Comparison of growth, nitrogen metabolism and organ weights in piglets and rats fed on diets containing *Phaseolus vulgaris* beans

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The effects of lectins in the diet have been mainly studied in rats. An important question is whether results obtained in rats can be extrapolated to larger animals like the pig. *Phaseolus vulgaris* beans are rich in toxic lectins. Therefore a study was carried out to compare the effects of diets containing 200 g *Phaseolus vulgaris* beans (raw or toasted)/kg in rats and piglets. Live-weight gain, nitrogen digestibility and N balance were much lower in piglets than in rats fed on diets containing raw beans. Live-weight gain and N balance were slightly negative in the piglets. When toasted beans were given, live-weight gain and N balance values were reduced in piglets but hardly at all in rats. Giving raw beans caused hypertrophy of the pancreas in the rats but in piglets the weight of the pancreas was reduced. Spleen weight was depressed in the piglets but not in the rats. Weight of liver was not affected in either animal species. When toasted beans were given no effects on the weights of pancreas, spleen or liver were found in piglets or rats. It was concluded that the piglet is much more sensitive to antinutritional factors in the *Phaseolus vulgaris* bean than the rat.

Antinutritional factors: Lectins: *Phaseolus vulgaris*: Piglet: Rat

Many seeds contain substances which are referred to as antinutritional factors (ANF) (Chubb, 1982). This term is used because these factors can disturb metabolic processes and reduce utilization of nutrients in the animal. These factors protect the seed against moulds, bacteria, birds, etc. (Ryan, 1978, 1983; Birk, 1987; Bond & Smith, 1989; Liener, 1989). The main ANF in legume seeds are lectins, trypsin inhibitors and tannins.

Lectins are proteins having an affinity for binding to sugars, and the glycocalyx of the gut wall contain sugars to which the lectin can bind. Due to this binding the gut wall may be severely damaged (Jaffe, 1980; Myer *et al.* 1982; Geer, 1983; King *et al.* 1983; Torres-Pinedo, 1983; Pusztai, 1987, 1989) and the absorption of nutrients is disturbed (Jaffe, 1980; Liener, 1986; Donatucci *et al.* 1987; van der Poel & Huisman, 1988). The excretion of brush border enzymes is affected (Kim *et al.* 1976; Nakata & Kimura, 1985; Rouanet *et al.* 1985; Kik *et al.* 1989) and the permeability of the gut wall is increased (Greer & Pusztai, 1985) and is accompanied by losses of endogenous protein (Pusztai *et al.* 1981).

Trypsin inhibitors are proteins which can form stable inactive complexes with the pancreatic enzymes trypsin (*EC* 3.4.21.4) and chymotrypsin (*EC* 3.4.21.1), resulting in reduced effectiveness of these enzymes (Liener & Kakade, 1980; Rackis & Gumbmann, 1981; Rackis *et al.* 1985), in a compensatory higher production of pancreatic enzymes (Liener & Kakade, 1980; Birk, 1989; Liener, 1989) and even hypertrophy and hyperplasia

of the pancreas in small animals (Liener & Kakade, 1980; Gumbmann *et al.* 1985; Gallaher & Schneeman, 1986; Birk, 1989).

Tannins are polyphenols; however, the way in which these components act in the animal is not exactly known. It has been shown that tannins form complexes with protein (feed protein and enzymes), carbohydrates and some minerals (Marquardt, 1989).

The modes of action of ANF have been studied mainly in rats and chickens but only a few studies have been carried out with pigs. We wished to know if the results obtained in rats can be extrapolated to the pig. Studies by Combs *et al.* (1967) and Yen *et al.* (1977) suggest that the rat and piglet respond differently to ANF in raw soya beans. Visitpanich *et al.* (1985) found similar relative growth depression of rats and piglets fed on pigeon pea (*Cajanus cajan*), but different effects were observed when chickpea (*Cicer arietinum*) was given. It has been reported frequently that the effect of trypsin inhibitors from soy-beans on the pancreas in small and large animals is different (Liener & Kakade, 1980).

There is not enough information about the sensitivity of various animal species to lectins. Therefore, the present study was carried out to compare effects of diets containing 200 g *Phaseolus vulgaris* beans/kg in rats and piglets. The most important ANF in these beans are the lectins (Bond & Smith, 1989). Since young animals would probably show the largest effects, it was decided to use early weaned piglets and young rats.

The object of the present study was to compare the effects of ANF in untreated and treated *Phaseolus vulgaris* beans on nitrogen digestibility, N utilization, weight gain and organ weights in piglets and rats.

MATERIAL AND METHODS

Diets

Three diets were formulated, a control diet containing no beans and two test diets containing 200 g *Phaseolus vulgaris* beans/kg. In one of the test diets raw *Phaseolus* beans were included, while the other test diet contained *Phaseolus* beans which had been toasted for 40 min. The composition of these diets is given in Table 1. A commercial batch of beans with a medium to high lectin content was selected.

A portion of these beans were steam-heated for 40 min at 104° and 19% moisture in the bean. Before autoclaving the beans were cracked in a hammermill. The chemical composition of the beans and the ANF content are described in Table 2. The diets were balanced for total protein, lysine, methionine + cystine, net energy, calcium and phosphorus (see Table 1).

To avoid spillage during feeding the diets were not given as a meal but in pelleted form. The pellet diameter for the piglets was 3 mm and for the rats 9 mm. Each of the three diets was given to fifteen piglets and fifteen rats.

Animals, experimental scheme and management of the growth experiment

The experiment was carried out with forty-five male piglets and forty-five male rats as shown in Fig. 1; fifteen rats or piglets received each diet. The piglets (fifteen litters of three piglets each) were of the crossbred Dutch Landrace × Dutch Yorkshire. The piglets were weaned at 2 weeks of age and were allowed adaptation to the metabolism cages for 1 week. During this period they received a starter diet consisting mainly of barley, maize, whey powder, herring meal, meat meal and enriched with vitamins and minerals. Two piglets were housed in each cage. After the adaptation period they were weighed and assigned to the treatments in such a way that each treatment comprised one piglet from each litter, and

Table 1. *Composition of the diets (g/kg)*

	Control diet	Test diets
Ingredients		
Phaseolus vulgaris beans	—	200
Skim-milk powder	50	50
Fish meal	12	35
Meat meal	39	39
Soya-bean meal (440 g crude protein (nitrogen × 6·25)/kg)	126	—
Barley	150	150
Wheat starch	264	218
Maize starch	264	218
Wheat bran	51	51
Beet molasses	19	17·5
Vitamin–mineral mixture*	10	10
$CaCO_3$	5	3
$CaHPO_4 \cdot 2H_2O$	6	5
$CaCl_2$	3	3
L-Lysine	0·8	—
DL-Methionine	2	5
Contents (calculated)		
Crude protein	184	182†
Net energy (MJ/kg)	10·0	10·0
Ash	56	55
Crude fat	30	31
Crude fibre	28	28
Lysine	10	10
Methionine + cystine	6·5	6·6
Calcium	9·9	9·7
Phosphorus	6·9	7·0

* Contributed the following nutrients (/kg diet): retinol 2·7 mg, cholecalciferol 45 μg, DL-α-tocopherol 40 mg, menadione 3 mg, riboflavin 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 μg, ascorbic acid 50 mg, $CuSO_4 \cdot 5H_2O$ 20 mg, $ZnSO_4 \cdot H_2O$ 200 mg, MnO 70 mg, $FeSO_4 \cdot 7H_2O$ 400 mg, $CoSO_4 \cdot 7H_2O$ 2·5 mg, $Na_2SeO_3 \cdot 5H_2O$ 0·2 mg, KI 0·5 mg.

† About 45 g originates from the beans and 137 g from other sources.

Table 2. *Chemical composition of the* Phaseolus vulgaris *beans (g/kg)*

	Raw beans	Toasted beans
Dry matter	891·5	893·6
Ash	48·0	48·2
Crude protein (nitrogen × 6·25)	226·6	230·2
Crude fat	20·0	20·5
N-free extract (crude fibre included)	59·69	59·47
Contents of antinutritional factors		
Haemagglutinins (HA)*	30	1·92
Trypsin inhibitors †	4·7	0·3
Protein dispersibility index ‡	36	22
Urease (*EC* 3.5.1.5) activity §	0·02	0·0

* Haemagglutination of rabbit erythrocyte; 1 HA, 1:1000 dilution step.

† mg inhibited trypsin/g product.

‡ Measure for the relative amount of dispersible protein in a product.

§ mg released ammonia N/min per g product.

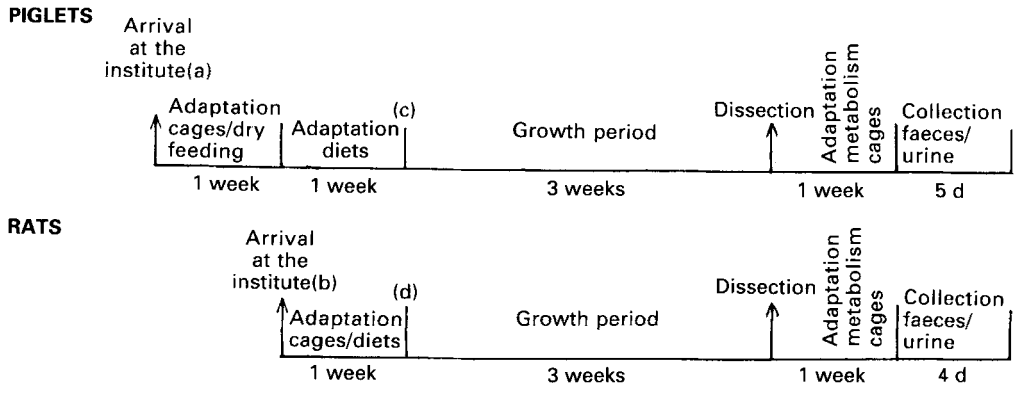

Fig. 1. Experimental scheme. (a) Age 2 weeks, (b) age 4 weeks, (c) age 4 weeks (mean live weight 4·5 kg), (d) age 5 weeks (mean live weight 39·7 g).

the treatments were further balanced for mean body-weight and animal variation per treatment. In the following week the control piglets were adapted to the control diet and the test piglets to a diet containing 200 g of a commercial batch toasted beans.

The piglets destined for the bean diets were adapted as follows: days 1 and 2, practical diet only; day 3, 750 g practical diet + 250 g commercial toasted bean diet/kg; day 4, 500 g practical diet + 500 g commercial toasted bean diet/kg; day 5, 250 g practical diet + 750 g commercial bean diet/kg; days 6 and 7, toasted commercial bean diet only. On day 8 the piglets on the diet with the commercial toasted beans were changed to their test bean diets, while the control piglets remained on the control diet. Growth, feed intake and feed conversion ratio (g feed intake/g weight gain) were measured weekly during the 3 weeks of study.

The rats were Wistar animals; forty-five rats were weaned at 4 weeks of age and placed in cages each containing three rats. In the first week they were adapted to the diets according to the same scheme as that used for the piglets. On day 7 they were distributed among the treatments according to the same criteria as those used for the piglets. From day 8 onwards growth, feed intake and feed conversion ratio were measured each week for 3 weeks.

The daily amount of feed offered to the piglets was restricted to about 4% of the body-weight, which is about 2·2 times their maintenance requirement for energy. The feed was given daily and adapted twice weekly to body-weight. Water was available *ad lib.* from nipple drinkers.

The feed consumption of the rats was restricted to 80% of the *ad lib.* amount consumed by a group of seven rats fed on the control diet. The feed was offered once daily. Water was available continuously from nipple drinkers. The piglets and rats were housed in two separate rooms with continuous artificial lighting at a low intensity. The room temperature for the piglets was between 25 and 28° and for the rats 21°.

Animals, experimental scheme and management in the N balance experiment

At the termination of the growth experiment seven piglets and seven rats from each treatment were chosen at random and placed individually in metabolism cages. After 1-week adaptation, faeces and urine were collected separately. The faeces of the piglets were collected twice daily for 5 d using special bags attached to the piglets around the anus. Urine flowed through a screen in the bottom of the cage and through a funnel into bottles containing 30 ml of a mixture containing 250 ml sulphuric acid (18 M) and 750 ml water/l.

Faeces and urine of the rats were collected separately by using metabolism cages. The urine was collected in bottles containing 1 ml of a mixture containing 250 ml H_2SO_4 (18 M) and 750 ml water/l. The faeces were frozen immediately after collection.

The collection periods were 5 d for the piglets and 4 d for the rats. The age of the animals at the start of the collection period was 56 d for the piglets and 63 d for the rats. Feeding levels for the piglets and rats during the N balance period were based on the formula used in the growth experiment.

Collection of organs

On the day following the termination of the growth period eight animals were taken at random from each group of piglets and rats for dissection of the liver, pancreas and spleen. The piglets and rats were anaesthetized using Fluothane®, nitrous oxide and oxygen. After anaesthesia the abdomen was opened, and the organs were removed quickly and weighed immediately. Just before dissection the animals were weighed; the organ weights were calculated relative to body-weight.

Chemical analyses

The dry matter (DM) content was determined by drying the samples to constant weight at 101°. Ash was determined by combustion at 550° for 4 h. N was analysed in fresh material using a Technicon AutoAnalyzer. After wet digestion with 2·0 M-potassium sulphate solution in 18 M-H_2SO_4 and selenium as catalyst, the N was bound by hypochlorite and phenol. The N complex was measured at 630 nm. Crude fat was analysed by treating with 3 M-hydrochloric acid for 1 h and drying for 3 h under vacuum at 100°, followed by 8 h extraction with diethyl ether. N-free extract was calculated as DM − (ash + (N × 6·25) + crude fat).

Lectins were measured by haemagglutination of erythrocytes according to Valdebouze *et al.* (1980), with modifications.

The content of trypsin inhibitors was analysed according to the method described by Kakade *et al.* (1974). The protein dispersibility index (PDI) was measured according to the American Oil Chemists' Society (1973).

The urease (*EC* 3.5.1.5) activity was measured as the release of ammonia-N (mg) during 1 min from a urea solution at 30° caused by addition of 1 g product.

Statistical analyses

The values for the various criteria are given as means and standard deviations. The differences between treatments were analysed by the Student's *t* test.

RESULTS

Growth, feed intake and feed conversion ratio

Weight gain, feed intake and feed conversion ratio of the piglets and rats are given in Table 3 and Fig. 2. In both species inclusion of beans in the diet reduced growth and increased feed conversion ratio. However, the negative effects were much greater in piglets than in rats. Piglets fed on the raw beans lost weight. In rats, when raw beans were given, growth was significantly ($P < 0·05$) reduced by 27% compared with the controls. Effects of the same magnitude were found for feed conversion ratio.

When the diet containing the toasted beans was given, growth was reduced by 19% ($P < 0·05$) in piglets; in rats the difference was 5% ($P < 0·05$) compared with the control. Feed conversion ratio in the piglets was 20% above the control value ($P < 0·05$) and in the rats 4% (not significant). Feed intake in piglets was much more depressed than in rats.

Table 3. *Growth, feed intake and feed conversion ratio (g feed/g weight gain) in piglets during 20 d and in rats during 21 d on diets containing raw and heated* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Diet	Growth (g/d) Mean	SD	%	Feed intake (g/d) Mean	SD	%	Feed conversion ratio Mean	SD	%
				Piglets					
1, Control	137·7[a]	19·3	100	239·5[a]	33·9	100	1·74[a]	0·12	100
2, 200 g unheated beans/kg	−36·0[b]	5·8	−26	96·5[b]	24·4	40	negative		
3, 200 g heated beans/kg (heated 40 min)	111·8[c]	20·7	81	231·6[a]	34·4	97	2·09[c]	0·11	120
				Rats					
1, Control	6·68[a]	0·24	100	19·33[a]	1·38	100	2·99[a]	0·21	100
2, 200 g unheated beans/kg	4·86[b]	0·20	73	17·54[b]	1·98	91	3·72[b]	0·32	124
3, 200 g heated beans/kg (heated 40 min)	6·32[c]	0·46	95	19·56[a]	1·40	101	3·10[a]	0·18	104

[a,b,c] Mean values in the same column of each animal species with unlike superscript letters differed significantly ($P < 0·05$).

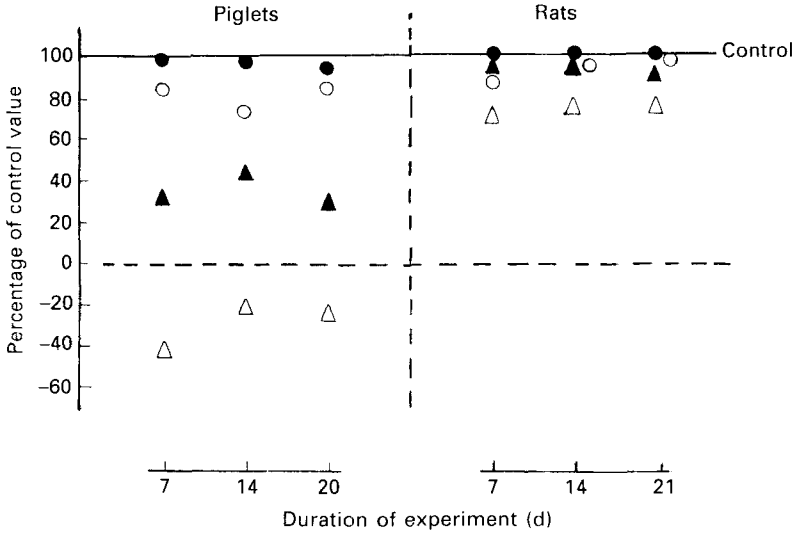

Fig. 2. Feed intake (▲, ●) and growth (△, ○) in piglets and rats fed on raw (▲, △) or toasted (●, ○) *Phaseolus vulgaris* bean diets. For details of animals and diets, see pp. 744–746 and Table 1.

Reduction in the feed intake when raw beans were given was 60% in piglets and 9% ($P < 0·05$) in rats. When the toasted beans were given, feed intake in the piglets was reduced by 3% (not significant), while in rats feed intake was not reduced.

N digestibility and N balance

In both species the faecal N digestibility of the bean diets was significantly ($P < 0·05$) lower than that of the control diet. The values for N digestibility of the diet containing raw beans were 37 and 13 units below control values in the piglets and the rats respectively. Thus N

Table 4. *Feed intake, apparent nitrogen digestibility of the diets and N balance of piglets and rats fed on diets containing raw and heated* Phaseolus vulgaris *beans*

(Mean values and standard deviations)

Diet	Feed intake (g/d)	N intake (g/d)	Apparent N digestibility (%)		N balance (g/d)			Retained N	
			Mean	SD	Mean	SD	% of control	% of total N intake	% of digested N
					Piglets				
1, Control	320[a]	9·35[a]	84·7[a]	2·2	4·72[a]	0·90	100	50·5	59·7
2, 200 g unheated beans/kg	174[b]	5·06[b]	47·6[b]	13·2	−0·33[b]	0·38	−7	−6·5	−14·3
3, 200 g heated beans/kg (heated 40 min)	299[a]	8·74[c]	76·5[c]	3·6	3·80[c]	0·42	57	43·5	56·7
					Rats				
1, Control	30·1[a]	0·89[a]	77·8[a]	1·0	0·25[a]	0·034	100	28·1	36·1
2, 200 g unheated beans/kg	24·8[b]	0·72[b]	64·5[b]	1·9	0·21[b]	0·042	84	29·0	45·0
3, 200 g heated beans/kg (heated 40 min)	28·9[a]	0·84[a]	71·6[c]	1·3	0·24[a]	0·031	96	28·5	39·8

[a,b,c] Mean values in the same column of each animal species with unlike superscript letters differed significantly ($P < 0·05$).

digestibility was more depressed in piglets than in rats (Table 4). The differences between the diets containing toasted beans and those of the control diet were 8 units ($P < 0·05$) and 6 units lower ($P < 0·05$) for the piglets and the rats respectively.

Results of the N balance (Table 4), expressed in g/d, showed the same pattern as that observed for weight gain. With the raw beans N balance and retained N, expressed as a percentage of (apparently) digested N, were slightly negative in the piglets. In rats the N balance was positive and about 16% ($P < 0·05$) lower than that for the control animals. In piglets fed on the toasted beans the N balance was about 19% ($P < 0·05$) below that for the controls; in rats the difference was not significant and only about 4%.

Organ weights

The weights of organs relative to body-weight are given in Table 5. No significant differences for the weights of the liver between treatments were observed in either species. The weights of the pancreas and spleen of the piglets fed on the raw beans were significantly lower than those in the control piglets and in the piglets fed on the toasted beans. The weights of the pancreas and the spleen of the piglets fed on the toasted beans were not different from those of the control animals. The weight of the pancreas of rats fed on the raw beans was significantly higher than that of the controls or the rats fed on the toasted beans. There were no differences in pancreas weight between the rats of the control group and those fed on the toasted beans. The relative weight of the spleen of the rats was not significantly different between the treatments.

DISCUSSION

In most studies reported in the literature very high levels of *Phaseolus vulgaris* beans are used. In our study relatively lower levels were used because in practical pig diets the inclusion levels are generally not higher than 100–200 g/kg diet. Inclusion of raw *Phaseolus*

Table 5. *Mean weights of organs in piglets and rats (g/kg body-weight)*
(Mean values and standard deviations)

Diet	Liver		Pancreas		Spleen	
	Mean	SD	Mean	SD	Mean	SD
			Piglets			
1, Control	25·2[a]	3·5	2·1[a]	0·4	3·4[a]	0·9
2, 200 g unheated beans/kg	23·5[a]	2·4	1·0[b]	0·3	1·4[b]	0·5
3, 200 g heated beans/kg (heated 40 min)	23·7[a]	2·2	2·0[a]	0·3	3·2[a]	1·0
			Rats			
1, Control	40·5[a]	4·3	3·4[a]	0·7	2·4[a]	0·7
2, 200 g unheated beans/kg	41·3[a]	3·5	6·1[b]	0·7	2·3[a]	0·7
3, 200 g heated beans/kg (heated 40 min)	41·7[a]	5·9	3·6[a]	0·6	2·5[a]	0·6

[a,b,c] Mean values in the same column of each animal species with unlike superscript letters differed significantly ($P < 0·05$).

vulgaris in the diet of piglets markedly reduced feed intake (60%) while in the rats the reduction was only 9% (Table 3). It is important to know whether the effect on feed intake can be attributed to the presence of a toxic factor in the bean or to palatability. In a study with pigs of approximately 16 weeks of age, King *et al.* (1983) found a marked reduction in feed intake and also weight loss when a diet containing 400 g *Phaseolus* beans/kg was given. In their experiment the control pigs were fed on the same low level of feed as the bean-fed pigs. Both the control pigs and the bean-fed pigs lost weight, but in the bean-fed pigs the weight loss was three times greater. This result indicates that a 'toxic' factor in the bean has played a role in addition to the effect of the reduced feed intake. In rats, Pusztai *et al.* (1981) found a reduced feed intake when purified lectins from the *Phaseolus* bean were included in the diet. This indicates that the reduction in feed intake may be specifically related to the lectins present in the *Phaseolus* bean.

The negative N balance (Table 4) in the piglets indicates that some body protein was lost due to the feeding of raw *Phaseolus* beans. The results for retained N show that more N was excreted in the urine than absorbed from the gut. This indicates that in the piglets not only was N absorption from the gut depressed, but also its deposition in the body. In rats, N balance was also depressed but the results of retained N show that deposition of absorbed N was not reduced.

The mechanism responsible for the disturbed N deposition in the piglets is not entirely clear. Pusztai *et al.*(1981) showed in a study with rats that lectins of the *Phaseolus* bean are responsible for negative N balance. In accordance with the results of feed intake N digestibility and N balance, the weight gain in the piglets fed on the raw *Phaseolus* beans was distinctly more depressed than that in the rats fed on the same diet. These piglets could not even maintain their body weight, whereas the rats were still gaining weight, although at a lower level (−27%) compared with the control rats.

The pancreatic hypertrophy in the rats (Table 5) was mainly related to the presence of trypsin inhibitors in the raw beans (Table 2), but de Oliveira *et al.* (1988) demonstrated that *Phaseolus* lectins may also cause pancreatic hypertrophy.

The observation that the pancreas weight in piglets fed on the raw beans was reduced (Table 5) seems to agree with the results of King *et al.* (1983) who observed degenerative changes in the pancreas cells of pigs fed on raw *Phaseolus* beans. A direct comparison with our study could not be made because the pancreas was not weighed in their study. Myer *et al.* (1982) also found a tendency for relatively lower pancreas weight in pigs fed on raw kidney beans. Green *et al.* (1986) demonstrated that growth of the pancreas can be inhibited when insufficient protein and amino acids are available. In our study the protein digestibility of the raw bean diet was markedly depressed in the piglets (Table 4). This may explain the reduced pancreas weights in the piglets. On the other hand, we also found severe damage of the small intestinal mucosa in the piglets fed on the raw *Phaseolus* beans (Kik *et al.* 1989), while in rats the gut wall damage was less severe. One can speculate that the lower pancreas weight of the piglets in our study may also be related to damage of the (cholecystokinin–pancreozymin) (CCK–PZ)-hormone producing intestinal endocrine cells, resulting in a depressed CCK–PZ hormone production and, hence, a decrease in pancreas mass. In the piglets fed on the raw beans the spleen weight was significantly lower compared with that of the piglets fed on the control diet and the diet containing the toasted beans.

A slight atrophy of the spleen was also reported by Myer *et al.* (1982) in pigs of about 25 kg fed on 150 g raw *Phaseolus* beans/kg in the diet. The weight of the spleen in the rats did not differ between the treatments.

It can be concluded that the piglet is much more sensitive to ANF in *Phaseolus vulgaris* beans than the rat. The reason for this species difference is not known. Some factors may have influenced the results; for example in many rat experiments the control diet contains 100 g casein protein/kg diet, and in the test diets half of the casein protein is replaced by bean protein (Pusztai *et al.* 1981; Greer, 1983; Greer & Pusztai, 1985) or bean protein is the only protein source (King *et al.* 1986; Aletor & Feguta, 1988). In the present study the protein level was 185 g/kg of which about 45 g were bean protein. Compared with the diets mentioned in the literature much more non-bean protein was present in our test diets. Thus in our studies the rats were less dependent on the bean protein. The protein levels in the diets and the inclusion level of beans in our study were based on practical maximal levels in diets for pigs. These factors may partly explain why in rats the effects were not so marked as often reported in literature. For comparison of effects between different animal species it is important that the design is monofactorial. Therefore, the present study was designed in such a way that inclusion of the beans was the only variable factor.

Another point is that in the present study the diets were balanced on the basis of the content of total protein and not on the content of digestible protein. The results in Table 4 show that the N digestibility of the bean diets was lower than that of the control diet. The difference in N digestibility between the rats and piglets fed on the diets containing the raw beans was marked (64·5 *v.* 47·6). It may be possible that the difference in digestibility can also explain some of the differences in ANF effects between rats and piglets observed in the present study.

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