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# Nutritional Evaluation of Biologically Treated White Kidney Beans (*Phaseolus vulgaris* L.) in Pigs: Ileal and Amino Acid Digestibility<sup>1</sup>

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**ABSTRACT:** We studied the effect of feeding young growing pigs a semisynthetic diet containing 7.5% white kidney beans—germinated (GB), pancreatin treated (PTB), or untreated (raw beans RB)—on protein and amino acid (AA) digestibilities at the terminal ileum. Eleven castrated male pigs (12.2 kg live weight) fitted with a post-valve T-cecal cannula and two blood catheters were used. The <sup>15</sup>N-isotope dilution method was used to determine the amount of endogenous protein passing the terminal ileum and the true ileal protein digestibility. Ileal crude protein losses in pigs fed the RB, GB, and PTB diets were 51.9, 27.4, and 51.1 g/kg of DMI, respectively. The

total amounts of AA passing the terminal ileum of the pigs fed the RB, GB, and PTB diets were 48.6, 21.4, and 42.2 g/kg DMI, respectively. The apparent ileal crude protein and AA digestibilities of the RB, GB, and PTB diets were 74, 87, and 75% and 76, 89, and 78%, respectively. True ileal protein digestibilities were 88, 93, and 93% for the RB, GB, and PTB diets, respectively. On the basis of this research, germination of white kidney beans improves the digestion of protein by decreasing the content of bean antinutritional factors and increasing the bean true ileal protein digestibility.

Key Words: Amino Acids, Ileum, Isotope Dilution, Nitrogen, Protein Digestion

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## Introduction

Use of white kidney beans (*Phaseolus vulgaris* L.) in nonruminants diets is limited because of factors that affect protein digestibility and animal growth. According to van der Poel (1990b), reduced ileal protein digestibility with feeding raw *Phaseolus vulgaris* L. depends on two main factors: 1) the inherent protein resistance to proteolysis and 2) interferences by antinutritive factors (ANF).

Beside removing ANF genetically, heat processing is an effective method for decreasing lectin and protease inhibitor activity (van der Poel, 1990a). Recent research on the effect of germination on ANF activity and the content of phaseolin, the main storage protein in *Phaseolus vulgaris* L., showed decreases in these components (Savelkoul et al., 1994). Effects of germination of legume seeds, including *Phaseolus*

*vulgaris* L. (Savelkoul et al., 1994), on in vitro protein digestibility have been found (Mostafa et al., 1987, Rahma et al., 1987).

An objective of this research was to study effects of germination on apparent ileal digestibility of nitrogen and amino acids and on endogenous losses in vivo in young pigs. In addition, we investigated the effect of pancreatin treatment of the bean on the ANF activity and in vivo digestibility of nitrogen and amino acids in white kidney beans.

## Materials and Methods

**Animals and Diets.** Experiments were approved by the Ethics Committee of the TNO Nutrition and Food Research Institute in Zeist, The Netherlands. Eleven Dutch Landrace × Dutch Yorkshire castrated male pigs, 10 wk old with an initial mean (±SE) live weight of 12.2 (±.4) kg, were used. The pigs were fitted with a post-valve T-cecal (PVTC) cannula, described by Van Leeuwen et al. (1991), that allow a quantitative collection of ileal digesta. Two silicone catheters were implanted, one into the external jugular vein (for taking blood samples) and the other one into the arteria carotis (for the infusion of [<sup>15</sup>N]leucine solu-

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Table 1. Ingredient composition (g/kg air-dry weight) of the basal diet

Ingredient	Composition
Casein	180
Cornstarch	470
Dextrose	150
Wheat bran	100
Soya oil	25
Premix <sup>a</sup>	10
Minerals <sup>b</sup>	60
DL-Methionine	1
L-Cystine	2
L-Threonine	1
Chromic oxide	1

<sup>a</sup>The vitamin/mineral mix provided the following (per kg of feed): 9,000 IU of vitamin A; 1,800 IU of vitamin D<sub>3</sub>; 40 mg of vitamin E; 1.36 mg of menadione as dimethyl-pyrimidinol bisulfite; 5 mg of riboflavin; 40 µg of cobalamine; 30 mg of niacin; 15 mg of d-pantothenic acid; 120 mg of choline chloride; 50 mg of ascorbic acid; 2 mg of thiamin; 3 mg of pyridoxine; .1 mg of d-biotin; 1 mg of folic acid; .38 mg of K (KI); .525 mg of Co (CoSO<sub>4</sub>); .06 mg of Se (Na<sub>2</sub>SeO<sub>3</sub>); 80 mg of Fe (FeSO<sub>4</sub>); 254 mg of Cu (CuSO<sub>4</sub>); 44 mg of Mn (MnO<sub>2</sub>); 72.8 mg of Zn (ZnSO<sub>4</sub>); 40 mg of tylosin.

<sup>b</sup>Contributed the following (per kg of feed): CaCO<sub>3</sub>, 14.5 g; monocalcium phosphate, 20.5 g; NaCl, 5 g; KHCO<sub>3</sub>, 16.5 g; NaHCO<sub>3</sub>, 2 g; MgO, 1.5 g.

tion) to measure endogenous <sup>15</sup>N losses with the <sup>15</sup>N-isotope dilution technique. The pigs were individually housed in smooth-walled metabolism cages in a temperature-controlled room (23 to 26°C, 50 to 70% relative humidity) described in Schulze et al. (1994).

Throughout the 9-d experimental period, the pigs were fed 92.5% of a basal semisynthetic diet (Table 1) with casein as the sole protein source, supplemented with 7.5% biologically treated or raw *Phaseolus vulgaris* L. beans. Biological treatment of the beans consisted of 1) 7-d germination or 2) 12-h pancreatin incubation (6% pancreatin; Merck, KGaA, D-64271 Darmstadt, Germany). White kidney beans were provided by Fr. Bakker Brothers (Noord-Scharwoude, the Netherlands). The raw white kidney beans were ground (1-mm mesh screen) before they were included in the experimental diet. Seeds were germinated up to 7 d in wet sand at 20°C under standard conditions (Van der Burg et al., 1983). After germination, bean seeds were harvested, washed, frozen in liquid N, lyophilized, and ground through a 1-mm mesh in a Retsch mill (Savelkoul et al., 1992b). After the raw seeds were ground through a 1-mm mesh screen in a Retsch mill, the bean flour was incubated for 12 h with 6% porcine pancreatin in an aqueous solution at room temperature. After the enzymatic treatment, the bean pulp was lyophilized and ground again in a Retsch mill (1-mm mesh screen).

The level of feed intake given to the pigs during the experiment provided about two times their maintenance requirement for energy. Pigs received about 500 g of feed per day. Chromic oxide (1 g/kg) was

included in the diet as an indigestible marker. The diet was mixed with water (1:2, wt/vol) immediately before feeding, and fresh water was available for 30 min after each meal. The chemical composition of the experimental diets and the levels of various ANF of the treated beans and the basal diet are given in Table 2.

**Experimental Procedure.** After insertion of the catheters, one in the carotid artery and one in the external jugular vein, pigs were allowed to recover for 1 d. The [<sup>15</sup>N]leucine solution was continuously infused with a peristaltic pump (5.04 mg of [<sup>15</sup>N]leucine (95% <sup>15</sup>N enrichment)·kg live weight<sup>-1</sup>·d<sup>-1</sup>) during the 9-d experimental period. The [<sup>15</sup>N]leucine was dissolved in a sterile nonpyrogenic physiological saline solution (Schulze et al., 1994). Ileal digesta were collected continuously directly into a bag fixed to the PVTC cannula for 12 h on d 7, 8, and 9 of the experimental period and stored frozen at -20°C. The bags were removed every 30 min. Prior to chemical analyses, the digesta were freeze-dried, ground (1-mm mesh screen), and pooled per pig. Blood samples were taken three times each day (0900, 1500, and 2100) from the external jugular vein with a syringe containing lithium heparin. Immediately after sampling, the blood was centrifuged at 3,000 × g and cooled to 50°C. The precipitate was discarded, and the supernatant was pooled each day for each pig and stored at -20°C. Prior to chemical analysis, pooled plasma samples were treated according to the procedure described by Schulze et al. (1994).

Nitrogen and DM were determined in the diet and freeze-dried ileal digesta following AOAC (1984) procedures. Chromium was determined in the diet and ileal digesta with the method of Bosch et al. (1988). Amino acids (AA) were determined by HPLC amino acid analyses in the diet and in ileal digesta, with the exception of methionine and cystine. They were determined following the hydrolysis with 6 M HCl for 22 h at 100°C according to the method of Slump (1969). Cystine and methionine were determined following oxidation with performic acid prior to acid hydrolysis (Moore, 1963). The various biologically treated beans and the basal diet were analyzed for total lectin contents using an ELISA method (Hendriks et al., 1987) and the amount of functional lectin using a functional lectin immunoassay (FLIA) according to Hamer et al. (1989). Trypsin inhibitor content (TIA) of the treated beans and the basal diet was determined according to the method described by Van Oort et al. (1989). The <sup>15</sup>N-enrichments of total N in ileal digesta, diet, and trichloroacetic acid (TCA)-soluble plasma were measured using a dual inlet isotope ratio mass spectrometer (VG Isotech, Fison Instruments, Middlewich, U.K.); 50 ± 10 µg N of freeze-dried ileal digesta and TCA-soluble blood plasma and of the diet were placed into tin capsules (8 × 5 mm, Fr. Van Loenen Instruments). The tin capsules were combusted in a Total Nitrogen Analyser

(Carlo Erba, ANA 1400, Fr. Carlo Erba, Milano, Italy), which was attached to the mass spectrometer. Samples were determined in duplicate, resulting in a precision of  $\pm .001$   $^{15}\text{N}$  atom percent excess.

Ileal digestibility of DM, crude protein ( $\text{N} \times 6.25$ ), and AA was calculated using the content of chromium in the diet relative to chromium in ileal digesta. The contribution of endogenous protein ( $\text{N} \times 6.25$ ) to total ileal protein was calculated from the ratio of  $^{15}\text{N}$ -enrichment excess in ileal digesta total N to that in the TCA-soluble plasma according to the equation given by Schulze et al. (1994). The true ileal protein digestibility was calculated from the apparent ileal protein digestibilities and the recovery of endogenous protein in ileal digesta.

**Data Analysis.** Daily amounts of DM, N, and AA at the terminal ileum and of AA per pig relative to feed DMI were calculated according to the following equation:

$$\begin{aligned} \text{Amount (g/kg DMI)} &= \text{nutrient concentration} \\ &\text{in ileal digesta (g/kg DM)} \\ &\times (\text{Diet chromium, g/kg DMI} / \\ &\text{Ileal chromium, g/kg DM}). \end{aligned}$$

The apparent digestibility coefficients (DC) were determined using the following equation (units are g/kg DM):

$$\text{DC (\%)} = \frac{\text{Dietary nutrient} - \text{Ileal nutrient}}{\text{Dietary nutrient}} \times 100.$$

Nitrogen and AA digestibility coefficients of the treated beans included in the experimental diets were calculated by difference.

Based on the daily N and DM digestibility coefficients, the effects of collection day, pig within treatment, and treatment were analyzed by analysis of variance (SAS-GLM Procedure, SAS, 1990) according to the following model:

$$Y_{ij} = \mu + T_i + A_k \times T_i + D_j + D_j \times T_i + e_{ijk}$$

in which  $Y_{ij}$  = dependent variable,  $\mu$  = overall mean,  $T_i$  = treatment ( $i = 1,2,3,4$ ),  $D_j$  = day of collection ( $j = 1,2,3$ ),  $A_k(T_i)$  = pig $_k$  in treatment $_i$  ( $k = 1,2,3$ ), and  $e_{ijk}$  = residual error. The effect of treatment was tested against pig within treatment. The day effect was tested against the residual error. The treatment effect of each individual AA and total AA digestibility coefficients of the diets were analyzed according to the following model:

$$Y_i = \mu + T_i + e_i$$

in which  $Y_i$  = dependent variable,  $\mu$  = overall mean,  $T_i$  = treatment ( $i = 1,2,3,4$ ), and  $e_i$  = residual error. When significant effects were obtained, differences between the treatment means were compared with Tukey's test (SAS, 1990).

Table 2. Dry matter, protein (g/kg air dry), and amino acid content (g/kg dry matter) of experimental diets

Item	Basal diet supplemented with beans <sup>a</sup>			Basal diet
	Untreated	Germinated	Pancreatin	
Dry matter	904.2	906.6	910.0	905.9
Protein	182.5	187.5	188.8	180.6
Indispensable amino acids				
Arginine	8.2	8.1	7.5	7.3
Histidine	5.8	6.0	6.3	5.7
Isoleucine	10.7	10.8	11.0	10.6
Leucine	18.8	18.8	19.1	18.7
Lysine	15.4	15.0	15.2	15.3
Methionine	6.1	6.2	5.8	6.1
Cysteine	3.7	3.2	3.4	3.4
Phenylalanine	10.4	10.2	10.6	9.7
Tyrosine	10.0	9.9	9.9	9.7
Threonine	10.0	10.0	10.0	9.7
Valine	13.8	13.8	14.0	13.8
Dispensable amino acids				
Alanine	7.3	7.1	7.3	6.7
Aspartic acid	16.2	18.0	16.0	14.8
Glutamic acid	44.4	43.5	44.0	44.7
Glycine	5.1	4.9	5.3	4.5
Proline	20.0	19.5	19.9	20.3
Serine	12.6	12.2	12.3	11.9

<sup>a</sup>*Phaseolus vulgaris* var. Processor.

Table 3. Content of trypsin inhibitor activity and lectins in biologically treated beans and the basal diet

Item	Beans <sup>a</sup>			Basal diet
	Untreated	Germinated	Pancreatin	
Trypsin inhibitor activity <sup>b</sup>	8.49	1.52	4.26	.17
Lectins (ELISA) <sup>c</sup>	116	5.70	107	ND <sup>e</sup>
Lectins (FLIA) <sup>d</sup>	74	3.70	61	ND

<sup>a</sup>*Phaseolus vulgaris* var. Processor.

<sup>b</sup>Trypsin inhibitor activity, in milligrams trypsin inhibited per gram of sample.

<sup>c</sup>Total amount of lectins, in milligram per gram of sample.

<sup>d</sup>Amount of functional lectins, in milligram per grams of sample.

<sup>e</sup>ND = nondetectable.

## Results

Throughout the feeding period and digestibility trial, all the pigs remained healthy and readily consumed the experimental diets. Only with the PBT diet was there a tendency for lower feed consumption. On the final day of the experiment, the mean ( $\pm$ SE) live weight of the pigs was 13.6 (SD =  $\pm$ .8) kg, resulting in an increase of 1.4 kg during the 9-d experimental period. Adequate homogenous samples of ileal digesta were collected from all but one pig during the collection periods. With one pig on the RB diet, in the second and third collection period,

however, insufficient sample was collected. Therefore, further calculations of flow rates and digestibility coefficients were performed using the first digesta collection data for this pig.

The contents of lectins and TIA in raw *Phaseolus vulgaris* L. were higher than in the other treatments (Table 3). The lectins (total and functional) and TIA levels decreased after 7-d germination by 95, 95, and 82%, respectively. However, incubating the beans with porcine pancreatin enzymes reduced the total amount of lectins (ELISA) and the amount of functional lectins (FLIA) and the TIA in the beans by only 8, 17.5, and 50%, respectively. The ANF level in the

Table 4. Mean dry matter, protein, and amino acid contents (g/kg dry matter intake) at the terminal ileum in pigs fed experimental diets

Item	Basal diet supplemented with beans <sup>a</sup>			Basal diet	SEM
	Untreated	Germinated	Pancreatin		
Number of pigs	3	3	3	2	
Dry matter	203.9 <sup>b</sup>	149.2 <sup>b</sup>	209.9 <sup>b</sup>	132.1 <sup>cd</sup>	38.29
Protein	51.94 <sup>b</sup>	27.38 <sup>c</sup>	51.13 <sup>b</sup>	27.88 <sup>c</sup>	7.313
Total	48.56 <sup>b</sup>	21.36 <sup>c</sup>	42.17 <sup>bd</sup>	22.17 <sup>cd</sup>	8.510
Indispensable amino acids					
Arginine	2.00 <sup>b</sup>	.90 <sup>c</sup>	1.70 <sup>bd</sup>	1.01 <sup>cd</sup>	.283
Histidine	1.35 <sup>b</sup>	.66 <sup>c</sup>	1.37 <sup>b</sup>	.68 <sup>bc</sup>	.279
Isoleucine	2.38 <sup>b</sup>	.99 <sup>c</sup>	2.04 <sup>b</sup>	.98 <sup>c</sup>	.383
Leucine	3.34 <sup>b</sup>	1.44 <sup>c</sup>	3.06 <sup>b</sup>	1.48 <sup>c</sup>	.532
Lysine	2.88 <sup>b</sup>	1.07 <sup>c</sup>	2.04 <sup>bc</sup>	1.11 <sup>bc</sup>	.733
Methionine	.48 <sup>b</sup>	.25 <sup>c</sup>	.43 <sup>bd</sup>	.28 <sup>cd</sup>	.072
Cysteine	1.10 <sup>b</sup>	.55 <sup>c</sup>	.98 <sup>bc</sup>	.60 <sup>bc</sup>	.234
Phenylalanine	2.09 <sup>b</sup>	.85 <sup>c</sup>	1.87 <sup>b</sup>	.88 <sup>c</sup>	.302
Tyrosine	1.61 <sup>b</sup>	.68 <sup>c</sup>	1.44 <sup>b</sup>	.75 <sup>c</sup>	.247
Threonine	3.06 <sup>b</sup>	1.54 <sup>c</sup>	2.90 <sup>b</sup>	1.70 <sup>bc</sup>	.583
Valine	2.96 <sup>b</sup>	1.35 <sup>c</sup>	2.70 <sup>b</sup>	1.40 <sup>c</sup>	.477
Dispensable amino acids					
Alanine	2.47 <sup>b</sup>	1.26 <sup>c</sup>	2.28 <sup>bd</sup>	1.34 <sup>cd</sup>	.404
Aspartic acid	4.77 <sup>b</sup>	2.01 <sup>c</sup>	4.24 <sup>b</sup>	2.06 <sup>c</sup>	.797
Glutamic acid	8.27 <sup>b</sup>	3.44 <sup>c</sup>	6.56 <sup>bc</sup>	3.40 <sup>c</sup>	1.621
Glycine	2.84 <sup>b</sup>	1.47 <sup>c</sup>	2.58 <sup>bc</sup>	1.50 <sup>bc</sup>	.599
Proline	3.15 <sup>b</sup>	1.29 <sup>b</sup>	2.77 <sup>b</sup>	1.34 <sup>b</sup>	.895
Serine	3.81 <sup>b</sup>	1.62 <sup>b</sup>	3.24 <sup>bd</sup>	1.68 <sup>cd</sup>	.678

<sup>a</sup>*Phaseolus vulgaris* var. Processor.

<sup>b,c,d</sup>Means in the same row followed by different letter differ ( $P < .05$ ).

basal diet was low TIA (Table 3). As expected, the ANF contents of the treatment diets markedly reflected the ANF contents of the added treated beans. The N, crude fat, and ash contents of the diets varied only slightly with the bean additions. The inclusion of 7.5% of the treated beans increased the crude fiber contents of the basal diet from 10.1 to 15.2, 20.5, and 14.5% in the RB, GB, and PBT diets, respectively.

The inclusion of raw and pancreatin-treated beans in the diets increased the passage of DM, protein, and AA at the terminal ileum compared with the diets supplemented with germinated beans or the basal diet (Table 4). The mean ileal apparent AA and N digestibility coefficients for the pigs fed the dietary treatments are shown in Table 5. The mean ileal apparent digestibility of N and AA were decreased ( $P < .05$ ) by adding raw and pancreatin treated beans compared with the basal diet. No differences ( $P > .05$ ) in mean ileal apparent N and AA digestibilities were observed when adding germinated beans compared with the basal diet.

The mean apparent ileal AA digestion coefficients were higher than for the mean apparent ileal N digestion coefficients. Apparent ileal digestibility of N and AA of the beans were increased ( $P < .05$ ) when the beans were germinated.

The loss of endogenous protein was higher when pancreatin-treated beans were used in the diets as

compared with the other diets (Table 6). Also, the true ileal protein digestibility of pancreatin-treated beans was higher than in germinated beans (Table 7).

## Discussion

Nutrient digestibility in legumes is influenced by species, source, level of inclusion in the diet, the contents of ANF, and the methods of storage and processing (Gupta, 1987). Low digestibilities of *Phaseolus vulgaris* L. beans can be partly due to the resistance of native bean storage proteins toward hydrolysis by digestive proteolytic enzymes such as pepsin and pancreatin (Liener and Thompson, 1980; Savelkoul et al., 1992b). Consequently, upgrading the protein digestibility of *Phaseolus vulgaris* L. beans by physical, chemical, and biological treatments should almost completely degrade the lectins and phaseolin to ensure increased digestibility results.

According to van der Poel et al. (1990a), upgrading beans by processing is possible by thermal treatments that are effective in decreasing the levels of lectins (Antunes and Sgarbieri, 1980) and the activity of protease inhibitors (Rackis et al., 1986). In addition, high-temperature steaming for a short time improved

Table 5. Mean apparent ileal dry matter, protein, and amino acid digestibility (%) in growing pigs fed experimental diets

Item	Basal diet supplemented with beans <sup>a</sup>			Basal diet	SEM
	Untreated	Germinated	Pancreatin		
Number of pigs	3	3	3	2	
Dry matter	79.6 <sup>b</sup>	85.1 <sup>bd</sup>	79.0 <sup>b</sup>	86.8 <sup>cd</sup>	3.83
Protein	74.3 <sup>b</sup>	86.8 <sup>c</sup>	75.3 <sup>b</sup>	86.0 <sup>c</sup>	3.61
Total	75.5 <sup>b</sup>	88.6 <sup>c</sup>	78.2 <sup>bd</sup>	87.6 <sup>cd</sup>	4.32
Indispensable amino acid digestibility					
Arginine	75.6 <sup>b</sup>	88.8 <sup>c</sup>	77.3 <sup>bd</sup>	86.2 <sup>cd</sup>	3.56
Histidine	76.5 <sup>b</sup>	88.9 <sup>c</sup>	78.1 <sup>bd</sup>	88.1 <sup>cd</sup>	4.80
Isoleucine	77.8 <sup>b</sup>	90.9 <sup>c</sup>	81.5 <sup>b</sup>	90.8 <sup>c</sup>	3.54
Leucine	82.2 <sup>b</sup>	92.3 <sup>c</sup>	84.0 <sup>b</sup>	92.1 <sup>c</sup>	2.81
Lysine	81.3 <sup>b</sup>	92.8 <sup>c</sup>	86.6 <sup>bc</sup>	92.7 <sup>bc</sup>	4.75
Methionine	92.2 <sup>b</sup>	96.0 <sup>c</sup>	92.7 <sup>bd</sup>	95.5 <sup>cd</sup>	1.15
Cysteine	69.9 <sup>b</sup>	82.9 <sup>b</sup>	71.3 <sup>b</sup>	82.3 <sup>b</sup>	6.57
Phenylalanine	79.9 <sup>b</sup>	91.7 <sup>c</sup>	82.3 <sup>b</sup>	90.9 <sup>c</sup>	2.95
Tyrosine	83.8 <sup>b</sup>	93.2 <sup>c</sup>	85.5 <sup>b</sup>	92.3 <sup>c</sup>	2.48
Threonine	69.2 <sup>b</sup>	84.6 <sup>cd</sup>	71.0 <sup>b</sup>	82.5 <sup>bd</sup>	5.85
Valine	78.6 <sup>b</sup>	90.2 <sup>c</sup>	80.7 <sup>b</sup>	89.9 <sup>c</sup>	3.45
Dispensable amino acid digestibility					
Alanine	66.1 <sup>bd</sup>	82.2 <sup>c</sup>	68.6 <sup>bd</sup>	80.0 <sup>cd</sup>	5.56
Aspartic acid	70.5 <sup>b</sup>	88.8 <sup>c</sup>	73.6 <sup>b</sup>	86.1 <sup>c</sup>	4.96
Glutamic acid	81.4 <sup>b</sup>	92.1 <sup>cd</sup>	85.1 <sup>bd</sup>	92.4 <sup>cd</sup>	3.67
Glycine	44.1 <sup>b</sup>	69.7 <sup>b</sup>	51.1 <sup>b</sup>	66.9 <sup>b</sup>	11.75
Proline	84.3 <sup>b</sup>	93.4 <sup>b</sup>	86.1 <sup>b</sup>	93.4 <sup>b</sup>	4.48
Serine	69.8 <sup>b</sup>	86.8 <sup>c</sup>	73.7 <sup>bd</sup>	85.9 <sup>cd</sup>	5.39

<sup>a</sup>*Phaseolus vulgaris* var. Processor.

<sup>b,c,d</sup>Means in the same row followed by different letter differ ( $P < .05$ ).

Table 6. Mean true<sup>a</sup> protein digestibility and endogenous ileal protein losses in pigs fed experimental diets

Item	Basal diet supplemented with beans <sup>b</sup>			SEM
	Untreated	Germinated	Pancreatin	
True protein digestibility	88.1 <sup>c</sup>	93.2 <sup>d</sup>	93.4 <sup>d</sup>	1.43
Endogenous protein				
DMI, g/kg	27.8 <sup>c</sup>	13.3 <sup>d</sup>	37.5 <sup>c</sup>	4.34
% of total ileal protein	53.4 <sup>c</sup>	48.4 <sup>c</sup>	73.5 <sup>d</sup>	4.54
g/100 g protein intake	13.8 <sup>c</sup>	6.4 <sup>d</sup>	18.1 <sup>e</sup>	2.13

<sup>a</sup>Directly determined real protein digestibility using the <sup>15</sup>N-isotope dilution method.

<sup>b</sup>*Phaseolus vulgaris* var. Processor.

<sup>c,d,e</sup>Means in the same row followed by different letter differ ( $P < .05$ ).

ileal N digestibility by conformational changes of storage protein, which was not observed with (prolonged) steaming at 102°C (van der Poel, 1990b). Technological treatments, however, may have some drawbacks because of fuel and labor requirements, maintenance of equipment, and potential overheating (Savelkoul et al., 1992a). Germination, an alternative method as applied here, increased the sensitivity of the beans toward proteolysis. Differences in the amino acid pattern after germination appeared mainly from degradation of phaseolin (Savelkoul et al., 1992b). Germination also decreased the ANF contents in the white kidney bean (Table 2). These results agree with reported decreases of the lectin content by 85%, a loss of functional lectins by 91%, and a decrease of TIA by 76% in beans after germination of 7 d (Savelkoul et al., 1994). According to Savelkoul et al. (1992a) the reason for the decreased lectin contents seemed to be the development of enzymes capable of degrading lectins during germination.

Savelkoul et al. (1994) showed an improvement of the in vitro N digestibility when germinated beans were compared with raw beans. The apparent ileal N and AA digestibilities increased with germinated bean included in the diets. There were no differences ( $P > .05$ ) when comparing the control with the GR diet and comparing the RB with the PTB diet. This study shows that pancreatin only partly reduced TIA and lectins. Germination for 7 d, however, reduced TIA and lectins to very low levels, and apparent digestibility rates of these beans were improved through germination. Furthermore, the sensitivity of phaseolin (the storage protein in beans) for proteolysis may have been increased. Romero and Ryan (1978) and Santoro et al. (1989) observed that purified phaseolin was strongly associated with the tissue of the gut wall, and this extended exposure time of the protein to the gut enzymes, partially degraded phaseolin, and rendered it susceptible to further proteolysis by trypsin and chymotrypsin. The negative apparent digestibility coefficients of the raw beans and pancreatin-treated beans may be related to 1) poor digestion of the bean protein, 2) an increased endogenous excretion, and 3) an effect of the bean inclusion on the digestibility of

the basal diet. Toasted beans with contents of ANF comparable to those in the PTB diet in our experiment were used by van der Poel et al. (1991a). The very low apparent N digestibility of the PT bean used in our experiment agrees very well with the results shown by van der Poel et al. (1991a). Similarly, the poor ileal N and AA digestibility of raw beans in our studies supports the observed negative apparent fecal N digestibility of raw beans in pigs by van der Poel et al. (1990c).

The decreased lectin content, which means loss of binding capacity of functional lectins toward brush border membranes, and TIA and the assumed increase of sensitivity of the phaseolin toward proteolysis in beans after germination resulted in a substantial improvement of the apparent ileal N and AA digestibility. This result agrees with data of increased apparent ileal N digestibility of short-term processed *Phaseolus vulgaris* L. beans at higher temperatures given by van der Poel et al. (1991b). Germination of *Phaseolus vulgaris* L. beans resulted in a considerable loss of amino acids, particularly glutamic acid, lysine, and the S-containing amino acids. This loss was still apparent when *Phaseolus vulgaris* L. beans were included in a pig diet at a rate of 75 g/kg and provided 10% of the total protein. The loss of amino acids is, however, more than compensated by the increased ileal protein digestion. This means that pigs lose more endogenous N than they can absorb from the N in

Table 7. Apparent and true ileal protein digestibilities of the treated beans<sup>a</sup>

Ileal protein digestibility	<i>Phaseolus vulgaris</i> var. Processor		
	Untreated	Germinated	Pancreatin treated
Apparent	-52.6	93.1	-6.9
True	-19.5	53.4	57.6

<sup>a</sup>Mean apparent and true ileal protein digestibilities of the treated bean were determined with the difference method using the mean digestibility values given in Tables 5 and 6 and assuming a true ileal protein digestibility of the basal diet of 98%.

beans. It also means that the net effect of germination is an improved apparent ileal AA absorption. Moreover, the pattern of amino acids is altered somewhat.

The negative apparent ileal protein digestibility of pancreatin-treated beans is similar to results reported by Huisman (1992) and van der Poel et al. (1991a) with toasted beans. The true ileal protein digestibility in pigs from pancreatin-treated beans was higher than that from germinated beans. This could be because pancreatin contains trypsin and chymotrypsin, which can bind to trypsin and chymotrypsin inhibitors and block the inhibitor activity. Endogenous protein losses in pigs were higher when pancreatin-treated beans were used instead of germinated beans. This means that in those beans, lectins and phaseolin were still present at considerable levels. It can be assumed that these are at least partly responsible for the high losses of endogenous protein. The beans were pretreated with pancreatin (12 h at room temperature), which could also simulate a germination period of 12 h when increased content of trypsin inhibitors was observed (Savelkoul et al., 1994) but also an increased susceptibility for protein hydrolysis (Savelkoul et al., 1992b). A higher trypsin inhibitor activity could have led to the excretion of more endogenous protein in the small intestine.

### Implications

This research shows that trypsin inhibitors can be partly eliminated from raw white kidney beans by pancreatin treatment. However, this biological treatment of the *Phaseolus vulgaris* L. bean provided no beneficial effect on apparent ileal nitrogen and amino acid digestibility because lectins are not eliminated by pancreatin treatment. Germination of the beans for 7 d improved the apparent ileal nitrogen and amino acid digestibility due to the degradation of lectins, trypsin inhibitors, and phaseolin. For efficient protein utilization of raw white kidney beans at 7.5% of a pig diet, processing by germination is recommended.

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