Artificial rearing of pigs

10. Effect of replacing dried skim-milk by a single-cell protein (Pruteen) on performance and digestion of protein

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1. Spray-dried diets were prepared containing (g/kg): dried skim-milk 665, dried whey 65, soya-bean oil 270 (diet U); or single-cell protein (Pruteen; SCP) 308, dried whey 440, soya-bean oil 252 (diet X). The diets had a crude protein (nitrogen × 6·25) content (g/kg) of 250 (diet U) and 240 (diet X), excluding nucleic acids (36 g/kg) in diet X.

2. The diets were reconstituted (200 g dry matter/l) and mixtures of diets U and X prepared to give diets supplying 0 (diet U), and approximately 400 (diet V), 600 (diet W) and 800 (diet X) g crude protein from SCP/kg total protein. All diets were supplemented with vitamins, and minerals to equalize the calcium, phosphorus, sodium and potassium concentrations.

3. Pigs weaned at 2 d of age were given the diets at hourly intervals on a scale based on live weight. At 28 d age the experiment was terminated and pigs killed 1 h after a feed for a study of protein digestion. Polyethylene glycol (PEG) was fed in the diets (0·5 g/l) for 24 h before slaughter.

4. Performance of pigs fed on diet V was as good as on the all-milk diet U. Greater levels of replacement by SCP (diets W and X) reduced performance. Mortality was greater on the all-milk diet, but protein source had no effect on the incidence of scouring. N retention (g/d per kg live weight) was similar for all diets but declined with age.

5. SCP appeared to stimulate secretion of pepsin and chymotrypsin, and reduced the pH value in digesta in the stomach. Enzyme adaptation may have been insufficient to digest high levels of SCP in the diet, and together with the decreased transit time observed using PEG as a marker, may account for the poorer performance when 600 or 800 g/kg milk protein was replaced.

6. Nucleic acids from SCP were metabolized and not retained for tissue synthesis. Allantoin excretion accounted for 75% of the theoretical maximum for complete excretion of nucleic acids, and uric acid excretion was also increased.

Previous attempts to replace milk protein in diets for baby pigs by alternatives have met with limited success. When half the protein was replaced by a fish protein concentrate, performance of artificially-reared pigs from 2–28 d of age was not affected, but total replacement reduced performance severely and adversely affected protein digestion (Newport, 1979*b*). In another experiment, soya-bean protein was a poorer alternative to milk protein for both partial and total replacement (Braude & Newport, 1978).

We have now evaluated a single-cell protein (Pruteen; Imperial Chemical Industries Ltd; SCP) as a substitute for milk protein in the diet of the artificially-reared pig. A preliminary report of this study has already been given (Newport & Keal, 1978). SCP is prepared by harvesting and spray-drying to a finely-ground powder the cells of the bacterium *Methylophilus methyltrophum*. The organism is grown in a medium containing methanol and ammonia. SCP has been shown to have a biological value for older pigs similar to that for white fish meal (Braude *et al.* 1977; Whittemore *et al.* 1976), or soya-bean meal (Roth & Kirchgessner, 1977*a*). These workers assumed that nucleic acids in SCP cannot be used for protein synthesis.

EXPERIMENTAL

Diets

Two spray-dried powders (U and X) were prepared by a mild-heat procedure. Their composition is given in Table 1. The powders were reconstituted in water (200 g dry matter/l),

Table 1. *Composition (g/kg) of the spray-dried powders*

	U	X
Ingredients:		
Dried skim-milk	665	—
Dried whey	65	440
Pruteen*	—	308
Soya-bean oil	270	252
Chemical analysis:		
Crude protein (CP) (nitrogen × 6·25)	251	274
Nucleic acid (NA)†	—	36
CP–NA	251	238
Non-casein-N (g/kg total N)	160	—
Total lipid	293	282
Ash	59	60
Calcium	8·8	4·9
Phosphorus	8·0	9·6
Sodium	4·6	8·7
Potassium	13·1	11·3
Calculated analysis:‡		
Lysine	19·3	18·8
Arginine	8·3	12·5
Methionine	5·4	6·3
Cystine	3·5	2·8
Histidine	6·1	4·8
Isoleucine	15·9	14·6
Leucine	22·8	22·2
Phenylalanine	10·3	10·0
Threonine	9·8	14·2
Tyrosine	8·8	8·4
Tryptophan	2·8	3·9
Valine	15·1	15·9

* Contained 60 g soya-bean oil/kg.
† Estimated by ICI Ltd.
‡ Calculated from analyses of dried skim-milk, and dried whey at NIRD; and Pruteen analysed by ICI Ltd.

Table 2. *Composition of the diets*

Diet ...	U	V	W	X
Milk-substitute powder* (g/l):				
Powder U	200	100	50	—
Powder X	—	100	150	200
Supplementary solutions (ml/l):				
Water-soluble vitamins†	6·6	6·6	6·6	6·6
Fat-soluble vitamins‡	1·0	1·0	1·0	1·0
$CaCl_2.6H_2O$ (548 g/l)	1·6	5·5	7·5	9·4
KCl (19 g/l)	—	1·8	2·7	3·6
$NaH_2PO_4.2H_2O$ (201 g/l)	8·0	4·0	2·0	—
NaCl (250 g/l)	5·8	3·0	1·4	—

All diets contained (g/kg DM): calcium 9·6, phosphorus 9·6, sodium 8·7, potassium 13·1.

* For details, see Table 1.

† Supplied (/kg diet dry matter): thiamine hydrochloride 1·65 mg, riboflavin 2·5 mg, pyridoxine hydrochloride 3·0 mg, nicotinic acid 20 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 DM): retinol 0·6 mg, cholecalciferol 5 μg, α-tocopherol 1·65 mg, menapthone 66 μg.

homogenized and pasteurized (Braude & Newport, 1973). Four liquid diets were given to pigs containing either powder U or X, or mixtures of both. A vitamin supplement was added to each of the four diets, together with appropriate mineral supplements to equalize the calcium, phosphorus, sodium and potassium contents of the diets. The composition of the diets is given in Table 2. Milk was the only source of protein in diet U. In diets V, W and X, SCP provided approximately 400, 600 and 800 g/kg total protein.

Experimental design

Litter-mate, 2-d-old pigs were allocated to the four diets (treatments) on the basis of live weight and sex. The pigs on different treatments were kept in separate but identical rooms, each containing four replicates. This procedure was repeated four times so that there were sixteen pigs/treatment. The pigs were on experiment until 28 d of age. The results were analysed after calculating missing values, due to the death of pigs after scouring.

Experimental procedure

The artificial rearing procedure was described by Braude, Mitchell *et al.* (1970). The pigs were fed at hourly intervals on the scale described by Braude & Newport (1973). Diets were reconstituted twice-weekly and stored at 5°. Fresh diet was given to the pigs each day, containing 0·5 ml formalin (400 g formaldehyde/l)/l.

Nitrogen retention was estimated from a collection period of 4 d duration as previously described (Braude *et al.* 1976). Collection periods were not made when pigs were scouring. Only urinary N was estimated, as the amount of faecal excretion was negligible.

Pigs were killed at 28 d of age, 1 h after a feed, by an intracardiac injection of sodium pentobarbitone. Polyethylene glycol (0·5 g/l) had been included in the diet for 24 h before slaughter. Digesta and organs were removed as described by Newport (1979*b*). The proximal and mid portions of the small intestine were each approximately 2·40 m, and the remainder of the small intestine (5·00 m) was considered to be the distal portion.

Analytical methods

Methods for the analysis of DM, total and non-protein-N, and total lipid were described by Braude, Mitchell *et al.* (1970) and Braude & Newport (1973). Non-casein-N was estimated by the method of Rowland (1938). Samples of diet were ashed, and Ca determined by atomic absorption spectroscopy, Na and K by atomic emission spectroscopy, and P by a colorimetric procedure (Cavell, 1955). Pepsin was analysed by the method of Anson (1938), and trypsin and chymotrypsin as described by Hummel (1959) with modifications (Newport, 1979*b*). The method of Smith (1958) was used for analysis of polyethylene glycol (PEG). The proportions of protein, peptides and amino acids in digesta were determined by filtration in Sephadex G-25 (Braude, Newport *et al.* 1970). Amino acids in SCP were analysed by cation-exchange chromatography following hydrolysis in 6M-hydrochloric acid for 24 h under N_2. Nucleic acids were analysed by the method of Gale & Folkes (1953).

RESULTS

Performance

Up to 400 g/kg dietary protein could be supplied by SCP without reducing the performance of the pigs compared with an all-milk protein (Table 3). Performance was reduced when 600 or 800 g/kg milk protein was replaced. Mortality was greater on the all-milk diet, but the number of days of scouring was similar for all treatments.

Table 3. *Performance of the pigs from 2–28 d of age*

(Mean values for sixteen pigs/treatment)

	Diet U	Diet V	Diet W	Diet X	SE of a mean (36 df)†	Statistical significance of differences between diets
Proportion of total protein (g/kg) supplied by SCP	0	400	600	800	—	–
Live-weight gain (g/d)	307	299	261	250	8·8	U > W, X***; V > W**, X***
Feed : gain (g dry matter consumed/g live-weight gain)	0·86	0·89	0·92	0·98	0·025	X > V*, U**
No. of deaths	5	2	2	0	—	—
No. of d scouring‡	2·9	1·9	1·6	2·7	—	—

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Missing values calculated for dead pigs.
‡ Mean no. of d scouring observed in each pig.

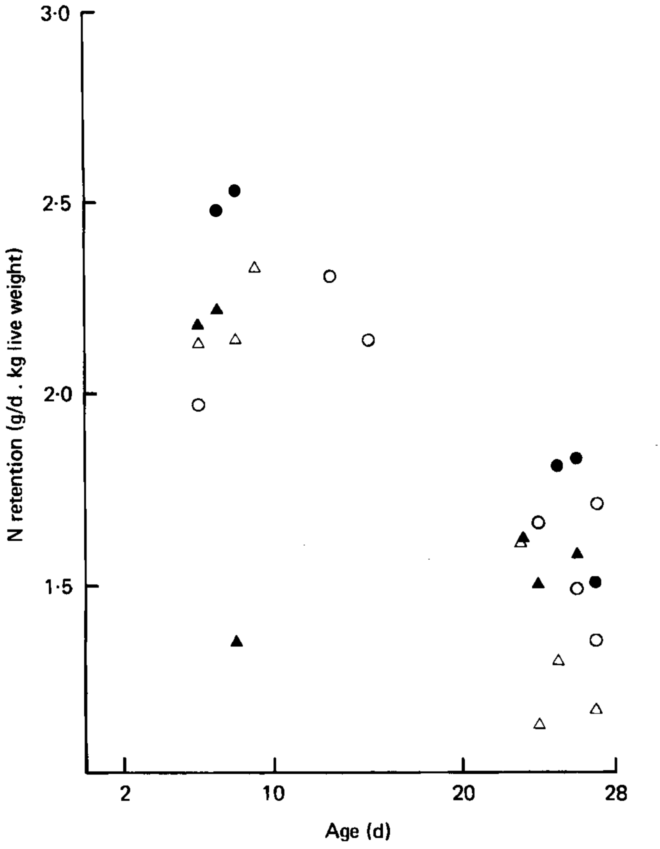

Fig. 1. Effect of age (d) and source of dietary protein on nitrogen retention (g/d per kg live weight) in pigs given diets in which a single-cell protein (Pruteen) replaced dried skim-milk. (For details of diets, see Table 2 and p. 161.) ○—○, Diet U; ●—●, Diet V; △—△, Diet W; ▲—▲, Diet X.

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Table 4. *Effect of replacing dried skim-milk by single-cell protein (Pruteen, SCP) and dried whey on the amount and pH of digesta in the stomach, and the composition of the digesta in the stomach and small intestine of 28-d-old pigs*

	No. of pigs/diet	Diet U	Diet V	Diet W	Diet X	SEM†	df	Statistical significance of differences between diets
Proportion of total protein (g/kg) supplied by SCP		0	400	600	800			
Stomach digesta:								
Total wt (g)	10	111·9	105·6	194·8	120·1	19·85	24	W > U, V, X***
Dry matter (DM) (g/kg)	10	199·2	134·8	140·4	116·8	15·23	22	U > V, W, X***
pH	16	4·38	2·94	3·92	3·67	0·286	36	U > X*, V***; W > V**; X> V*
Total N (g/kg)	6	11·58	6·28	5·30	5·37	0·729	15	U > V, W, X***
Small intestine digesta:								
Total N (mg)	6	382	448	513	329	49·4	15	W > U*, X**; V > X*
Non-protein-N (g/kg total N)	6	540	583	688	610	64·5	15	W > U*

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

† One missing value was calculated for diets U, V and W (total weight and DM), and two missing values for diet X (DM). Means for pH values include five (diet U) and two (diets V and W) missing values.

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Table 5. *Effect of replacing dried skim-milk by single-cell protein (Pruteen; SCP) and dried whey on the concentrations of pepsin in stomach digesta and tissue, concentrations of trypsin and chymotrypsin in the pancreas and amounts in digesta from the small intestine of 28-d-old pigs*

	No. of pigs/diet	Diet U	Diet V	Diet W	Diet X	SEM†	df	Statistical significance of differences between diets
Proportion of total protein (g/kg) supplied by SCP	—	0	400	600	800	—	—	—
Pepsin in:								
Digesta (μg/g)	10	54·5	73·6	77·9	115·9	17·59	24	X > W, V*; > U**
Tissue (mg/g)	10	1·64	1·85	1·79	1·80	0·183	23	NS
Trypsin in:								
Pancreas (mg/g)	8	2·15	1·32	1·31	2·08	0·224	18	U, X > V, W**
Total digesta (mg)	9	9·2	8·2	8·3	11·6	2·11	21	NS
Chymotrypsin in:								
Pancreas (mg/g)	8	4·32	2·32	2·30	2·70	0·380	18	U > V, W, X***
Total digesta (mg)	9	15·6	22·8	22·3	22·8	3·07	21	U < V, W, X*

NS, not significant ($P > 0·05$).
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Three or four missing values were calculated for each variate.

Table 6. *Effect of replacing dried skim-milk by single-cell protein (Pruteen; SCP) and dried whey on the recovery of polyethylene glycol (g/kg intake) in digesta of 28-d-old pigs*

(Mean values for six pigs/diet)

	Diet				SE of a mean (13 df)†	Statistical significance of differences between diets
	U	V	W	X		
Proportion of total protein (g/kg) supplied by SCP	0	400	600	800		
Small intestine: Proximal	2·8	4·7	3·7	3·3	0·96	NS
Small intestine: Mid	7·9	5·5	5·9	10·4	2·61	NS
Small intestine: Distal	64·7	32·1	38·4	41·7	8·95	U > V**, > W, X*

NS, not significant ($P > 0·05$).

* $P < 0·05$, ** $P < 0·01$.

† Includes one missing value each for diets V and W.

N retention

Regression analysis of the N retention data in Fig. 1 showed no differences between diets ($P > 0·05$), but N retention declined with age. Omitting the value of 1·35 g/d per kg live weight for an 8-d-old pig given diet X did not affect the statistical conclusions.

Digestion in the stomach

Replacing dried skim-milk by SCP increased the amount of digesta in the stomach when 600 g/kg milk protein was replaced, but other levels of replacement had little effect (Table 4). The DM, N content and pH of the digesta were reduced when SCP was included in the diet. Pepsin concentrations in the digesta were greater in pigs given diets containing SCP, but no differences were found in the levels in the stomach tissue (Table 5).

Digestion in the small intestine

Some increase in the amount of total N was found in the digesta in the small intestine of pigs given diets containing SCP, but no consistent trend was observed (Table 4). The proportion of total N present as non-protein-N was increased when SCP was given in the diet, but only at 600 g/kg replacement was the increase statistically significant ($P < 0·05$).

Protein and large peptides accounted for only 50–100 mg/g total N as determined by filtration in Sephadex G-25 of digesta from one pig given diet U or X. In both instances, similar patterns were found in both the proximal and distal regions of the small intestine.

Concentrations of trypsin in the pancreas and amounts in the digesta were lower at intermediate levels of replacement by SCP (diets V and W) compared with the all-milk or total replacement (diets U and X respectively) (Table 5). Differences were only significant ($P < 0·01$) in the pancreas. Chymotrypsin concentrations were depressed by SCP in the pancreas, but in the digesta the amount of the enzyme was greater than in pigs given the all-milk diet.

Rate of passage of digesta

The amount of PEG recovered in the proximal and mid regions of the small intestine did not exceed 10 mg/g ingested, and recoveries were similar for all diets (Table 6). In the distal portion the amount of PEG recovered was lower in pigs receiving diets containing SCP.

Table 7. *Effect of replacing dried skim-milk by single-cell protein (Pruteen; SCP) and dried whey on intake of nucleic acids and nucleic acid metabolism in baby pigs*

(Mean values for six pigs/treatment)

Diet ...	U		X	
Proportion of total protein (g/kg) supplied by SCP	0		800	
	Mean	SE	Mean	SE
Nucleic acid-N intake (g/d)	0·26	0·041	1·06	0·180**
Urine (g/d):				
Uric acid (g/d)	1·02	0·393	2·67	0·519*
Allantoin (g/d)	0·77	0·148	1·66	0·397*
Blood plasma:				
Uric acid (mg/ml)	0·45	0·118	0·79	0·178 NS
Allantoin (μg/ml)	6·14	0·641	10·04	0·871**

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

Nucleic acid metabolism

The increased nucleic acid intake of pigs given a diet containing a large proportion of SCP (diet X) was reflected by an increase in the daily excretion of uric acid and allantoin, and an increased concentration of allantoin in blood plasma (Table 7).

DISCUSSION

Although the performance of pigs given diets supplying 600 or 800 g protein from SCP/kg was not as good as milk protein, it was still satisfactory and the incidence of scouring was no worse than on the all-milk diet (Table 3). Previous experiments (Newport, 1979*a*, *b*; Newport *et al.* 1979) have produced low mortality with all-milk diets, but with considerable variation between replicates. The small number of replicates in any single experiment could therefore give the occasional abnormally high mortality seen in this experiment.

The similar values obtained for N retention/kg live weight (Fig. 1) suggest that the proportion of carcass protein was unaffected when SCP was substituted for milk protein. This contrasts with a previous experiment where fish, as a substitute for milk protein, reduced N retention (Newport, 1979*b*).

The DM content in the digesta from the stomach of 28-d-old pigs was reduced when diets containing SCP were given (Table 4). These diets, unlike milk protein, did not coagulate in the stomach. Aside from the anomalous values for diet W, the total amount of DM in the stomach was reduced by SCP, suggesting that stomach emptying may be more rapid. The lower recoveries of PEG from pigs given the SCP diet compared with milk protein (Table 6) suggested that more marker had passed into the large intestine or had been excreted in pigs given SCP, and provides indirect evidence of a shorter transit time. Maner *et al.* (1962) also found a decrease in transit time in 4-week-old pigs when milk protein (casein) was replaced by soya-bean protein.

Intestinal proteolysis of SCP may require increased secretion of chymotrypsin. The amount of the enzyme was greater in the digesta, and the lower pancreatic concentrations suggest a response to an increased demand for secretion (Table 5). Trypsin was unaffected by protein source. In young pigs with pancreatic cannulas Pekas *et al.* (1966) reported an increased rate of proteolytic enzyme secretion when milk protein was replaced by soya-bean protein although other studies on pancreatic tissue or digesta from slaughtered pigs did not suggest stimulation of secretion by non-milk proteins (Pond *et al.* 1971; Newport, 1979*b*).

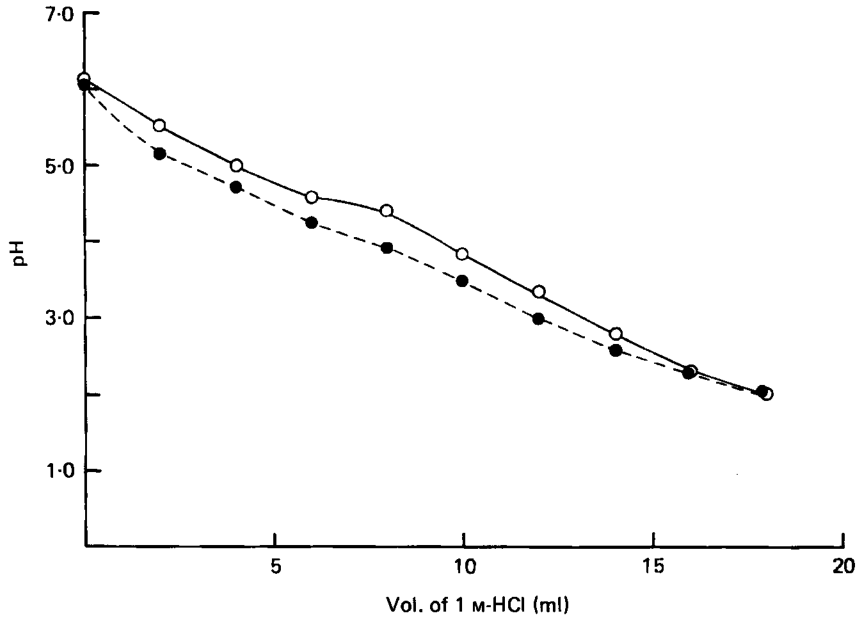

Fig. 2. Buffering capacity of all-milk-protein diet (diet U; ○—○) and diet in which a single-cell protein (Pruteen) replaced dried skim-milk (diet X; ●—●). For details of diets, see Table 2 and p. 161.

The reduced pH of the stomach digesta from pigs given the SCP diet (X) compared with milk protein (diet U) (Table 4) probably reflects the reduced buffering capacity of diet X (Fig. 2) rather than an increase in acid secretion. However, the greater pepsin activity in pigs given diet X (Table 5) does suggest an increase in pepsin secretion.

As in previous experiments with pigs (Seve, 1976; Roth & Kirchgessner, 1977*a*; Zimmerman & Tegbe, 1977) ingestion of nucleic acids increased the rate of allantoin excretion (Table 7). The increase was equivalent to approximately 75% of the theoretical conversion of nucleic acids to allantoin, and together with the increase in uric acid excretion indicate that little, if any, salvage of nucleic acid-N occurred. Urea excretion was not estimated in this experiment, but Roth & Kirchgessner (1977*b*) found this to be a further route for the loss of nucleic acid-N. Although D'Mello *et al.* (1976) and Roth & Kirchgessner (1977*a*) proposed that some nucleic acid-N may be salvaged from diets with adequate amino acid contents, only when the diet is deficient in total N (Whittemore *et al.* 1978) or non-essential amino acids (Peers, 1977) does the evidence seem convincing.

Compared with milk protein, SCP has an adequate amino acid composition to meet the requirements of the baby pig (Table 1), and differences in amino acid composition cannot explain the poorer performance of pigs given high levels of SCP in the diet. The amounts of N in the diets available for body synthesis were also similar as the results confirmed that nucleic acid-N was not utilized, a factor which had been allowed for in the design of the experiment.

Increased activities of pepsin and chymotrypsin in the digesta (Table 5) suggest some adaptation in secretion when SCP is introduced in the diet. Possibly maximum adaptation had occurred at 400 g protein replaced/kg, and the poorer performance with 600 or 800 g protein replaced/kg reflect a decrease in efficiency of protein digestion. The indications of a decreased transit time (Table 6) would also favour a lowering in efficiency. The proportion of N soluble in trichloroacetic acid (Table 4), or of peptides determined by filtration through

Sephadex gel in the digesta of the small intestine do not confirm this hypothesis, but these peptides may be resistant to the terminal stages of digestion. Alternatively, protein digestion of milk and SCP may be equally efficient in the 28-d-old pig in which the studies of digestion were made, and poorer performance reflects less efficient digestion in the younger pig.

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