Artificial rearing of pigs

13. Effect of replacement of dried skim-milk by a functional fish-protein concentrate on the performance of the pigs and digestion of protein

BY M. J. NEWPORT AND H. D. KEAL

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, Berkshire

(*Received 3 March 1982 – Accepted 28 May 1982*)

1. Pigs weaned at 2 d of age were given diets in which a functional fish-protein concentrate (FFPC) supplied 0 (diet U), 350 (diet H), 525 (diet I) or 700 (diet G) g crude protein (nitrogen × 6·25, CP)/kg total CP. The remainder of the CP was supplied by dried skim-milk and whey. The diets were reconstituted (200 g dry matter (DM)/l, from two spray-dried powders (diets U and G) and these were mixed in the proportions 1:1 and 1:3 (v/v) to give diets H and I respectively. All four diets were given at hourly intervals on a scale based on live weight, and supplemented with vitamins and minerals. All diets contained the same amounts of calcium, phosphorus, sodium and potassium.

2. In an experiment to 28 d of age with ten pigs/diet, performance was inversely related to the amount of FFPC in the diet. One pig (diet I) and four pigs (diet G) died after scouring. In healthy pigs, N retention (g/d per kg live weight) was not affected by partial replacement of dried skim-milk (diet H).

3. Digestion of protein was studied in a further experiment with eight pigs/diet, killed at 7 d of age and at 1 h after a feed. The amount, pH, DM and total N contents of the digesta in the stomach were also inversely related to the amount of FFPC in the diet.

4. FFPC had no consistent effect on proteolytic enzymes. Greater amounts of trypsin and chymotrypsin were present in the distal compared with the proximal region of the small intestine, but source of dietary protein had no effect.

5. The proportion of non-protein-N in the total N in digesta from the small intestine was lower in pigs receiving FFPC suggesting that this protein may be more resistant to proteolysis than milk proteins. The apparent absence of an increase in enzyme secretion when FFPC was given could, therefore, explain the decrease in performance.

6. Some general effects of non-milk proteins from this series of experiments in the baby pig are discussed. It is suggested that performance may be inversely related to the rate of gastric emptying, as indicated by the amount of DM in the stomach. Unlike milk, other sources of protein did not coagulate in the stomach, and reduced the pH, DM and total N contents of the digesta. Giving diets containing a single-cell protein did appear to stimulate proteolytic enzyme secretion, unlike fish or soya-bean proteins.

In a previous study with pigs weaned at 2 d of age total replacement of dried skim-milk by a fish-protein concentrate gave poor performance and reduced nitrogen retention (Newport, 1979), but there was little effect when only half the dried skim-milk was replaced. In calves, a product described as a functional fish-protein concentrate (FFPC) gave much improved apparent digestibilities and retentions of N compared with previous studies on other preparations from fish, although milk protein was still superior (Opstvedt *et al.* 1978). FFPC is a spray-dried product with good water-holding and emulsifying characteristics to which the improvement in N digestibility and retention were attributed, and the authors also speculated that these properties may lead to longer abomasal retention as found for milk.

It was therefore of interest to examine FFPC as a source of protein in a milk-substitute for pigs weaned at 2 d of age to determine whether a similar improvement in N utilization would occur as in calves, and to obtain some direct information on its digestion, particularly the amounts retained in the stomach compared with milk protein. Four diets were fed in which milk was the only source of protein or 350, 500 or 700 g/kg crude protein (N × 6·25, CP) replaced from FFPC.

Table 1. *Composition (per l) of diets supplying milk (diet U) or fish (diet G) protein*

	Diet	
	U	G
Dried skim-milk (g)	132·0	—
Dried whey (g)	14·0	106·0
Functional fish-protein concentrate (g)*	—	40·0
Soya-bean oil (g)	54·0	54·0
Butylated hydroxytoluene (mg)	3·0	3·0
Mineral solutions (ml)		
$CaCl_2$ (106 g/l)	—	20·0
$NaH_2PO_4.2H_2O$ (121 g/l)	—	20·0
K_2SO_4 (34 g/l)	20·0	—
NaCl (166 g/l)	20·0	—
Water-soluble-vitamin solution (ml)†	10·0	10·0
Fat-soluble-vitamin solution (ml)‡	1·8	1·8

Both diets contained (g/kg dry matter): crude protein (N × 6·25) 235, total lipid 305, calcium 9·1, phosphorus 8·0, sodium 8·7, potassium 15·4.

* Obtained from Norwegian Herring Oil and Meal Industry Research Institute, Bergen, Norway. Contains (g/kg): dry matter 950, crude protein 840, ash 110, total lipid 40, calcium 13, phosphorus 13.

† Supplied/kg diet dry matter: thiamin hydrochloride 1·65 mg, riboflavin 2·5 mg, pyridoxine hydrochloride 3·0 mg, nicotinic acid 20·0 mg, calcium pantothenate 11·7 mg, cyanocobalamin 16·5 μg, biotin 50 μg, folic acid 0·5 mg, ascorbic acid 30 mg, choline chloride 1·1 g.

‡ Supplied/kg diet dry matter: retinol 0·6 mg, cholecalciferol 5 μg, α-tocopherol 1·65 mg, menaphthone 66 μg.

EXPERIMENTAL

Experimental design and procedures

Litter-mate 2-d-old pigs were allocated to four diets. Initial weight and sexes were equalized between diets. There were ten pigs/diet in a feeding experiment of 26 d duration, and seven pigs/diet in a slaughter experiment in which the pigs were killed at 7 d of age for a study of protein digestion as described by Newport (1980).

The pigs were given the diets on a scale based on live weight at hourly intervals from an automatic feeder. The feeding scale and details of the rearing procedure have been previously described (Braude *et al.* 1970; Braude & Newport, 1973).

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

Diets

Two diets were prepared supplying protein either entirely from milk (diet U) or 700 g/kg total protein from FFPC (diet G). Their compositions are given in Table 1 and 2.

Whey, or skim-milk and whey, were mixed with soya-bean oil and butylated hydroxytoluene, and spray-dried using a mild-heat treatment. Both powders were reconstituted in water, and the spray-dried FFPC mixed into the appropriate powder. The reconstituted diets, containing 200 g dry matter (DM)/l, were homogenized at a pressure of 176 kg/cm^2 and pasteurized at 72° for 17 s. Batches of diet were prepared twice-weekly and stored at 5°. Solutions containing minerals, water-soluble and fat-soluble vitamins (Table 1) were added daily to diets U and G and as a preservative (/l), 0·5 ml of a solution containing 400 g formaldehyde/l. Diets supplying 350 (diet H) or 525 (diet I) g CP/kg total CP from FFPC were prepared by mixing diets U and G in the proportions of 1:1 and 1:3 (v/v) respectively.

Table 2. *Amino acid content (g/kg dry matter) of diets supplying milk (diet U) or fish (diet G) protein*

Amino acid	Diet U	Diet G	Amino acid	Diet U	Diet G
Lysine	17·4	19·0	Tryptophan*	2·8	3·5
Arginine	8·6	10·8	Valine	14·7	10·3
Methionine + cystine	7·8	7·6	Aspartic acid	18·6	21·4
Histidine	6·0	4·7	Serine	13·7	9·5
Isoleucine	12·0	9·9	Glutamic acid	45·6	34·3
Leucine	22·7	18·2	Proline	22·3	9·1
Phenylalanine	11·2	8·3	Glycine	4·9	9·6
Threonine	10·9	10·5	Alanine	8·3	11·9
Tyrosine	11·8	7·1			

* Calculated from National Research Council (1969) values for dried skim-milk and dried whey, and from analysis on functional fish-protein at the Norwegian Herring Oil and Meal Industry Research Institute.

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 of diet were ashed before the determination of calcium, sodium and potassium by atomic absorption spectroscopy and phosphorus by a colorimetric procedure described by Cavell (1955). The amino acid composition of the diets was determined by hydrolysis and ion-exchange chromatography using methods given in Braude *et al.* (1977). 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), standing for 16 h at 5°, and the removal of the precipitated protein by filtration through Whatman no. 542 filter paper.

Pepsin (*EC* 3.4.23.1) activities in stomach tissue and digesta were 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 assayed by the method of Hummel (1959), but after activation of pancreatic homogenates with enterokinase (*EC* 3.4.21.9) (Miles Labs Ltd, Stoke Poges). Activities of both trypsin and chymotrypsin were calculated by comparison with purified enzymes (*EC* 3.4.21.4 and 3.4.21.1 respectively) (Koch-Light Labs, Colnbrook, Bucks.).

RESULTS

Performance and N retention

Between 2 and 7 d of age, performance was inversely related to the fish-protein content of the diets (Table 3). The same effect continued throughout the experiment to 28 d of age, but with a greater deterioration in performance relative to other diets when dried skim-milk was totally replaced by the fish protein. Scouring and mortality were also greater on this diet.

N retention, estimated in eight 20-d-old pigs given diets U or H, was $2{\cdot}02 \pm 0{\cdot}173$ and $2{\cdot}13 \pm 0{\cdot}224$ g/d per kg live weight respectively. This difference was not significant ($P > 0{\cdot}05$).

Table 3. *Effect of replacing dried skim-milk by a functional fish-protein concentrate (FFPC) and dried whey on the mortality rate and performance of pigs from 2 to 28 d of age*

(Mean values for ten pigs/diet)

						Statistical significance of effects of proportion of FFPC	
Diet ...		U	H	I	G	Linear effect (per g/kg)	Quadratic effect (per (g/kg)²)
Proportion of protein (g/kg) supplied by FFPC ...		0	350	525	700	—	—
Age (d)	Mortality ...	0	0	0	1		
2–7	Live-wt gain (g/d)	179	146	103	85	−0·139*** ±0·0273	NS
	Feed: gain†	0·98	1·10	1·59	1·97	0·00141*** ±0·000271	NS
	Mortality	0	0	1	4	—	—
2–28	Live-wt gain (g/d)	315	245	211	96	−0·290*** ±0·0264	−0·00041** ±0·000126
	Feed: gain†	0·96	1·13	1·24	2·57	0·00195*** ±0·000418	0·0000065** ±0·000002

NS, not significant ($P > 0·05$), ** $P < 0·01$, *** $P < 0·001$.

† g dry matter consumed/g gain.

Missing values were calculated for all dead pigs, and also for pigs from 2 to 7 d of age which died later in the experiment.

Table 4. *Effect of replacing dried skim-milk by a functional fish-protein concentrate (FFPC) and dried whey on the amount, pH and composition of digesta from the stomach and the concentrations of pepsin in the digesta and stomach tissue*

(Mean values for seven pigs/diet)

					Statistical significance of effects of proportion of FFPC	
Diet ...	U	H	I	G	Linear effect (per g/kg)	Quadratic effect (per (g/kg)²)
Proportion of protein (g/kg) supplied by FFPC	0	350	525	700	—	—
Digesta						
Total (g)	124·7	104·6	102·0	72·7	−0·067* ±0·0236	NS
pH	5·1	4·8	4·6	3·9	−0·00165*** ±0·00028	0·0000032* ±0·00000133
Dry matter (g/kg)	240	194	144	124	−0·171** ±0·0549	NS
Total nitrogen						
mg/g	12·1	6·3	5·5	4·3	−0·0111** ±0·00332	NS
mg/g dry matter	48·2	32·8	37·8	42·6	NS	NS
Pepsin (units/g)	18·9	10·5	12·7	15·8	NS	NS
Tissue						
Pepsin (units/g)	270	147	239	299	NS	NS

NS, not significant ($P > 0·05$).

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Digestion in the stomach

The amount, DM and N content, and pH of the digesta were inversely related to the amount of fish protein in the diet (Table 4). The source of protein in the diet had no consistent effect on the concentration of pepsin in the digesta or tissue.

Table 5. *Effect of replacing dried skim-milk by a functional fish-protein concentrate (FFPC) and dried whey on the amount of total nitrogen and proportion of non-protein-N in the small intestine of 7-d-old pigs*

(Mean values for seven pigs/diet)

Diet ...	U	H	I	G	Linear effect of proportion of FFPC (per g/kg)
Proportion of protein (g/kg) supplied by FFPC	0	350	525	700	
Total N (mg)					
Proximal	28·8	23·8	13·5	27·2	
Mid	44·8	31·7	39·4	31·5	
Distal	38·5	42·4	60·7	47·8	
Mean	37·4	32·6	37·9	35·5	NS
Non-protein-N (g/kg total N)					
Proximal	445	317	344	341	
Mid	400	431	382	362	
Distal	511	360	439	323	
Mean	452	369	388	342	−0·144** ± 0·0467

NS, not significant ($P > 0·05$), ** $P < 0·01$.
Site differences were significant ($P < 0·05$); distal > mid > proximal for total N, but were not significant ($P > 0·05$) for non-protein-N.

Table 6. *Effect of replacing dried skim-milk by a functional fish-protein concentrate (FFPC) and dried whey on concentrations of trypsin and chymotrypsin in the pancreas, and amounts in the small intestine of 7-d-old pigs*

(Mean values for seven pigs/diet)

Diet ...	U	H	I	G
Proportion of protein (g/kg) supplied by FFPC	0	350	525	700
Pancreas				
Trypsin (mg/g)	0·95	0·46	0·83	0·50
Chymotrypsin (mg/g)	1·25	1·17	1·37	1·04
Trypsin (mg) in small intestine				
Proximal	0·42	0·33	0·21	0·80
Mid	0·23	0·29	0·19	0·97
Distal	1·18	0·92	1·80	1·98
Chymotrypsin (mg) in small intestine				
Proximal	0·80	0·75	0·15	0·67
Mid	0·68	1·09	0·66	0·90
Distal	1·64	1·50	2·63	2·88

There were no significant ($P > 0·05$) linear or quadratic effects of level of FFPC in the diet.
Trypsin and chymotrypsin were both greater ($P < 0·05$) in the distal site compared with proximal or mid sites.

Digestion in the small intestine

Dietary source of protein had no effect on the total N in the small intestine, but the proportion of non-protein-N was decreased by FFPC (Table 5). The amount of total N did increase distally.

FFPC had no consistent effect on the concentrations of trypsin or chymotrypsin in the pancreas, or amounts of these two enzymes in the small intestine (Table 6). The amounts of trypsin and chymotrypsin were increased in the distal portion of the small intestine.

DISCUSSION

Effects of fish proteins

Studies in calves have shown that FFPC was much superior to previous preparations of fish protein for inclusion into milk-substitutes, and this was attributed to an improvement in water-holding and emulsification capacity (Opstvedt *et al.* 1978). However, performance and apparent digestibility and retention of N were decreased when dried skim-milk was replaced by FFPC. Intermediate levels of replacement were not examined.

In contrast to the experiments with calves, the present experiment with baby pigs indicated that FFPC was more poorly utilized than a fish-protein concentrate studied in an earlier experiment (Newport, 1979), and was markedly inferior to milk protein (Table 3). Caution is needed in such a comparison, as the performance of pigs receiving the milk-protein (control) diet differed considerably between the two experiments, that in the present experiment with FFPC being more similar to previous experience (Newport, 1980; Newport & Keal, 1980). The concentrations of some essential amino acids were slightly reduced when FFPC replaced dried skim-milk (Table 2). The significance of these reductions is uncertain as the requirements of baby pigs for amino acids are tentative due to the limited amount of experimental results (Agricultural Research Council, 1981).

Although N retention was estimated in only three pigs given the all-milk diet, the mean value was very similar to previous results in pigs of the same age (Newport, 1980) and so it may be concluded that N retention/kg live weight was not affected by supplying approximately one-third of the protein from FFPC in healthy pigs. This suggests that the composition of the carcass is unaffected by protein source. The incidence of scouring and soft faeces made meaningful results impossible to obtain from the other two diets under our conditions. In calves Opstvedt *et al* (1978) found no effect of FFPC on N retained/kg live weight.

As FFPC affected performance throughout the experiment, and digestive ability is more limited in the immediate neonatal period (Aumaitre, 1972; Cranwell *et al.* 1980), studies on digestion were made in pigs killed at 7 d of age. The amount of DM in the stomach was inversely related to the amount of FFPC in the diet (Table 4) and suggests an increased rate of gastric emptying. When dried skim-milk was totally replaced this amount of DM was considerably greater than in the experiment with the fish-protein concentrate (Newport, 1979) which suggests that FFPC may be retained for a longer period in the stomach. The effects of fish protein on the composition of the digesta in the stomach were similar in both experiments.

Longer gastric retention should favour a more complete digestion of the protein. However, the amounts of trypsin and chymotrypsin in the small intestine (Table 6) were several-fold less than in the experiment with a fish-protein concentrate (Newport, 1979) suggesting that pancreatic enzyme secretion may have been lower. A lower rate of secretion may still be adequate for digestion of milk protein but inadequate for FFPC. Some support for this hypothesis can be found in the lower proportions of non-protein-N in the total N of the digesta (Table 5) from pigs given FFPC. These differences in amounts of intestinal proteolytic enzymes further underline the hazards of comparing these two experiments.

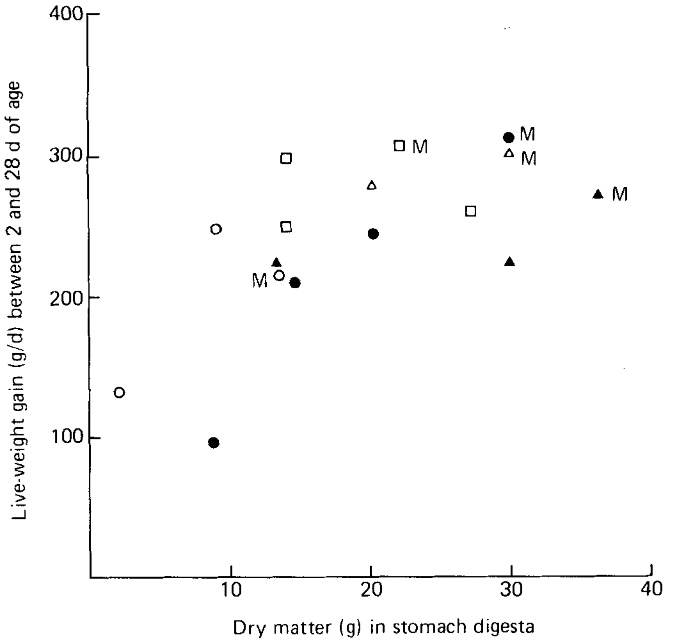

Fig. 1. Relationship between growth rate and amount of digesta in the stomach from studies on replacement of milk protein by alternative sources. Alternative protein sources were: ○, fish-protein concentrate (Newport, 1979); ●, functional fish-protein concentrate (present experiment); △, isolated soya-bean protein (Newport, 1980); ▲, soya-bean protein concentrate (Newport & Keal, 1982); □, single-cell protein (Newport & Keal, 1980). M, control diets with all-milk protein.

Effects of non-milk proteins on protein digestion

This experiment concludes a series (Newport, 1979; Newport, 1980; Newport & Keal, 1980; Newport & Keal, 1982) in which a major objective was to attempt to relate the effects of non-milk proteins on performance to limitations of, or changes in, the digestive system. In all these experiments coagulation in the stomach of diets containing non-milk proteins did not occur, or was poorer than with an all-milk-protein diet. Thus, non-milk proteins gave a decrease in the percentages of DM and total N in the digesta in the stomach. The pH value of the digesta was also decreased, presumably due to the lowered buffering effect with the reduction in DM content of the digesta to below that in the diet. In the diet, non-milk proteins either slightly increased (Newport, 1980) or decreased (Newport & Keal, 1980) the buffering capacity.

In this series of experiments pigs were slaughtered under standardized conditions and, therefore, the amount of DM in the stomach may be an indicator of the rate of gastric emptying. The results indicate a relationship between growth rate and the amount of DM in the stomach (Fig. 1), and suggest that gastric emptying rate may be inversely related to performance in the neonatal pig. The relationship is more apparent in the present experiment with FFPC and in an experiment with a fish-protein concentrate (Newport, 1979) when pigs were slaughtered at 7 d of age, than in other experiments in which pigs were slaughtered at 28 d of age. Maner *et al.* (1962) also found that differences in transit time between protein sources disappeared with age.

Experiments with a single-cell protein (Newport & Keal, 1980) gave only a small depression in growth rates even when dried skim-milk was totally replaced, but there were considerable differences in amounts of digesta in the stomach at 28 d of age (Fig. 1). This suggests that the relationship of growth rate with the amount of digesta DM in the stomach may be curvilinear. Similar results were obtained when up to half of the milk protein was replaced by soya-bean proteins (Newport, 1980; Newport & Keal, 1982) but total replacement with soya-bean protein gave a high mortality. Thus, this hypothesis (Fig. 1) could be further examined by experiments with intermediate levels of soya-bean proteins selected to give moderate or poor growth.

The growth of pigs receiving the all-milk-protein (control) diet in the experiment with the fish-protein concentrate (Newport, 1979) was poorer than in other experiments (Fig. 1). This may be due to the lower CP content of the control diet than in other experiments. Another factor contributing to the variability of results is the incidence of scouring, which differed between experiments, and may have influenced growth rates. However results from all control groups support the curvilinear nature of the response shown in Fig. 1.

Although Pekas *et al.* (1966) observed that proteolytic enzyme secretion in pancreatic juice was greater for soya bean than for milk protein, in our series of experiments, the single-cell protein (Newport & Keal, 1980) was the only source of protein to have any consistent effect on proteolytic enzymes, with increases in the concentration of pepsin in digesta from the stomach and amount of chymotrypsin in the small intestine. In contrast, the amounts of trypsin and chymotrypsin in the small intestine were reduced when a fish-protein concentrate was given (Newport, 1979). In general, the amounts of trypsin or chymotrypsin in digesta were unrelated to the concentration of these enzymes in the pancreas. These results do suggest that the single-cell protein may stimulate physiological mechanisms leading to an increased secretion of proteolytic enzymes and could account for the improved performance of the pigs compared with other alternatives to milk protein.

The functional fish-protein was kindly donated by the Norwegian Herring Oil and Meal Industry Research Institute, and the spray-dried powders were prepared by Volac Ltd, Royston, Herts.

The authors thank J. A. Williams for the care of the pigs and preparation of diets and Mrs G. L. Howarth for chemical and enzyme analyses.

REFERENCES

Agricultural Research Council (1981). *The Nutrient Requirements of Pigs*. Farnham Royal: Commonwealth Agricultural Bureaux.
Anson, M. L. (1938). *J. gen. Physiol.* **22**, 79.
Aumaitre, A. (1972). *Wld Rev. Anim. Prod.* **8**, 54.
Braude, R., Keal, H. D. & Newport, M. J. (1976). *Br. J. Nutr.* **35**, 253.
Braude, R., Keal, H. D. & Newport, M. J. (1977). *Br. J. Nutr.* **37**, 187.
Braude, R., Mitchell, K. G., Newport, M. J. & Porter, J. W. G. (1970). *Br. J. Nutr.* **24**, 501.
Braude, R. & Newport, M. J. (1973). *Br. J. Nutr.* **29**, 447.
Cavell, A. J. (1955). *J. Sci. Fd Agric.* **6**, 479.
Cranwell, P. D., Shaughnessy, J. J. & Smith, R. E. (1980). *Proc. Nutr. Soc.* **39**, 93A.
Hummel, B. C. W. (1959). *Can. J. Biochem. Physiol.* **37**, 1393.
Maner, J. H., Pond, W. G., Loosli, J. K. & Lowrey, R. S. (1962). *J. Anim. Sci.* **21**, 49.
National Research Council (1969). *Tables of Feed Composition*. Washington DC: National Academy of Sciences.
Newport, M. J. (1979). *Br. J. Nutr.* **41**, 103.
Newport, M. J. (1980). *Br. J. Nutr.* **44**, 171.
Newport, M. J. & Keal, H. D. (1980). *Br. J. Nutr.* **44**, 161.
Newport, M. J. & Keal, H. D. (1982). *Br. J. Nutr.* **48**, 89.
Opstvedt, J., Sobstad, G. & Hansen, P. (1978). *J. Dairy Sci.* **61**, 72.
Pekas, J. C., Thompson, A. M. & Hays, V. W. (1966). *J. Anim. Sci.* **25**, 113.

Printed in Great Britain