Effect of condensed tannins in hulls of faba beans (*Vicia faba* L.) on the activities of trypsin (*EC* 2.4.21.4) and chymotrypsin (*EC* 2.4.21.1) in digesta collected from the small intestine of pigs

BY A. J. M. JANSMAN[1,2], H. ENTING[1]*, M. W. A. VERSTEGEN[1]
AND J. HUISMAN[2]

[1] *Agricultural University, Department of Animal Nutrition, Haagsteeg 4, 6708 PM Wageningen, The Netherlands* [2] *TNO Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology (ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands*

The effects of condensed tannins in hulls of faba beans (*Vicia faba* L.) on the activities of trypsin (*EC* 3.4.21.4) and chymotrypsin (*EC* 3.4.21.1) in digesta obtained from the small intestine of pigs were studied. Using four castrated male pigs (mean body weight 83 kg) fitted with both a simple T-cannula in the duodenum and a post-valvular T-cannula at the terminal ileum, two experimental diets were tested in a Latin square design. The low-tannin diet (LT) contained 200 g faba bean hulls (cv. Blandine)/kg with a low content of condensed tannins (< 0·1% catechin equivalents). The high-tannin diet (HT) contained 200 g faba bean hulls (cv. Alfred)/kg with a content of condensed tannins of 3·5% catechin equivalents. Spot samples of fresh duodenal digesta were taken daily at fifteen time points between 08.00 and 20.00 hours on four consecutive days. Ileal digesta were collected nearly quantitatively on the same days between 08.00 and 20.00 hours over periods of 2 h. Trypsin and chymotrypsin activities in duodenal digesta did not differ between treatments at any time point ($P > 0.05$). In ileal digesta of pigs given diet HT the mean activity of trypsin was reduced ($P < 0.05$). The activity of chymotrypsin in ileal digesta did not differ between treatments. Trypsin activity:chymotrypsin activity was somewhat lower in ileal digesta of pigs receiving the HT diet ($P < 0.10$). The apparent ileal digestibility of crude protein ($N \times 6.25$) was lower for the HT than for the LT diet (0·614 *v.* 0·728; $P < 0.05$). Condensed tannins are probably responsible for the lower activity of trypsin in ileal digesta of pigs fed on high-tannin faba bean hulls. Various explanations for the absence of effects of condensed tannins on enzyme activity in duodenal digesta are discussed.

Condensed tannins: Trypsin: Chymotrypsin: Pigs

Faba beans (*Vicia faba* L.) are of interest as a protein supplement for pig diets (Thacker & Bowland, 1985). However, the nutritive value of faba beans is lower than predicted on the basis of their chemical composition (Fowler, 1980). This is thought to be due to the presence of several antinutritional factors. Protease inhibitors (Abbey *et al.* 1979; Griffiths, 1981, 1984), haemagglutinins (lectins; Marquardt *et al.* 1974), vicine, convicine (Marquardt, 1989) and condensed tannins (Marquardt *et al.* 1977) have been found in faba beans. Among these, condensed tannins appear to be particularly important in relation to the nutritive value of faba beans (Griffiths, 1981; Marquardt & Bell, 1988). In faba beans these polyphenolic compounds are found in the hulls of coloured-flowering varieties (Griffiths & Jones, 1977; Ward *et al.* 1977; Newton & Hill, 1983).

* Present address: CLO-Institute for Animal Nutrition 'De Schothorst', P.O. Box 533, 8200 AM Lelystad, The Netherlands.

Dietary inclusion of significant levels of high-tanning faba beans or their hulls reduced body weight gain, impaired feed conversion efficiency and reduced the apparent digestibility of crude protein in rats (Moseley & Griffiths, 1979; Griffiths & Moseley, 1980), chickens (Marquardt *et al.* 1977; Longstaff & McNab, 1991) and pigs (Jansman *et al.* 1993). The negative effects of condensed tannins are thought to arise from their interactions with dietary and endogenous proteins such as digestive enzymes (Leinmüller & Menke, 1990; Salunkhe *et al.* 1990). Condensed tannins can inhibit the activity of digestive enzymes both *in vitro* (Griffiths, 1981; Oh & Hoff, 1986; Horigome *et al.* 1988) and *in vivo* (Griffiths & Moseley, 1980; Horigome *et al.* 1988; Longstaff & McNab, 1991). However, Mole & Waterman (1987), Blytt *et al.* (1988) and Butler (1989) indicated that antinutritional effects of dietary condensed tannins are not due to binding and inhibition of digestive enzymes.

In most studies dealing with the antinutritional effects of tannins, rats and chickens are used. In the present experiment the effect of condensed tannins in faba beans on the activities of trypsin (*EC* 3.4.21.4) and chymotrypsin (*EC* 3.4.21.1) was studied in digesta of pigs obtained from two sites in the small intestine.

MATERIALS AND METHODS
Animals and housing

Four castrated male pigs (Dutch Landrace × Dutch Yorkshire) with a mean body weight of 83·0 kg were housed individually in metabolism cages at an ambient temperature of 24° and a relative humidity of 60%. At a body weight of about 40 kg the pigs had been surgically fitted with a post-valvular T-caecum (PVTC) cannula (van Leeuwen *et al.* 1991). In addition, a simple T-cannula was positioned approximately 100 mm caudal of the stomach pylorus, opposite the bile and pancreatic ducts.

Experimental diets

Two diets containing hulls of two different varieties of faba beans were formulated. The basis of the diets consisted of barley, maize and dried skimmed milk. The low-tannin (LT) diet contained 200 g of hulls of faba beans of the white-flowering cv. Blandine/kg with a low content of condensed tannins. The high-tannin (HT) diet contained the same level of hulls of cv. Alfred with a high tannin content. The hulls were prepared as described by Jansman *et al.* (1993).

The composition of the diets and some analytical data are given in Table 1. The diets were balanced with regard to net energy, total content of lysine, methionine and cystine, threonine, tryptophan, isoleucine, vitamins and minerals. Crude protein (N × 6·25) content was slightly higher in the HT diet (166 g/kg) than in the LT diet (158 g/kg). The level of crude fibre was lower in the HT diet than in the LT diet (104 *v.* 126 g/kg). The content of condensed tannins, analysed according to the method of Kuhla & Ebmeier (1981), was below the lowest detection limit (< 0·10%) for the LT diet and was 0·68% catechin equivalents for the HT diet. The activity of trypsin inhibitors in the diets, as determined by the method of van Oort *et al.* (1989), appeared to be similarly low for both diets. Cr_2O_3 (2·5 g/kg) was added to the diets as a digestibility marker.

The pigs were fed two equal portions daily, 1050 g per feeding time, at 08.00 and 20.00 hours. The unpelleted feed was mixed with water (1:2, w/v) just before feeding.

Digesta collection procedures

The pigs were adapted to the experimental diets during a period of 10 d. Two pigs were randomly assigned to the LT diet, and the other two pigs received the HT diet. In the first collection period of 4 d (P1) about 50 g fresh duodenal digesta was obtained at each time

Table 1. *Composition (g/kg) of the low-tannin (LT) and high-tannin (HT) experimental diets*

Diet...	LT	HT
Barley	300·0	300·0
Maize	206·8	206·8
Maize gluten meal (590 g crude protein/kg)	38·0	38·0
Dried skimmed milk	100·0	100·0
Meat meal	40·0	40·0
Vicia faba hulls (cv. Blandine)	200·0	
Vicia faba hulls (cv. Alfred)		200·0
Soya-bean oil	52·0	52·0
Vitamin/mineral mix*	10·0	10·0
Cane molasses	20·0	20·0
Limestone	12·0	12·0
$CaHPO_4.2H_2O$	5·5	5·5
NaCl	3·0	3·0
$KHCO_3$	3·0	3·0
$NaHCO_3$	4·0	4·0
DL-Methionine	0·7	0·7
L-Lysine HCl	3·5	3·5
L-Threonine	0·8	0·8
L-Tryptophan	0·5	0·5
L-Isoleucine	0·2	0·2
Calculated and analysed contents		
Net energy† (kJ/kg)	9012	9012
Dry matter‡	947·8	938·0
Crude protein‡	158·2	165·9
Ether extract†	74·5	74·5
Crude fibre‡	125·8	104·3
Ash†	60·4	60·4
Ca†	9·9	9·9
P†	6·2	6·2
Condensed tannins§	< 0·10	0·68
Trypsin inhibitor activity‖	0·32	0·27

* The vitamin/mineral mix supplied (per kg diet): vitamin A 3·10 mg; cholecalciferol 45 μg; vitamin E 40 mg; riboflavin 5 mg; nicotinamide 30 mg; d-pantothenic acid 12 mg; choline chloride 150 mg; cyanocobalamin 40 μg; menadione 3 mg; ascorbic acid 50 mg; pteroymonoglutamic acid 0·3 mg; $CuSO_4.5H_2O$ 100 mg; $ZnSO_4.H_2O$ 200 mg; MnO_2 70 mg; $FeSO_4.7H_2O$ 400 mg; $CoSO_4.7H_2O$ 2·5 mg; $Na_2SeO_3.5H_2O$ 0·2 mg; KI 0·5 mg; tylosin 20 mg.

† Calculated content.
‡ Analysed content.
§ % Catechin equivalents (analysed).
‖ mg Trypsin inhibited/g diet (analysed).

(08.00, 08.30, 09.00, 10.00 hours and subsequently every hour up to 20.00 hours) by collecting outflow from the duodenal cannula. Each time the cannula was opened for a maximum of 5 min. Fresh digesta samples were weighed and their pH was measured. They were pooled per time point per animal over 4 d and stored at $-20°$.

Ileal digesta were collected on the same days between 08.00 and 20.00 hours over periods of 2 h. A PVC tube connected the cannula with a container in which digesta were collected. The container was cooled with ice. Ileal digesta were pooled immediately per animal per 2 h period over 4 d. Samples were then stored at $-20°$. The pH of the pooled samples was measured afterwards.

After the first collection period the animals were given the other diet (change-over). During the next period of 10 d the pigs were adapted to the diets, then a second collection period followed. This period (P2) also lasted 4 d. The procedures for digesta collection and sampling were as in P1. Afterwards, representative parts of the ileal digesta and all duodenal digesta samples were freeze-dried.

For the determination of the apparent ileal digestibility of dry matter (DM) and crude protein (CP) of the diets, freeze-dried ileal digesta per 2 h collection period were pooled per animal for each of the collection periods.

Samples of feed and freeze-dried digesta were ground in a laboratory mill with a 1 mm screen. Digesta samples were stored under N_2 in small air-tight plastic flasks at $-20°$ until analysis.

Chemical analyses

The diets were analysed for DM and N contents by standard procedures and for Cr_2O_3 using atomic absorption spectroscopy. The DM content of duodenal digesta was determined from the weight loss during freeze-drying. The N content of duodenal digesta was analysed in the freeze-dried samples. Fresh ileal digesta were analysed for dry matter and N content. In the pooled samples of freeze-dried ileal digesta per animal for each of the collection periods, DM, N and Cr_2O_3 contents were determined. The CP content of samples was calculated as $N \times 6.25$.

Trypsin and chymotrypsin activities in freeze-dried duodenal and ileal digesta were determined spectrophotometrically according to Bergmeyer (1974). Trypsin activity was determined using α-N-toluene-p-sulphonyl-L-arginine methyl ester (10 mM) as substrate in Tris-HCl buffer (46 mM, pH 8·1, 11·5 mM-$CaCl_2$) at 25°. Chymotrypsin activity was measured using N-benzoyl-L-tyrosine ethyl ester (0·96 mM) as substrate in Tris-HCl buffer (80 mM, pH 7·8, 0·1 M-$CaCl_2$), also at 25°. Activity is defined as μmol substrate converted by the enzyme/min. The activity is expressed as units (U)/g freeze-dried sample.

Statistical analyses

The results for digesta for each individual time point or collection period and the overall mean values per animal over 4 d were analysed statistically according to the following model (SAS-GLM procedure; Statistical Analysis System, 1990):

$$y_{ijkl} = \mu + \text{period}_i + \text{diet}_j + \text{animal}_k + e_{ijkl},$$

where y_{ijkl} is the dependent variable, μ is the overall mean, period_i is the collection period (1 or 2), diet_j is the diet (1 or 2), animal_k is the effect of the kth animal (1–4), and e_{ijkl} is the residual error.

The factor 'period' had no significant effect on either of the variables analysed. The results in Tables 2 and 3 are presented as least-square means for diets with the standard errors of models, which did not include 'period' as a factor. Results in Figs. 1–8 are shown as means with their standard deviations.

Small-scale experiment with pigs with pancreatic duct cannulas

A small-scale experiment with a change-over design was conducted in three pigs fitted with pancreatic duct cannulas (body weight about 40 kg; cannulation according to Hee *et al.* 1985) and fed on diets containing (g/kg diet): skimmed milk powder 180, faba bean cotyledons 300, maize starch 327 and faba bean hulls with either a low or a high content of condensed tannins 100, as the major ingredients (1100 g/day). After 10 d adaptation to

Table 2. *Mean values for dry matter and crude protein contents, pH and enzyme activity of duodenal digesta of pigs fed on a diet containing hulls of faba beans* (Vicia faba *L.; 200 g/kg*) *with either a low* (LT) *or high* (HT) *content of condensed tannins*†

(Values are means for sixty determinations)

Diet...	LT	HT	SE	Statistical significance
Dry matter (g/kg)	93·7	92·4	2·9	NS
Crude protein (g/kg FDM)	167·3	178·1	2·3	**
pH	4·94	5·06	0·06	NS
Trypsin activity (U/g FDM)	156·7	142·6	20·2	NS
Chymotrypsin activity (U/g FDM)	38·3	38·0	6·6	NS
Trypsin:chymotrypsin activity ratio	4·20	4·18	0·31	NS

FDM, freeze-dried matter; NS, not significant.
** $P < 0.01$.
† For details of diets and procedures, see Table 1 and pp. 628–630.

the diets, pancreatic secretion was measured quantitatively on four consecutive days for 12 h/d (08.00–20.00 hours). Activities of trypsin and chymotrypsin were measured in representative samples of pancreatic juice using the methods of Bergmeyer (1974).

RESULTS

General remarks

Statistical analysis of the experimental data (DM content and pH of digesta, trypsin and chymotrypsin activities and their activity ratio in freeze-dried digesta, and CP content of digesta) for each time point of collection of duodenal digesta revealed a significant animal effect in nine of the ninety cases (six variables × fifteen time points). For data on ileal digesta per 2 h collection period, a significant animal effect was found in five of the thirty cases (five variables × six collection periods of 2 h). For the mean values per animal over 4 d, a significant animal effect was found only for the DM content of fresh duodenal and ileal digesta.

Duodenal digesta

Mean size of the samples of duodenal digesta at each time point of sampling was 49·1 and 47·7 g for treatments LT and HT respectively. DM content of the duodenal digesta did not differ (Table 2). Between 08.00 and 20.00 hours, 6·9 and 6·7% of total DM of one meal (1050 g) was sampled via the duodenal cannula for the LT and the HT treatments respectively. Mean values for CP content, pH and activity of trypsin and chymotrypsin in duodenal digesta are given in Table 2. Mean CP content of freeze-dried duodenal digesta was higher for the HT treatment ($P < 0.01$). Mean pH of duodenal digesta was similar for treatments LT and HT. The course of the pH over the 12 h period of sampling was similar for both diets (Fig. 1). Values were highest just after and before feeding (pH 5·5–6·0). Lowest values were measured 2–3 h after feeding (pH 4–4·5).

For both treatments, trypsin and chymotrypsin activities in freeze-dried duodenal digesta varied considerably over the 12 h of sampling. Neither overall mean values (Table 2) nor individual values per time point (Figs. 2 and 3) differed significantly between treatments. Standard deviations for the measurements on enzyme activities were relatively high, particularly for samples collected between 12.00 and 20.00 hours.
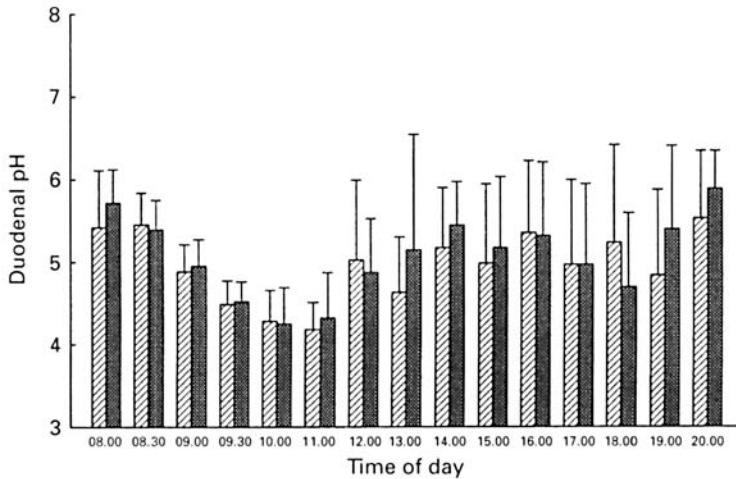
Fig. 1. pH of duodenal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Values are means and standard deviations for four pigs.
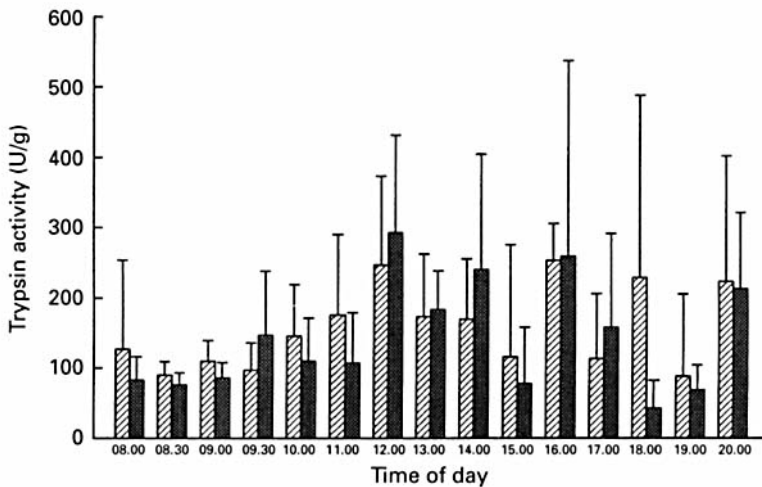

Fig. 2. Trypsin activity (U/g) in freeze-dried duodenal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Values are means and standard deviations for four pigs.

Mean values for trypsin activity:chymotrypsin activity in freeze-dried duodenal digesta did not differ significantly between diets. Values tended to increase during the first hours after feeding and to decrease later until a subsequent feeding (Fig. 4).

Ileal digesta

The total amount of fresh digesta collected at the terminal ileum per 12 h did not differ between treatments (2119 and 2037 g for diets LT and HT respectively). The quantity of fresh digesta collected per 2 h varied between 265 and 432 g for diet LT and between 268 and 391 g for diet HT. DM content of ileal digesta was slightly lower for treatment HT ($P < 0.05$; Table 3). The CP content in ileal digesta (on a DM basis) was relatively constant
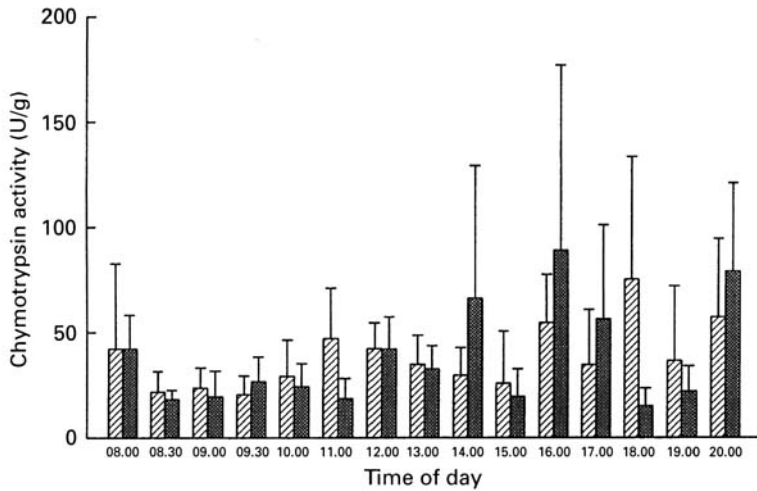
Fig. 3. Chymotrypsin activity (U/g) in freeze-dried duodenal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Values are means and standard deviations for four pigs.
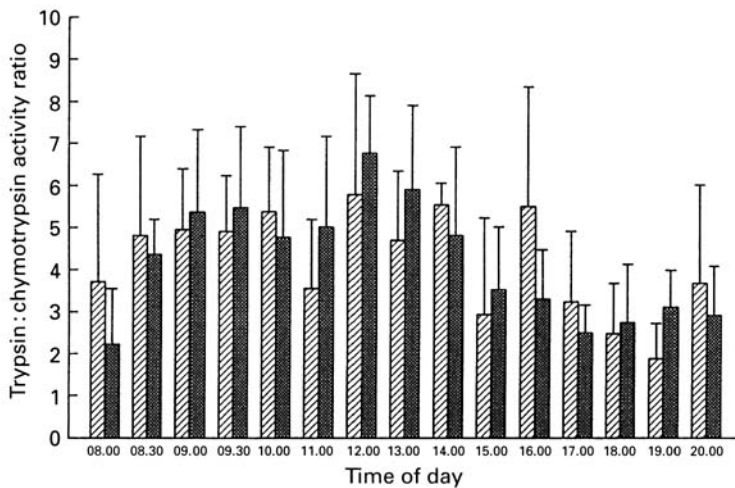

Fig. 4. Ratio of the activity between trypsin and chymotrypsin in freeze-dried duodenal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Values are means and standard deviations for four pigs.

over the day (Fig. 5). Overall, and during each of the 2 h periods, the CP content was higher for pigs receiving the HT diet ($P < 0.05$; Table 3 and Fig. 5). The pH of ileal digesta for both treatments was similar (Table 3). Overall, and between 12.00 and 20.00 hours, the trypsin activity in ileal digesta was significantly lower for the HT treatment (Table 3 and Fig. 6). Chymotrypsin activity in freeze-dried ileal digesta did not differ between the diets, except for a lower activity found 6–8 h postprandially for the HT diet ($P < 0.05$; Table 3 and Fig. 7). Between 14.00 and 18.00 hours the trypsin:chymotrypsin activity ratio was significantly lower in ileal digesta for the HT treatment ($P < 0.05$; Fig. 8). Overall, and in digesta collected over most of the other 2 h periods, the ratio tended to be lower for the HT diet.

Table 3. *Mean values for dry matter and crude protein contents, pH and enzyme activity in ileal digesta and apparent ileal digestibility values (DC) for dry matter (DM) and crude protein (CP) in pigs fed on a diet containing hulls of faba beans* (Vicia faba *L; 200 g/kg) with either a low (LT) or high (HT) content of condensed tannins†*

(Values are means for twenty-four determinations)

Diet...	LT	HT	SE	Statistical significance
Dry matter (g/kg)	122·8	109·9	3·6	*
Crude protein (g/kg DM)	118·3	176·0	3·6	***
pH	7·18	7·15	0·05	NS
Trypsin activity (U/g FDM)	110·2	76·7	5·7	**
Chymotrypsin activity (U/g FDM)	27·9	25·8	6·0	NS
Trypsin:chymotrypsin activity ratio	4·22	3·11	0·65	NS
DC_{DM}	0·571	0·570	0·014	NS
DC_{CP}	0·728	0·614	0·013	*

FDM, freeze-dried matter; NS, not significant.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
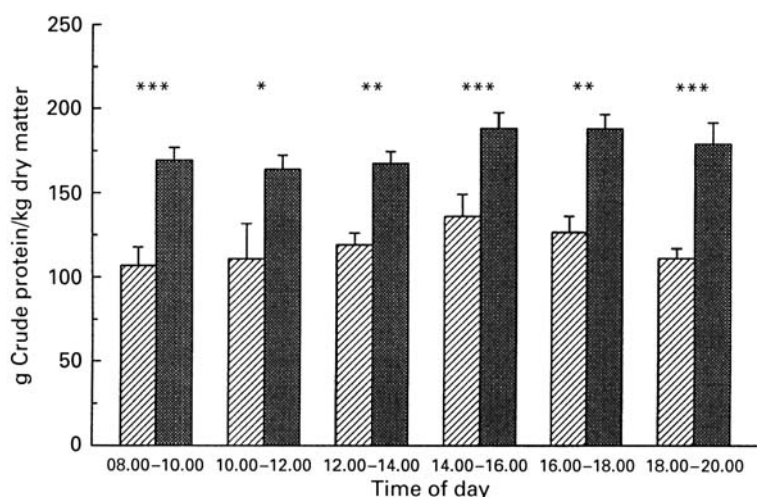† For details of diets and procedures, see Table 1 and pp. 628–630.

Fig. 5. Crude protein content of ileal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Differences between treatments * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values are means and standard deviations for four pigs.

The apparent ileal digestibility of DM, calculated using the marker (Cr_2O_3) ratio, was similar for both diets. For CP the ileal digestibility value was 11 units lower for the HT treatment ($P < 0.05$; Table 3).

Pancreatic enzyme secretion in pigs with pancreatic duct cannulas

Total pancreatic secretion did not differ between treatments (1363 and 1317 g per 12 h respectively). Mean output of enzymes (U/12 h), measured in only one of the two experimental periods, was 126925 and 115265 for trypsin and 60686 and 53210 for chymotrypsin in pigs given the low- and high-tannin faba-bean hulls respectively.
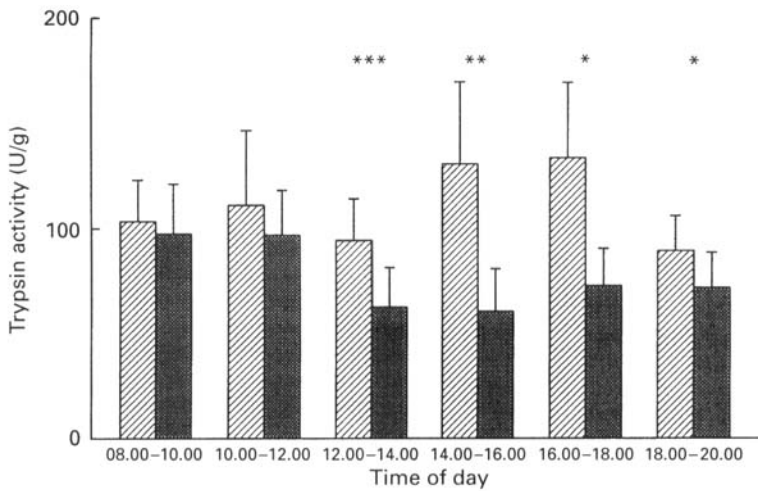
Fig. 6. Trypsin activity (U/g) in freeze-dried ileal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Differences between treatments * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Values are means and standard deviations for four pigs.
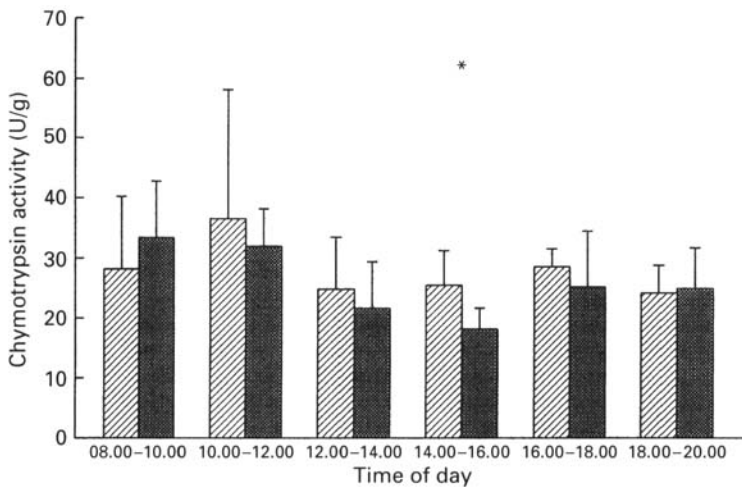

Fig. 7. Chymotrypsin activity (U/g) in freeze-dried ileal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Difference between treatments * $P < 0.05$. Values are means and standard deviations for four pigs.

DISCUSSION

General

Composition and enzyme activity of digesta in the small intestine of pigs vary widely over the day (Braude *et al.* 1976; Low *et al.* 1978; Low, 1979). Therefore, single-spot sampling of digesta or digesta collection over a short period of time will not provide representative samples. The use of re-entrant cannulas allows quantitative collection of digesta at various sites of the digestive tract in pigs. However, re-entrant cannulation has some drawbacks in terms of practical application and maintenance of a normal physiological status of the intestines. This was shown by Köhler *et al.* (1992) in pigs fitted with a re-entrant cannula
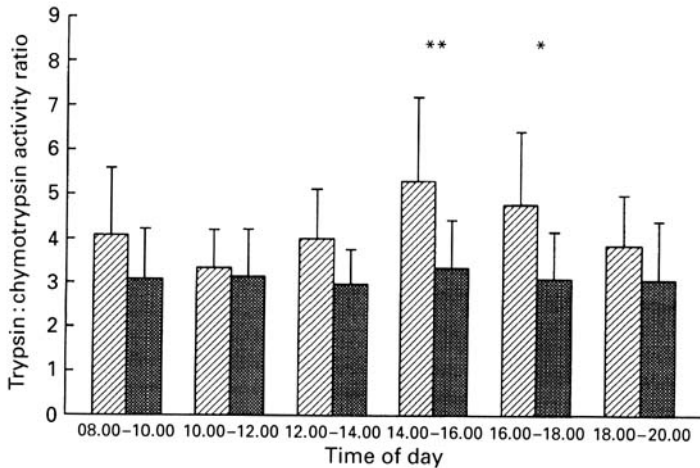
Fig. 8. Trypsin:chymotrypsin activity ratio in freeze-dried ileal digesta of pigs fed on a diet containing 200 g/kg of low-tannin (▨) or high-tannin (▨) faba-bean (*Vicia faba* L.) hulls. Difference between treatments * $P < 0.05$; ** $P < 0.01$. Values are means and standard deviations for four pigs.

in the terminal ileum. Therefore, in the present study frequent spot sampling of duodenal digesta via a T-cannula has been used to study composition and enzyme activity of digesta in the proximal part of the duodenum of pigs. Both DM content (results not shown) and pH of duodenal digesta in our study followed the same pattern over the day as found by Braude *et al.* (1976) with quantitative collection. Therefore, we assumed that our samples of duodenal digesta were representative. The PVTC cannula at the terminal ileum of pigs allows almost complete collection of ileal digesta (van Leeuwen *et al.* 1991). Therefore, digesta collected via this cannula over 12 h is assumed to be representative of the digesta reaching the terminal ileum.

To study the effects of condensed tannins in faba beans, hulls of faba beans with a different tannin content were included in the diets. In faba beans condensed tannins are found in the hulls of the seeds (Bos & Jetten, 1989). Furthermore, hulls of faba beans have a low CP content and consist mainly of non-starch polysaccharides, cellulose in particular (Cerning *et al.* 1975). The slightly higher content of crude fibre and the lower level of CP in the LT diet (Table 1) are associated with small differences in the levels of fibre and protein between the two hull fractions. The content of condensed tannins can be considered as the major difference between the diets.

Digesta composition and pancreatic enzyme secretion

The fall in pH of duodenal digesta in the first hours after feeding (Fig. 1) is associated with the high rate of gastric emptying shortly after feeding as found by Braude *et al.* (1976). Mean pH values of duodenal digesta in our study were similar to those found by Braude *et al.* (1976) for duodenal digesta collected quantitatively over 24 h. The relatively low DM content of duodenal digesta for both treatments is related to endogenous secretions in the proximal part of the digestive tract in the form of saliva, gastric juice, bile and pancreatic juice (Braude *et al.* 1976). In our study the DM content of duodenal digesta was not influenced by the type of faba-bean hulls included in the diets.

The mean CP content of duodenal digesta (on a DM basis; Table 2) was almost equal to the level in the diets (Table 1). Some authors have found an increase in protein flow at the duodenal level in pigs compared with protein intake, as a result of endogenous protein

secretion in the proximal digestive tract. This phenomenon has been reviewed by Low (1979). N absorption before the proximal duodenum was not observed in pigs (Zebrowska *et al.* 1983).

In our study activities of digestive enzymes in duodenal digesta fluctuated markedly over the day. Similar observations were made by Low (1982). This is probably related to within-day fluctuations in the secretion of pancreatic juice and/or the concentration of digestive enzymes in the juice and the dilution of enzymes by digesta flow. Corring & Saucier (1972) and Partridge *et al.* (1982) showed that both secretion of pancreatic juice and its composition vary widely over the day and that both are affected by the composition of the diet.

Information on the possible effects of dietary tannins on the secretory activity of the pancreas is scarce. Marquardt *et al.* (1977), Longstaff & McNab (1991) and Jansman *et al.* (1993) did not observe a change in pancreas weight in chickens and pigs, after feeding diets with tannin-rich hulls from faba beans.

It was concluded from the small-scale experiment with pigs with pancreatic duct cannulas that condensed tannins of faba beans do not affect the volume of pancreatic secretion and the pancreatic output of trypsin and chymotrypsin in pigs.

The small difference in crude fibre content between the diets (Table 1) probably does not affect digestive enzyme secretion by the pancreas. Mosenthin & Sauer (1991) found that inclusion of Alphafloc or straw at a level of 100 g/kg diet did not affect pancreatic enzyme output in pigs.

Enzyme activity in small intestinal digesta

Enzyme activity in digesta is influenced by physico–chemical conditions in digesta, dietary components, and denaturation and autodigestion of enzymes (Low, 1982). The latter two, however, do not seem to be very important in the proximal duodenum. Low (1982) reported that total enzyme activity (U) in duodenal digesta of pigs was similar to the total activity found in the jejunum. Among the dietary components, fibre may reduce the activity of digestive enzymes due to adsorption of enzymes to the fibre matrix, as shown *in vitro* by Schneeman (1978), and due to the effect of fibre-associated components (pectins, gums) on viscosity and pH (Isaksson *et al.* 1982). The pH of duodenal digesta in our study differed only slightly between treatments.

Corring *et al.* (1972), who measured total pancreatic output, and Low (1982), who measured enzyme activity in total duodenal digesta (U), found an increased activity of both trypsin and chymotrypsin in the first hours after feeding. The low activity of both enzymes, expressed as U/g freeze-dried matter (U/g FDM), in duodenal digesta in the first hours after feeding in our study may be the result of a dilution of pancreatic enzymes due to an increased flow of digesta during this period. However, duodenal digesta flow itself could not be measured in our study. At the ileal level the enzyme activities (U/g FDM) (Figs. 6 and 7) were more steady over the day. The same was observed by Low (1982).

In several studies condensed tannins in diets have reduced the activities of digestive enzymes in digesta obtained from various sites of the intestinal tract of rats and chickens. Horigome *et al.* (1988) found reduced activities of trypsin and α-amylase (*EC* 3.2.1.1) in the upper, middle and lower small intestine of rats after feeding tannin-rich extracts from various fodder plants. Similar results were obtained by Griffiths & Moseley (1980) after feeding rats tannin-rich faba-bean hulls. Longstaff & McNab (1991) found reduced activities of trypsin and lipase (*EC* 3.1.1.3) in small intestinal digesta of chickens given high-tannin faba-bean hulls compared with low-tannin faba-bean hulls. Yuste *et al.* (1992) found reduced activities of trypsin, α-amylase and lipase in jejunal digesta of young chickens after feeding tannin-containing faba-bean hulls or tannin-rich extracts from the

same hulls. Formation of tannin–enzyme complexes may explain the effects of dietary tannins on enzyme activity. Addition of polyvinylpyrrolidone (PVP), a potent tannin binder, to digesta extracts of tannin-fed rats restored trypsin activity to values of the control group. This indicates that the enzyme–tannin complex formation is reversible (Griffiths, 1980). In our study no differences between treatments were found with regard to the activities of trypsin and chymotrypsin in duodenal digesta.

Trypsin activity in ileal digesta (U/g FDM) was 70 and 54% of the level measured in duodenal digesta for the LT and HT diets respectively. For chymotrypsin activity the corresponding values were 73 and 68%. Using apparent ileal DM digestibility values from Table 3 for estimating ileal DM flow, the total activity of trypsin (U/24 h) was 30 and 23% of the estimated activity in the duodenum for the LT and HT treatments respectively. Corresponding values for chymotrypsin activity were 31 and 29% respectively. These values are in agreement with estimates made by Low (1982) using the re-entrant cannulation technique for quantitative digesta collection at different sites of the small intestine.

Trypsin activity was significantly reduced in ileal digesta of pigs fed on the high-tannin hulls. There may thus be a difference in response to trypsin activity with regard to tannins at different sites of the digestive tract in pigs. These can be related to several factors, such as differences in solubility of tannins at various sites, the nature and quantity of dietary or other endogenous components in digesta which may bind to tannins, the length of the interval between enzyme activation and encounter of tannins available for interaction, and differences in physico-chemical circumstances. With respect to the latter, Hagerman & Butler (1978), found in *in vitro* studies that maximum precipitation of tannin–protein complexes occurred at a pH close to the isoelectric point (pI) of the proteins involved. Jones & Mangan (1977) observed that the major part of the fraction 1 leaf protein of lucerne (*Medicago sativa* L.) that was complexed with condensed tannins from sainfoin (*Onobrychis viciifolia* Scop.) in the rumen of sheep was released in the proximal duodenum at a pH of 2·5. Therefore, condensed tannins may be available for enzyme binding in the proximal duodenum. However, the pH as measured in duodenal digesta of pigs could still be relatively low for maximum protein binding since pI values for the porcine zymogens, trypsinogen (*EC* 3.4.21.4) and chymotrypsinogen A (*EC* 3.4.21.1) are 7·5 and 7·2, respectively (Walsh & Wilcox, 1970). The pH of ileal digesta in pigs was shown to be slightly higher than 7. This may be favourable for tannin–enzyme interactions.

Tannins have a different binding affinity for various proteins (Asquith & Butler, 1986). Thus, they may affect the activity of various enzymes to a different extent. From our study it may be concluded that condensed tannins in faba-bean hulls have a higher affinity for trypsin than for chymotrypsin in ileal digesta of pigs. This can be inferred from the tendency towards a reduction in the trypsin:chymotrypsin activity ratio in ileal digesta of pigs fed on the HT diet. A low affinity of tannins for lipase relative to trypsin and α-amylase was suggested by Horigome *et al.* (1988) to explain the difference in effects of condensed tannins in digesta of rats on these enzymes.

The mean digestibility of DM and CP of the diets was low. This may be due to the indicator method used. The relatively low recovery of the marker as found in the present study may be related to the double cannulation of the pigs, the method of sample collection and sample treatments. The apparent ileal digestibility of CP was significantly reduced in the pigs receiving the high-tannin diet. A decrease in digestibility of CP was also found in other studies with chickens and pigs fed on diets containing faba-bean tannins (Martin-Tanguy *et al.* 1977; Lacassagne *et al.* 1988; Longstaff & McNab, 1991; Jansman *et al.* 1993).

Both trypsin and chymotrypsin are important for the breakdown of both dietary and

endogenous proteins into peptides, and also for the activation of various digestive enzymes (Rinderknecht, 1986). Therefore, the reduced apparent ileal digestibility of protein in high-tannin diets in non-ruminant animals may be related to a reduced activity of important digestive enzymes. However, various authors (Low, 1982; Zebrowska *et al.* 1983) have suggested that the total amount of protease secreted by the pancreas of pigs exceeds by far the amount required for complete hydrolysis of dietary proteins. This indicates that a reduction in activity of proteolytic enzymes in digesta obtained from a particular site of the digestive tract may have limited consequences for the animal's capacity to degrade dietary proteins through the whole digestive tract. Binding of tannins to dietary proteins or an increased excretion of endogenous proteins therefore seems a more logical explanation for the reduced apparent digestibility of protein in high-tannin diets. Moreover, Partridge *et al.* (1982) determined that the amount of N secreted by the pancreas of pigs (body weight 50 kg) represents only 3–6 % of total daily N throughput in the duodenum. The low enzyme protein:total protein ratio in digesta *in vivo* led Blytt *et al.* (1988) to suggest that the main antinutritional effects of dietary tannins are not due to inhibition of digestive enzymes.

REFERENCES

Abbey, B. W., Norton, G. & Neale, R. J. (1979). Effects of dietary proteinase inhibitors from field bean (*Vicia faba* L.) and field-bean meal on pancreatic function of the rat. *British Journal of Nutrition* **41**, 39–45.

Asquith, T. N. & Butler, L. G. (1986). Interactions of condensed tannins with selected proteins. *Phytochemistry* **25**, 1591–1593.

Bergmeyer, H. U. (1974). *Methoden der Enzymatischen Analyse* (Methods of Enzymatic Analysis), 3rd ed. Weinheim: Verlag Chemie.

Blytt, H. J., Guscar, T. K. & Butler, L. G. (1988). Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. *Journal of Chemical Ecology* **14**, 1455–1465.

Bos, K. D. & Jetten, J. (1989). Determination of tannins in faba beans. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 285–296 [J. Huisman, A. F. B. van der Poel, and I. E. Liener, editors]. Wageningen, The Netherlands: PUDOC.

Braude, R., Fulford, R. J. & Low, A. G. (1976). Studies on digestion and absorption in the intestines of growing pigs. Measurements of the flow of digesta and pH. *British Journal of Nutrition* **36**, 497–511.

Butler, L. G. (1989). Sorghum polyphenols. In *Toxicants of Plant Origin: IV. Phenolics*, pp. 95–121 [P. R. Cheeke, editor]. Boca Raton, Florida: CRC Press.

Cerning, J., Saposnik, A. & Guilbot, A. (1975). Carbohydrate composition of horsebeans (*Vicia faba*) of different origins. *Cereal Chemistry* **52**, 125–138.

Corring, T., Aumaitre, A. & Rérat, 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, Biophysique* **12**, 109–124.

Corring, T. & Saucier, R. (1972). Sécrétion pancréatique sur porcs fistulés. Adaption à la teneur en protéins du régime (Pancreatic secretion of the fistulated pig. Adaptation to protein content in the diet). *Annales de Biologie Animale, Biochimie, Biophysique* **12**, 233–241.

Fowler, V. R. (1980). The value of field beans (*Vicia faba* L.) and other legume seeds for the feeding of pigs. In *Vicia faba: Feeding Value, Processing and Viruses*, pp. 31–43 [D. A. Bond, editor]. The Hague, The Netherlands: Martinus Nijhoff.

Griffiths, D. W. (1980). The role of field bean polyphenolics in digestive enzyme inhibition. In *Vicia faba: Feeding Value, Processing and Viruses*, pp. 145–157 [D. A. Bond, editor]. The Hague, The Netherlands: Martinus Nijhoff.

Griffiths, D. W. (1981). The polyphenolic content and enzyme inhibiting activity of testa from bean (*Vicia faba*) and pea (*Pisum* spp.) varieties. *Journal of the Science of Food and Agriculture* **32**, 797–804.

Griffiths, D. W. (1984). The inhibition of digestive enzymes by polyphenolic compounds. In *Nutritional and Toxicological Aspects of Food Safety*, pp. 509–517 [M. Friedman, editor]. New York: Plenum Press.

Griffiths, D. W. & Jones, D. I. H. (1977). Cellulase inhibition by tannins in the testa of field beans (*Vicia faba*). *Journal of the Science of Food and Agriculture* **28**, 983–989.

Griffiths, D. W. & Moseley, G. (1980). The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *Journal of the Science of Food and Agriculture* **31**, 255–259.

Hagerman, A. E. & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of the Science of Food and Agriculture* **26**, 809–812.

Hee, J. H., Sauer, W. C., Berzins, R. & Ozimek, L. (1985). Permanent re-entrant diversion of porcine pancreatic secretions. *Canadian Journal of Animal Science* **65**, 451–457.

Horigome, T., Kumar, R. & Okamoto, K. (1988). Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. *British Journal of Nutrition* **60**, 275–285.

Isaksson, G., Lundquist, I. & Ihse, I. (1982). Effect of dietary fiber on pancreatic enzyme activity in vitro. *Gastroenterology* **82**, 918–924.

Jansman, A. J. M., Verstegen, M. W. A. & Huisman, J. (1993). Effects of dietary inclusion of hulls of faba beans (*Vicia faba* L.) with a low and high tannin content of condensed tannins on digestion and some physiological parameters in piglets. *Animal Feed Science and Technology* **43**, 239–257.

Jones, W. T. & Mangan, J. L. (1977). Complexes of the condensed tannin of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with sub-maxillary mucoprotein and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Köhler, T., Verstegen, M. W. A., Mosenthin, R., Wensing, T., den Hartog, L. A. & Huisman, J. (1992). Effect of ileo-rectal anastomosis and post-valve T-caecum cannulation on growing pigs. *British Journal of Nutrition* **68**, 305–315.

Kuhla, S. & Ebmeier, C. (1981). Untersuchungen zum Tanningehalt in Ackerbohnen (Determination of the tannin content of faba beans). *Archiv für Tierernährung* **33**, 47–56.

Lacassagne, L., Francesch, M., Carré, B. & Melcion, J. P. (1988). Utilization of tannin-containing and tannin-free faba beans (*Vicia faba*) by young chicks: effects of pelleting feeds on energy, protein and starch digestibility. *Animal Feed Science and Technology* **20**, 59–68.

Leinmüller, E. & Menke, K. H. (1990). Tannine in Füttermitteln für Wiederkäuer, I. Chemische Eigenschaften und Reaktionen mit Macromolekülen (Tannins in feedstuffs for ruminants. I. Chemical properties and reactions with macromolecules). *Übersicht der Tierernährung* **18**, 91–114.

Longstaff, M. & McNab, J. M. (1991). The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *British Journal of Nutrition* **65**, 199–216.

Low, A. G. (1979). Studies on digestion and absorption in the intestines of growing pigs. 6. Measurements of the flow of nitrogen. *British Journal of Nutrition* **41**, 137–146.

Low, A. G. (1982). The activity of pepsin, trypsin and chymotrypsin during 24 h periods in the small intestine of growing pigs. *British Journal of Nutrition* **48**, 147–159.

Low, A. G., Partridge, I. G. & Sambrook, I. E. (1978). Studies on digestion and absorption in the intestines of growing pigs. 2. Measurements of the flow of dry matter, ash and water. *British Journal of Nutrition* **39**, 515–526.

Marquardt, R. R. (1989). Dietary effects of tannins, vicine and convicine. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 141–155 [J. Huisman, A. F. B. van der Poel and I. E. Liener, editors]. Wageningen, The Netherlands: PUDOC.

Marquardt, R. R. & Bell, J. M. (1988). Future potential for use of pulse crops in animal feeds. In *World Crops: Cool Season Food Legumes*, pp. 421–444 [R. J. Summerfield, editor]. Dordrecht, The Netherlands: Martinus Nijhoff/Dr W. Junk Publishers.

Marquardt, R. R., Campbell, L. D., Stothers, S. C. & McKirdy, J. A. (1974). Growth response of chicks and rats fed diets containing four cultivars of raw and autoclaved faba beans. *Canadian Journal of Animal Science* **54**, 177–182.

Marquardt, R. R., Ward, A. T., Campbell, L. D. & Cansfield, P. E. (1977). purification and characterization of a growth inhibitor in faba beans (*Vicia faba* L. var minor). *Journal of Nutrition* **107**, 1313–1324.

Martin-Tanguy, J., Guillaume, J. & Kossa, A. (1977). Condensed tannins in horse bean seeds: chemical structure and apparent effects on poultry. *Journal of the Science of Food and Agriculture* **28**, 757–765.

Mole, S. & Waterman, P. G. (1987). A critical analysis of techniques for measuring tannins in ecological studies. II. Techniques for biochemically defining tannins. *Oecologia* **72**, 148–156.

Moseley, G. & Griffiths, D. W. (1979). Varietal variation in the anti-nutritive effects of field beans (*Vicia faba*) when fed to rats. *Journal of the Science of Food and Agriculture* **30**, 772–778.

Mosenthin, R. & Sauer, W. C. (1991). The effect of source of fiber on pancreatic secretions and on amino acid digestibility in the pig. *Journal of Animal Physiology and Animal Nutrition* **65**, 45–52.

Newton, S. D. & Hill, G. D. (1983). The composition and nutritive value of field beans. *Nutrition Abstracts and Reviews, Series B* **53**, 99–115.

Oh, H. I. & Hoff, J. E. (1986). Effect of condensed grape tannins on the *in vitro* activity of digestive proteases and activation of their zymogens. *Journal of Food Science* **51**, 577–580.

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.

Rinderknecht, H. (1986). Pancreatic secretory enzymes. In *The Exocrine Pancreas: Biology, Pathobiology, and Diseases*, pp. 163–183 [V. L. W. Go, editor]. New York: Raven Press.

Salunkhe, D. K., Chavan, J. K. & Kadam, S. S. (1990). Nutritional consequences of dietary tannins. In *Dietary Tannins: Consequences and Remedies*, pp. 113–146 [D. K. Salunkhe, J. K. Chavan and S. S. Kadam, editors]. Boca Raton, Florida: CRC Press.

Sambrook, I. E. (1981). Studies on the flow and composition of bile in growing pigs. *Journal of the Science of Food and Agriculture* **32**, 781–791.

Schneeman, B. O. (1978). Effect of plant fiber on lipase, trypsin and chymotrypsin activity. *Journal of Food Science* **43**, 633–634.

Statistical Analysis Systems (1990). Cary, NC: SAS Institute Inc.

Thacker, P. A. & Bowland, J. P. (1985). Faba beans: an alternative protein supplement for use in pig diets. *Pig News and Information* **6**, 25–30.

van Leeuwen, P., van Kleef, D. J., van Kempen, G. J. M., Huisman, J. & Verstegen, M. W. A. (1991). The post valve T-caecum cannulation technique in pigs applied to determine the digestibility of amino acids in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* **65**, 183–193.

van Oort, M. G., Hamer, R. J. & Slager, E. A. (1989). The trypsin inhibitor assay: improvement of an existing method. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 110–113 [J. Huisman, A. F. B. van der Poel and I. E. Liener, editors]. Wageningen, The Netherlands: PUDOC.

Walsh, K. A. & Wilcox, P. E. (1970). Serine proteases. In *Methods in Enzymology. Volume XIX. Proteolytic Enzymes*, pp. 31–112 [G. E. Perlmenn and L. Lorand, editors]. New York: Academic Press.

Ward, A. T., Marquardt, R. R. & Campbell, L. D. (1977). Further studies on the isolation of the thermolabile growth inhibitor from the faba bean (*Vicia faba* L. var. minor). *Journal of Nutrition* **107**, 1325–1334.

Yuste, P., Longstaff, M. & McCorquodale, C. (1992). The effect of proanthocyanidin-rich hulls and proanthocyanidin extracts from bean (*Vicia faba* L.) hulls on nutrient digestibility and digestive enzyme activities in young chicks. *British Journal of Nutrition* **67**, 57–65.

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.