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Response of Nutrient Digestibilities 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 dietary soybean trypsin inhibitor (SBTI) content on nutrient and energy digestibilities in growing pigs. Six barrows, average initial body weight (BW) $47 \cdot 8 \pm 4 \cdot 0$ kg, were fitted with a simple T-cannula at the distal ileum and fed two diets according to a crossover design. Two maize starch-based diets were formulated to contain 200 g crude protein (CP) kg^{-1} from either Nutrisoy (a food-grade defatted soy flour) or autoclaved Nutrisoy. The contents of SBTI in the Nutrisoy and autoclaved Nutrisoy diets were 13·4 and 3·0 g kg^{-1}, respectively. The experiment consisted of two periods of 12 days each. The average BW at the start of the first and second experimental periods were $53 \cdot 3 \pm 3 \cdot 7$ and $61 \cdot 0 \pm 5 \cdot 1$ kg, respectively. The average BW at the conclusion of the experiment was $71 \cdot 8 \pm 7 \cdot 6$ kg. The ileal digestibilities of dry matter (DM), organic (OM), energy, CP and all amino acids measured were higher ($P < 0 \cdot 01$) in pigs fed the autoclaved Nutrisoy diet. The energy digestibility increased from 66·0 to 77·9%, and the CP digestibility increased from 37·4 to 77·1%. The increases in ileal digestibilities of the indispensable amino acids ranged from 27·0 (methionine) to 49·2 (leucine) percentage units. The increases in ileal digestibilities of the dispensable amino acids ranged from 30·2 (aspartic acid) to 50·8 (tyrosine) percentage units. The faecal digestibilities of all criteria measured were also higher ($P < 0 \cdot 01$) in pigs fed the autoclaved Nutrisoy diet. Furthermore, there was a greater ($P < 0 \cdot 01$) net disappearance (g kg^{-1} DM intake) of DM, OM, CP, energy and all amino acids in the large intestine of pigs fed the Nutrisoy diet. In conclusion, feeding diets containing high levels of SBTI decreased both ileal and faecal digestibilities of all criteria measured. The formation of SBTI-enzyme complexes is likely to be responsible for the reduction in protein digestion and amino acid absorption.

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Key words: pigs; soybean trypsin inhibitors; amino acids; digestibility

INTRODUCTION

Feeding raw soybean or soybean trypsin inhibitors (SBTI) to chicks (Chernick *et al* 1948; Yen *et al* 1973; Herkelman *et al* 1993) and rats (Rackis 1972; Rackis *et al* 1979; Liener and Kakade 1980) causes hypertrophy of the pancreas, hypersecretion of pancreatic enzymes and a reduction in nutrient and energy digestibilities. Studies with pigs fed raw soybean did not show pancreatic hypertrophy (Yen *et al* 1977) and hypersecretion of pancreatic enzymes (Li *et al* 1997). Studies with growing pigs (Li *et al* 1997) showed that hypersecretion of pancreatic juice occurred only in terms of volume, but not total enzyme activities. A decrease in performance (eg

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Yen *et al* 1974; Cook *et al* 1988) and in faecal crude protein (CP) and energy digestibilities ($P < 0.05$) was also observed in pigs fed raw soybean (Combs *et al* 1967).

The objectives of this study (a follow up to that of Li *et al* 1997) were to determine the effects of feeding diets containing Nutrisoy and autoclaved Nutrisoy, which contain relatively high and low levels of SBTI, respectively, on ileal as well as faecal digestibilities of energy and nutrients in growing pigs.

EXPERIMENTAL PROCEDURES

Animals and diets

Six PIC (Pig Improvement Canada) barrows (Camborough × Canabrid), average initial body weight (BW) 47·8 ± 4·0 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 temperature-controlled barn (20 ± 1°C). The pigs were given *ad libitum* access to a grower diet containing 160 g CP kg^{-1} (Sauer *et al* 1983). Water was available from a low-pressure drinking nipple.

Four days later, the barrows were fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer (1976). The cannulas were modified according to De Lange *et al* (1989). The barrows were returned to the metabolism crates after surgery and fasted that same day. The next day they were given approximately 200 g of a starter diet containing 180 g CP kg^{-1} (Sauer *et al* 1983). The feed allowance was gradually increased until the pigs consumed the diet at a daily rate of 4% of their individual BW. The barrows were allowed a 9-day recuperation period. A detailed description of pre- and post-operative care was previously presented by Li *et al* (1994).

Two maize starch-based diets were formulated to contain 200 g CP kg^{-1} (dry matter (DM) basis) from either Nutrisoy or autoclaved Nutrisoy. The preparation of autoclaved Nutrisoy, formulation of the experimental diets and chemical composition of autoclaved Nutrisoy and Nutrisoy were presented previously (Li *et al* 1997).

The experiment was carried out according to a two-period crossover design (Petersen 1985). Each experimental period lasted 12 days. The pigs were fed twice daily, equal amounts, at 08:00 and 20:00 h, at a daily rate of 4% of BW which was determined at the beginning of each experimental period. The average BW of the pigs at the start of the first and second experimental periods was 53·3 ± 3·7 and 61·0 ± 5·1 kg, respectively. The average BW at the conclusion of the experiment was 71·8 ± 7·6 kg. The average daily gain (ADG) and feed/gain ratio (F/G) were determined for each pig during each experimental period. The collection of faeces was initiated at 08:00 h on day 8 of each experimental period and continued for 48 h. Ileal digesta were collected for a total of 24 h from 08:00 to 20:00 h on day 10 and from 20:00 h on day 11 to 08:00 h on day 12.

The procedure for the collection of digesta and faeces was described previously by Li *et al* (1994). Faeces and digesta were frozen at −20°C immediately after collection. The samples were pooled, leaving one sample of faeces and digesta for each pig for each experimental period. 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 the guidelines established by CCAC (1980).

Chemical analyses

Samples of digesta and faeces were freeze-dried and ground in a Wiley mill through a 0·8-mm mesh screen before analysis. Analyses for DM, organic matter (OM), energy and CP were conducted according to AOAC (1990) procedures. Analysis of chromic oxide was carried out using procedures described by Fenton and Fenton (1979). Amino acids in digesta and faeces were analysed according to principles outlined by Jones and Gilligan (1983) using a Varian 5000 high performance liquid chromatography system (Varian Canada, Mississauga, ON, Canada). This procedure was described in more detail by Sedgwick *et al* (1991) and Li and Sauer (1994).

Statistical analysis

Data were subjected to analysis of variance using the General Linear Model procedure of SAS (1990) according to a two-period crossover design (Petersen 1985). The statistical model included dietary treatments (df = 1), experimental periods (df = 1) and the interactions of treatment and period (df = 1) as sources of variation. For parameters where the interaction was not significant, the variation was combined into the error term. Least square means of treatments and periods were compared using the probability of difference (PDIFF) procedure (SAS 1990).

RESULTS AND DISCUSSION

All pigs consumed their meal allowances within 30 min after feeding throughout the experiment. Post-mortem examinations conducted at the conclusion of the experiment showed no intestinal adhesions or other abnormalities.

The incorporation of autoclaved Nutrisoy compared with Nutrisoy into the maize starch-based diet increased ($P < 0{\cdot}01$) the apparent ileal digestibilities of DM, OM, energy, CP and all amino acids (Table 1). The digestibility of methionine in the diets was corrected for methionine that was supplemented (0·15%) which is completely absorbed in the small intestine (Huisman J unpublished results, 1996). The energy digestibility increased from 66·0 to 77·9% and the CP digestibility increased from 37·4 to 77·1%. The increase in digestibility ranged from 27·0 (methionine) to 49·2 (leucine) percentage units for the indispensable amino acids. The increase in digestibility ranged from 30·2 (aspartic acid) to 50·8 (tyrosine) percentage units for the dispensable amino acids.

With the exception of methionine (58·9%), the apparent ileal digestibilities of the indispensable amino acids in the Nutrisoy diet were uniformly low, ranging from 37·1 (leucine) to 45·4% (arginine; Table 1). These results are different from studies with many other protein-containing ingredients including soybean meal and peas. For soybean meal-based diets, the apparent ileal digestibilities of arginine and lysine are relatively high, whereas that of threonine is relatively low (eg Li and Sauer 1994). Similar findings were also reported in studies with peas (eg Fan *et al* 1994). Low (1980) reported that arginine and lysine would be expected to appear first after enzymatic hydrolysis and threonine last, based on the known specificity of the proteases and peptidases involved in protein digestion. With the exception of lysine, this pattern was also observed for the autoclaved Nutrisoy diet. The relatively lower lysine digestibility may have resulted from the process of autoclaving which may reduce its digestibility as a result of the Maillard reaction (Hurrell and Carpenter 1977). From the aforementioned results, it can be speculated that SBTI and trypsin (also chymotrypsin) combined to form complexes which resulted in an overall decrease in the efficiency of protein digestion in the Nutrisoy diet. The enzyme–inhibitor complex is very strong due to the close complementary fit of these two interacting molecules. The binding force is further reinforced by a large number of non-covalent hydrophobic and hydrogen

TABLE 1

Effect of experimental diet on ileal and faecal digestibilities (%) of nutrients and energy in growing pigs

Items	*Ileal digestibilities*			*Faecal digestibilities*		
	Nutrisoy	*Autoclaved Nutrisoy*	*SE*[a]	*Nutrisoy*	*Autoclaved Nutrisoy*	*SE*[a]
Dry matter	64·3[c]	73·9[b]	1·11	87·2[c]	89·7[b]	0·47
Organic matter	67·0[c]	76·2[b]	0·95	89·2[c]	91·3[b]	0·43
Crude protein	37·4[c]	77·1[b]	2·69	77·3[c]	90·2[b]	1·38
Energy	66·0[c]	77·9[b]	1·12	87·6[c]	90·9[b]	0·60
Amino acids						
Indispensable						
Arginine	45·4[c]	90·0[b]	2·34	84·8[c]	95·6[b]	1·02
Histidine	43·9[c]	82·5[b]	2·88	85·1[c]	94·7[b]	1·19
Isoleucine	40·4[c]	86·3[b]	2·81	74·2[c]	91·3[b]	1·70
Leucine	37·1[c]	86·3[b]	2·43	75·2[c]	91·8[b]	1·67
Lysine	40·8[c]	79·6[b]	3·09	79·6[c]	90·2[b]	1·58
Methionine[d]	58·9[c]	85·9[b]	3·87	72·2[c]	89·4[b]	2·57
Phenylalanine	39·1[c]	87·8[b]	1·98	77·0[c]	92·8[b]	1·55
Threonine	36·5[c]	73·3[b]	3·96	72·4[c]	88·9[b]	2·01
Valine	38·2[c]	83·6[b]	2·46	73·6[c]	90·9[b]	1·84
Dispensable						
Alanine	43·4[c]	81·1[b]	2·78	71·9[c]	89·3[b]	2·29
Aspartic acid	42·4[c]	72·6[b]	1·92	81·1[c]	92·5[b]	1·48
Cysteine	35·5[c]	67·7[b]	4·43	76·8[c]	85·6[b]	1·23
Glutamic acid	48·6[c]	83·7[b]	1·60	86·0[c]	95·4[b]	1·03
Glycine	29·4[c]	70·2[b]	4·18	75·4[c]	89·7[b]	1·97
Serine	36·8[c]	80·6[b]	2·16	80·2[c]	92·9[b]	1·36
Tyrosine	34·1[c]	84·9[b]	3·06	72·9[c]	91·4[b]	2·27

[a] Standard error of the mean ($n = 6$).

[b,c] Means in the same row, within ileal or faecal digestibilities, with different superscripts differ ($P < 0{\cdot}01$).

[d] Digestibility after correction for dietary supplementation of methionine.

TABLE 2
Effect of experimental diet and experimental period on the net disappearance (g kg^{-1} DM intake) of nutrients and energy in the large intestine of growing pigs

Items	*Dietary treatment*			*Experimental period*		
	Nutrisoy	*Autoclaved Nutrisoy*	*SE*[a]	*Period 1*	*Period 2*	*SE*[a]
Dry matter	229·2[b]	157·4[c]	9·21	179·1	7·5	9·21
Organic matter	209·9[b]	143·3[c]	7·25	163·5	89·7	7·25
Crude protein	81·0[b]	27·1[c]	3·19	46·5[c]	61·7[b]	3·19
Energy (MJ kg^{-1})	4·1[c]	2·5[c]	0·17	2·9	3·3	0·17
Amino acids						
Indispensable						
Arginine	5·3[b]	0·7[c]	0·21	2·4[c]	3·6[b]	0·21
Histidine	1·9[b]	0·6[c]	0·09	1·2	1·3	0·10
Isoleucine	3·2[b]	0·5[c]	0·19	1·3[c]	2·4[b]	0·19
Leucine	5·7[b]	0·8[c]	0·20	2·4[c]	4·0[b]	0·20
Lysine	4·7[b]	1·2[c]	0·22	2·6	3·3	0·22
Methionine[d]	2·8[b]	0·3[c]	0·09	1·2[c]	2·0[b]	0·09
Phenylalanine	3·8[b]	0·5[c]	0·12	1·6[c]	2·6[b]	0·11
Threonine	2·6[b]	1·1[c]	0·21	1·4[c]	2·2[b]	0·21
Valine	3·3[b]	0·7[c]	0·12	1·4[c]	2·6[b]	0·12
Dispensable						
Alanine	2·4[b]	0·7[c]	0·13	1·1[c]	2·0[b]	0·13
Aspartic acid	7·6[b]	4·0[c]	0·26	4·8[c]	6·9[b]	0·26
Cysteine	3·6[b]	0·6[c]	0·13	1·5[c]	2·7[b]	0·13
Glutamic acid	13·1[b]	4·1[c]	0·33	6·9[c]	10·3[b]	0·33
Glycine	3·9[b]	1·6[c]	0·28	2·3	3·2	0·28
Serine	4·0[b]	1·1[c]	0·13	2·1[c]	3·0[b]	0·13
Tyrosine	2·1[b]	0·3[c]	0·10	0·9[c]	1·5[b]	0·09

[a] Standard error of the mean ($n = 6$).
[b,c] Means in the same row, within treatment or periods, with different superscript letters differ ($P < 0{\cdot}01$).
[d] Net disappearance after correction for dietary supplementation of methionine.

bonds (Blow *et al* 1974; Sweet *et al* 1974). Based on the very low ileal amino acid digestibilities in the Nutrisoy diet, only small amounts of free trypsin and chymotrypsin may have been available for digestion.

The incorporation of autoclaved Nutrisoy compared with Nutrisoy also increased ($P < 0{\cdot}01$) the apparent faecal digestibilities of all parameters measured (Table 1). The energy digestibility increased from 87·6 to 90·9% and the CP digestibility increased from 77·3 to 90·2%. The increase in digestibility ranged from 9·6 (histidine) to 17·3 (valine) percentage units for the indispensable amino acids. The increase in digestibility ranged from 8·8 (cysteine) to 18·5 (tyrosine) percentage units for the dispensable amino acids.

As pointed out previously, the ileal rather than faecal analysis method should be used to determine amino acid digestibilities in feedstuffs for pigs because of the modifying action of the microflora in the large intestine (Sauer and Ozimek 1986). The differences in amino acid digestibilities between dietary treatments measured with the ileal analysis method were considerably larger than those measured with the faecal analysis method (Table 1), which shows that the ileal analysis method is more sensitive than the faecal analysis method for detecting differences in amino acid digestibilities. The improved sensitivity of the ileal analysis method was also observed by Sauer *et al* (1977) and Vandergrift *et al* (1983) in studies with feedstuffs that were processed differently. In contrast, the digestibility of energy should be determined with the faecal analysis method, because there can be considerable disappearance of energy in the large intestine in the form of volatile fatty acids. The extent of the disappearance is dependent on diet composition (eg Sauer *et al* 1980; Fan *et al* 1994).

Compared with pigs fed the autoclaved Nutrisoy diet, pigs fed the Nutrisoy diet had a lower ADG and higher F/G ratio. The ADG for pigs fed the Nutrisoy and autoclaved Nutrisoy diets were 523 and 780 g, respectively. In the same order of the dietary treatments, the F/G ratio was 5·0 and 2·8, respectively. Some caution should be exercised in the interpretation of the performance results, as these are based only on a 14-day

TABLE 3
Effects of experimental period on ileal and faecal digestibilities (%) of nutrients and energy in growing pigs

Items	*Ileal digestibilities*			*Faecal digestibilities*		
	Period 1	*Period 2*	*SE*[a]	*Period 1*	*Period 2*	*SE*[a]
Dry matter	69·6	68·6	1·11	87·6[c]	89·3[b]	0·47
Organic matter	72·1	71·1	0·95	89·4[c]	91·1[b]	0·43
Crude protein	59·7	54·8	2·69	82·5	85·1	1·38
Energy	72·6	71·3	1·13	88·5	90·0	0·60
Amino acids						
Indispensable						
Arginine	70·6	64·8	2·34	88·4	92·0	1·02
Histidine	62·5	64·0	2·88	88·1	91·7	1·19
Isoleucine	66·8	59·8	2·81	80·2	85·3	1·69
Leucine	64·7	59·0	2·43	80·9	86·0	1·67
Lysine	61·3	59·1	3·09	82·8	87·0	1·57
Methionine[d]	74·8	70·0	3·88	77·1[b]	84·5[a]	2·57
Phenylalanine	66·1	60·8	1·98	82·5	87·3	1·55
Threonine	58·3	51·5	3·97	78·5	82·8	2·01
Valine	64·9	56·9	2·46	79·9	84·6	1·84
Dispensable						
Alanine	65·3	59·2	2·78	78·1	83·1	2·29
Aspartic acid	61·6[b]	53·3[c]	1·92	85·6	88·0	1·48
Cysteine	57·8	45·3	4·75	76·9[c]	85·5[b]	1·23
Glutamic acid	69·7[b]	62·5[c]	1·60	89·4	91·9	1·03
Glycine	52·9	46·7	4·17	80·7	84·4	1·97
Serine	62·0	55·4	2·16	84·8	88·3	1·36
Tyrosine	62·5	56·5	3·06	79·9	84·4	2·27

[a] Standard error of the mean ($n = 6$).
[b,c] Means in the same row, within ileal or faecal digestibilities, with different superscripts differ ($P < 0{\cdot}05$).
[d] Digestibility after correction for dietary supplementation of methionine.

measurement. However, the differences in performance reflect differences in energy and amino acid digestibilities between dietary treatments. The results are in agreement with those reported in the literature in which diets containing raw soybean were compared with soybean meal. A decrease in performance, nitrogen retention and nutrient digestibilities were reported in studies with young (Yen *et al* 1974; Cook *et al* 1988), growing (Combs *et al* 1967) and growing-finishing pigs (Jimenez *et al* 1963; Young 1967) when diets containing raw soybean were fed.

There was a higher ($P < 0{\cdot}01$) net disappearance (g kg^{-1} DM intake) of DM, OM, CP, energy and all amino acids in the large intestine of pigs fed the Nutrisoy than with the autoclaved Nutrisoy diet (Table 2). For CP, the net disappearance was 53·9 g greater; for energy, the net disappearance was 1·6 MJ kg^{-1} greater. The disappearance of energy in the large intestine corresponded to 21·8% of the energy intake in pigs fed the Nutrisoy and only 13·3% in pigs fed the autoclaved Nutrisoy diet, respectively. For the indispensable amino acids, the differences in net disappearance between dietary treatments ranged from 1·3 (histidine) to 4·9 g (leucine) and for the dispensable amino acids from 1·7 (alanine) to 9·0 g (glutamic acid). The larger disappearance of amino acids in the large intestine of pigs fed the Nutrisoy as opposed to the autoclaved Nutrisoy diet will have no effect on the protein utilisation by the pig. Zebrowska *et al* (1973) showed that microbial fermentation of undigested protein (and unabsorbed amino acids and peptides) in the large intestine yielded nitrogen-containing compounds, mainly in the form of ammonia. Once absorbed, ammonia will be converted to urea and excreted in urine. However, the higher disappearance of energy in the large intestine in pigs fed the Nutrisoy diet will be of partial benefit, because microbial fermentation of undigested energy sources in the large intestine will yield volatile fatty acids which will be used by the pig as supplementary energy source. In terms of energy utilisation, these results indicate a compensatory effect by the action of microflora. This compensatory effect will likely be important in pigs fed diets high in non-starch polysaccharides, in particular when dietary energy supply is limiting.

There was a greater ($P < 0{\cdot}05$) net disappearance of CP and most of the amino acids during period 2 (Table 2). With the exception of aspartic and glutamic acid, there were no differences ($P > 0{\cdot}05$) in the apparent ileal digestibilities of all amino acids between experimental periods. With the exception of cysteine and methionine, there were no differences between periods in the faecal digestibilities of all amino acids (Table 3). These results are in agreement with previous studies using pigs of the same BW range that showed no period effects on apparent ileal (eg Fan *et al* 1994) or faecal digestibilities (eg Imbeah and Sauer 1991).

As shown in a previous study (Li *et al* 1997), the feeding of Nutrisoy compared with autoclaved Nutrisoy did not affect ($P > 0{\cdot}05$) the total activities of the exocrine pancreatic enzymes in growing pigs, indicating the absence of hypersecretion of pancreatic enzymes, which was demonstrated in studies with chicks (eg Chernick *et al* 1948) and rats (eg Rackis *et al* 1979). The large increase in the recovery of amino acids in ileal digesta when the Nutrisoy diet was fed is likely caused by the formation of complexes between SBTI and trypsin and chymotrypsin. The formation of these complexes will increase both endogenous (of animal origin) and exogenous (of dietary origin) recoveries of amino acids. The increased endogenous recovery of amino acids likely results from a decrease in autodigestion of these enzymes in the small intestine. The increased exogenous recovery of amino acids results from a decrease in digestion of exogenous protein due to a decrease in the available (not complexed) supply of trypsin and chymotrypsin.

In conclusion, the ileal as well as faecal digestibilities of energy and amino acids were considerably lower in pigs fed the Nutrisoy compared with the autoclaved Nutrisoy diet. There was also a greater disappearance of energy and amino acids in the large intestine of pigs fed the Nutrisoy diet. The lower ileal digestibilities in pigs fed the Nutrisoy diet are likely due to the formation of complexes between SBTI and trypsin and chymotrypsin, which results in a decrease in the available supply of these enzymes and a decrease in the efficiency of protein digestion. Further studies may be warranted to partition the effect of SBTI on exogenous and endogenous amino acid losses.

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