Effects of diets containing casein and rapeseed on enzyme secretion from the exocrine pancreas in the pig

BY P. VALETTE[1], H. MALOUIN[2], T. CORRING[1], L. SAVOIE[2], A. M. GUEUGNEAU[1] AND S. BEROT[1]

[1] *Laboratoire d'Ecologie et de Physiologie du Système Digestif, INRA-CRJ, 78352 Jouy en Josas cédex, France*

[2] *Departement de nutrition humaine et de consommation FSAA, Université Laval, Québec, Canada G1K 7P4*

(*Received 6 March 1990 – Accepted 22 May 1991*)

The effect of dietary protein on enzyme activity of pancreatic juice was studied in ten growing, castrated, Large White male pigs. Animals, fitted with permanent cannulas in the pancreatic duct and in the duodenum, were divided into two groups receiving either casein or rapeseed concentrate as a protein source. After a 15 d adaptation period to the experimental diet, the volume of pancreatic secretion was significantly higher, whereas the protein concentration was lower in the casein group compared with the rapeseed group. No statistical difference was observed in the daily protein output between groups. Total secreted activities of carboxypeptidase A (*EC* 3.4.17.1), and elastase (*EC* 3.4.21.36) were higher in the casein group during the nocturnal period, whereas total activities of trypsin (*EC* 3.4.21.4), chymotrypsin (*EC* 3.4.21.1), carboxypeptidase B (*EC* 3.4.17.2) and amylase (*EC* 3.2.1.1) in pancreatic secretions during the post-prandial periods were increased by the ingestion of the rapeseed diet. It is concluded that the pancreatic enzyme secretion is sensitive to the nature of the protein ingested.

Dietary proteins: Casein: Rapeseed: Pancreatic juice: Pigs

Several reports have demonstrated that dietary composition can modulate pancreatic secretion. The activities of proteolytic enzymes in either pancreatic tissue or juice are modified by the protein content of the diet, while amylase (*EC* 3.2.1.1) and lipase (*EC* 3.1.1.3) activities are respectively dependent on the carbohydrate and lipid contents (Corring, 1980). Kinetic studies have shown that these variations in pancreatic tissue enzyme concentrations are observed within 24 h of a sudden change in diet composition and that steady-state levels were attained about 5 or 6 d after the dietary change (Ben Abdeljlil & Desnuelle, 1964).

Each enzyme behaves differently, depending on the amount and the type of substrate ingested. In rat (Keim, 1986), pig (Corring, 1979) or man (Bucko *et al.* 1982), trypsin (*EC* 3.4.21.4) and chymotrypsin (*EC* 3.4.21.1) activities increase with a protein-rich diet, chymotrypsin being more sensitive to this dietary modulation. Giorgi *et al.* (1985) have shown that the intrapancreatic activity of carboxypeptidase A (*EC* 3.4.17.1) remains unaffected by an increase from 150 to 700 g/kg in dietary protein content, while trypsin, chymotrypsin and elastase (*EC* 3.4.21.36) activities increase 1·4, 2·8 and 2·0 times respectively and concluded that adaptability to dietary protein seemed to be a characteristic of the serine protease family.

Pancreatic proteolytic enzyme secretion is also sensitive to the type of protein source in the diet. According to Snook & Meyer (1964), a diet containing 150 g whole-egg protein/kg results in an increase in the synthesis and secretion of trypsin and chymotrypsin when

Table 1. *Composition of experimental diets (g/kg dry matter (DM))**

	Casein	Rapeseed
Casein	139	—
Rapeseed concentrate	—	203
Peanut oil	60	30
Maize starch	655	656
Purified cellulose	60	25
Vermiculite	50	50
Mineral mix†	25	25
Vitamin mix‡	10	10
Antioxidant (butyl hydroxytoluene)	1	1
Crude fibre content	60·6	62·0
Total protein content	120	120
Total lipid content	60	30
Total starch content	65·5	66·2

* Containing as dry matter (g/kg fresh matter): casein 892·6, rapeseed 886·9; energy content (kJ/g DM): casein 18·0, rapeseed 17·6, as determined with the automatic adiabatic calorimeter (Gallenkemp, UK).

† Supplying (/kg DM): dicalcium phosphate 10 g, sea salt 3 g, potassium chloride 4 g, magnesium carbonate 2 g, iron sulphate 500 mg, manganese sulphate 160 mg, copper sulphate 40 mg, zinc sulphate 440 mg, potassium iodide 450 μg, sodium selenite 600 μg.

‡ Supplying (/kg DM): retinol 1·5 mg, cholecalciferol 25 μg, vitamin E 22 mg, vitamin K (menadione) 4·4 mg, thiamin hydrochloride 3·3 mg, riboflavin 6 mg, niacin 36 mg, calcium pantothenate 30 mg, pyridoxine hydrochloride 3 mg, pteroyl monoglutamic acid 2 mg, inositol 200 mg, p-aminobenzoic acid 20 mg, biotin 300 μg, cyanocobalamin 30 μg, choline concentrate 6 g.

compared with a 150 g casein/kg diet. It was postulated that the response to whole-egg protein was related to the high biological quality of this protein and to its content of egg-white trypsin inhibitor (Snook, 1969). Johnson *et al.* (1977) have shown that when low-quality proteins are fed, no enzymic response occurs even with a high-protein diet and they have concluded that proteins of a high quality are necessary to elevate enzyme synthesis.

The aim of the present experiment was to study the effect of two different dietary proteins, casein and rapeseed, which have a high biological yield but specific hydrolysis kinetics (Savoie *et al.* 1988), on the enzyme activity of exocrine secretions from the pig pancreas.

METHODS

Animals and diets

Ten growing, castrated, Large White male pigs were divided into two groups fed on a diet containing either casein protein (sodium caseinate; Union des Caséineries Coopératives de Charente Poitou; 863 g protein/kg dry matter (DM)) or rapeseed-concentrate protein (Protein concentrate 00 Tandem; Cetiom; 592 g protein/kg DM) as a protein source. The composition of the diets is given in Table 1. The two diets were isonitrogenous and they were made isoenergetic in gross energy by adjusting the fat and cellulose contents. The feed was provided in two equal meals of 800 g DM each (food–water; 1:1, w/v) at 09.00 and 16.00 hours.

Experimental design

The pigs were adapted to their experimental diets for 8 d before surgery. The mean live weight was 38·5 (SE 3·4) kg for the group fed on the casein diet and 36·9 (SE 5·1) kg for the group fed on the rapeseed diet. Under halothane anaesthesia animals were prepared for complete collection of pancreatic juice, by a direct cannulation of the pancreatic duct, and

for its subsequent return into the duodenum (Corring *et al.* 1972). This involved the insertion of a polyethylene catheter into the pancreatic duct immediately above its point of entry into the duodenum. The duct was then tightly closed around the catheter by a suture. A second catheter was inserted into the duodenum, close to the normal point of entry of pancreatic juice. Both catheters were exteriorized through stab wounds in the flank. The macroscopic and histological examinations of the pancreas from pigs fitted with such a pancreatic fistula for up to 1 month did not reveal any abnormality (values not shown).

After surgery, pigs were kept in individual metabolism-type cages and given their diets during a 7 d recovery period followed by a 5 d experimental period.

Measurement of pancreatic secretion

The catheters for pancreatic juice collection and return were connected to an apparatus which re-introduced the secreted pancreatic juice immediately into the duodenum and gave automatically a precise record of its volume (Juste *et al.* 1983). During the recovery period, pancreatic juice was re-introduced entirely into the duodenum and, during the experimental period, a sample (100 ml/l) was withdrawn continuously for analysis. Hourly samples were pooled in three periods: post-prandial secretions from 09.00 to 16.00 hours (period 1) and from 16.00 to 23.00 hours (period 2) and basal secretion from 23.00 to 09.00 hours (period 3). These periods were chosen to determine the possible effect of digestive state on the pancreatic secretion. The samples were stored at $-20°$ until analysis.

Analysis

Total proteins were determined according to Lowry *et al.* (1951). Lipase activity was measured by titrimetry at pH 9 according to the method of Rathelot *et al.* (1975). Amylase activity was determined according to the modified method of Metais & Bieth (1968) on a biological sample containing a high amylase activity (Corring & Saucier, 1972). Chymotrypsinogen (Reboud *et al.* 1962) and proelastase (Gertler & Hofmann, 1970) were activated with a trypsin solution (1 mg trypsin/ml 0·001 M-hydrochloric acid) added at 60 mg/g total protein in the juice sample. Trypsinogen (Reboud *et al.* 1962) and procarboxypeptidases A and B (Yamasaki *et al.* 1963) were activated with the same trypsin solution added at 100 mg/g total protein in the juice sample. Chymotrypsin and trypsin activities were determined by titrimetry at pH 7·9 on *N*-acetyl-L-tyrosine ethyl ester (ATEE) and *N*-benzoyl-L-arginine ethyl ester (BAEE) substrates, respectively (Reboud *et al.* 1962). Elastase activity was measured by titrimetry at pH 7·9 on N-acetyl L-alanyl L-alanine methyl ester substrate according to the method of Gertler & Hofmann (1970). Carboxypeptidase A activity was determined by titrimetry at pH 7·9 on hippuryl-phenyl lactic acid (HPLA) substrate according to the method of Yamasaki *et al.* (1963). A spectrophotometric method was used for the determination of carboxypeptidase B (*EC* 3.4.17.2) activity according to the method of Folk *et al.* (1960). All enzymic activities were expressed as total activities secreted in pancreatic juice (μmol substrate hydrolysed/min).

Statistical analysis

Differences between means were assessed by Student's *t* test following analysis of variance (Steel & Torrie, 1980). These calculations were performed with a statistical software (Genstat, developed by the Rothamsted Experimental Station, Harpenden, Herts, UK).

RESULTS

All feed offered was consumed by the animals and the average weight gain of all animals was approximately 15 kg during the first 4 weeks after surgery.

The volume of pancreatic juice collected over 24 h was significantly higher (+29%, $P < 0.05$) in the casein-fed animals (Table 2). Differences between casein- and rapeseed-fed animals were observed at each of the three experimental periods, being significant for the first post-prandial period (+22%, $P < 0.05$) and the nocturnal period (+40%, $P < 0.05$). The mean daily concentration of total protein in pancreatic juice was not significantly different between casein- and rapeseed-fed pigs (Table 2), although total protein concentration was significantly ($P < 0.05$) lower in the casein-fed pigs during the period 09.00 to 16.00 hours.

The total protein output per 24 h in pancreatic juice was not significantly affected by the ingested protein (Table 2).

The activities of the proteolytic enzymes, secreted in pancreatic juice, were not affected in the same manner by the two protein diets (Table 3). The activities of trypsin, chymotrypsin and carboxypeptidase B secreted in pancreatic juice were higher in rapeseed-fed animals with these effects being significant ($P < 0.05$) in the first post-prandial period for trypsin and during the first two post-prandial periods and the 24 h mean, for chymotrypsin and carboxypeptidase B. This effect of the rapeseed diet on the secreted activities of the three enzymes appeared to be diminished during the nocturnal period. The mean daily total secreted activities of elastase and carboxypeptidase A were lower on the rapeseed diet principally due to a significant ($P < 0.05$) decrease in the secreted activities of these enzymes during the nocturnal period.

Total secreted lipase activity was not significantly modified by the ingested protein. But, the daily total activity of amylase was significantly higher in rapeseed-fed pigs (+63%, $P < 0.05$), values being significantly higher in each of the two post-prandial periods (Table 3).

DISCUSSION

The present study demonstrates that the volume and enzyme concentration of pancreatic juice secretion can be influenced by the consumption of diets containing different proteins.

The primary response was a 29% increase in pancreatic juice daily flow-rate in the casein group. The mechanisms responsible for this increased secretory rate are not known but may be related to the buffer capacity of protein sources. O'Hare *et al.* (1984) showed that the buffer capacity varied from one protein to another. For example, casein, a phosphoserine-rich protein, has a higher buffering capacity than rapeseed concentrate whose mineral content decreases during preparation (Berot & Briffaud, 1983). In order to attain optimum pH conditions for enzyme activation and activity, the pancreas may have to secrete more bicarbonate with a casein diet, which would explain the increase in water secretion.

Our study showed that total protein secretion in pancreatic juice was not modified by the nature of dietary protein ingested by the pig. These findings are consistent with those of Partridge *et al.* (1982) and Zebrowska *et al.* (1983) who demonstrated, in the pig, that the daily amount of secreted protein remains constant whatever the source of dietary protein (e.g. barley, wheat and fish protein or barley and soya-bean protein). Our results concerning enzyme activities do not support those obtained by Partridge *et al.* (1982) and Zebrowska *et al.* (1983). They demonstrated that the consumption of diets differing in protein source did not significantly modify the activities of trypsin, chymotrypsin, carboxypeptidase A and carboxypeptidase B secreted in pancreatic juice. However, comparisons of the present work with previous studies is complicated by the use of two semi-purified diets in our studies, whereas Partridge *et al.* (1982) and Zebrowska *et al.* (1983) have examined a purified diet containing casein as protein source and a practical growing-pig diet based on barley, wheatings and fish meal. The difference in physical form between the diets in the previous work could be responsible for some modifications of the pancreatic secretion (Malagelada,

Table 2. *Pancreatic juice volume, protein concentration and output in the pancreatic juice of pigs adapted to casein or rapeseed-concentrate diet*†

(Mean values with their standard errors for five pigs given the diets for five experimental days per pig)

		Casein		Rapeseed	
Diets	Experimental periods	Mean	SE	Mean	SE
Volume (ml)	p 1	585	39	460*	32
	p 2	526	49	479	28
	p 3	1238	142	736*	62
	24 h‡	2383	215	1677*	109
Protein concentration (mg/ml)	p 1	7·06	0·76	12·79*	1·30
	p 2	8·04	0·85	11·28	1·18
	p 3	4·63	0·66	5·30	0·46
	24 h‡	6·11	0·79	8·76	0·98
Protein output (g)	p 1	4·50	0·43	5·87	0·72
	p 2	4·09	0·37	5·62	0·74
	p 3	4·60	0·42	4·22	0·57
	24 h‡	13·22	0·98	15·58	2·00

p 1, 09.00–16.00 hours; p 2, 16.00–23.00 hours; p 3, 23.00–09.00 hours.

* Mean values were significantly different from those for casein diet: * $P < 0·05$.

† For details of diets, see Table 1 and for details of procedures, see pp. 216–217.

‡ For volume and protein output, 24 h period is the sum of p 1, p 2 and p 3; for protein concentration, 24 h period is the mean of p 1, p 2 and p 3.

1980). The results indicate that the activities of trypsin, chymotrypsin and carboxypeptidase B secreted in pancreatic juice are increased on the rapeseed diet during the post-prandial periods but that elastase and carboxypeptidase A are decreased during the nocturnal period on this diet relative to pigs fed on the casein diet. Our data do not allow an explanation of these results.

As regards the other enzymes, amylase activity was higher in the rapeseed-fed pigs. An increase in amylase activity has been previously described after a low-protein high-carbohydrate diet in the pig (Corring & Saucier, 1972) and in the rat (Temler *et al.* 1984). The starch content of the rapeseed diet is not very different from that of the casein diet and, therefore, cannot explain the higher amylase activity in pancreatic juice of animals fed on the rapeseed diet. Lipase activity was not significantly affected by casein or rapeseed diet even though the casein diet contained twice as much lipid as the rapeseed diet in order to make the diets isoenergetic. This result is not in agreement with previous studies which showed an increase in lipase activity when dietary lipid intake is increased in pigs and rats (Mourot & Corring, 1979; Sabb *et al.* 1986).

In published studies, authors have compared protein sources in which biological values were different (i.e. casein, zein or wheat gluten) (Johnson *et al.* 1977) or protein sources which contained trypsin inhibitor (Green & Nasset, 1983; Richter & Schneeman, 1987), the latter being a potential activator of pancreatic secretion in several species (Schneeman *et al.* 1977; Struthers & McDonald, 1983; Temler *et al.* 1984). To our knowledge, no study has been performed where both proteins studied are of high biological value.

The total amount of pancreatic proteins secreted during 24 h was not significantly different in the two groups, but the enzyme activities, secreted in pancreatic juice, were significantly modified by the type of protein ingested. It appears that the pancreatic proteolytic 'digestive' activity was modified by the dietary protein, which is interesting

Table 3. *Total enzyme activities in the pancreatic juice of pigs adapted to casein or rapeseed-concentrate diet*† (*μmol substrate hydrolysed/min*)

(Mean values with their standard errors for five pigs given the diets for five experimental days per pig)

Diet	Experimental periods	Trypsin (*EC* 3.4.21.4)		Chymotrypsin (*EC* 3.4.21.1)		Elastase (*EC* 3.4.21.36)		Carboxypeptidase A (*EC* 3.4.17.1)		Carboxypeptidase B (*EC* 3.4.17.2)		Lipase (*EC* 3.1.1.3)		Amylase (*EC* 3.2.1.1)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Casein	p 1	20·9	2·2	149·2	10·1	9·60	0·91	89·5	5·1	5·93	0·74	158·9	22·0	8250	1873
	p 2	19·0	2·0	136·0	11·4	7·28	0·75	82·7	7·4	4·69	0·61	146·9	18·3	7805	691
	p 3	21·8	2·0	167·3	13·3	10·65	1·01	105·2	8·9	6·67	0·67	163·6	17·2	7892	732
	24 h‡	62·0	5·5	453·3	26·0	27·56	2·26	279·8	15·7	17·40	2·19	473·1	50·5	23948	1749
Rapeseed	p 1	33·2*	4·8	238·0*	26·8	9·84	1·08	83·2	10·5	10·08*	2·00	158·2	20·0	14690*	2555
	p 2	28·9	4·6	232·3*	29·4	7·57	0·80	83·6	12·6	9·31*	2·02	152·6	21·0	14351*	2698
	p 3	22·7	3·3	180·3	22·7	7·78*	1·03	66·4*	8·4	6·86	1·30	119·0	14·2	10960	2031
	h‡	83·8	10·9	647·4*	75·4	24·62	2·77	232·3	30·5	26·16*	5·52	429·8	55·4	39825*	7352

p 1, 09.00–16.00 hours; p 2, 16.00–23.00 hours; p 3, 23.00–09.00 hours.
Mean values were significantly different from those for casein diet: * $P < 0{\cdot}05$.
† For details of diets, see Table 1 and for details of procedures, see pp. 216–217.
‡ 24 h period is the sum of p 1, p 2 and p 3.

from a nutritional point of view. However, the present work does not indicate whether this effect was due to a change in enzyme biosynthesis or proteolytic activity of the enzyme. The results of the present study have relevance to the use of in vitro techniques to study the rates of proteolysis of different dietary proteins since the type of protein in the basal diet fed to pigs donating pancreatic juice may influence the rates of hydrolysis of different proteins. It is not known to what extent the differences in enzyme activities between casein and rapeseed pancreatic juices are sufficient to modify the in vitro hydrolysis kinetics of these proteins.

The authors wish to thank Georgette Brachet and F. Cointepas for animal care. This work was supported by a grant from 'Programme France–Québec sur les biotechnologies'.

REFERENCES

Ben Abdeljlil, A. & Desnuelle, P. (1964). Sur l'adaptation des enzymes exocrines du pancréas à la composition du régime. (The adaptation of the exocrine enzymes of the pancreas to the composition of the diet.) *Biochimica et Biophysica Acta* **81**, 136–149.

Berot, S. & Briffaud, J. (1983). Parameters for obtaining concentrates from rapeseed and sunflower meal. *Qualitas Plantarum: Plant Foods for Human Nutrition* **33**, 237–242.

Bucko, A., Kopec, Z., Oveocka, M. & Grunt, J. (1982). Adaptability of pancreatic enzymes activity to various food nutritive values in man. Influence of high protein diet. *Die Nährung* **26**, 59–64.

Corring, T. (1979). Sécrétion du pancréas exocrine et régulations nutritionelles. (Pancreatic exocrine secretion and dietary regulation.) *Médecine et Nutrition* **XI**, 25–30.

Corring, T. (1980). The adaptation of digestive enzymes to the diet: its physiological significance. *Reproduction, Nutrition, Développement* **20**, 1217–1235.

Corring, T., Aumaître, A. & Rerat, A. (1972). Fistulation permanente du pancréas exocrine chez le porc. Application: réponse de la sécrétion pancréatique au repas. (Permanent pancreatic fistulation in the pig. Secretory response to meal ingestion.) *Annales de Biologie Animale, Biochimie et Biophysique* **12**, 109–124.

Corring, T. & Saucier, R. (1972). Sécrétion pancréatique sur porc fistulé: adaptation à la teneur en protéines du régime. (Pancreatic secretion of the fistulated pig. Adaptation to protein content of the diet.) *Annales de Biologie Animale, Biochimie et Biophysique* **12**, 233–241.

Folk, J. E., Piez, K. A., Carroll, W. R. & Gladner, J. A. (1960). Carboxypeptidase B. VI. Purification and characterization of the porcine enzyme. *Journal of Biological Chemistry* **235**, 2272–2277.

Gertler, A. & Hofmann, T. H. (1970). Acetyl-L-alanyl-L-alanine methyl ester: a new highly specific elastase substrate. *Canadian Journal of Biochemistry* **48**, 384–386.

Giorgi, D., Renaud, W., Bernard, J. P. & Dagorn, J. C. (1985). Regulation of proteolytic enzyme activities and mRNA concentrations in rat pancreas by food content. *Biochemical and Biophysical Research Communications* **127**, 937–942.

Green, G. M. & Nasset, E. S. (1983). Role of dietary protein in rat pancreatic enzyme secretory response to a meal. *Journal of Nutrition* **113**, 2245–2252.

Johnson, A., Hurwitz, R. & Kretchmer, N. (1977). Adaptation of rat pancreatic amylase and chymotrypsinogen to changes in diet. *Journal of Nutrition* **107**, 87–96.

Juste, C., Corring, T. & Le Coz, Y. (1983). Bile restitution procedure for studying bile secretion in fistulated pigs. *Laboratory Animal Sciences* **33**, 199–202.

Keim, V. (1986). Rapid adaptation of pancreatic enzyme secretion in the conscious rat. II. Effects of fasting and dietary modulation. *Annals of Nutrition and Metabolism* **30**, 113–119.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Malagelada, J. R. (1980). Pathophysiological responses to meals in the Zollinger–Ellison syndrome. 2. Gastric emptying and its effect on duodenal function. *Gut* **21**, 98–104.

Metais, P. & Bieth, J. (1968). Détermination de l'α-amylase par une microtechnique. (Determination of α-amylase using a microtechnique.) *Annales de Biologie Clinique* **26**, 133–142.

Mourot, J. & Corring, T. (1979). Adaptation of the lipase-colipase system to dietary lipid content in pig pancreatic tissue. *Annales de Biologie Animale, Biochimie et Biophysique* **19**, 119–124.

O'Hare, W. T., Curry, M. C. & Allen, J. C. (1984). Effect of buffering capacity on a commonly used assay of protein digestibility. *Journal of Food Science* **49**, 498–499.

Partridge, I. G., Low, A. G., Sambrook, I. E. & Corring, T. (1982). The influence of diet on the exocrine pancreatic secretion of growing pigs. *British Journal of Nutrition* **48**, 137–145.

Rathelot, J., Julien, R., Canioni, P., Coeroll, C. & Sarda, L. (1975). Studies on the effect on bile salt and colipase on enzymatic lipolysis. Improved method for the determination of pancreatic lipase and colipase. *Biochimie* **57**, 1117–1122.

Reboud, J. P., Ben Abdeljlil, A. & Desnuelle, P. (1962). Variations de la teneur en enzymes du pancréas de rat en fonction de la composition des régimes. (Variations of the enzymic content of rat pancreas as a function of the composition of the diet.) *Biochimica et Biophysica Acta* **58**, 326–337.

Richter, B. D. & Schneeman, B. O. (1987). Pancreatic response to long-term feeding of soy protein isolate, casein or egg-white in rats. *Journal of Nutrition* **117**, 247–252.

Sabb, J. E., Godfrey, P. M. & Brannon, P. M. (1986). Adaptive response of rat pancreatic lipase to dietary fat: effects of amount and type of fat. *Journal of Nutrition* **116**, 892–899.

Savoie, L., Galibois, I., Parent, G. & Charbonneau, R. (1988). Sequential release of amino acids and peptides during in vitro digestion of casein and rapeseed proteins. *Nutrition Research* **8**, 1319–1326.

Schneeman, B. O., Chang, I., Smith, L. B. & Lyman, R. L. (1977). Effect of dietary amino acids, casein, and soybean trypsin inhibitor on pancreatic protein secretion in rats. *Journal of Nutrition* **107**, 281–288.

Snook, J. T. (1969). Factors in whole egg protein influencing dietary induction of increases in enzyme and RNA levels in rat pancreas. *Journal of Nutrition* **97**, 286 294.

Snook, J. T. & Meyer, J. H. (1964). Response of digestive enzymes to dietary protein. *Journal of Nutrition* **82**, 409 414.

Steel, R. C. D. & Torrie, J. H. (1980). *Principles and Procedures of Statistics*, 2nd ed. New York: McGraw-Hill.

Struthers, B. J. & McDonald, J. R. (1983). Comparative inhibition of trypsins from several species by soybean trypsin inhibitors. *Journal of Nutrition* **113**, 800–804.

Temler, R. S., Dormond, C. A., Simon, E., Morel, B. & Mettraux, C. (1984). Response of rat pancreatic proteases to dietary proteins, their hydrolysates and soybean trypsin inhibitor. *Journal of Nutrition* **114**, 270–278.

Yamasaki, M., Brown, J. R., Cox, D. I., Greenshields, R. N., Wade, R. D. & Neurath, H. (1963). Procarboxypeptidase A-S6. Further studies of its isolation and properties. *Biochemistry* **2**, 859–866.

Zebrowska, T., Low, A. G. & Zebrowska, H. (1983). Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pig. *British Journal of Nutrition* **49**, 401–410.

Printed in Great Britain