## Artificial rearing of pigs

# 12. Effect of replacement of dried skim-milk by either a soya-protein isolate or concentrate on the performance of the pigs and digestion of protein

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1. Pigs (sixteen/diet) were weaned at 2 d of age and given liquid diets (200 g dry matter/1) during a 26 d experiment. The pigs were fed on a scale based on live weight. Dried skim-milk was the only source of protein in diet U and was partially or totally replaced by a soya-bean isolate (diet B) or a concentrate (diets C and D). Soya-bean protein provided 500, 700 or 350 g/kg total crude protein (nitrogen  $\times 6.25$ ) in diets B, C and D respectively.

2. Performance was similar for diets B and D, but poorer than that of pigs given diet U. The apparent digestibility and retention of N of these diets was similar. Pigs given diet C scoured severely and twelve died.

3. Protein digestion was studied in pigs given diets U, B and D, killed at 28 d of age, at the termination of the feeding experiment. The dry matter content and proportion of N in the digesta in the stomach were reduced in pigs given soya-bean protein. Pepsin concentrations in digesta and stomach tissue were unchanged.

4. The concentrations of trypsin and chymotrypsin in the pancreas were greater in pigs given the soya-bean protein concentrate compared with milk protein, but only the increase in trypsin was significant (P < 0.01). Digesta from the small intestine of pigs given the soya-bean-protein isolate contained less chymotrypsin (P < 0.05). There were no differences in the proportion of non-protein-N in the total N in the digesta, suggesting that proteolysis of the milk and soya-bean proteins were equally efficient by 28 d of age.

The replacement of 370 g/kg protein in a diet containing dried skim-milk as the only source of protein by an isolated soya-bean protein did not affect performance of pigs from 2–28 d of age, although some decrease in nitrogen retention (g/d per kg live weight) was found (Newport, 1980). In 28-d-old pigs, inclusion of this isolated soya-bean protein did not affect the amount of digesta in the stomach but did reduce the pH and dry matter (DM) and total N contents of the digesta. The chymotrypsin activity of the pancreas was also reduced. Total replacement of the dried skim-milk by the isolated soya-bean protein led to severe scouring, and death of fourteen out of eighteen pigs.

In preruminant calves, products obtained by extraction of soya beans can produce an allergic response in gastro-intestinal function resulting in some inhibition of abomasal emptying, poorer absorption of N and an increase in the blood levels of antibodies against soya-bean protein (Smith & Sissons, 1975; Smith *et al.* 1979). These effects were greater for soya-bean flours which contain approximately 550 g crude protein/kg, than for a highly-extracted protein isolate containing approximately 900 g crude protein/kg. A soya-bean protein concentrate containing approximately 700 g crude protein/kg did not produce any allergic responses.

As the soya-protein isolates would be unlikely to be an economical source of protein for neonatal pigs, a feeding experiment and digestion study has been made of the lesshighly-extracted soya-bean protein concentrate in comparison with a soya-bean protein isolate and milk. The protein concentrate was the same product as had been used in the experiments with calves described previously.

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	Diet				
	U	В	С		
Ingredients (g/kg)					
Dried skim-milk	660	197			
Dried whey	70	390.3	457-3		
Soya-bean protein isolate <sup>†</sup>	_	140			
Soya-bean protein concentrate1			270		
Soya-bean oil	270	270	270		
DL-methionine		2.7	2.7		
Chemical analysis (g/kg)					
Crude protein (nitrogen $\times 6.25$ )	241.9	240.0	240.0		
Total lipid	282.2	308.6	312.7		

Table 1. Composition of the spray-dried diets

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

<sup>‡</sup> Danpro A; Aarhus Oliefabrik A/S, DK-8100 Aarhus C, Denmark.

## EXPERIMENTAL

#### Diets and feeding scale

Three diets were prepared by spray-drying mixtures of skim-milk, whey and soya-bean oil. Two dried powders were dry-blended with DL-methionine and either a soya-bean protein isolate (diet B) or a concentrate (diet C) (Table 1). A mild-heat process was used for the spray-drying to prevent denaturation of the whey proteins in the skim-milk. These diets were reconstituted in water (200 g DM/1), homogenized and pasteurized, and supplemented with vitamins (Braude & Newport, 1973; Newport & Keal, 1980). After supplementing diets B and C with solutions containing 548 g CaCl<sub>2</sub>.  $6H_2O/1$  and 201 g NaH<sub>2</sub>PO<sub>4</sub>.  $2H_2O/1$ , all three diets contained 9.5 g Ca/kg DM and 8.2 g P/kg DM. A fourth diet (D) was prepared by mixing equal volumes of diets U and C.

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 four treatments (diets) on the basis of live weight and sex. Pigs on different treatments were kept in four separate rooms, each containing four pigs. Sixteen pigs/treatment were used in an experiment from 2 to 28 d of age. One pig given diet U and two pigs given diet D died after scouring, and missing values were calculated before statistical analysis. Pigs given diet C were excluded from the statistical analysis as only four survived.

## N retention and procedure at slaughter

The apparent digestibility and retention of N were estimated using a collection period of 4 d duration (Braude *et al.* 1976). A comparison of the digestion of the protein sources was made in 28-d-old pigs given diets U, B or D and killed 1 h after a feed (Newport, 1980). Analyses of digesta and tissues were not carried out on all replicates; selections were made at random. The four surviving pigs on diet C were excluded from this study as their growth rate was abnormally low.

#### Analytical methods

Determination of DM, total N and lipid have been described by Braude *et al.* (1970) and Braude & Newport (1973). Non-protein-N (NPN) was determined as total N in the supernatant fraction after the addition of an equal volume of trichloroacetic acid (200 g/l) to the digesta (Newport, 1980).

Pepsin was determined 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 activated in homogenates of pancreas with enterokinase (Miles Labs Ltd, Stoke Poges), and determined in pancreas and digesta by the method of Hummel (1959). The activities of both trypsin and chymotrypsin were calculated by comparison with purified enzymes (Koch-Light Labs, Colnbrook, Bucks).

#### RESULTS

#### Performance

Partial replacement of dried skim-milk (diet U) with a soya-bean concentrate (diet D) reduced growth rate during the period from 2 to 7 d of age, but both the concentrate and the isolate (diet B) reduced growth rate from 2 to 28 d of age (Table 2). The ratio, feed intake: weight gain (feed:gain) was also greater for pigs receiving the soya-bean protein concentrate. There were some refusals of the diets containing soya-bean protein in the period 2 to 7 d of age. The number of days on which scouring was observed was similar for all three diets. When dried skim-milk was totally replaced by the concentrate (diet C), twelve pigs out of sixteen died after severe scouring. From 2 to 28 d of age, the four survivors had a mean growth rate of  $33 \pm 4.6$  g/d and their feed: gain value was  $4.66 \pm 0.811$ .

## N retention

N retention (g/d per kg) decreased with age (P < 0.05), but differences between treatments (diets U, B and D) were not significant (P > 0.05) (Fig. 1). The apparent digestibility of N did not change with age, and mean values ( $\pm$ SE) at 16 d of age were  $0.986 \pm 0.0038$ ,  $0.983 \pm 0.0023$  and  $0.985 \pm 0.0032$  for pigs receiving diets U, B and D respectively.

U —	B Isolate	D Concentrate	se of a mean (27 df)	Statistical significance of differences between diets
0	500	350		
16	16	16		_
1	0	2	_	_
107	100	74	10.7	U > D*
1.73	1.31	1.77	0.273	NS
274	225	225	9.4	$U > B, D^{***}$
0.97	1.00	1.05	0.028	D > A*
3·4±0·77	2·6±0·54	$3.0\pm0.62$		NS
	$ \begin{array}{c} U \\ - \\ 0 \\ 16 \\ 1 \\ 107 \\ 1.73 \\ 274 \\ 0.97 \\ 3.4 \pm 0.77 \end{array} $	$\begin{array}{c ccc} U & B \\ \hline - & Isolate \\ \hline 0 & 500 \\ \hline 16 & 16 \\ 1 & 0 \\ 107 & 100 \\ 1 \cdot 73 & 1 \cdot 31 \\ 274 & 225 \\ 0 \cdot 97 & 1 \cdot 00 \\ 3 \cdot 4 \pm 0 \cdot 77 & 2 \cdot 6 \pm 0 \cdot 54 \\ \hline \end{array}$	$\begin{array}{c ccccc} U & B & D \\ \hline - & Isolate & Concentrate \\ \hline 0 & 500 & 350 \\ \hline 16 & 16 & 16 \\ 1 & 0 & 2 \\ 107 & 100 & 74 \\ 1.73 & 1.31 & 1.77 \\ 274 & 225 & 225 \\ 0.97 & 1.00 & 1.05 \\ 3.4 \pm 0.77 & 2.6 \pm 0.54 & 3.0 \pm 0.62 \\ \hline \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 2. Effect of replacing dried skim-milk by sova-bean protein on performance of pigs

NS, not significant (P > 0.05).

\* P < 0.05, \*\*\* P < 0.001.

† g Dry matter consumed/g live-weight gain.



Fig. 1. Effect of age (d) on nitrogen retention (g/d per kg live weight) in pigs fed on a dried skim-milk diet (diet U,  $\bigcirc -\bigcirc$ ) or diets with 500 g/kg protein replaced by an isolated soya-bean protein (diet B,  $\triangle -\triangle$ ) or 350 g/kg protein replaced by a soya-bean protein concentrate (diet D,  $\bigcirc -\bigcirc$ ).

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

Mean values	for	thirteen	pigs,	(treatment)
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Diet Type of soya product	<u>U</u>	B Isolate	D Concentrate	se of a mean (21 df)	Statistical significance of differences between diets
Proportion of protein (g/kg) supplied by soya bean	0	500	350		
Digesta:					,
Total wt (g)	159-3	130.3	202.2	27.7	NS
Dry matter (g/kg)	227	103	149	14-1	U > B***, D**, D > B*
рНа	4.49	3.85	4.52	0.264	NS
Total nitrogen (g/kg)	14-14	3.96	6·79	1.57	U > B***, D**
Non-protein-N (g/kg total N)	169	344	215	26.0	B > D**, A***
Pepsin (units/g)	194	184	209	28.2	NS
Tissue:					
Pepsin (units/g)	2624	3805	3326	514.6	NS
NS, not significant $(P > 0.05)$ .		* $P < 0.05$ ,	** $P < 0.01$ ,	*** <i>P</i> < 0.00	D1.

			10)		
Diet	U	B	D		
Type of soya product		Isolate	Concentrate	se of a mean (10 df)	
Proportion of protein (g/kg) supplied by soya bean	0	500	350		
Pancreas:					
No. pigs	6	6	6	_	
Trypsin (mg/g)	0.70	0.99	1.48	0.160	D > U**
Chymotrypsin (mg/g)	1.53	1.76	2.14	0.327	NS

 Table 4. Effect of replacing dried skim-milk by soya-bean protein on the concentrations of trypsin and chymotrypsin in the pancreas of 28-d-old pigs

 (Mean values for six pigs)

NS, not significant (P > 0.05). \*\*P < 0.01.

Table 5. Effect of replacing dried skim-milk by either an isolate or a concentrate of soya-bean
protein on the amount of total nitrogen, proportion of non-protein-N and amounts of trypsin
and chymotrypsin in the small intestine of 28-d-old pigs

Diet	U	В	D		urr
Type of soya product	_	Isolate	Concentrate	e	
Proportion of protein (g/kg) supplied by soya-bean	0	500	350	Sites	Treatments
No. of pigs	8	8	8		
Total N (mg):					
Proximal	109	101	91	ſ	
Mid	122	117	101	{ 18·0 (14)***	15.7 (12)
Distal	278	366	325	t	
Non-protein-N (g/kg total N):					
Proximal	443	312	262	ſ	
Mid	409	421	365	41.2 (14)	23.3 (12)
Distal	301	512	412		
No. pigs	10	10	10		
Trypsin (mg):					
Proximal	0.6	0.7	1.1	ſ	
Mid	0.3	0.0	2.4	{ 1·36 (18)*	0.82 (15)
Distal	10.3	4·2	8.1	l i	
Chymotrypsin (mg):					
Proximal	2.4	1.2	4.6	ſ	
Mid	2.8	1.0	3.2	↓ 1·31 (18)***	0.67 (15)*
Distal	18.6	10.3	14.3	l	

(Values in parentheses are df<sup>†</sup>)

• P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

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† Results for total N and non-protein-N contained two missing values and there were three missing values in the trypsin and chymotrypsin results.

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## Digestion in the stomach

Substitution of soya-bean protein for milk protein did not significantly (P > 0.05) affect the total amount or the pH value of digesta remaining in the stomach 1 h after a feed, but reductions were found in the concentrations of DM and total N (Table 3). The proportion of total N present as NPN was increased in pigs given the soya-bean-protein isolate. Concentrations of pepsin in tissue or digesta were not affected by dietary protein source.

## Digestion in the small intestine

The concentration of trypsin in the pancreas was increased in pigs given the soya-bean-protein concentrate (Table 4), but this was not reflected in the amount of trypsin in the small intestine (Table 5). A smaller amount of chymotrypsin was present in the digesta when the soya-bean-protein isolate was given. There were no significant effects of diet on the amount of total N or proportion of NPN in the digesta. Greater amounts of total N, trypsin and chymotrypsin were present in the distal portion of the small intestine compared with that in the proximal or mid portions. Statistically significant interactions (P < 0.05) of treatment × sites were found in NPN and chymotrypsin

#### DISCUSSION

Pigs scoured severely and mortality was high when dried skim-milk powder was totally replaced by the soya-bean protein concentrate (diet C). Partial replacement by this concentrate (diet D) or a soya-bean protein isolate (diet B) reduced the performance from 2 to 28 d of age and with the concentrate, also growth rate during the initial 5 d of the experiment (Table 2). The proportion of soya-bean-protein isolate in diet B was greater than in a previous experiment (Newport, 1980) and this could account for the greater growth depression in the present experiment.

Scouring and mortality restricted studies of the apparent digestibility and retention of N to the milk-protein diet (diet U) and the partially-substituted diets (diets B and D). N retention on the latter two diets compared with milk protein was not reduced, unlike the previous experiment (Newport, 1980). It is possible that an error may have arisen in the present experiment due to calculation of N intake from analysis of the dried powders rather than of the reconstituted liquids. However, such an error would have little effect on the digestibility of N, which was similar for all three diets, as the amounts of faeces were very small.

The effects of partial replacement by isolated soya-bean protein on the amount and composition of digesta in the stomach were similar to those in the previous experiment (Newport, 1980; Table 3). The reduction in DM content of the digesta was smaller in pigs given the soya-bean protein concentrate than when the soya-bean protein isolate was given, and the pH value of the digesta was similar to that in pigs given the all-milk-protein diet. The difference in stomach pH between the two types of soya-bean products may reflect the greater amount of digesta in the stomachs of pigs given the soya-bean-protein concentrate. The total N as a proportion of the DM in the stomach was lower when soya-bean protein was given, suggesting a more rapid emptying of this type of protein than of milk protein. The results indicate that the soya-bean protein isolate did not inhibit stomach emptying as had been reported in preruminant calves (Smith & Sissons, 1975; Smith *et al.* 1979). However, a different isolate was used in the calf experiments and soya-bean concentrate tended to increase the amount of digesta in the stomach compared with milk protein, although differences were not statistically significant (P > 0.05).

The greater proportion of total N present as NPN in the digesta of the stomach (Table

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3) probably reflects the greater proportion in the diets containing soya-bean protein (251 and 214 g NPN/kg total N in diets B and D respectively) compared with milk protein (103 g NPN/kg total N in diet U). The similarity in pepsin concentrations in the digesta and tissue, confirming previous findings (Newport, 1980), would also suggest that proteolysis in the stomach was not affected in the 28-d-old pig.

Unlike the previous experiment, the concentration of chymotrypsin in the pancreas was not reduced in pigs receiving isolated soya-bean protein (Table 4), indeed there was a trend toward the concentrations of both trypsin and chymotrypsin to increase, and a larger increase in these concentrations occurred when the soya-bean protein concentrate was given. These effects are consistent with the previous observations (Newport, 1980) of higher concentrations in the pancreas of survivors given the diet with total replacement of dried skim-milk by isolated soya-bean protein.

Although the amount of chymotrypsin in the small intestine was reduced in pigs given the soya-bean isolate, but not when given the concentrate, no differences in the proportion of total N present as NPN were found (Table 5). The latter suggests that proteolysis was unimpaired, and is supported by previous observations (Newport, 1980) when only half as much chymotrypsin was present in digesta without apparently reducing intestinal proteolysis.

In the 28-d-old pig, soya-bean protein whether in the form of an isolate or concentrate was digested as well as milk protein as shown by the measurements of apparent digestibility, and observations on proteolysis in slaughtered pigs. The more rapid emptying of soya-bean protein from the stomach, if it occurs in the younger pig, may impair protein digestion particularly as proteolytic enzyme and acid secretion would be lower (Aumaitre, 1972; Cranwell *et al.* 1976).

The amount of diet offered also seems to be an important factor in the utilization of diets containing soya-bean proteins, and the lower amount of feed offered may explain why Sewell *et al.* (1953) and Cunningham & Brisson (1957) were able to rear pigs from 2 d of age with diets containing soya-bean as the only source of protein.

This and the previous experiment (Newport, 1980) suggest that approximately one-third of the protein in a diet with milk as the only source of protein can be replaced by a soya-bean-protein isolate without affecting the performance, but a reduction in performance was found with a soya-bean-protein concentrate. At the level of feed intake necessary for rapid growth, total replacement of skim-milk resulted in high mortality with both the isolate and concentrate. An increase in the rate of stomach emptying may be contributary to the effect of total replacement of skim-milk, but it is an unsatisfactory explanation of the effect of the lower level of inclusion of soya-bean protein as no differences in apparent digestibility were observed.

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