Artificial rearing of pigs

11. Effect of replacement of dried skim-milk by an isolated soya-bean protein on the performance of the pigs and digestion of protein

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(*Received* 7 *January* 1980 – *Accepted* 28 *February* 1980)

1. Pigs (twenty-one/diet) were weaned at 2 d of age and given liquid diets (200 g dry matter/l) at hourly intervals during a 26 d experiment. The pigs were fed on a scale based on live weight. The diets contained (g/kg DM): dried skim-milk 730 (diet A), dried whey 508·5, isolated soya-bean protein 218, DL-methionine 3·5 (diet S), and soya-bean oil 270 (diets A and S). Diet T contained equal proportions of diets A and S. Soya-bean supplied 0, 370 and 740 g crude protein (nitrogen × 6·25)/kg total crude protein in diets A, T and S respectively.

2. Performance was similar for both diets A and T ($P > 0.05$). Pigs given diet S scoured severely, and fourteen died. The survivors grew very poorly. Nitrogen retention (g/d per kg live weight) was greater for diet A compared with diet T ($P < 0.01$), and decreased with age ($P < 0.001$).

3. Protein digestion was examined in the pigs killed at 28 d of age. The amount of soya-bean protein in the diet did not affect the amount of digesta in the stomach, but soya-bean protein decreased the pH, DM and total N content of the digesta ($P < 0.01$), and increased, though not significantly ($P > 0.05$), pepsin activity in the digesta and stomach tissue. Acid secretion into the stomach appeared to be enhanced in pigs given a diet containing soya-bean protein.

4. Amounts of trypsin, chymotrypsin, total N and proportion of non-protein-N in the digesta from the small intestine were similar for both diets A and T. The amounts for both diets were greater in the distal compared with the proximal region of the small intestine ($P < 0.05$). Chymotrypsin activity in the pancreas was reduced ($P < 0.05$) in pigs given diet T, although this reduction did not seem to impair digestion in 28-d-old pigs. Trypsin activity in the pancreas was similar for both diets A and T.

5. It seems likely that the neonatal pig does not have the digestive capacity to tolerate the large daily intakes of soya-bean protein when dried skim-milk was totally replaced in the diet (diet S). When half the dried skim-milk was replaced, protein digestion was not impaired in 28-d-old pigs.

Before artificial rearing of pigs could become commercially viable, a suitable replacement for dried skim-milk as a source of protein would be necessary (Newport, 1979). Products from soya beans could provide a satisfactory source of protein for pigs reared from 2 d of age.

It has been established that pigs can be reared from 2 or 3 d of age using diets containing an isolated soya protein supplemented with methionine (Sewell *et al.* 1953; Cunningham & Brisson, 1957), but milk protein supports better growth (Maner *et al.* 1961; Schneider & Sarett, 1966). Dried skim-milk was also superior to soya-bean meal for pigs weaned either at 10 (Hays *et al.* 1959) or 21 d of age (Kellogg *et al.* 1964). Jones *et al.* (1977) reported that performance of pigs given a soya-bean flour diet from 21 d of age was equal to that obtained with milk protein. The performance of pigs given diets containing soya-bean protein improves with age and may be associated with the increase in apparent digestibility (Hays *et al.* 1959; Combs *et al.* 1963).

In this experiment an isolated soya-bean protein rather than a less refined product was studied so that the effects of protein would be apparent without the complications of additional carbohydrates and other material.

Table 1. *Composition of the spray-dried diets*

	Diet	
	A	S*
Ingredients (g/kg):		
Dried skim-milk	730	—
Dried whey	—	508·5
Isolated soya-bean protein†	—	218
Soya-bean oil	270	270
DL-methionine	—	3·5
Chemical analysis (g/kg):		
Crude protein (nitrogen × 6·25)	256·8	264·4
Total lipid	295·4	293·0
Calcium	7·6	3·2
Phosphorus	7·5	6·2
Methionine + cystine (calculated)	0·95	0·95

* Supplemented with solutions after reconstitution (see p. 172) which provided 4·4 and 1·3 g Ca and P/kg respectively.

† Supro 710; Ralston Purina Company, Checkerboard Square, St Louis, Missouri, USA.

EXPERIMENTAL

Diets and feeding scale

Two diets were prepared, their composition is given in Table 1. Diet A was prepared by spray-drying a mixture of skim-milk and soya-bean oil. A mixture of skim-milk, whey and soya-bean oil was spray-dried, and then mixed with the isolated soya-bean protein (diet S). A mild-heat process was used for the spray-drying to prevent denaturation of the whey proteins in the skim-milk. These two diets were reconstituted in water (200 g dry matter/l), homogenized and pasteurized, and supplemented with vitamins as previously described (Braude & Newport, 1973; Newport & Keal, 1980). DL-methionine (3·5 g/kg) was added to diet S before reconstitution. Diet S, after reconstitution, was supplemented with (/l) 8·8 ml of a solution containing 548 g $CaCl_2.6H_2O$/l and 6·5 ml of a solution containing 201 g $NaH_2PO_4.2H_2O$/l. A third diet (diet T) was prepared by mixing equal volumes of diets A and S.

The pigs were fed at hourly intervals on a scale described by Braude & Newport (1973).

Experimental design and statistical analysis

Litter-mate, 2-d-old pigs were allocated to one of three treatments (diets) on the basis of live weight and sex. The pigs on different treatments were kept in separate rooms, each containing usually four replicates. In total, twenty-one pigs/treatment were placed on an experiment from 2–28 d of age. During the experiment, three litter-mate blocks were lost after all three pigs had died following a period of scouring. In the remaining eighteen blocks, four pigs given diet T, and fourteen pigs given diet S, died after scouring. Missing values were calculated for pigs given diet T, and statistical analysis was carried out on values for diets A and T only. The mean value, with standard error, was calculated for the four pigs surviving on diet S.

Nitrogen retention and procedure at slaughter

N retention was estimated from a collection period of 4 d duration, as previously described (Braude *et al.* 1976). Collection periods were not attempted when pigs were scouring, and

Table 2. *Effect of replacing dried skim-milk by an isolated soya-bean protein and dried whey on performance*

	Diet ...	A	T	Difference A – T ± SE (13 df)*		S	
	Proportion of protein (g/kg) supplied by soya-bean ...	0	370			740	
Age (d)	No. of pigs ...	18	14		SE	4	SE
2–7	Live-wt gain (g/d)	131	122	9	6·9	93	23·1
	Feed:gain†	0·86	1·58	−0·72	0·726	2·32	0·655
2–28	Live-wt gain (g/d)	303	279	24	13·2	67	3·1
	Feed:gain†	0·87	0·91	−0·04	0·024	2·98	0·340

* No significant differences ($P > 0·05$) were found.
† Dry matter (kg) consumed per kg live-weight gain.

spillage of diet into the urine further reduced the number. Two collection periods were made from some of the pigs.

At 28 d of age, the pigs were killed 1 h after a feed by an intracardiac injection of sodium pentobarbitone. The pancreas and stomach were removed, the digesta emptied from the stomach and its pH value determined with a glass electrode. The small intestine was removed, cut into three portions, two proximal portions each of 1·20 m and the remainder. Each portion was flushed with a solution of sodium chloride (9 g/l) at approximately 20° and the effluent collected. The stomach wall, pancreas and digesta were stored at −20° until analysed.

Analytical methods

Methods for the determination of DM, total N and total lipid have been described by Braude *et al.* (1970) and Braude & Newport (1973). Samples were ashed and calcium determined by atomic absorption spectroscopy. Phosphorus was determined after ashing by colorimetric estimation of the phosphovanadomolybdate complex as described by Cavell (1955). Non-protein-N was determined as total N in the supernatant fraction following the addition of an equal volume of trichloroacetic acid (200 g/l) to the digesta, storing for 16 h at 5°, and removing the precipitated protein by filtration through filter paper (Whatman No. 542). The pepsin content of the stomach tissue and digesta was assayed by the method of Anson (1938). One unit of pepsin activity was equivalent to an increase in extinction at 280 nm of 0·001/min at 37°. Trypsin and chymotrypsin were estimated in the pancreas and digesta in the small intestine as described by Hummel (1959). The pancreatic enzymes were assayed after activation with enterokinase (Miles Labs Ltd, Stoke Poges). The activities of both trypsin and chymotrypsin were calculated by comparison with purified enzymes (Koch-Light Labs, Colnbrook, Bucks).

RESULTS

Performance

The performance of the pigs was not affected ($P > 0·05$) by the replacement of half the dried skim-milk by soya-bean protein and dried whey (Table 2). However, showing very poor growth when soya-bean protein provided 740 g/kg dietary protein (diet S) only four of the twenty-one pigs started on the experiment survived. Mortality was associated with severe scouring.

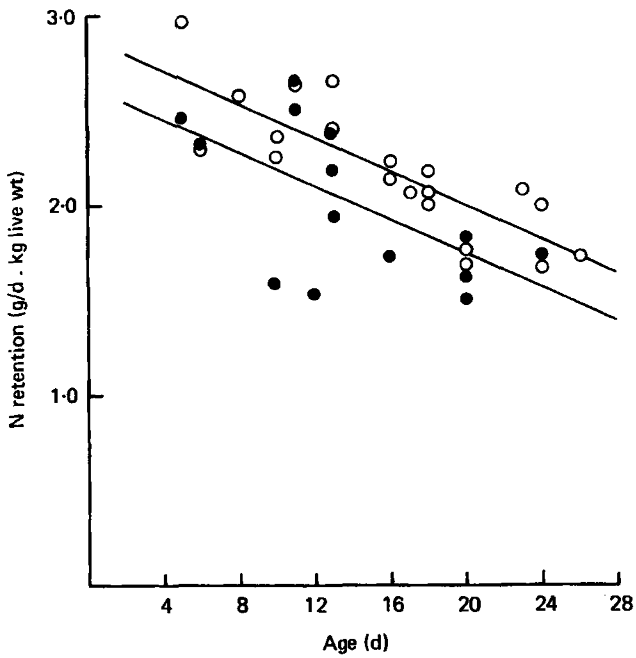

Fig. 1. Effect of age (d) on N retention (g/d per kg live weight) in pigs fed on a dried skim-milk diet (diet A, ○—○), and a diet in which the skim-milk was replaced by an isolated soya-bean protein (diet T, ●—●). For details of diets, see Table 1 and p. 172. Residual standard deviations were 0·195 (18 df) and 0·343 (12 df) for diets A and T respectively.

Table 3. *Effect of replacing dried skim-milk by an isolated soya-bean protein and dried whey on the composition and pH value of the digesta in the stomach, and the concentrations of pepsin in the stomach tissue and digesta*

Diet ...	A	T	Difference A–T ±SE (14 df)		S	
Proportion of protein (g/kg) supplied by soya-bean ...	0	370			740	
No. of pigs† ...	18	14			4	
				SE		SE
Digesta:						
Total wt (g)	107·8	128·9	−21·1	13·96	182·7	33·37
Dry matter (g/kg)	278	157	121**	26·12	145	10·1
pH	4·33	3·68	0·65**	0·176	4·40	0·235
Total nitrogen (g/kg)	15·91	7·04	8·87**	1·80	6·27	0·325
Non-protein-N (g/kg total N)	137	280	−143**	31·7	309	46·1
Pepsin (units/g)	171	221	−50	33·9	290	65·8
Tissue:						
Pepsin (units/g)	3932	4021	−89	397·2	4509	501·4

** $P < 0.01$.

† Total and non-protein N analysed in twelve (diet A), and ten (diet T) pigs; SE of difference based on 7 df.

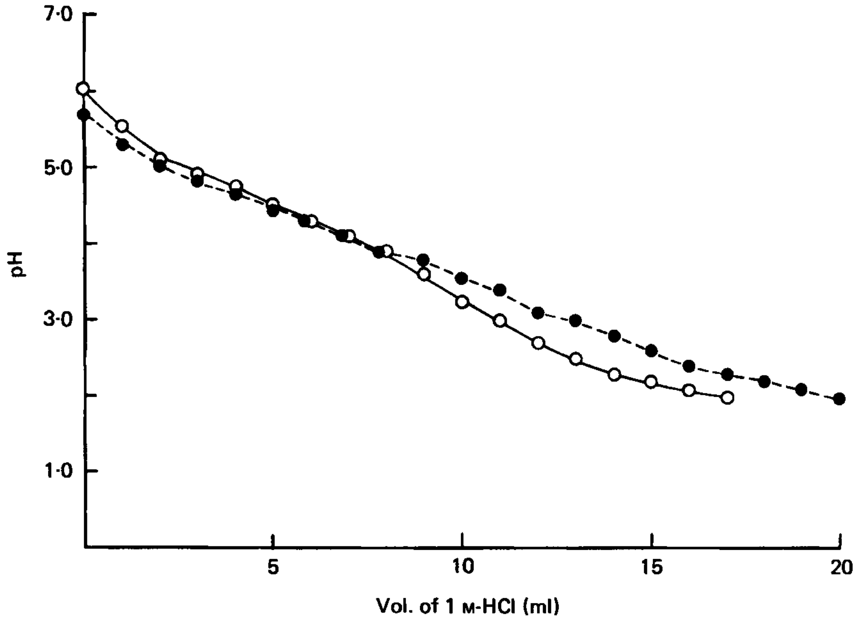

Fig. 2. Buffering capacity of a diet containing dried skim-milk (diet A, ○—○) and a diet in which the skim-milk was replaced by an isolated soya-bean protein (diet S, ●—●). For details of diets, see Table 1 and p. 172.

Nitrogen retention

N retention (g/d per kg live weight) was reduced ($P < 0.01$) when half the dried skim-milk was replaced by soya-bean protein, and declined with age ($P < 0.001$) at a similar rate for both diets (Fig. 1).

Digestion in the stomach

The amount of digesta in the stomach was similar for both the all-milk diet (A) and partial replacement with soya-bean protein (diet T) (Table 3). DM, total N and pH were all reduced in the digesta of pigs given soya-bean protein. The proportion of non-protein-N was increased ($P < 0.01$), and some increase in pepsin activity in the digesta and tissue was also found, although this was not significant ($P > 0.05$). Total replacement of skim-milk (diet S) compared with partial replacement increased the amount of digesta and its pH value, and also pepsin activity, but the increase was significant only for the pH value.

The buffering capacity of diet S was greater than diet A below pH 3·9 (Fig. 2).

Digestion in the small intestine

Partial replacement with soya-bean protein had little effect on the total amount of N or the proportion of non-protein-N in the small intestine (Table 4). When all dried skim-milk was replaced a smaller amount of total N was found ($P < 0.001$). There was a significant increase ($P < 0.05$) in the amount of N and proportion of non-protein-N in the distal portion compared with the proximal portion.

Partial replacement of dried skim-milk reduced the chymotrypsin activity in the pancreas, but the mean activities of both trypsin and chymotrypsin were increased, though not significantly, by total replacement (Table 5). Replacement of milk protein did not significantly affect the amount of enzyme in the digesta. The distal portion of the small intestine contained more enzyme ($P < 0.05$) than the proximal portion.

Table 4. *Effect of replacing dried skim-milk by an isolated soya-bean protein and dried whey on the amount of total nitrogen, and proportion of non-protein N, in the small intestine of 28-d-old pigs*

Diet ...	A	T	Mean ± SED (12 df)*		S	
Proportion of protein (g/kg) supplied by soya-bean ...	0	370			740	
No. of pigs ...	7	6			4	
					Mean	SE
Total N (mg):						
Proximal	105	75	90	43·5	79	29·7
Mid	122	195	159		62	4·6
Distal	235	244	240		103	29·5
Total	462	514			244	47·3
Difference (A–T) ± SE	−52 ± 68·1 (5 df)					
Non-protein-N (g/kg total N):						
Proximal	470	368	419	84·2	366	51·8
Mid	354	521	438		488	64·3
Distal	646	629	638		428	85·8

* Differences were not significant ($P > 0·05$).

Table 5. *Effect of replacing dried skim-milk by an isolated soya-bean protein and dried whey on concentrations of trypsin and chymotrypsin in the pancreas, and amounts in the small intestine of 28-d-old pigs*

Diet ...	A	T			S	
Proportion of protein (g/kg) supplied by soya-bean ...	0	370			740	
Pancreas:						
No. of pigs ...	18	14	Difference A − T ± SE (14 df)		4	
					Mean	SE
Trypsin (mg/g)	2·24	2·30	−0·06 ± 0·268		4·56	1·472
Chymotrypsin (mg/g)	3·40	2·50	0·90* ± 0·329		3·84	0·990
Trypsin (mg) in small intestine:			Mean ± SED (12 df)			
No. of pigs ...	7	5			4	
Proximal	0·82	0·50	0·66	0·373	0·37	0·125
Mid	1·24	1·39	1·31		0·62	0·217
Distal	2·54	2·18	2·36		1·27	0·565
Total	4·60	4·07			2·26	0·860
Difference (A − T) ± SE	0·53 ± 1·936 (4 df)					
Chymotrypsin (mg) in small intestine:						
No. of pigs ...	7	6			4	
Proximal	1·45	0·46	0·96	0·440	0·49	0·066
Mid	1·43	1·98	1·71		0·47	0·157
Distal	2·76	3·67	3·22		1·45	0·851
Total	5·64	6·11			2·41	0·985
Difference (A − T) ± SE	0·47 ± 1·459 (4 df)					

* $P < 0·05$.

DISCUSSION

The extreme mortality associated with total replacement of milk by soya-bean protein indicates severe digestive difficulty or a toxic effect. However, digestion seems a more likely problem as the manufacturers claimed negative activity in the protein isolate for both soya-bean trypsin inhibitor and urease. Replacing half the dried skim-milk by the soya-bean protein isolate did not affect over-all performance, although there was an indication of depression in feed:gain ratio during the first week of life (Table 2) and N retention (g/d per kg live weight) was reduced (Fig. 1).

Sewell *et al.* (1953) and Cunningham & Brisson (1957) were able to rear pigs from 2 d of age using soya bean as the only source of dietary protein. The neonatal pig may be more tolerant of the protein isolates used in those experiments than the particular isolate used in this experiment. It is also probable that the level of feed intake influences the tolerance, especially if digestive capacity is limited, and daily intakes in the earlier experiments were only approximately half those in this experiment.

The amounts of digesta in the stomach of pigs given the all-milk diet were similar to that from pigs in which half the milk protein was replaced by soya-bean protein (Table 3). This suggests a similar rate of stomach emptying for both diets. A similar result was found when a single-cell protein was used as an alternative to milk protein (Newport & Keal, 1980), but fish protein (Newport, 1979) appeared to increase stomach emptying. However, the latter result may be due to age as pigs on the fish-protein experiment were killed at 6 d of age, compared with the 28 d of age of the pigs fed on either soya-bean or single-cell protein.

The increased pepsin activity in the stomach digesta, although not statistically significant, may account for the larger proportion of non-protein-N when pigs received soya-bean protein in the diet. A small increase in activity was also found when single-cell protein replaced dried skim-milk (Newport & Keal, 1980). Interpretation of the results from pigs fed on the all-soya-bean diet (diet S) is difficult as growth was so poor, and physiological development may have been impaired. However, the results suggest a possible secretory response of pepsin by the stomach to the soya-bean protein, as both the concentration in the digesta and the total amount of digesta were greater than from either diets A or T. The lower pH value of the digesta from pigs given diet T compared with diet A certainly indicated greater acid secretion in pigs given soya-bean protein (Table 3), especially as soya-bean diets had a greater buffering capacity at the pH of the digesta (Fig. 2).

The depression in pancreatic chymotrypsin activity when milk protein was partially replaced (diet T) appears anomalous (Table 5), and cannot be explained by increased secretion, as the amounts in the digesta were only marginally greater than for the all-milk diet. The depression could indicate an inhibitory effect of the soya-bean protein on the enzyme. However, the proportion of non-protein-N in the distal small intestine (Table 4) would suggest that the diet with half the milk protein replaced was digested with equal efficiency compared with the all-milk diet in the 28-d-old pig, and therefore pancreatic proteases were not a limiting factor in protein digestion. Pond *et al.* (1971) reported no difference in pancreatic concentrations of trypsin and chymotrypsin from 23-d-old pigs given diets containing casein or soya-bean protein.

The amounts of trypsin and chymotrypsin in the digesta from the small intestine were not affected by partial replacement of dried skim-milk by soya-bean protein (Table 5), implying that secretion was not affected. It seems likely therefore that intestinal digestion of protein does not require enhanced pancreatic secretion when only moderate amounts of soya-bean protein are included, although as the only source of protein soya bean has been shown to increase pancreatic secretion of proteolytic enzymes in 45-d-old pigs (Pekas *et al.* 1966).

Neonatal pigs may not be capable of increased pancreatic secretion, until several weeks of age, which could explain the high mortality in this experiment when 2-d-old pigs were given diets supplying 740 g/kg protein from soya-bean, and also account for the similar performance when given milk or soya-bean from 21 d of age, having first been weaned at d of age onto a milk diet (Jones *et al.* 1977).

Further studies with diets supplying between 370 and 740 g/kg of the protein from soya bean for pigs weaned at 2 d of age would indicate the limit of tolerance to the protein at this age. The hypothesis that limitations of protein digestion are responsible for the adverse effect of large proportions of soya-bean protein in the diet could be explored by further studies in pigs shortly after weaning.

The author thanks H. D. Keal and J. A. Williams for the care of the pigs and preparation of diets, Philippa Marshall for chemical and enzyme analyses, and Mrs R. J. Fulford for statistical advice.

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Printed in Great Britain