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Comparison of growth performance and nutrient retention of weaner pigs given diets based on casein, free amino acids or conventional proteins

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In two experiments the potential value of diets based on casein or free amino acids (FAA) for amino acid utilization experiments were examined. In Expt 1 the optimum dietary electrolyte balance (dEB) for a casein-based diet was estimated by supplemention with 10 or 20 g $NaHCO_3$/kg, to produce diets containing 64, 183 or 302 mmol/kg. In addition, piglet growth performance and efficiency of nutrient deposition of piglets given the casein diets were compared with two multiple protein source diets; Supercreep, a commercial multiple protein source diet or CFS (casein–fish–soyabean–sugar) or a FAA-based diet. Expt 2 was designed to compare piglet response to FAA diet stored at −15° with twice daily feeding, with FAA diet stored at ambient temperature (13–30°) and offered *ad libitum*. A CFS diet was used as a positive control and the experiment was conducted over the 10–20 kg growth phase. Expt 1 used forty-eight piglets weaned at 20–22 d of age and allocated to one of six treatments formulated to contain at least 0·84 g lysine/MJ digestible energy in a randomized block design. Piglets given the CFS and Supercreep diets produced superior growth rates (518, 491 g/d) to those given a FAA diet (353 g/d) or casein diet containing 0, 10 or 20 g $NaHCO_3$/kg respectively (365, 417, 390 g/d) between 5 and 20 kg live weight. Piglets given the casein and FAA diets had higher amino acid digestibilities than those given the Supercreep and CFS diets. The increase in the dEB of the casein diet from 64 to 183 mmol/kg improved piglet growth performance between 5 and 20 kg by 14 %. All piglets given casein diets had similar ileal and faecal digestibilities, empty-body compositions, nutrient deposition rates and retention ratios. The results of Expt 2 showed that there was no beneficial effect on piglet performance of storing the FAA diet at −15° and feeding twice daily. Based on the results of these two experiments, neither the casein (0, 10, 20 g $NaHCO_3$/kg) nor FAA diets were suitable for estimating amino acid utilization by the piglet. There remain unidentified factors which limit the growth performance of piglets given the casein and FAA diets.

Casein: Amino acids: Pigs

A diet formulated on an ideal protein basis, with protein digestibility close to 1, would provide an excellent reference diet for the study of amino acid utilization. This reference diet could be used to identify causes of inefficient utilization of amino acids in diets containing traditional protein sources. The efficiency of utilization of amino acids is typically measured using N balance and/or empty-body retention. Yet there are no empty-body estimates in the literature for the efficiency of utilization of diets (which have been formulated on an ideal protein basis) that contain highly digestible protein sources.

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Diets containing casein and/or free amino acids (FAA) as their sole source of N are known to have apparent N digestibilities approaching 1·0 (Kies *et al.* 1986; Chung & Baker, 1992). This makes both casein and FAA potentially good sources of amino acids for diets to be used in amino acid utilization studies.

Previous work (Officer, 1995) has shown that casein–FAA-based diets, with a dietary electrolyte balance (dEB) of 28 mmol/kg, produce poor piglet growth. Patience *et al.* (1987) found that growth performance and feed intake of 8–12-week-old piglets declined when the dEB fell below 0 mmol/kg. The piglets used by Officer (1995) started consuming the casein–FAA diet from 3 weeks of age when $NaHCO_3$ concentrations in the intestine are low. Although the concentration of $NaHCO_3$ rapidly increases over the first 8 weeks of a piglet's life (Harada *et al.* 1988), the effect of $NaHCO_3$ supplementation on piglet performance is unclear. A subsequent preliminary study was conducted to measure the effect of $NaHCO_3$ supplementation on piglet growth from 3 weeks of age. The addition of 12 g $NaHCO_3$/kg to the casein–FAA diet improved piglet weight gain and feed intake (Officer, 1995). Therefore, further experimentation was required to estimate the optimum dEB of the casein diet so that piglet performance could be maximized.

A FAA diet developed by Chung & Baker (1991), also known as the Illinois ideal protein (IIP) diet, showed considerable potential for use in amino acid utilization studies. Although the efficiency of N retention by piglets given IIP was not tested in empty-body studies, the growth performance of piglets from 10 kg live weight was in excess of 500 g/d over a 2-week period. In a N balance study, 92 % of N in IIP was digestible and 86 % of this digestible N was retained by the pig (Chung & Baker, 1992). The IIP diet appears to provide promise for use in amino acid utilization studies of the young pig and has two major advantages over the casein–FAA combination. First, the exact amount of amino acids required by the piglets can be added to the diet without having to conduct an amino acid analysis. This is advantageous in terms of accuracy and analytical cost. Second, the digestibility of all amino acids in the FAA diet can be assumed to be 1·0.

Chung & Baker (1991) stored their FAA diet at −20° and gave piglets their daily allocation in two meals. This is in contrast to the ambient storage temperature and *ad libitum* feeding regimen used in all experiments at this Institute. Chung & Baker (1991) developed their storage and feeding protocol to prevent unfavourable chemical reactions occurring in the FAA diet. The importance of storage temperature and feeding regimen needs to be examined if the FAA diet is to be used in future experiments on amino acid utilization.

Before diets based on either FAA or casein can be used as reference diets in amino acid utilization experiments, the performance of piglets given these diets needs to be compared with research and commercial diets known to promote excellent piglet growth, but which are in some way unsuitable for use in amino acid utilization studies. Two diets, CFS (casein–fish meal–soybean meal with sucrose base) and Supercreep (Bunge Meat Industries, Corowa, NSW, Australia), have shown merit as possible positive controls against which other purified diets can be compared (Officer, 1995).

The present paper contains two experiments, the first of which determined the optimum electrolyte balance for a casein diet and compared this diet with research (IIP and CFS) and commercial (Supercreep) controls known to promote excellent piglet growth. The main aim of this experiment was to determine whether either the casein diet or the IIP diet was suitable for use in future amino acid utilization experiments. The second experiment examined the effect of storage temperature and feeding regimen of a FAA diet on piglet performance.

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EXPERIMENTAL

Expt 1. Comparison of growth performance and nutrient retention of piglets given diets with casein, free amino acid and conventional protein sources

Diets. The treatments were a commercial Supercreep, Chung & Baker's (1992) IIP diet (FAA), the CFS diet, and three casein diets with 0, 10 and 20 g $NaHCO_3$/kg (Tables 1 and 2). The casein diets (diets 4–6) contained a lactic acid casein with free amino acids added to obtain an ideal amino acid ratio. The ratios of amino acids relative to lysine were taken from published data (Agricultural Research Council, 1981; Zhang *et al.* 1984; Black *et al.* 1986; National Research Council, 1988; Wang & Fuller, 1989) and were: lysine 100, methionine 32, methionine + cysteine 63, threonine 65, valine 75, isoleucine 60, leucine 110, phenylalanine 61, phenylalanine + tyrosine 120, histidine 42, tryptophan 19 and arginine 43. The essential : non essential amino acid ratio of 46 : 54 was adjusted by the addition of a combination of glutamic acid and glycine. The casein diets were formulated with 97 % of amino acid N assumed to be truly digestible (Kies *et al.* 1986). Diets 5 and 6 were the same as diet 4 except that they had 10 and 20 g $NaHCO_3$/kg added.

The diets were formulated to similar digestible energy (DE) contents which, with the exception of the FAA diet, were all in excess of 15 MJ/kg to allow unrestricted energy intake (Campbell & Dunkin, 1983). The carbohydrate sources used were lactose and sucrose (150 g/kg each) plus a variable level of wheat starch (Chung & Baker, 1990). Wheat starch ("Unigel", NB Love, Noorooka, Queensland, Australia), rather than maize

Table 1. *Expt 1. Ingredient and nutrient composition of Supercreep and casein–fish meal–soyabean (CFS) diets (g/kg, air-dry)*

Supercreep (diet 1)		CFS (diet 3)	
Ingredients		Ingredients	
Groats	482·3	Casein	104·0
Uncle Toby's cereals	150·0	Fish meal	104·0
Soyabean meal	51·7	Soyabean meal	104·0
Meat meal	60·0	Sucrose	643·2
Fish meal	75·0	Soyabean oil	15·6
Blood meal	20·0	Dicalcium phosphate	15·6
Skimmed milk	80·0	Mineral premix*	3·6
Whey powder	40·0	Vitamin premix*	1·6
Water	10·0	L-Valine	0·06
Tallow	27·0	DL-Methionine	0·28
Salt	2·0	L-Threonine	0·24
L-Threonine	0·13	L-Histidine	0·21
Creep premix	1·75	L-Leucine	0·05
Endox	0·20	L-Tryptophan	0·06
Neomycin SO_4	0·25		
Fuzone 200	0·50		
Composition†		Composition†	
DE (MJ/kg)	15·8	DE (MJ/kg)	15·8
Crude protein	230	Crude protein	227
Lysine	13·4	Lysine	15·5
Lysine (g/MJ DE)	0·85	Lysine (g/MJ DE)	0·98

DE, estimated digestible energy.

* Premix 3 contributed (mg/kg diet): Fe 60, Zn 100, Mn 30, Cu 5, I 2, NaCl 2·8 g, Se 0·15, retinol equivalent 960 μg, cholecalciferol 12 μg, α-tocopherol 20, thiamin 1·5, riboflavin 3, nicotinic acid 14, pantothenic acid 10, pyridoxine 2·5, cyanocobalamin 15 μg, pteroylmonoglutamic acid 2, chlorine 500, ascorbic acid 10, biotin 0·1.

† Formulated composition.

Table 2. *Expt 1. Composition of free amino acid (FAA) and casein-based diets (g/kg air dry)*

Diet no.... Components	2 FAA	4 Casein	5 Casein 10 $NaHCO_3$	6 Casein 20 $NaHCO_3$
Casein*	–	144·2	144·2	144·2
L-Glutamic acid	105·4	64·0	64·0	64·0
L-Glycine	12·0	10·3	10·3	10·3
L-Proline	4·0	0	0	0
Essential amino acids†	80·8	16·3	16·3	16·3
Starch	328·6	308·9	292·4	275·8
Sugar	150	150	150	150
Lactose	150	150	150	150
Soyabean oil	50	55	61·5	68
Solka floc	30	30	30	30
$NaHCO_3$	12	–	10	20
Vitamins + minerals‡	76·3	70·5	70·5	70·5
Fuzone	0·5	0·5	0·5	0·5
Neomycin SO_4	0·25	0·25	0·25	0·25
Ethoxyquin	0·125	0·125	0·125	0·125
Composition				
DE (MJ/kg)	14·3	15·5	15·5	15·5
dEB (mmol/kg)	109	64	183	302
Crude protein	151	184	184	184
EAA : NEAA	46 : 54	46 : 54	46 : 54	46 : 54
Total lysine	12	13·4	13·4	13·4
Lysine (g/MJ DE)	0·84	0·84	0·84	0·84

DE, digestible energy; dEB, dietary electrolyte balance (Na + K − Cl); EAA : NEAA, essential : non essential amino acid ratio.

* Composition of lactic acid casein (g/kg air dry): crude protein 822, dry matter 890, gross energy (MJ/kg) 21·4, lysine 69·3, methionine 27, cystine 1·1, threonine 42·6 valine 69·7, isoleucine 54·4, leucine 90·5, phenylalanine 55·8, tyrosine 50·9, histidine 27·1, tryptophan 10·8, arginine 39·1, glutamic acid 220·3, proline 99, aspartic acid 69·3, serine 58·7, alanine 28·8, glycine 16·8.

† Contributed (g/kg) for diets 2, 4, 5 and 6 respectively: L-lysine-HCl 15·0, 2·811, 2·811, 2·811; DL-methionine, 3·6, 0·401, 0·401, 0·401; L-cysteine, 3·6, 4·002, 4·002, 4·002; L-threonine 7·8, 3·525, 3·525, 3·525; L-valine 8·16, 0·019, 0·019, 0·019; L-isoleucine 7·2, 0·215, 0·215, 0·215; L-leucine 12·0, 1·709, 1·709, 1·709; L-tyrosine 5·358, 0·004, 0·004, 0·004; L-phenylalanine 6·042, 0·706, 0·706, 0·706; L-histidine 3·84, 1·733, 1·733, 1·733; L-tryptophan 2·16, 0·992, 0·992, 0·992; L-arginine-HCl 6·073, 0·153, 0·153, 0·153.

‡ Contributed (g/kg): choline chloride 2, dicalcium phosphate 47, K_2SO_4 9, $MgSO_4$ 4·5, salt 8·8, 3, 3, 3 for diets 2, 4, 5 and 6 respectively; (mg/kg) Fe 83, Zn 50, Mn 211, Cu 4, I 2, Co 0·2, Mo 3, B 1·6, Se 0·3, retinol equivalent 1·56, cholecalciferol 15 μg, α-tocopherol 20, thiamin-HCl 20, riboflavin 10, nicotinic acid 50, pantothenic acid 30, pyridoxine 6, cyanocobalamin 0·04, pteroylmonoglutamic acid 4, ascorbic acid 250, biotin 0·6, menadione 2.

starch, was used because of its availability. The lactose was white edible grade and the sucrose was unprocessed raw. Soyabean oil was the only source of essential fatty acids in the diet. An antioxidant, ethoxyquin (125 mg/kg), and a dietary fibre source, solka floc (30 g/kg), were included in diets 2, 4, 5 and 6. Both Fuzone 200 (furazolidone 200 mg/g) and neomycin sulfate (neomycycin 700 mg/g) were added to control *Campylobacter* and *E. coli*.

Animals and procedures. Forty-eight male Large White piglets, weaned at 20–22 d of age, were allocated to six dietary treatments. Each treatment comprised eight piglets in a randomized block design. Each piglet was assigned to a block on the basis of its 3 week weight and, where possible, litter of origin. In addition five piglets were killed at 3 weeks of age for measurement of initial body composition.

Each piglet was individually housed in a raised 0·90 × 0·48 m cage with wire mesh floor and fed *ad libitum*. Feed consumption and weight gain were measured weekly. Feed spillage was collected weekly from plastic trays located below the front of each experimental cage. Water was provided by a single bite nipple at the rear of the pen. House

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temperature was set at 28° (air speed 0·3 m/s) and was maintained until the youngest piglet had spent 7 d at that setting. The temperature was then reduced by 1°/week to 25°. There was a 14 d spread in starting date of the experiment. The spread in starting date for piglets within blocks was only 4 d.

Ileal and faecal nutrient digestibilities was made using the inert marker, Cr_2O_3, added to the diet (2 g/kg) at least 4 d before digestibility measurements were required. A grab sample of faeces was taken for analysis just before slaughter. Ileal collections were taken immediately post-slaughter from the region 0·15–1·5 m anterior to the ileocaecal junction. Each ileal sample was immediately frozen (−15°) before being freeze-dried, ground and then chemically analysed.

The piglets were killed by captive bolt pistol, to minimize shedding of the gastro-intestinal mucosa into the ileum (Badaway *et al.* 1957) and then bled. The gastrointestinal contents of all piglets were removed, and the blood, carcass and viscera combined before being frozen. The frozen empty-bodies of piglets were minced, mixed, subsampled, freeze dried and ground before chemical analysis.

The following factors were used in calculations; 6·38 to convert casein N to protein and 6·25 for all other N sources (Agricultural Research Council, 1981). The energy (MJ) stored in carcass protein was calculated as kg carcass protein × 24·2 (Jordan & Brown, 1970). Energy stored as fat was calculated as carcass energy minus protein energy, the difference of which was divided by 39·7 (Burlacu *et al.* 1973). Amino acid compositions of the initial slaughter group and those on the experimental treatments were made from a composite sample of mince from the piglets on that treatment.

All results were analysed using the generalized linear models procedure of the statistical package SAS (Statistical Analysis Systems, Inc., 1989). Least significant differences (5 %) were used to separate treatment means.

Expt 2. Effect of storage temperature and feeding regimen of a free amino acid diet on piglet growth

Diets. The two diets were formulated to 0·84 g available lysine/MJ DE. The ingredients and composition of the CFS and FAA diets are detailed in diet 3 of Table 1 and diet 2 of Table 2.

Animals and procedures. This experiment compared ambient storage (22·3 (SD 2·2)°, range 13–30°) and *ad libitum* feeding with −15° storage and twice daily feeding of a FAA diet. A diet containing intact proteins (CFS) was offered *ad libitum* and used as a control. Piglets were grown from 10–20 kg live weight.

Half the FAA diet was stored at ambient temperature (13–30°) for up to 28 d, the remainder at −15°. The piglets (six per treatment) were allowed to adjust to the experimental treatments from 1 week post weaning (35 d of age) until they reached 10 kg live weight. Piglet performance was then assessed between 10 and 20 kg live weight. The individual cages, allocation, feeding, weighing and reject collection procedures were as described in Expt 1. The results were examined using the generalized linear models procedure of SAS (Statistical Analysis Systems, Inc., 1989) with least significant differences (5 %) being used to separate treatment means.

Chemical analyses

The methods of the Association of Official Analytical Chemists (1984) were used for proximate analyses. Gross energy was determined by adiabatic bomb calorimetry. Cr in

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ileal digesta was measured by atomic absorption spectrophotometry (Kimura & Miller, 1957) under nitrous oxide–acetylene, having been digested with nitric and perchloric acids.

All amino acid samples were hydrolysed at 110° for 24 h with constant-boiling-point HCl under N_2. The amino acids in the casein, ileal digesta, faeces and empty-body were separated by reverse-phase chromatography and measured after reaction with phenylisothiocyanate, using α-amino butyric acid as the internal standard. Methionine and cystine were not analysed by performic acid oxidation and levels of these two amino acids may be underestimated. Tryptophan was determined by HPLC following LiOH alkaline hydrolysis (Degussa AG, 1986).

RESULTS

Expt 1. Comparison of growth performance and nutrient retention of piglets given diets with casein, free amino acid and conventional protein sources

There were marked differences in piglet weight gains between treatments after only 7 d (Table 3). Over the 5–10 kg growth phase the Supercreep and CFS diets produced piglet weight gains and feed conversion efficiencies which were superior ($P < 0.001$) to all but those on the diet with casein plus 10 g $NaHCO_3$/kg. The addition of 10 g $NaHCO_3$/kg to the casein treatment tended to improve piglet performance compared with the casein ($P = 0.065$) diet and was superior to the FAA treatment. As the piglets grew, the differences between the casein and FAA treatments diminished. In contrast, the differences between the Supercreep and CFS treatments and all other treatments increased.

The ileal and faecal digesibilities of N, dry matter and energy were higher ($P < 0.001$) in the casein and FAA diets than either the Supercreep or CFS diet (Table 4). The determined DE contents of the casein diets were 0·2–0·4 MJ/kg higher than was assumed during formulation. The FAA diet contained 0·9 MJ DE/kg more than was formulated. The DE of the CFS and Supercreep diets were similar to their formulated values. The ileal amino acid digestibilities of the casein and FAA diets were generally greater ($P < 0.001$ for all amino acids except arginine and proline) than the CFS and Supercreep diets (Table 5). The digestibilities of glycine and proline were lower and more variable than the digestibilities of all the other amino acids.

The bodies of piglets given the Supercreep and FAA diets contained more energy with a higher proportion of fat ($P < 0.01$) than those on either the CFS or casein plus 10 g $NaHCO_3$/kg diets (Table 6). The amount of empty-body protein was similar across treatments, although those piglets on the FAA diet tended ($P < 0.08$) to contain less protein.

Both the CFS and Supercreep diets gave 24–41 % greater ($P < 0.001$) rates of energy and 21–64 % faster ($P < 0.001$) protein deposition rates than the other treatments. Piglets given the CFS treatment deposited more protein ($P < 0.001$) and less fat ($P < 0.05$) than those on the Supercreep treatment. Piglets given the FAA diet deposited 20–23 % less protein ($P < 0.001$) than those given the $NaHCO_3$-supplemented casein diets.

Piglets given the Supercreep and CFS diets retained more energy and protein per unit DE consumed than those given the casein treatments, which in turn had higher energy and protein retention ratios than the FAA-fed animals ($P < 0.001$). The ratio protein retained : protein consumed was highest in piglets given the CFS diet ($P < 0.05$). Both the CFS- and Supercreep-fed piglets retained a greater proportion of dietary ileal digestible N (0·82 and 0·78) compared with those given casein or FAA diets (0·61 and 0·59) respectively ($P < 0.001$).

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Table 3. *Expt 1. Comparison of 5–20 kg performance of weaner pigs given diets containing casein, free amino acids (FAA) or conventional protein*†

(Values are means for eight piglets per dietary group)

Diet no.... Variable	1 Supercreep	2 FAA	3 CFS	4 Casein	5 Casein 10 $NaHCO_3$	6 Casein 20 $NaHCO_3$	Statistical significance of effect of diet	SEM (edf 33)
Week 1 (age 4 weeks)								
Feed intake (g/d)	196	173	222	218	254	211	NS	29·3
Gain (g/d)	195^{a}	83^{bc}	163^{ab}	86^{c}	197^{a}	139^{abc}	*	29·9
Total (5–10 kg)								
Feed intake (kg)	5·2^{d}	8·4^{a}	5·7^{cd}	8·0^{ab}	6·6^{bcd}	6·9^{bc}	***	0·60
(g/d)	358	390	388	384	388	378	NS	19·0
Gain (g/d)	318^{a}	220^{c}	321^{a}	236^{bc}	281^{ab}	260^{bc}	***	17·6
FCR	1·1^{c}	1·9^{a}	1·2^{bc}	1·7^{ab}	1·4^{bc}	1·5^{b}	***	0·12
Total (10–20 kg)								
Feed intake (kg)	14·1^{c}	18·9^{a}	13·9^{c}	16·8^{bc}	16·0^{bc}	16·5^{b}	***	0·59
(g/d)	890^{ab}	854^{abc}	923^{a}	792^{c}	813^{bc}	809^{bc}	**	32·2
Gain (g/d)	652^{a}	488^{b}	711^{a}	500^{b}	538^{b}	527^{b}	***	27·0
FCR	1·4^{c}	1·7^{a}	1·3^{c}	1·6^{b}	1·5^{b}	1·5^{b}	***	0·05
Total (5–20 kg)								
LW	20·3	20·9	20·6	20·6	20·5	20·7	NS	0·30
EBW	19·0	19·7	19·6	19·4	19·5	19·6	NS	0·29
LW:EBW	0·94	0·95	0·95	0·94	0·95	0·95	NS	0·004
Feed intake (kg)	19·4^{c}	27·3^{a}	19·6^{c}	24·8^{b}	22·6^{b}	23·4^{b}	***	0·97
(g/d)	633^{ab}	627^{ab}	659^{a}	585^{c}	617^{ab}	593^{bc}	*	19·0
Gain (g/d)	491^{a}	353^{c}	518^{a}	365^{c}	417^{b}	390^{bc}	***	16·9
FCR	1·3^{c}	1·8^{a}	1·3^{c}	1·6^{ab}	1·5^{b}	1·5^{b}	***	0·06

FCR, feed conversion ratio; LW, live weight; EBW, empty-body weight; CFS, casein–fish meal–soyabean diet.

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.

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

† For details of diets, see Tables 1 and 2.

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Table 4. *Expt 1. Apparent ileal and faecal digestibility of nitrogen, dry matter and energy from diets containing casein, free amino acids (FAA) or conventional proteins, fed to pigs*†

(Values are means for eight piglets per dietary group)

Diet no.... Variable	1 Supercreep	2 FAA	3 CFS	4 Casein	5 Casein 10 $NaHCO_3$	6 Casein 20 $NaHCO_3$	Statistical significance of effect of diet	SEM (edf 24)
Diet components								
Ileal								
N	0.73^{b}	0.92^{a}	0.77^{b}	0.91^{a}	0.93^{a}	0.92^{a}	***	0.025
Dry matter	0.66^{b}	0.89^{a}	0.85^{a}	0.88^{a}	0.89^{a}	0.84^{a}	***	0.023
Faecal								
N	0.83^{b}	0.97^{a}	0.84^{b}	0.95^{a}	0.97^{a}	0.97^{a}	***	0.023
Dry matter	0.86^{b}	0.92^{a}	0.92^{a}	0.92^{a}	0.92^{a}	0.91^{a}	***	0.009
DE (MJ/kg)	15.9	15.2	15.8	15.9	15.8	15.7	NS	0.19

DE, digestible energy; CFS, casein–fish meal–soyabean diet.

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

*** $P < 0.001$.

† For details of diets, see Tables 1 and 2.

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Table 5. *Expt 1. Apparent ileal amino acid digestibility of diets containing casein, free amino acids (FAA) or conventional proteins*†

(Values are means for eight piglets per dietary group)

Diet no....	1	2	3	4	5	6	Statistical significance	
Variable	Supercreep	FAA	CFS	Casein	Casein 10 $NaHCO_3$	Casein 20 $NaHCO_3$	of effect of diet	SEM (edf 24)
Essential amino acids								
Lysine	0·81^{c}	0·98^{a}	0·87^{b}	0·98^{a}	0·98^{a}	0·98^{a}	***	0·016
Methionine	0·81^{c}	0·95^{a}	0·89^{b}	0·95^{a}	0·96^{a}	0·95^{a}	***	0·017
Threonine	0·73^{b}	0·94^{a}	0·78^{b}	0·92^{a}	0·94^{a}	0·93^{a}	***	0·027
Valine	0·77^{b}	0·95^{a}	0·83^{b}	0·93^{a}	0·94^{a}	0·93^{a}	***	0·023
Isoleucine	0·77^{b}	0·96^{a}	0·84^{b}	0·92^{a}	0·94^{a}	0·93^{a}	***	0·024
Leucine	0·80^{b}	0·96^{a}	0·85^{b}	0·96^{a}	0·96^{a}	0·96^{a}	***	0·020
Tyrosine	0·79^{c}	0·95^{a}	0·87^{b}	0·95^{a}	0·96^{a}	0·96^{a}	***	0·021
Phenylalanine	0·81^{b}	0·96^{a}	0·85^{b}	0·96^{a}	0·97^{a}	0·96^{a}	***	0·019
Histidine	0·81^{b}	0·96^{a}	0·86^{b}	0·96^{a}	0·97^{a}	0·97^{a}	***	0·016
Arginine	0·81^{b}	0·92^{a}	0·85^{ab}	0·89^{a}	0·92^{a}	0·92^{a}	*	0·028
Non-essential amino acids								
Glutamic acid	0·82^{b}	0·98^{a}	0·85^{b}	0·98^{a}	0·99^{a}	0·98^{a}	***	0·014
Proline	0·79	0·68	0·85	0·81	0·90	0·95	NS	0·086
Glycine	0·69^{b}	0·89^{a}	0·62^{b}	0·88^{a}	0·88^{a}	0·89^{a}	***	0·039

CFS, casein–fish meal–soyabean diet.

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.

* $P < 0·05$, *** $P < 0·001$.

† For details of diets, see Tables 1 and 2.

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Table 6. *Expt 1. Empty-body protein, energy and fat concentrations, deposition rates and efficiencies of retention of piglets given diets containing casein, free amino acids (FAA) or conventional proteins*†

(Values are means for eight piglets per dietary group)

Diet no.... Variable	1 Supercreep	2 FAA	3 CFS	4 Casein	5 Casein 10 $NaHCO_3$	6 Casein 20 $NaHCO_3$	Statistical significance of effect of diet	SEM (edf 33)
Composition‡								
Energy (MJ/kg)	10·1[a]	10·0[a]	9·2[b]	9·6[ab]	9·1[b]	9·7[ab]	**	0·21
Protein (kg/kg)	0·160	0·150	0·166	0·162	0·158	0·161	NS	0·0036
Fat (kg/kg)	0·156[ab]	0·161[a]	0·131[c]	0·144[bc]	0·134[c]	0·146[bc]	**	0·0056
Deposition rates								
Energy (MJ/d)	4·8[a]	3·6[b]	4·6[a]	3·4[b]	3·6[b]	3·7[b]	***	0·23
Protein (g/d)	75·3[b]	50·8[d]	83·4[a]	57·4[cd]	62·4[c]	60·9[c]	***	2·51
Fat (g/d)	65·5[a]	50·9[b]	53·2[b]	44·1[b]	47·8[b]	47·9[b]	*	4·56
Retentions (ratios)								
GE : DEI (MJ/MJ)	0·49[a]	0·36[c]	0·44[ab]	0·38[c]	0·38[c]	0·40[bc]	***	0·019
CP : DEI (g/MJ)	7·6[a]	5·1[c]	8·1[a]	6·3[b]	6·5[b]	6·6[b]	***	0·230
CP : CPI (kg/kg)	0·57[b]	0·54[b]	0·63[a]	0·55[b]	0·57[b]	0·57[b]	*	0·020
CP : IDPI (kg/kg)	0·78[a]	0·59[b]	0·82[a]	0·60[b]	0·61[b]	0·62[b]	***	0·023

CFS, casein–fish meal–soyabean diet; GE, gross energy; DEI, digestible energy intake; CP, crude protein; CPI, crude protein intake; IDPI, ileal digestible protein intake.

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.

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

† For details of diets, see Tables 1 and 2.

‡ Composition of piglets (empty-body-weight basis).

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Table 7. *Expt 2. Effect of storage temperature and feeding regimen on piglet performance**

(Values are means for six piglets per treatment)

Diet...	CFS	FAA	FAA	
Feeding regimen...	*Ad libitum*	*Ad libitum*	Twice daily	SEM
Storage temperature...	13–30°	13–30°	−15°	(edf 17)
Time pre-trial (d)	3·5[a]	7·0[b]	9·2[c]	0·66
Feed intake (g/d)	1008[a]	710[b]	689[b]	51·0
Gain (g/d)	798[a]	463[b]	413[b]	41·8
FCR	1·26[a]	1·56[ab]	1·69[b]	0·13

FAA, free amino acid; CFS, casein–fish meal–soyabean diet; FCR, feed conversion ratio.

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

* For details of diets, see Tables 1 and 2.

Expt 2. Effect of storage temperature and feeding regimen of a free amino acid diet on piglet growth

Piglets given the FAA diet were slower to reach 10 kg live weight than those given the CFS diet ($P < 0.05$; Table 7). With the exception of the feed conversion ratio (FCR) of piglets receiving the FAA *ad libitum*, the feed intake, weight gain and FCR for piglets given the FAA diets were inferior to those given the control diet and were unaffected by method of storage or feeding regimen ($P < 0.05$).

DISCUSSION

The growth rates of piglets given the casein and FAA diets were disappointing when compared with the excellent weight gains of those given the CFS and Supercreep diets. The 10–20 kg weight gains of piglets given the CFS and Supercreep diets were similar to those observed by Campbell *et al.* (1988). The piglets given the FAA diet (Expt 1) exhibited approximately 10 % lower gains than those reported by Chung & Baker (1992). The effects of acid–base balance, storage temperature and feeding regimen and other factors on performance of piglets given the casein and FAA diets are discussed.

Acid–base balance for casein diets

Piglets given a casein diet containing 183 mmol/kg of dEB grew 14 % faster than those given a diet containing 64 mmol/kg. The beneficial effect provided by adding 10 g $NaHCO_3$/kg diminished as the piglets grew, which may indicate that the piglets adapted over time to the acid–base balance of the diet they were receiving. Alternatively, as the piglets' production of bicarbonate increased with age, their response to bicarbonate supplementation declined.

The improvement in the growth rates of piglets given the modified casein diets was insufficient to match the performance of piglets given either the CFS diet or the Supercreep diet. The growth rates of piglets given the Supercreep and CFS diets were between 17 and 24 % greater than those on the best casein-based diet. Both the determined DE content and ileal amino acid digestibilities of the casein diets were close to their formulated values and therefore unlikely to be the cause of reduced growth.

Patience *et al.* (1987) examined a range of acid–base ratios from −85 to 341 mmol/kg (dEB) for 8–12-week-old piglets using a diet based on maize and soyabean meal. Piglets showed signs of metabolic acidosis, including reduced blood pH and bicarbonate, when the

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dEB was less than 175 mmol/kg. Yet, impaired growth and feed intake were not evident until dEB fell below 0 mmol/kg. The results of this experiment show that impaired growth may occur at higher dEB (e.g. 64 mmol/kg) than was measured by Patience *et al.* (1987).

In his review of acid–base balance Patience (1990) states "changes in acid–base balance influence the metabolic fate of many amino acids". This means that although the three casein diets had similar DE contents and amino acid digestibilities, the higher dEB of the $NaHCO_3$-supplemented treatments probably improved the efficiency with which the absorbed amino acids were metabolized. There was a trend ($P > 0{\cdot}05$) for piglets given the $NaHCO_3$-supplemented treatments to deposit more protein (g/d) than those given the plain casein diet. This trend may have been clearer if the pigs had been killed at 10 rather than 20 kg.

Casein and free amino acid diets: causes of poor nitrogen retention

The poor retention of N by pigs given the casein and FAA diets compared with the Supercreep and CFS diets can be partly explained by the inefficient use of FAA by the pig when they are consumed in large quantities. Fickler *et al.* (1994) showed that piglets (10–15 kg) given Chung & Baker's (1991) FAA diet performed poorly compared with those given a grain, soyabean meal, fish meal and skimmed-milk-powder diet. Piglets given the FAA diet had 24 % lower growth rates, 14 % lower absolute N retention (N balance) despite consuming similar levels of energy (Fickler *et al.* 1994). The reduction in N retention measured by Fickler *et al.* (1994) is confirmed here. The efficiency of protein retention (Table 6, crude protein : crude-protein intake) for piglets given the FAA diet in Expt 1 was 14 % lower than for those piglets given the CFS diet. It appears that when FAA constitute 35 % or more of N in a diet, as they did in the casein and FAA diets, they are utilized inefficiently.

The poor efficiency of N retention of the casein and FAA-fed piglets was not the result of a reduced lysine : DE ratio. The ileal digestible lysine : DE ratios determined for the diets were CFS 0·91, casein 0·83, FAA 0·79 and Supercreep 0·73. With the possible exception of the Supercreep diet, these values compare favourably with a requirement of about 0·8 g/MJ DE for piglets of this strain (Officer, 1994). The piglets given Supercreep consumed less digestible lysine/MJ DE than the casein and FAA piglets but exhibited higher efficiencies of retention. This suggests that the digestible amino acid ratios did not reflect amino acid availability.

With the exception of the three casein diets, all diets were formulated to different amino acid ratios. The Supercreep and CFS diets were included in Expt 1 to give the authors reference points against which the casein and FAA diets could be compared. Whilst the casein and FAA diets were formulated to similar lysine : DE ratios they had different amino acid balances. This means that some of the variation in the efficiency of amino acid use may be attributed to these different amino acid ratios.

The DE content for the FAA diet was 0·9 MJ/kg (6 %) higher than the formulated value. The estimate for DE content of the FAA diet was made from the lower (14 MJ/kg) of two metabolizable energy (ME) contents determined by Chung & Baker (1991). With hindsight the higher ME estimate of 14·4 MJ/kg would have been more appropriate. As a consequence, the amino acid content per unit DE was lower in the FAA diet than in all but the Supercreep diet. The higher DE of the FAA diet meant that these piglets were receiving between 7 and 9 % less lysine per unit DE than piglets given the casein diets. If the FAA and casein diets had contained similar lysine : DE ratios, the differences in deposition rates and efficiency of protein retention may not have occurred.

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The results of Expt 2 show that the poor growth of piglets given the FAA diets cannot be attributed to the storage temperature and feeding regimen employed. Chemical reactions within the FAA diet stored at ambient temperature do not appear to have caused the lower piglet performance. The results of experiments using liquid amino acid and glucose mixtures heated to 100° show that there is potential for L-cysteine to be used as an inhibitor of Maillard reactions (Friedman & Molnar-Perl, 1990). The presence of free L-cysteine in the FAA diet may explain why no difference in piglet performance was measured between the two storage and feeding regimens.

Apart from the differences in protein source the diets also differed in carbohydrate source. For example, the casein diet contained a combination of starch, sucrose and lactose, while the CFS diet contained only sucrose. The importance of the different carbohydrate sources used for feed intake, weight gain and nutrient utilization needs further evaluation.

Effect of methodology on nitrogen retention estimates

The efficiencies of N retention for the casein and FAA diets were low compared with estimates made by N balance (Moughan *et al.* 1987; Chung & Baker, 1992). The difference in methodology is important because Newport & Henschel (1984) showed that N balance overestimates N retention in the carcass by 16–25 %. If the N retention : N intake for Chung & Baker's (1992) FAA diet (called IIP) is a 25 % overestimate, then the efficiency of N retention in the carcass of pigs given IIP should be 0·59. Despite the fact that this 0·59 estimate of the efficiency of N retention is still 9 % higher than what was measured in Expt 1, most of the difference between these results and those found in the literature appears to be methodological.

Overall, the results indicate that 183 mmol/kg dEB in the casein diet is adequate. Even though increasing the casein diet dEB from 63 to 183 mmol/kg improved the growth rates of piglets, this formulation did not result in growth rates equivalent to those observed for CFS- and Supercreep-fed piglets. The growth performance of piglets fed on the FAA was also inferior to that of those fed on the control diets. The poor growth of piglets given the FAA diets cannot be attributed to the storage temperature and feeding regimen employed or to reduced amino acid digestibility. This indicates that the casein and FAA diets are not suitable in their current form to be used as a base diet for amino acid utilization studies. Further experimentation is required to evaluate the effect of carbohydrate source on piglet growth performance.

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REFERENCES

Agricultural Research Council (1981). *The Nutrient Requirements of Pigs*. Slough: Commonwealth Agricultural Bureaux.

Association of Official Analytical Chemists (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed. Washington, DC: Association of Official Analytical Chemists.

Badawy, A. M., Campbell, R. M., Cuthbertson, D. P. & Fell, B. F. (1957). Changes in the intestinal mucosa of the sheep following death by humane killer. *Nature* **180**, 756–757.

Black, J. B., Campbell, R. G., Williams, I. H., James, K. J. & Davies, G. T. (1986). Simulation of energy and amino acid utilisation in the pig. *Research and Development in Agriculture* **3**, 121–145.

Burlacu, G., Baia, G., Ionila, D., Moisa, D., Tascenco, V., Visan, I. & Stoica, I. (1973). Efficiency of the utilization of the energy of food in piglets after weaning. *Journal of Agricultural Science, Cambridge* **81**, 295–302.

Campbell, R. G. & Dunkin, A. C. (1983). The influence of dietary protein and energy intake on the performance, body composition and energy utilisation of pigs growing from 7 to 19 kg. *Animal Production* **36**, 185–192.

Campbell, R. G., Taverner, M. R. & Rayner, C. J. (1988). The tissue and dietary protein and amino acid requirements of pigs from 8·0 to 20·0 kg live weight. *Animal Production* **46**, 283–290.

Chung, T. K. & Baker, D. H. (1990). Development of a purified crystalline amino acid diet for young swine. *Journal of Animal Science* **68**, 389–390.

Chung, T. K. & Baker, D. H. (1991). A chemically defined diet for maximal growth of pigs. *Journal of Nutrition* **121**, 979–984.

Chung, T. K. & Baker, D. H. (1992). Ideal amino acid pattern for 10-kilogram pigs. *Journal of Animal Science* **70**, 3102–3111.

Degussa AG (1986). Determination of tryptophan in raw materials and feedstuffs after alkaline hydrolysis and HPLC detection. *Analysis*, A5. Hanau: Degussa AG.

Fickler, J., Roth, F. X. & Kirchgessner, M. (1994). Use of a chemically defined amino-acid diet in a N-balance experiment with piglets. 1. Communication on the importance of non-essential amino acids for protein retention. *Journal of Animal Physiology and Animal Nutrition* **72**, 207–214.

Friedman, M. & Molnar-Perl, I (1990). Inhibition of browning by sulphur amino acids. 1. Heated amino acid–glucose systems. *Journal of Agricultural and Food Chemistry* **38**, 1642–1647.

Harada, E., Kiriyama, H., Kobayashi, E. & Tsuchita, H. (1988). Postnatal development of biliary and pancreatic exocrine secretion in piglets. *Comparative Biochemistry and Physiology* **91A**, 43–51.

Jordan, J. W. & Brown, W. O. (1970). The retention of energy and protein in the baby pig fed on cows' milk. In *Energy Metabolism of Farm Animals*, pp. 161–164 [A. Schurch and C. Wenk, editors]. Zurich: Juris Druck and Verlag.

Kies, A. K., Moughan, P. J. & Smith, W. C. (1986). The apparent and true ileal digestibility of nitrogen and amino acids in lactic casein for the growing pig. *Animal Feed Science and Technology* **16**, 169–178.

Kimura, F. T. & Miller, V. I. (1957). Improved determination of chromic oxide in cow feed and faeces. *Agriculture, Food and Chemistry* **5**, 216.

Moughan, P. J., Smith, W. C. & Cornwell, J. K. (1987). Determination of the biological value of a protein source with supposedly ideal amino acid balance (A. R. C. 1981) for the young pig (10–20 kg liveweight). *Journal of the Science of Food and Agriculture* **38**, 91–96.

National Research Council (1988). *Nutrient Requirements of Swine*, 9th ed. Washington, DC: National Academy Press.

Newport, M. J. & Henschel, M. J. (1984). Evaluation of the neonatal pig as a model for infant nutrition: effects of different proportions of casein and whey protein in milk on nitrogen metabolism and composition of digesta in the stomach. *Pediatric Research* **18**, 658–662.

Officer, D. I. (1994). Growth response of weaner piglets given soybean–sugar diets to dietary lysine. *Proceedings of the Nutrition Society of Australia* **18**, 168.

Officer, D. I. (1995). Development of a synthetic diet for studies on amino acid utilization in the weaner pig. Master of Rural Science Thesis, University of New England.

Patience, J. F. (1990). A review of the role of acid–base balance in amino acid nutrition. *Journal of Animal Science* **68**, 398–408.

Patience, J. F., Austic, R. E. & Boyd, R. D. (1987). Effect of dietary electrolyte balance on growth and acid–base status in swine. *Journal of Animal Science* **64**, 457.

Statistical Analysis Systems Institute, Inc. (1989). *SAS/STAT User's Guide* (Release 6.09). Cary, NC: SAS Institute Inc.

Wang, T. C. & Fuller, M. F. (1989). The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *British Journal of Nutrition* **62**, 77–89.

Zhang, Y., Partridge, I. G., Keal, H. D. & Mitchell, K. G. (1984). Dietary amino acid balance and requirements for pigs weaned at 3 weeks of age. *Animal Production* **39**, 441.

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