and were not significantly different ($P > 0.05$) between diets. Daily food intakes (days 10−23, mean ± SE) were 7.0 ± 0.1, 7.2 ± 0.1, 7.1 ± 0.1, and 7.1 ± 0.2 g for diets PF1, PF2, PF3, and PF4, respectively, and were not significantly different ($P > 0.05$) between diets.

Starch Digestibility. Ileal and fecal measures of starch digestibility were close to 100% (99.2−100%) for all of the diets.

Nitrogen and Amino Acid Flows in the Ileal Digesta. The endogenous AA flows at the terminal ileum are given in **Table 4**.

There was a significant effect ($P < 0.05$) of diet on the endogenous ileal AA flows for most of the amino acids except for alanine, proline, glycine, and histidine. When there was a statistically significant effect of the diet, the endogenous ileal AA flows for rats fed diet PF3 were significantly ($P < 0.05$) higher than those for rats fed the controls PF1 and PF4 and for rats fed diet PF2 except for the amino acids threonine and tyrosine, for which endogenous flows from rats fed diets PF2 and PF3 were not significantly different ($P > 0.05$). Endogenous ileal AA flows were not different ($P > 0.05$) among rats fed the controls PF1 and PF4 and rats fed diet PF2 except for the amino acids threonine and tyrosine, for which endogenous flows from rats fed diet PF2 were higher than rats fed the controls PF1 or PF4.

Endogenous ileal total N flows, determined on the composite ileal digesta samples, were 0.073, 0.070, 0.089, and 0.069 mg/100 mg of dry matter intake (DMI) for rats fed diets PF1, PF2, PF3, and PF4, respectively. As observed for the endogenous ileal AA flows, the endogenous ileal N flow for rats fed diet PF3 was numerically higher than those for rats fed diet PF1, PF2, or PF4.

Nitrogen and Amino Acid Flows in the Feces. The endogenous AA and N flows in the feces are given in **Table 5**. As observed for the ileal measures, the endogenous fecal AA flows for rats fed diet PF3 were significantly ($P < 0.05$) higher than those for rats fed diet PF2 or the controls PF1 and PF4.

Table 3. Amylase Inhibitor Activity, Trypsin Inhibitor Activity, and Lectin (PHA) Content in the Commercial *P. vulgaris* Extract before and after Autoclaving

	undenatured PVE	autoclaved PVE[a]	overall SE
amylase inhibitor activity (AIU/g of DM)[b]	89.9	3.6	3.8
lectin (mg of PHA/g of DM)[c]	14.0	nd[d]	0.6
trypsin inhibitor activity (TIU $\times 10^{-3}$/g of DM)[e]	56.9	21.3	0.7

[a] aPVE. [b] AIU, amylase inhibitor unit, $n = 4$. [c] PHA, *P. vulgaris* lectin, $n = 4$. [d] nd, not detectable. [e] TIU, trypsin inhibitor unit, $n = 6$.

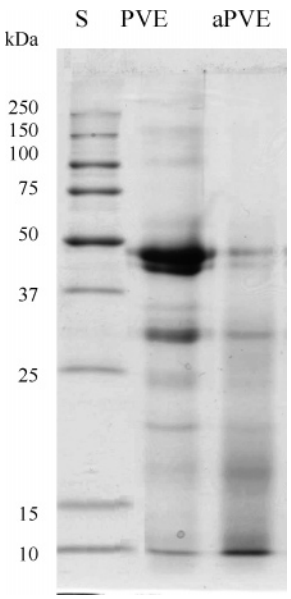

Figure 1. Protein profile of extracts from a crude commercial *P. vulgaris* extract (PVE) and an autoclaved PVE (aPVE). Aliquots from each extract containing 200 μg of soluble proteins were subjected to gel electrophoresis in the presence of sodium dodecyl phosphate followed by coloration with Coomassie Brilliant blue R-250 (*13*). Electrophoresis was run at 40 mA for 1.15 h. A molecular mass standard (S) was included (10–250 kDa).

Fecal endogenous AA flows were not significantly ($P > 0.05$) different among rats fed the controls PF1 and PF4 and rats fed diet PF2. The endogenous fecal N flow for rats fed diet PF3 was significantly ($P < 0.001$) higher than for rats fed diet PF1, PF2, or PF4. The endogenous fecal N flow for rats fed diet PF2 was significantly ($P < 0.001$) higher than for rats fed the controls PF1 and PF4. The endogenous fecal N losses for rats fed diet PF1 or PF4 were not significantly ($P > 0.05$) different.

DISCUSSION

The primary aim of the present study was to assess the effects of a commercial *P. vulgaris* extract given at recommended concentrations on ileal endogenous protein losses in the growing rat. The current work showed that PVE, when included in the diet at 1.1%, was not only ineffective in reducing starch digestibility but also led to increased ileal and fecal endogenous protein losses. The ineffectiveness of a commercial PVE was reported earlier (*21*) and shown to be due to insufficient anti-amylase activity (*22*).

The present study did not show any specific effect of PVE either on body weight loss or on voluntary food intake, as previously observed with rats fed a commercial soybean starch stopper product (*23*). This finding is consistent with the absence

Table 4. Mean Endogenous Ileal Amino Acid Flows ($n = 8$) at the Terminal Ileum of Growing Rats Fed Diet PF1, PF2, PF3, or PF4[a]

	diet[b] (mg/100 mg of dry matter intake)					
	PF1	PF2	PF3	PF4	overall SE	significance[c]
Asp	0.049a	0.049a	0.060b	0.049a	0.002	**
Thr	0.034a	0.035ac	0.040bc	0.034a	0.002	*
Ser	0.022a	0.023a	0.029b	0.022a	0.001	***
Glu	0.049a	0.048a	0.060b	0.046a	0.002	**
Pro	0.031	0.029	0.033	0.029	0.002	NS
Gly	0.065	0.059	0.068	0.055	0.005	NS
Ala	0.018	0.019	0.022	0.018	0.001	NS
Val	0.017a	0.018a	0.022b	0.017a	0.001	**
Ile	0.011a	0.012a	0.015b	0.011a	0.001	**
Leu	0.018a	0.020a	0.025b	0.018a	0.001	**
Tyr	0.010a	0.011ac	0.012bc	0.009a	0.001	**
Phe	0.010a	0.011a	0.014b	0.010a	0.001	***
His	0.021	0.021	0.025	0.021	0.001	NS
Lys	0.015a	0.016a	0.019b	0.015a	0.001	*
Arg	0.012[a]	0.011a	0.015b	0.011a	0.001	**

[a] PF1, PF2, and PF3 contained 0, 4, and 11 g/kg of commercial PVE. PF4 contained 11 g/kg of autoclaved PVE. [b] Means within a row with a different letter are significantly ($P < 0.05$) different. [c] ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

Table 5. Mean Endogenous Amino Acid and Total Nitrogen Flows ($n = 8$) in the Feces of Growing Rats Fed Diet PF1, PF2, PF3, or PF4[a]

	diet[b] (mg/100 mg of dry matter intake)					
	PF1	PF2	PF3	PF4	overall SE	significance[c]
Asp	0.063a	0.066a	0.080b	0.067a	0.002	***
Thr	0.027a	0.028a	0.034b	0.028a	0.001	***
Ser	0.026a	0.027a	0.033b	0.027a	0.001	***
Glu	0.074a	0.079a	0.097[b]	0.076a	0.003	***
Pro	0.024a	0.025bd	0.031c	0.025ad	0.001	**
Gly	0.032a	0.033a	0.040b	0.033a	0.001	***
Ala	0.038a	0.040a	0.049b	0.041a	0.002	*
Val	0.029a	0.031a	0.038b	0.030a	0.001	***
Ile	0.023a	0.024a	0.029b	0.024a	0.001	***
Leu	0.037a	0.039a	0.048b	0.038a	0.001	***
Tyr	0.019a	0.021ac	0.025b	0.020ac	0.001	***
Phe	0.021a	0.023a	0.028b	0.021a	0.001	***
His	0.015a	0.015a	0.019b	0.014a	0.001	***
Lys	0.031a	0.034a	0.041b	0.033a	0.001	***
Arg	0.024a	0.024a	0.030b	0.024a	0.001	***
N	0.089a	0.105b	0.116c	0.095a	0.003	***

[a] PF1, PF2, and PF3 contained 0, 4, and 11 g/kg of commercial PVE. PF4 contained 11 g/kg of autoclaved PVE. [b] Means within a row with a different letter are significantly ($P < 0.05$) different. [c] ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

of a PVE effect on starch digestibility, presumably due to its amylase inhibitor activity (89.8 units/g), which is in the lower range of that determined on other commercial *P. vulgaris* extracts (44.3−327.6 units/g) (*3*). Moreover, αAI was found to be unstable in the stomach and active only after preincubation with amylase in the absence of starch (*5*), which cannot occur when PVE is ingested within a starchy meal.

Our results showed that endogenous protein losses increased in animals fed the PVE-supplemented diets, from determinations both at the terminal ileum and in the feces. Although ileal endogenous losses are considered to be more representative of the gut endogenous losses, as the metabolic interference of the colonic bacteria is reduced, endogenous fecal losses, thus, are expected to follow a similar trend, giving supplementary information on the effect of dietary factors on endogenous protein losses. Endogenous protein losses, determined after

administration of a protein-free diet, are known to be somewhat underestimated (*24*). This method, however, is still useful and widely used for evaluating relative effects of dietary factors, such as in the present study. The PVE used in this study was included in the protein-free diets at low but nutritionnally relevant concentrations (0.4 and 1.1% in PF2 and PF3, respectively). The actual consumption of PVE (30 and 75 mg/day/rat for diets PF2 and PF3, respectively) was equivalent to −36 and +53% of the average recommended consumption for a human subject (1.9 and 4.6 g/day, respectively). The actual consumption of PVE was less than first planned, due to a lower daily food intake than that reported by Butts et al. (*18*), but did not affect the objectives of this study. Diets PF2 and PF3 were not strictly protein-free but contained 0.45 and 0.59% crude protein, respectively. It can be shown from first principles (Deglaire, unpublished data) that such amounts of dietary protein would make only a negligible contribution to the ileal AA flows. When rats were fed diet PF4, which contained the same protein concentration as diet PF3 but from inactivated (autoclaved) PVE, ileal and fecal endogenous amino acid losses were not significantly different from those determined in rats fed the protein-free diet (PF1). This confirms that protein from PVE in diets PF2 and PF3 was a negligible proportion of the ileal and fecal protein losses and that AA and N determined in the digesta or in the feces could reasonably be considered as endogenous.

When rats were fed the diet containing 1.1% PVE (PF3), they exhibited higher ileal and fecal endogenous AA and total N losses than rats fed the protein-free diet (PF1) or the diet containing 1.1% aPVE (PF4). This accords with the observations of Costa de Oliveira et al. (*8*), who reported higher endogenous fecal protein losses in rats fed a diet containing 10% *P. vulgaris.* The diet used by Costa de Oliveira is similar to the present diet at 1.1% PVE (PF3), as 1 g of PVE is a water extract of 12 g of raw *P. vulgaris*.

As shown on the SDS-PAGE profile, the present PVE was not a purified extract and contained a large number of different proteins, the qualitative distribution of which was similar to the one found in raw *P. vulgaris* (*3*). This has been previously observed in at least four other commercial PVEs (*3*). The signal observed at 30 kDa is characteristic for lectins, and the one at 10 kDa is typical for the tryspin inhibitor (8 kDa) (*22*). The strong signals observed between 43 and 48 kDa appear to belong to phaseolin, the main storage protein reported to have subunits between 43 and 53 kDa (*25*) and which represents nearly 80% of the total bean proteins (*26*). Some of the signals observed at 33 and 45 kDa might be subunits, reported to be at 12.4, 15.2, 33.6, and 45 kDa (*27*). The profile of aPVE confirms that proteins, more specifically ANF, were denatured by thermal treatment, as observed with the measure of activities and concentrations. Trypsin inhibitor activity was not completely inactivated by the autoclaving step, which is in agreement with previous observations on autoclaved *P. vulgaris* (*9*). The trypsin inhibitor of most legume seeds is known to be difficult to completely inactivate by heat treatment, but is usually reduced to nonharmful concentrations (*28*) with autoclaving. Phaseolin, the concentration of which was not determined here, is known to have an improved digestibility and thus a denatured structure after thermal treatment (*29*), which is further indication of the association of the signals between 43 and 48 kDa with phaseolin subunits.

The observed effect of PVE on the ileal and fecal endogenous nitrogen and amino acid losses is most likely due to the ANF, especially trypsin inhibitor and lectins, known to be the major antinutritional factor present in *P. vulgaris* (*3*, *4*). Lectins have been shown to bind to the microvilli of rat mucosa cells, leading to a degradation of brush border cells and to a faster renewal of cells. One result of this is cellular hyperplasia and increased gut endogenous losses as reviewed by Vasconcelos and Oliveira (*30*). Trypsin inhibitors from legume seeds were shown to stimulate pancreatic secretions (*5*), resulting in an increase of gut endogenous protein losses. More generally, ANF may adversely affect protein digestibility and AA availability (*31*) and are reported to enhance endogenous ileal protein losses in pigs (*6*, *7*). Phaseolin's effect on the gut is controversial: Santoro et al. (*32*) reported an increase in the small intestinal dry weight and of fecal losses of endogenous N in rats, whereas Montoya et al. (*33*) did not find any detrimental effect.

The total dietary fiber content was high in both PVE and aPVE, and dietary fiber may influence gut endogenous losses. Garcia (*34*) reported a value close to 20% in other varieties of raw *P. vulgaris*. The fiber content was not different between PVE and aPVE, however, which means that the level of dietary fiber was not responsible for the higher N and AA losses.

When rats were fed diet PF2, containing 0.4% PVE, most endogenous fecal and ileal AA flows were not different ($P > 0.05$) from those of rats fed the control diets (PF1 and PF4), except for the ileal AA threonine and tyrosine. This suggests the presence of a threshold concentration of PVE, and probably of ANF, before major effects on endogenous protein losses are found. PVEs from other manufacturers can be found with higher ANF concentrations, as reported by Liener (*3*), and are thus likely to enhance endogenous losses over and above that found with the present PVE.

In conclusion, the PVE (crude extract of *P. vulgaris*), when ingested at 1.1% of the diet, increased ileal and fecal protein losses in the growing rat, but did not affect carbohydrate digestion. The consumption of the present commercial *P. vulgaris* extract is not without physiological effect, at least in the growing rat. This is likely to be due to the ANF, especially trypsin inhibitor and lectin. The replacement of gut proteins arising due to enhanced losses of gut endogenous proteins is energy demanding, and thus the PVE may have some effect on overall animal energetics. The potentially harmful effects of the *P. vulgaris* extract, when consumed at a nutritional level, on gut tissue integrity need to be investigated further.

ABBREVIATIONS USED

αAI, α-amylase inhibitor; AA, amino acid(s); ANF, antinutritional factors; DMI, dry matter intake; PVE, *Phaseolus vulgaris* extract; aPVE, autoclaved PVE; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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