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Response of Pancreatic Secretions to Feeding Diets with Low and High Levels of Soybean Trypsin Inhibitors in Growing Pigs

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Abstract: Studies were carried out to determine the effect of soybean trypsin inhibitors (SBTI) on exocrine pancreatic secretions in growing pigs. Six barrows with an average initial body weight (BW) of 27·1 ± 1·4 kg were fitted with permanent pancreatic re-entrant cannulas and fed two diets according to a cross-over design. Two maize starch-based diets were formulated to contain 200 g kg^{-1} crude protein from either Nutrisoy (food grade defatted soy flour) or autoclaved Nutrisoy. The concentrations of SBTI in Nutrisoy and autoclaved Nutrisoy diets were 13·4 and 3·0 g kg^{-1}, respectively. The experiment consisted of two periods of 9 days each. The average BW at the start of the first and second experimental periods was 33·5 ± 2·7 and 37·2 ± 3·7 kg, respectively. The average BW at the conclusion of the experiment was 41·8 ± 3·9 kg. The volume of pancreatic secretion was higher ($P < 0·01$) when the Nutrisoy, as opposed to the autoclaved Nutrisoy diet was fed (3804 vs 2634 ml $(24\ h)^{-1}$). The concentration of nitrogen and protein and specific activities (units $litre^{-1}$) of amylase, chymotrypsin and trypsin were lower ($P < 0·05$) in pancreatic juice of pigs fed the Nutrisoy diet. There were no differences ($P > 0·05$) in the total secretions of nitrogen (g $(24\ h)^{-1}$) and total activities (units $(24\ h)^{-1}$) of amylase, lipase, chymotrypsin and trypsin in pancreatic juice of pigs fed the Nutrisoy and autoclaved Nutrisoy diets. However, the total secretion of protein was slightly higher (25·7 vs 22·8 g $(24\ h)^{-1}$; $P < 0·05$) in pancreatic juice of pigs fed the autoclaved Nutrisoy diet, which corresponded with the increase in the secretion of protein-bound amino acids. There was also an increase in the total secretion of free amino acids in pancreatic juice. These studies show no effect of SBTI on the total enzyme activities in pancreatic juice of growing pigs. © 1998 SCI.

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Key words: pigs; soybean trypsin inhibitors; pancreatic enzymes

INTRODUCTION

Feeding raw soybean or soybean trypsin inhibitors (SBTI) elicited adverse nutritional, biological and physiological responses in rats (Liener and Kakade 1980), chickens (Yen *et al* 1973) and pigs (Yen *et al* 1974). SBTI inactivate trypsin and chymotrypsin by forming complexes with these enzymes (Blow *et al* 1974). Rats and chickens fed SBTI or raw soybean also respond by hypersecretion of pancreatic enzymes and hypertrophy of the pancreas. Trypsin, which contains 8·7% cystine, accounts for half of the cystine secreted in pancreatic juice of rats fed raw soybean (Barnes *et al* 1965a). Hypersecretion of pancreatic enzymes results in a higher demand for cystine by the pancreas, which may create a deficiency of the sulphur-containing amino acids (Barnes *et al* 1965b).

There is a scarcity of information on the effect of feeding raw soybean products or SBTI on pancreatic secretions in pigs. Therefore, studies were carried out to

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determine the effect of feeding diets containing Nutrisoy (a food grade defatted soy flour) and autoclaved Nutrisoy, which have a relatively high and low content of SBTI, respectively, on the pancreatic secretions in growing pigs prepared for total collection of pancreatic juice using the 'Pouch' method (Hee *et al* 1985).

EXPERIMENTAL PROCEDURES

Animals and diets

Six PIC (Pig Improvement Canada) barrows (Camborough × Canabrid) with an average initial body weight (BW) of 27·2 ± 1·6 kg were obtained from the University of Alberta Swine Research Unit. The barrows were housed individually in stainless-steel metabolism crates (length 140 cm; height 85 cm; width 80 cm) in a barn with automatic temperature control (25 ± 1°C) and given *ad libitum* access to a grower diet containing 160 g crude protein (CP) kg^{-1} (Sauer *et al* 1983). Water was available from a low-pressure drinking nipple.

Nine days later, the barrows (average BW 31·5 ± 2·7 kg) were fitted with pancreatic re-entrant cannulas. Procedures for pre-operative care, surgery and post-operative care were carried out according to Hee *et al* (1985). The pancreatic re-entrant cannula was constructed according to Hee *et al* (1985) with modifications described by Ozimek *et al* (1986). The barrows were immediately returned to the metabolism crates after surgery and fasted that same day. The next day they were given approximately 100 g of a starter diet containing 180 g CP kg^{-1} (Sauer *et al* 1983), twice daily at 08:30 and 20:30 h. The daily feed allowance was gradually increased until the pigs consumed the amount of diet equivalent to 5% of BW, which was determined at the beginning of each experimental period. The barrows were allowed a 9-day recuperation period before the beginning of the first experimental period. One of the pigs did not recover from surgery.

Two maize starch-based Nutrisoy diets (Table 1) were formulated to contain 200 g CP kg^{-1} from either Nutrisoy or autoclaved Nutrisoy. The Nutrisoy was autoclaved with an Amsco 3201 Gravity Sterilizer (American Sterilizer Company, Erle, PA, USA) using steam at 120°C, 32 psi, for 15 min followed by 5 min drying. Autoclaving reduced the content of SBTI in Nutrisoy. The chemical composition of the experimental diets and Nutrisoy and autoclaved Nutrisoy are presented in Table 2. After autoclaving, the Nutrisoy flour turned into hard clumps and was subsequently ground through a 2-mm mesh screen before incorporation into the diet. Dextrose (100 g kg^{-1}) was included in the diet to improve palatability. Solkafloc (60 g kg^{-1}) and canola oil (50 g kg^{-1}) were also included in the diets.

TABLE 1
Formulation of the Nutrisoy and autoclaved Nutrisoy diets (g kg^{-1})

Ingredients	*Nutrisoy*	*Autoclaved Nutrisoy*
Nutrisoy[a]	350·0	—
Nutrisoy (autoclaved)	—	350·0
Corn starch	400·0	400·0
Canola oil	50·0	50·0
Dextrose	100·0	100·0
Solkafloc	60·0	60·0
Iodized salt[b]	3·0	3·0
Mineral–vitamin premix[c]	10·0	10·0
Biophos[d]	16·0	16·0
Calcium carbonate[e]	5·0	5·0
Chromic oxide	3·0	3·0
Antibiotics[f]	1·5	1·5
DL-Methionine	1·5	1·5

[a] Defatted soy flour. Supplied by Archer Daniels Midland Co, Decatur, IL, USA.
[b] Provided (g kg^{-1}) NaCl, 990 and I, 0·15. Supplied by Sifto Canada Inc, Mississauga, ON, Canada.
[c] Provided the following (kg^{-1} diet): vitamin A, 7500 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K_3, 2 mg; vitamin B_{12}, 0·03 mg; riboflavin, 12 mg; niacin, 40 mg; pantothenic acid, 25 mg; choline, 600 mg; biotin, 0·25 mg; folic acid, 1·6 mg; thiamin, 3·0 mg; Ethoxyquin, 5 mg; Fe, 150 mg; Mn, 20 mg; Zn, 120 mg; Cu, 125 mg; I, 0·02 mg; Se, 0·3 mg. Supplied by Hoffmann-LaRoche Ltd 2455 Meadowpine Blvd, Mississauga, ON, Canada.
[d] Provided (g kg^{-1}) available phosphorous, 150–180; calcium, 240. Supplied by Continental Lime Ltd, Exshaw, AB, Canada.
[e] Provided (g kg^{-1}) calcium, 380. Supplied by Continental Lime Ltd, Exshaw, AB, Canada.
[f] Veterinary LS-20 premix, provided the following (kg^{-1} diet): lincomycin hydrochloride, 22 mg; spectinomycin sulphate, 22 mg; mineral oil USP, 10 mg. Supplied by the Upjohn Co, Animal Health Division, Orangeville, ON, Canada.

Vitamins and minerals were supplemented to meet or exceed NRC (1988) standards.

The experiment was carried out according to a two-period crossover design (Petersen 1985). Each experimental period lasted 9 days. The second experimental period was initiated immediately after the conclusion of the first period. The pigs were fed twice daily, equal amounts, at 08:30 and 20:30 h, equivalent to 5% of BW. The average BW at the start of the first and second experimental periods was 33·5 ± 2·7 and 37·2 ± 3·7 kg, respectively. The average BW at the conclusion of the experiment was 41·8 ± 3·9 kg. Pancreatic juice was collected for 24 h from 08:30 to 20:30 h on day 7 (day collection) and from 20:30 h on day 8 to 08:30 h on day 9 (night collection) of each experimental period. Collection, sampling and subsequent return of

TABLE 2
Analysed chemical and amino acid composition (g kg^{-1})[a] of Nutrisoy, autoclaved Nutrisoy and the experimental diets

Items	*Nutrisoy*	*Autoclaved Nutrisoy*	*Nutrisoy diet*	*Autoclaved Nutrisoy diet*
Dry matter	926·2	926·3	919·4	916·7
Crude protein	574·7	572·4	202·7	206·5
Energy (MJ kg^{-1})	20·1	20·1	18·8	18·8
Neutral-detergent fibre	73·0	75·3	80·3	80·3
Acid-detergent fibre	40·4	41·2	65·5	65·5
Ether extract	12·1	9·4	61·4	59·1
Soybean trypsin inhibitors	38·4	9·3	13·3	3·3
Amino acids				
Indispensable				
Arginine	38·0	37·0	13·8	13·9
Histidine	13·6	13·3	5·0	5·0
Isoleucine	24·6	24·5	9·1	9·4
Leucine	39·9	39·8	14·9	15·5
Lysine	33·5	30·9	12·1	11·7
Methionine	9·3	9·6	7·7	7·7
Phenylalanine	26·6	26·5	10·0	10·4
Threonine	19·1	19·0	7·2	7·3
Valine	24·4	24·4	9·1	9·5
Dispensable				
Alanine	22·4	22·4	8·5	8·6
Aspartic acid	51·7	51·3	19·6	19·7
Cysteine	8·6	9·5	10·0	8·6
Glutamic acid	91·7	90·9	34·9	36·2
Glycine	22·4	22·4	8·4	8·6
Serine	24·7	24·6	9·4	9·6
Tyrosine	15·3	15·6	5·2	5·5

[a] Dry matter basis.

pancreatic juice was carried out according to procedures described by Hee *et al* (1985). The hourly volume of pancreatic juice was recorded during each 12-h collection period. A sample of 10% of the hourly volume was taken and the remainder was made up to its original volume with saline and returned to the pig. The samples were frozen immediately after collection and stored at −20°C.

The experimental proposal and surgical procedures were reviewed and approved by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta. The animals were cared for in accordance with guidelines established by CCAC (1980).

Chemical analyses

Samples of the diets were taken each time the meal allowances were weighed and pooled for each dietary treatment. Samples of diets were ground in a Wiley mill (Arther H. Thomas Co, Philadelphia, PA, USA) through a 0·8-mm mesh screen before analysis. Analyses for dry matter, nitrogen and ether extract were carried out according to AOAC (1990). Analyses for neutral-detergent fibre and acid-detergent fibre were carried out according to procedures outlined by Goering and van Soest (1970). The gross energy content in the diets and Nutrisoy was determined with an AC-300 Leco Automatic Calorimeter (Leco Corporation, St Joseph, MO, USA). The procedures for amino acid analysis of the diets were described previously (Li *et al* 1994). Tryptophan and proline were not measured.

SBTI contents in Nutrisoy and diets were determined according to procedures based on Hamerstrand *et al* (1981) and Kakade *et al* (1969) with modifications described by Li (1996).

The SBTI concentration in the samples was calculated based on the amount of trypsin units (TU) inhibited. One TU is arbitrarily defined as the increase of 0·01 absorbance unit at 410 nm in 10 ml of incubation mixture. The trypsin inhibitor activity is defined as the amount of TU inhibited under conditions described by Kakade *et al* (1969). Kakade *et al* (1969), using purified trypsin, found that 1 μg 'pure' trypsin has an activity of 1·9 TU, which is equivalent to 0·019 absorbance units. For each microgram pure trypsin inhibited, there will be a decrease of 0·019 absorbance units in the sample tested. Based on these findings, the SBTI content was

calculated using the following formula:

$$\text{SBTI (mg g}^{-1}\text{ sample)} = (A_{standard} - A_{sample})/0{\cdot}019 \times (1\text{ mg}/1000\ \mu\text{g}) \times \text{dilution factor/sample weight (g)}$$

where $A_{standard}$ is the absorbance of trypsin standards and A_{sample} is the absorbance of samples.

For the determination of nitrogen, protein, amino acid concentration and enzyme activities, the hourly samples of pancreatic juice were thawed at 4°C and pooled within pig within each 12-h collection. Nitrogen in pancreatic juice was measured with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St Joseph, MO, USA). Pancreatic juice was weighed (100 mg) into an aluminium sample vial and loaded on the automatic sampling rotator. Protein concentration in pancreatic juice was determined according to the method described by Lowry *et al* (1951).

The free amino acid concentration in pancreatic juice was determined according to the following procedure. Vials containing 2 ml of pooled undiluted pancreatic juice were placed in a rack in an ice bath, and 500 μl of pancreatic juice from each vial were pipetted into 10 × 75 mm disposable borosilicate glass tubes and 100 μl of internal standard solution (320 μmol DL-8-aminobutyric acid dissolved in deionized water) was added to each test-tube. The contents were mixed using a Vortex Genie™ (Scientific Industries, Bohemia, NY, USA). Proteins were precipitated by adding 400 μl of 5% (w/v) trichloroacetic acid. The contents were mixed again several times and centrifuged at 1100*g* at 4°C for 15 min to pellet the precipitated proteins. After centrifugation, 100 μl of the supernatant was used for amino acid analysis.

The amino acid concentration in pancreatic juice was determined following acid hydrolysis. Undiluted pancreatic juice (500 μl) was pipetted into 13 × 100 mm screw-capped test-tubes, mixed with 6 ml 6 M HCL, flushed with nitrogen, capped and hydrolysed at 110°C for 24 h. Both total and free amino acids were analysed according to Jones and Gilligan (1983) using a Varian 5000 high-performance liquid chromatography system (HPLC, Varian Canada, Mississauga, ON, Canada). The procedure was described in more detail by Gabert *et al* (1996a).

Protein-bound amino acids were calculated as the difference between total and free amino acids. During hydrolysis, glutamine and asparagine are converted to glutamic and aspartic acid, respectively. Therefore, protein-bound glutamic and aspartic acid were calculated by subtracting free glutamic acid plus free glutamine and free aspartic acid plus free asparagine from total glutamic and aspartic acid, respectively.

Amylase (EC 3.2.1.1) activity in pancreatic juice was determined according to procedures described by Rick and Stegbauer (1974) and the *Enzyme Manual* (WBC 1988). Lipase (EC 3.1.1.1) activity was determined according to Schmidt *et al* (1974). Activities of trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were determined according to Rick (1974a,b) following activation of chymotrypsinogen and trypsinogen to chymotrypsin and trypsin, respectively, by enterokinase (Sigma; enteropeptidase, EC 3.4.21.9; code E0632). The activation procedure was carried out according to Glazer and Steer (1977) with modifications described by Gabert *et al* (1996b). Undiluted pancreatic juice (0·2 ml) was added to 1·8 ml of buffer (pH 8·1) containing 100 μg ml^{-1} bovine serum albumin (Sigma; code A3059), 50 mmol $CaCl_2$ and 50 mmol Tris-[hydroxymethyl]-aminomethane (Sigma; code T1378). Enterokinase was dissolved in deionized water (10 mg ml^{-1}) and centrifuged at 15 600 *g* at 5°C for 15 min to remove cell debris. Activation was initiated by adding 0·2 ml of diluted pancreatic juice to 0·2 ml supernatant which contained the enterokinase followed by incubation at 5°C for 3 and 120 h for chymotrypsin and trypsin, respectively.

Calculations and statistical analysis

The 12-h flow of nitrogen, protein and total protein-bound and free amino acids were calculated by multiplying the concentrations of these by the volume of pancreatic juice secreted per 12 h. One unit of enzyme activity is defined as the amount of enzyme that hydrolyzes 1 μmol substrate in 1 min at 25°C. The specific activities of enzymes in pancreatic juice are expressed as units per litre (U $litre^{-1} \times 10^{-3}$). Total enzyme activities were calculated as the product of specific activities and volume of pancreatic juice secreted in 12 h. Total flows of nitrogen, protein and total protein-bound and free amino acids and total enzyme activities are expressed on a 24-h basis. Previous studies showed no differences ($P > 0{\cdot}05$) in pancreatic secretions between 12-h collections during day and night in pigs that were fed twice daily at 12-h intervals (Hee *et al* 1988a; Mosenthin and Sauer 1991).

Data were subjected to analysis of variance according to procedures described by Petersen (1985) using the General Linear Model procedure of SAS (1990). The statistical model included dietary treatments (df = 1), experimental periods (df = 1) and interactions of dietary treatments and experimental periods (df = 1) as sources of variation. For parameters where the interaction was not significant, the variation was removed from the model. The least squares means of dietary treatments and experimental periods were compared using the probability of difference (PDIFF) procedure (SAS 1990).

RESULTS AND DISCUSSION

Except one animal which did not recover from surgery, the pigs remained healthy and usually consumed their

meal allowances within 1 h of feeding. Post-mortem examinations conducted at the conclusion of the experiment revealed no intestinal adhesions and/or other abnormalities.

Chemical and amino acid composition of the experimental diets and Nutrisoy are presented in Table 2. Autoclaving did not affect the amino acid content, but reduced the SBTI content in Nutrisoy from 38·5 to 9·3 g kg^{-1}. The SBTI contents in the Nutrisoy and autoclaved Nutrisoy diets were 13·3 and 3·3 g kg^{-1}, respectively.

The incorporation of Nutrisoy compared with autoclaved Nutrisoy into the corn starch-based diet increased ($P < 0·01$) the total volume of pancreatic juice secretion (Table 3). There was a decrease ($P < 0·05$) in the concentration of nitrogen, but not total secretion, in pancreatic juice of pigs fed the Nutrisoy compared with the autoclaved Nutrisoy diet. These results are in agreement with those reported by Zebrowska *et al* (1985) who observed an increase ($P < 0·01$) in the volume of pancreatic juice secretion when raw as opposed to heat-treated soybean meal (2908 vs 1710 ml $(24\ h)^{-1}$) was fed to growing pigs. They also observed no effect ($P > 0·05$) on total nitrogen secretion. There was a slight increase ($P < 0·05$) in the total secretion of protein in this study.

The inclusion of Nutrisoy compared with autoclaved Nutrisoy into the diet decreased the specific activity of amylase ($P < 0·01$), chymotrypsin ($P < 0·01$) and trypsin ($P < 0·05$), but not of lipase (Table 3). There was no effect ($P > 0·05$) on the total enzyme activities. These results are in agreement with studies reported by Zebrowska *et al* (1985) who observed no effect ($P > 0·05$) on the total activities of trypsin, chymotrypsin and amylase when heat-treated as opposed to raw soybean meal was included in corn starch-based diets. However, the results of these studies contradict those reported by Ozimek *et al* (1985), who observed higher total pancreatic enzyme activities in pigs that were fed corn starch-based diets containing Nutrisoy and autoclaved Nutrisoy (160 g CP kg^{-1}). However, the previous results did not take into account the effect of BW which has been shown to affect the pancreatic enzyme secretions.

This study showed no effect of SBTI on the total activities of the exocrine pancreatic enzymes in pigs. However, studies with chicks and rats fed SBTI (or raw vs heat-treated soybean meal) showed an increase in pancreatic enzyme secretions and hypertrophy of the pancreas (Liener and Kakade 1980). In these species, according to a negative feedback regulation mechanism proposed by Green and Lyman (1972), trypsin (and also chymotrypsin) are inactivated by the formation of a complex with trypsin inhibitors. The inhibition of trypsin and chymotrypsin activities will trigger an increased secretion of cholecystokinin, which stimulates the secretion of pancreatic enzymes. Some researchers (Schingoethe *et al* 1970) suggested that there was a direct relationship between the size of the pancreas (in relation to BW) and the sensitivity of response to SBTI or raw soybean. It was postulated that species with a pancreas weight exceeding 0·3% of BW (which include

TABLE 3
Effect of experimental diet on the secretion volume, nitrogen and protein contents and enzyme activities of pancreatic juice in growing pigs

Diets		*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*[a]
Volume	ml $(24\ h)^{-1}$	$3803·6^{D}$	$2633·1^{E}$	104·38
Nitrogen	g $litre^{-1}$	$1·3^{e}$	$2·0^{d}$	0·08
	g $(24\ h)^{-1}$	5·0	5·2	0·16
Protein	g $litre^{-1}$	$6·2^{e}$	$10·3^{d}$	0·58
	g $(24\ h)^{-1}$	$22·8^{e}$	$25·7^{d}$	1·26
Enzyme activities				
Amylase	Specific[b]	$107·4^{E}$	$159·5^{D}$	0·61
	Total[c]	414·3	420·8	18·68
Lipase	Specific[b]	32·7	36·3	2·07
	Total[c]	108·1	95·6	9·22
Chymotrypsin	Specific[b]	$42·1^{E}$	$60·3^{D}$	1·38
	Total[c]	139·0	154·0	6·96
Trypsin	Specific[b]	$46·0^{e}$	$73·5^{d}$	6·62
	Total[c]	168·5	178·9	10·60

[a] Standard error of the mean ($n = 10$).
[b] U $litre^{-1} \times 10^{-3}$.
[c] U $(24\ h)^{-1} \times 10^{-3}$.
[d,e] Means in the same row with different superscript letters differ ($P < 0·05$).
[D,E] Means in the same row with different superscript letters differ ($P < 0·01$).

rats and chicks) are more prone to pancreatic hypersecretion and hypertrophy than species in which the ratio of pancreas to BW is less than 0·3%. This, in turn, may affect the requirement of the sulphur-containing amino acids because the exocrine pancreatic enzymes contain high concentrations of cystine. Species in which the size of the pancreas is relatively large (in relation to BW) may have a higher requirement of the sulphur-containing amino acids, especially when these species are fed SBTI or raw soybean products.

The effect of experimental period on the parameters measured is presented in Table 4. The average BW of the pigs were 35·4 and 39·5 kg during periods 1 and 2, respectively. The total volume of secretion and nitrogen and protein outputs were higher ($P < 0·05$) during period 2. There were no differences ($P > 0·05$) in the concentrations of nitrogen and protein in pancreatic juice between experimental periods. Both specific ($P < 0·01$) and total activities ($P < 0·05$) of amylase were higher during period 2. The total activity of chymotrypsin was higher ($P < 0·05$) during period 2. These results confirm those of Weström *et al* (1988), who showed that, as BW increased, pancreatic secretion increased. Gabert *et al* (1996b) also reported an increase in secretion volume, protein output and total enzyme activities with increasing BW in pigs. The increase in secretion volume, protein and total enzyme activities are likely related to feed intake, which can be a confounding factor in measurement of pancreatic secretions. Another study (Mosenthin and Sauer 1991), however, did not show a period effect.

Little attempt has been made to compare results from the present study with those reported in the literature, because of differences in BW between pigs. In addition, Imbeah *et al* (1988) pointed out that a direct comparison of results, as opposed to relative differences, is very difficult for reasons of differences in feed intake, feeding regimen, diet composition and procedures for enzyme analysis and techniques used to collect pancreatic juice. It is of utmost importance to consider the technique used to collect pancreatic juice. The direct cannulation (eg Corring 1975) and the 'Pouch' technique or a modification thereof (eg Hee *et al* 1985) allow for total collection of pancreatic juice and the measurement of total enzyme activities. On the other hand, the slaughter technique in which enzyme activities are measured in digesta collected from the small intestine, will provide specific activities. Results expressed in total rather than specific activities are a better reflection of the effect of dietary treatments on pancreatic enzyme secretion. Differences in specific activities may simply result from the dilution effect of the volume of pancreatic juice as was previously discussed by Hee *et al* (1988a,b).

The concentration (mmol litre^{-1}) of each amino acid in the total, free and protein-bound amino acid pools

TABLE 4

Effect of experimental period on the secretion volume, nitrogen and protein contents and enzyme activities of pancreatic juice in growing pigs

Items		*Experimental periods*		
		1	*2*	*SE*[a]
Volume	ml (24 h)$^{-1}$	2731·6[e]	3505·1[d]	104·38
Nitrogen	g litre^{-1}	1·7	1·7	0·08
	g (24 h)$^{-1}$	4·9[e]	5·8[d]	0·16
Protein	g litre^{-1}	7·9	8·6	0·58
	g(24 h)$^{-1}$	21·4[e]	27·4[d]	1·26
Enzyme activities				
Amylase	Specific[b]	130·4[E]	136·5[D]	0·61
	Total[c]	371·6[e]	463·6[d]	18·68
Lipase	Specific[b]	38·1	30·9	2·07
	Total[c]	106·5	103·9	9·24
Chymotrypsin	Specific[b]	49·8	52·6	0·38
	Total[c]	138·6[e]	171·6[d]	6·96
Trypsin	Specific[b]	66·4	53·1	6·62
	Total[c]	179·6	167·8	10·60

[a] Standard error of the mean ($n = 10$).
[b] U litre$^{-1} \times 10^{-3}$.
[c] U (24 h)$^{-1} \times 10^{-3}$.
[d,e] Means in the same row with different superscript letters differ ($P < 0·05$).
[D,E] Means in the same row with different superscript letters differ ($P < 0·01$).

TABLE 5
Effect of experimental diet on amino acid concentration (mmol litre^{-1}) in pancreatic juice in growing pigs

Amino acids	*Total amino acids*			*Free amino acids*			*Protein-bound amino acids*		
	Nutrisoy	*Autoclaved Nutrisoy*	*SE*[a]	*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*	*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*
Indispensable									
Arginine	1·9^{e}	3·5^{d}	0·30	0·6	1·2	0·17	1·3^{e}	2·3^{d}	0·18
Histidine	1·0^{e}	1·7^{d}	0·17	0·2^{e}	0·5^{d}	0·05	0·7	1·3	0·14
Isoleucine	2·7^{e}	4·6^{d}	0·37	0·6^{e}	1·2^{d}	0·12	2·0^{e}	3·4^{d}	0·29
Leucine	3·7^{e}	6·2^{d}	0·55	1·2^{e}	2·4^{d}	0·24	2·5^{e}	3·8^{d}	0·45
Lysine	2·3^{e}	4·6^{d}	0·49	0·8	2·1	0·31	1·5^{e}	2·5^{d}	0·35
Methionine	2·5^{e}	3·1^{d}	0·74	0·1^{e}	0·3^{d}	0·04	2·3	2·8	0·76
Phenylalanine	1·8^{e}	3·2^{d}	0·29	0·5^{e}	1·1^{d}	0·13	1·3	2·1	0·20
Threonine	2·7^{e}	4·8^{d}	0·43	0·4	0·8	0·09	2·3^{e}	4·1^{d}	0·37
Valine	3·5^{e}	6·2^{d}	0·49	0·5	1·1	0·13	3·0^{e}	5·1^{d}	0·40
Subtotal	22·1	37·9		4·9	11·8		16·9	27·4	
Dispensable									
Alanine	0·6^{e}	6·2^{d}	0·55	0·7	1·4	0·18	2·9	4·8	0·42
Aspartic acid[b]	5·6^{e}	9·3^{d}	0·62	0·1	0·2	0·03	5·2^{e}	8·5^{d}	0·55
Asparagine				0·1	0·2	0·04			
Cysteine	4·8^{e}	6·1^{d}	0·28	0·5	0·3	0·28	4·3^{e}	5·8^{d}	0·39
Glutamic acid[c]	4·3^{e}	7·4^{d}	0·63	0·3^{e}	0·7^{d}	0·07	3·7^{e}	6·2^{d}	0·55
Glutamine				0·5^{e}	1·0^{d}	0·10			
Glycine	5·1^{e}	9·3^{d}	0·78	0·3	0·6	0·09	4·9^{e}	8·7^{d}	0·71
Serine	3·9^{e}	6·8^{d}	0·57	0·4	0·9	0·13	3·5^{e}	5·9^{d}	0·47
Tyrosine	1·9^{e}	3·3^{d}	0·26	0·6	1·3	0·16	1·3	2·0	0·18
Subtotal	29·2	48·4		3·5	6·6		25·8	41·9	
Total	51·3	86·3		8·4	18·4		42·7	69·3	

[a] Standard error of the mean ($n = 10$).
[b] Protein-bound aspartic acid is calculated as total aspartic acid − (free asparagine + free aspartic acid).
[c] Protein-bound glutamic acid as total glutamic acid − (free glutamine + free glutamic acid).
[d,e] Values in the same row, within total, free and protein-bound amino acids, with different superscript letters differ ($P < 0·05$).

TABLE 6
Effect of experimental diet on total amino acid secretion (mmol (24 h)$^{-1}$) in pancreatic juice collected from growing pigs

Amino acids	*Total amino acids*			*Free amino acids*			*Protein-bound amino acids*		
	Nutrisoy	*Autoclaved Nutrisoy*	*SE*[a]	*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*	*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*
Indispensable									
Arginine	7·3^{e}	8·6^{d}	0·27	2·5	2·8	0·34	4·9^{e}	5·8^{d}	0·39
Histidine	3·6^{e}	4·2^{d}	0·12	0·8^{e}	1·1^{d}	0·04	2·9	3·2	0·15
Isoleucine	10·0^{e}	11·3^{d}	0·19	2·1^{e}	2·8^{d}	0·13	7·9^{e}	8·5^{d}	0·22
Leucine	13·7^{e}	15·2^{d}	0·39	4·2^{e}	5·6^{d}	0·24	9·5	9·5	0·50
Lysine	8·7^{e}	11·0^{d}	0·38	2·9^{e}	4·7^{d}	0·28	6·2	6·4	0·30
Methionine	8·4	9·2	0·65	0·3	0·8	0·04	8·1	8·4	0·63
Phenylalanine	6·7^{e}	7·9^{d}	0·25	1·7^{e}	2·5^{d}	0·13	5·0	5·4	0·22
Threonine	10·1^{e}	11·7^{d}	0·26	1·2^{e}	1·8^{d}	0·06	8·9^{e}	10·0^{d}	0·24
Valine	13·3^{e}	15·2^{d}	0·27	1·9^{e}	2·5^{d}	0·10	11·4^{e}	12·6^{d}	0·25
Subtotal	81·8	94·3		17·6	24·6		64·8	69·8	
Dispensable									
Alanine	13·6^{e}	15·3^{d}	0·37	2·3^{e}	3·2^{d}	0·15	11·3	12·1	0·32
Aspartic acid[b]	21·0^{E}	23·2^{D}	0·17	0·3	0·3	0·05	19·6^{e}	21·2^{d}	0·19
Asparagine				0·3	0·4	0·04			
Cysteine	18·4	15·7	1·34	1·8	2·2	0·08	16·3	13·9	1·79
Glutamic acid[c]	16·1^{e}	18·1^{d}	0·38	1·1^{e}	1·6^{d}	0·08	14·1	15·4	0·39
Glutamine				1·8^{e}	2·4^{d}	0·11			
Glycine	19·5^{e}	22·8^{d}	0·57	0·9	1·3	0·10	18·5^{e}	21·5^{d}	0·48
Serine	14·9^{e}	16·8^{d}	0·39	1·5	2·1	0·13	13·4	14·7	0·32
Tyrosine	7·3^{e}	8·1^{d}	0·16	2·1^{e}	2·8^{d}	0·14	5·2	5·2	0·17
Subtotal	110·8	120·1		12·1	16·3		98·4	104·0	
Total	192·6	214·4		29·7	40·9		163·2	173·8	

[a] Standard error of the mean ($n = 10$).
[b] Protein-bound aspartic acid is calculated as total aspartic acid − (free asparagine + free aspartic acid).
[c] Protein-bound glutamic acid as total glutamic acid − (free glutamine + free glutamic acid).
[d,e] Values in the same row, within total, free and protein-bound amino acids, with different superscript letters differ ($P < 0·05$).
[D,E] Values in the same row, within total, free and protein-bound amino acids, with different superscript letters differ ($P < 0·01$)

was higher in pancreatic juice collected from pigs fed autoclaved Nutrisoy (Table 5), which corresponds to the higher protein concentration (Table 3). In the free amino acid pool, of the indispensable amino acids, the highest concentrations were observed for leucine and lysine. In the protein-bound pool, the highest concentrations were found for aspartic acid, cysteine, glutamic acid, glycine and serine. The high concentrations of these protein-bound amino acids reflect their relatively high concentrations in the pancreatic enzymes. For example, chymotrypsin contains relatively high concentrations of aspartic acid, glycine and alanine (Charles *et al* 1967), while trypsin contains relatively high concentrations of aspartic acid, glutamic acid, glycine and serine (Charles *et al* 1963). Amylase and lipase also contain relatively high concentrations of some of the aforementioned amino acids (Cozzone *et al* 1970; Winkler *et al* 1990).

The effect of experimental diet on the total amino acid secretion (mmol $(24\ h)^{-1}$) in pancreatic juice is presented in Table 6. The increase in total secretion of each of the amino acids reflects the increase in protein secretion when autoclaved Nutrisoy rather than Nutrisoy is fed. For most amino acids in the free and protein-bound pools, the increase is significant ($P < 0{\cdot}05$). The free amino acid pool in pancreatic juice makes up a substantial proportion of the total amino acid pool ranging from 1·3 (aspartic acid) to 36·8% (leucine) in pigs fed the autoclaved Nutrisoy diet and from 1·4 (aspartic acid) to 30·6% (leucine) in pigs fed the Nutrisoy diet. Furthermore, the content of the indispensable amino acids is relatively high in the free amino acid pool. On the other hand, the content of the dispensable amino acids is relatively high in the protein-bound amino acid pool.

In conclusion, feeding diets containing high and low levels of SBTI (simulated by feeding Nutrisoy and autoclaved Nutrisoy) to pigs did not affect ($P > 0{\cdot}05$) the total activities of the exocrine pancreatic enzymes. Therefore, the detrimental effect of SBTI does not appear to be the result of hypersecretion of pancreatic enzymes *per se*.

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REFERENCES

AOAC 1990 *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Arlington, VA, USA.

Barnes R H, Fiala G, Kwong E 1965a Prevention of coprophagy in the rat and the growth stimulating effects of methionine, cystine, and penicillin when added to diets containing untreated soybeans. *J Nutr* **85** 127–131.

Barnes R H, Kwong E, Fiala G 1965b Effect of penicillin added to an untreated soybean diet on cystine excretion in feces of the rat. *J Nutr* **85** 123–126.

Blow D M, Janin J, Sweet R M 1974 Mode of action of soybean trypsin inhibitor (Kunitz) as a model for specific protein–protein interactions. *Nature* (London) **249** 54–57.

CCAC 1980 *Guide to the Care and Use of Experimental Animals* (Vol 1) (with addendum). Canadian Council on Animal Care, Ottawa, ON, Canada.

Charles M, Rovery M, Guidoni A, Desnuelle P 1963 Sur le trypsinogène et la trypsine de porc. *Biochim Biophys Acta* **69** 115–129.

Charles M, Gratecos D, Rovery M, Desnuelle P 1967 Le chymotrypsinogène A de porc: purification et études de quelques propriétés. *Biochim Biophys Acta* **140** 395–409.

Corring T 1975 Adaptation de la sécrétion du pancreas exocrine au régime alimentaire chez le porc. Physiologie comparée, étude expérimentale et mécanismes. These Doctorat D'etat Science, Paris, France.

Cozzone P, Paséro L, Marchis-Mouren G 1970 Characterization of porcine pancreatic isoamylases: separation and amino acid composition. *Biochim Biophys Acta* **200** 590–593.

Gabert V M, Sauer W C, Li S, Fan M Z 1996a Exocrine pancreatic secretions in young pigs fed diets containing faba beans (*Vicia faba*) and peas (*Pisum sativum*). Concentrations and flows of total, protein-bound and free amino acids. *J Sci Food Agric* **70** 256–262.

Gabert V M, Sauer W C, Li S, Fan M Z 1996b Exocrine pancreatic secretions in young pigs fed diets containing faba beans ((*Vicia faba*) and peas (*Pisum sativum*). Nitrogen, protein and enzyme secretions. *J Sci Food Agric* **70** 247–255.

Glazer G, Steer M L 1977 Requirements for activation of trypsinogen and chymotrypsinogen in rabbit pancreatic juice. *Anal Biochem* **77** 130–140.

Goering H K, van Soest P J 1970 *Forage Fibre Analysis* (Agric. Handbook No 379). USDA, Washington, DC, USA.

Green G M, Lyman R L 1972 Feedback regulation of a pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats. *Proc Soc Exp Biol Med* **140** 6–12.

Hamerstrand G E, Black L T, Glover J D 1981 Trypsin inhibitors in soy products: modification of the standard analytical procedure. *Cereal Chem* **58** 42–45.

Hee J, Sauer W C Berzins R, Ozimek L 1985 Permanent reentrant diversion of pancreatic secretions. *Can J Anim Sci* **65** 451–457.

Hee J, Sauer W C, Mosenthin R 1988a The effect of frequency of feeding on the pancreatic secretions in the pig. *J Anim Physiol Anim Nutr* **60** 249–256.

Hee J, Sauer W C, Mosenthin R 1988b The measurement of pancreatic secretions in the pig with the pouch technique. *J Anim Physiol Anim Nutr* **60** 241–248.

Imbeah M, Sauer W C, Mosenthin R 1988 The prediction of the digestible amino acid supply in barley–soybean meal or canola meal diets and pancreatic enzyme secretion in pigs. *J Anim Sci* **66** 1409–1417.

Jones B N, Gilligan J P 1983 *o*-Phthaldialdehyde precolumn derivatization and reverse-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J Chromatogr* **266** 471–482.

Kakade M L, Simons N, Liener I E 1969 An evaluation of natural vs synthetic substrate for measuring the antitryptic activity of soybean samples. *Cereal Chem* **46** 518–522.

Li S 1996 Enzyme supplementation and exocrine pancreatic enzyme secretions in pigs. PhD thesis, University of Alberta, Edmonton, Canada.

Li S, Sauer W C, Hardin R T 1994 Effect of dietary fibre level on amino acid digestibility in young pigs. *Can J Anim Sci* **74** 327–333.

Liener I E, Kakade M L 1980 Protease inhibitors. In: *Toxic Constituents of Plant Foodstuffs* ed Liener I E. Academic Press, New York, USA, pp 7–71.

Lowry O H, Rosebrough N J, Farr A L, Randall R J 1951 Protein measurement with the Folin phenol reagent. *J Biol Chem* **193** 265–275.

Mosenthin R, Sauer W C 1991 The effect of source of fibre on pancreatic secretions and on amino acid digestibility in the pig. *J Anim Phys Anim Nutr* **65** 45–52.

NRC 1988 *Nutrient Requirements of Swine* (9th edn). National Academy Press, Washington, DC, USA.

Ozimek L, Sauer W C, Ozimek G, Conway D M 1985 The effect of soybean protease inhibitors on ileal and faecal digestibilities and pancreas function. *Feeder's Day Report* (University of Alberta, Edmonton, AB, Canada) **64** 63–64.

Ozimek L, Sauer W C, Mosenthin R 1986 Permanent re-entrant diversion of bovine pancreatic secretions. *J Anim Sci* **63**(Suppl 1) 408.

Petersen R G 1985 *Design and Analysis of Experiments*. Marcel Dekker, New York, USA, pp 305–306.

Rick W 1974a Trypsin: measurement with N_{α}-*p*-toluene-sulphonyl-arginine methyl ester as substrate. In: *Methods of Enzymatic Analysis* (Vol. 2), ed Bergmeyer H U. Verlag Chemie Weinheim, Academic Press, New York, USA pp 1021–1024.

Rick W 1974b Chymotrypsin: measurement with *N*-benzoyl-L-tyrosine ethyl ester as substrate. In: *Methods of Enzymatic Analysis* (Vol 2), ed Bergmeyer H U. Verlag Chemie Weinheim, Academic Press, New York, USA, pp 1009–1012.

Rick W, Stegbauer H P 1974 α-Amylase measurement of reducing groups. In: *Methods of Enzymatic Analysis* (Vol 2), ed Bergmeyer H U. Verlag Chemie Weinheim, Academic Press, New York, USA, pp 885–890.

SAS 1990 *SAS*/STAT User's Guide (Release 6.08). SAS Institute, Cary, NC, USA.

Sauer W C, Jorgensen H, Berzins R 1983 A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. *Can J Anim Sci* **63** 233–237.

Schingoethe D J, Gorrill A D L, Thomas J W, Yang M G 1970 Size and proteolytic enzyme activity of the pancreas of several species of vertebrate animals. *Can J Physiol Pharmacol* **48** 43–49.

Schmidt F H, Stork H, von Dahl K 1974 Lipase: photometric assay. In: *Methods of Enzymatic Analysis* (Vol 2), ed Bergmeyer H U. Verlag Chemie Weinheim, Academic Press, New York, USA pp 819–823.

Steel R G D, Torrie J H 1980 *Principles and procedures of statistics: A Biometrical Approach* (2nd edn). McGraw-Hill, New York, USA.

WBC 1988 *Worthington Enzyme Manual*, ed Worthington C C. Worthington Biochemical Corporation, Freehold, NJ, USA.

Weström B R, Pierzynowski S G, Karlsson B W, Svendsen J 1988 Development of the exocrine pancreatic function: response to food and hormonal stimulation in pigs from birth up to after weaning. In: *Digestive Physiology in the Pig*, eds Buraczewska L, Buraczewski S, Pastuszewska B & Zebrowska T. Institute of Animal Physiology and Nutrition, Polish Academy of Science, Jablonna, Poland, pp 36–43.

Winkler F K, D'Arcy A, Hunziker W 1990 Structure of human pancreatic lipase. *Nature* **343** 771–774.

Yen J T Jensen A H, Hymowitz T, Baker D H 1973 Utilization of different varieties of raw soybeans by male and female chicks. *Poultry Sci* **52** 1875–1882.

Yen J T, Hymowitz T, Jensen A H 1974 Effects of soybeans of different trypsin inhibitor activities on performance of growing swine. *J Anim Sci* **38** 304–309.

Zebrowska T, Tanksley Jr T D, Knabe D A 1985 The influence of differently processed soybean meals on the exocrine pancreatic secretion in growing pigs. In: *Digestive Physiology in the Pig*, eds Just A, Jorgensen H & Fernandez J A. National Institute of Animal Science, Copenhagen, Denmark, pp 149–151.