Efficiency of use of nitrogen from dried microbial cells after a period of N deprivation in growing pigs

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1. Semi-synthetic diets, with dried microbial cells (Pruteen) as the nitrogen source, were used to measure N retention in 50 kg pigs which had been given only sufficient N (5·3 g/d) to maintain N equilibrium for the previous 12 d. Control pigs were given 33·2 g N/d.

2. Metabolic faecal N losses were 1·62 g/d (1·2 g/kg dry matter eaten) and endogenous urinary losses were 3·90 g/d.

3. Realimentation of N-deprived pigs was achieved with diets providing 20·0, 33·2 and 67·4 g N/d and daily rates for N retention were 11·2, 17·8 and 25·9 g respectively; the corresponding value for control pigs was 15·0 g. 'Catch-up' protein growth was demonstrated in pigs given both 33·2 and 67·4 g N/d. In the former instance, this was associated with an increase in the efficiency of utilization of dietary N.

4. The biological value of the protein in Pruteen was 0·85, and it appeared that under conditions of increased demand for N the pig could utilize some of the nucleic acid-N fraction of this protein source.

Reduced growth consequent upon food deprivation may be subsequently made good by 'catch-up' growth (Allden, 1970; Fowler, 1976). There is dispute, however, as to the tissues involved in 'catch-up' growth and whether or not there is a change in metabolic efficiency. Fowler (1976) distinguishes between 'target' and 'variable' fat, the latter being a function of the level of energy supply in excess of the physiological needs for growth of lean and essential tissues. Growth fluctuations in 'variable' fat, dictated by changes in food supply, are of less significance than those in lean tissue. If protein retention in lean growth reacts to nutrient supply in the same way as 'variable' fat, 'catch-up' growth in all tissues would follow automatically upon realimentation. The contrary is more likely to be the situation however, as protein growth is also subject to physiological control.

Kielanowski (1969) is of the opinion that enhanced protein retention can be an integral part of 'catch-up' growth, and this has recently been demonstrated by Fowler (1976). Other recent reports of 'catch-up' growth (e.g. Laksesvela, 1976; Neilsen, 1976) are more difficult to interpret as the tissues concerned are not identified, and responses could have been exclusively in the form of 'variable' fat.

'Catch-up' protein growth, where treated animals show enhanced protein retention greater than that of non-deprived control animals, implies either an increase in protein supply above the control level or an increase in the efficiency of use of the same protein supply. The latter phenomenon would require an improvement in effective biological value (BV) of dietary protein. Millward, Garlick, James, Sender & Waterlow (1976) have expressed the belief that the decrease in nitrogen excretion after N deprivation is partly as a result of a reduction in the proportion of the flux excreted. It appears from the results of Millward, Garlick & Nnanyelugo (1974) that although refeeding effects an increase in flux, there is a delay of approximately 14 d in the instance of the rat, during which it is conceivable that protein retention may occur concomitant with a less than commensurate excretion rate, thus enabling an increase in effective BV.

Information about the efficiency of protein use as evidenced by BV is a prime considera-

Table 1. *Chemical composition (g/kg dry matter (DM)) of dietary ingredients*

Ingredient	DM (g/kg)	Gross energy	Nitrogen	Fat	Fibre	Ash
Dried microbial cells*	893	22·57	122·55	86·00	16·40	88·19
Maize starch	864	17·08	0·50	0·81	5·60	1·52
Sucrose	999	16·40	0·17	2·00	4·00	0·36
Glucose	911	15·52	0·17	2·40	2·80	0·64
Cellulose†	936	17·24	0·21	0·0	35·21	4·24
Maize oil	0	39·70	0·0	99·92	0·0	0·0
Mineral and vitamin mixture‡	910	—	1·82	3·60	4·80	895·55

* Pruteen; ICI Ltd (Agricultural Division), Billingham, Cleveland.

† Solka-floc; Johnson, Jorgenson and Wettre Ltd, London EC4M 7HA.

‡ 1065C; Vitriton Ltd, Stamford, Lincs PE9 2RA. To supply (/kg diet); calcium 11·8 g, phosphorus 8·0 g, sodium chloride 5·0 g, potassium 5·0 g, magnesium 400 mg, iron 60 mg, zinc 60 mg, manganese 20 mg, copper 10 mg, cobalt 0·5 mg, iodine 0·8 mg, thiamin 4·0 mg, riboflavin 5·0 mg, nicotinamide 30·0 mg, pantothenic acid 10·0 mg, pyridoxine 2·5 mg, pteroylmonoglutamic acid 2·0 mg, choline 1000 mg, cyanocobalamin 20·0 μg, retinol 901 μg, biotin 200 μg, cholecalciferol 25·0 μg, D-α-tocopherol 10·0 mg, menaphthone 120 μg, butylated hydroxytoluene 0·125 g.

tion in the evaluation of a new food protein. There are few estimates of BV for the dried microbial cell (DMC) protein source used here, and those are not wholly in agreement. Schulz & Oslage (1976) determined a BV of 0·79 with rats, whereas D'Mello, Peers & Whittemore (1976) found 0·68 with young growing pigs. The potential utilization by pigs of the non-amino nucleic acid-N fraction of DMC (comprising 0·19 of the total DMC N), remains unresolved (D'Mello *et al.* 1976), but nevertheless is of relevance to BV determinations.

N retention and efficiency of use of protein from DMC are studied here by conventional balance techniques after 10 d deprivation and 20 d realimentation at three levels of N supply. The evidence presented will show that enhanced N retention can occur after N deprivation and that this can be consequent upon an increase in efficiency of N use.

EXPERIMENTAL

Diets

Four semi-synthetic diets were compounded from the ingredients whose composition is shown in Table 1. The protein source was DMC (Pruteen; ICI Ltd (Agricultural Division), Billingham, Cleveland), which is the flash-dried product of the culture of *Methylophilus methylotrophus* on methanol. The amino acid composition of the N in DMC is given in Table 2. Ingredients were compounded in the proportions indicated in Table 3, to form four diets of differing N content. Determined N contents were 3·92, 14·78, 24·28 and 49·98 g/kg dry matter (DM) for diet nos. 1–4 respectively. All diets were fed at the rate of 750 g twice daily together with 1·5 l water at each feed. There were no refusals. Daily intakes of DM, gross energy (GE) and N are presented in Table 4. Diet no. 1 was formulated to maintain the animals in N equilibrium, while diet no. 3 was calculated to approach the animal's requirement.

Procedures

Twenty-three Large White × Landrace barrows of 49·2 ± 1·32 (mean ± SE) kg live weight were used for conventional N balance determinations using metabolic crates which allowed quantitative feeding and the separate collection of faeces and urine. Bulk 10 d or 5 d collections of excreta were preserved at pH 3–3·5 by addition of dilute sulphuric acid, and analysed for N by the Kjeldahl technique and for GE by adiabatic bomb calorimetry.

All pigs were given diet no. 3 for 3 d before the start of the experiment, and then six

Table 2. *Amino acid composition (g/16 g nitrogen) and N contents (g/kg* DM*) of dried microbial cells**

Amino acid	Source: D'Mello, Peers & Whittemore (1976)	ICI Ltd (1976)	ICI Ltd (unpublished results)	Schulz & Oslage (1976)
Aspartic acid	9·2	8·6	8·3	—
Threonine	4·9	4·6	4·2	4·2
Serine	3·2	3·4	3·4	—
Glutamic acid	12·7	9·8	9·7	—
Glycine	7·0	5·1	5·3	5·0
Alanine	7·4	6·9	7·2	—
Valine	6·0	5·3	5·1	5·2
Cystine	0·8	0·6	—	3·6
Methionine	2·3	2·5	2·1	
Isoleucine	4·1	4·4	4·2	4·4
Leucine	6·9	6·8	6·9	7·0
Tyrosine	4·1	3·1	2·8	—
Phenylalanine	5·2	3·5	3·2	—
Lysine	5·6	6·3	5·8	5·9
Histidine	1·9	2·0	1·7	1·7
Arginine	4·6	4·5	4·4	4·5
Tryptophan	0·9	1·0	—	—
N component				
Total N	129·9	128·0	127·0	131·0
Nucleic acid-N	24·2	24·0	24·3	—
N recovered as amino acids	96·3	104·0	—	—
Ammonia	—	0·08	0·15	—

DM, dry matter.
* Pruteen; ICI Ltd (Agricultural Division), Billingham, Cleveland.

Table 3. *Ingredients (g/kg fresh weight) and chemical composition of experimental diets fed to pigs*

Ingredients	Diet no. 1	2	3	4
Dried microbial cells*	24·75	95·50	185·00	370·00
Maize starch	725·25	657·50	565·00	380·00
Sucrose	50·00	50·00	50·00	50·00
Glucose	50·00	50·00	50·00	50·00
Cellulose	30·00	30·00	30·00	30·00
Maize oil	50·00	50·00	50·00	50·00
Vitamins and minerals†	70·00	70·00	70·00	70·00
Chemical composition				
DM (g/kg)	904·3	902·2	910·7	917·5
Gross energy (MJ/kg DM)	16·22	17·14	17·49	18·77
Nitrogen (g/kg DM)	3·92	14·78	24·28	49·98

DM, dry matter.
* Pruteen; ICI Ltd (Agriculture Division), Billingham, Cleveland.
† For details, see Table 1.

Table 4. *Daily intakes of dry matter* (DM), *gross energy* (*GE*) *and nitrogen by pigs given experimental diets of differing nitrogen content**

	Diet no.			
	1	2	3	4
Fresh wt (g/d)	1500·0	1500·0	1500·0	1500·0
DM (g/d)	1356·0	1353·3	1366·3	1376·3
GE (MJ/d)	21·98	23·19	23·90	25·84
N (g/d)	5·31	20·00	33·17	67·41

* For details, see Tables 1–3.

Table 5. *Design of experiment to determine the effect of feeding diets of differing nitrogen content** (*diet nos.* 1–4) *to pigs after a period of N deprivation*

Day of experiment	Balance period no.	Dietary treatment				
		3-3	1-1	1-2	1-3	1-4
1–2	—	3	1	1	1	1
3–12	1	3	1	1	1	1
13–14	—	3	1	2	3	4
15–19	2	3	1	2	3	4
20–24	3	3	1	2	3	4
25–29	4	—	—	—	3†	4†
30–34	5	—	—	—	3†	4†
No. of pigs		6	5	4	4	4

* For details of diets, see Tables 1–4.
† Two pigs for each dietary treatment.

pigs were allocated to diet no. 3 and the remainder to diet no. 1 for a preliminary feeding period of 2 d. From the third to the twelfth day, a 10 d balance period (balance period no. 1) was completed, after which six pigs remained on diet no. 3 (treatment 3-3), five pigs remained on diet no. 1 (treatment 1-1), while four pigs were each allocated to diet nos. 2 (treatment 1-2), 3 (treatment 1-3) and 4 (treatment 1-4) for a further preliminary feeding period of 2 d. There were two 5 d balance periods from the fifteenth to the twenty-fourth day of the trial; balance period no. 2 days 15–19, balance period no. 3 days 20–24. Two pigs each remained on diet nos. 3 and 4 for a further two 5 d balance periods; balance period no. 4 days 25–29, balance period no. 5 days 30–34 (see Table 5).

RESULTS

Daily N losses from pigs given diet no. 1 (dietary treatment 1-1: balance period no. 1, seventeen pigs; balance periods nos. 2 and 3, five pigs) were (mean ± SE) 1·62 ± 0·089 g in faeces and 3·90 ± 0·147 g in urine. These were assumed to be measurements of metabolic faecal N (MFN) and endogenous urinary N (EUN) losses. Equivalent losses for pigs given diet no. 3 (dietary treatment 3-3: balance period no. 1, six pigs; balance periods nos. 2 and 3, six pigs) were (mean ± SE) 2·65 ± 0·126 g and 15·48 ± 0·972 g.

Table 6 gives the N retention and digestibilities in balance period no. 1 (days 3–12) for pigs given diet nos. 1 (5·31 g N/d) and 3 (33·2 g N/d). The N retention found for pigs given diet no. 1 (−0·32 ± 0·439 g/d) showed the animals to be close to N balance. Apparent N digestibility ((N intake − faecal N) ÷ N intake) of diet no. 1 was reduced due to the high loading of MFN in comparison to N intake; determination of true digestibility ((N intake -

Table 6. *Nitrogen retention and digestibility of gross energy (GE) and N in balance period no.* 1 *(days* 3–12 *of the experiment)*† *for pigs given diet nos.* 1 *and* 3. *Diet nos.* 1 *and* 3 *contained* 3·9 *and* 24·3 *g N/kg dry matter respectively*‡

(Mean values with their standard errors for seventeen animals (diet no. 1) and six animals (diet no. 3))

	Diet				Statistical significance of difference between treatments
	1		3		
	Mean	SE	Mean	SE	
N retention (g/d)	−0·32	0·317	15·06	0·534	***
Digestibility					
GE (apparent)	0·95	0·003	0·95	0·005	NS
N (apparent)	0·65	0·013	0·91	0·022	***
(true)	0·96	0·013	0·96	0·022	NS

NS, not significant.
*** $P < 0·001$.
† For details of experimental procedures, see Table 5.
‡ For details of diets, see Tables 1–4.

Table 7. *Nitrogen balance and digestibility of gross energy (GE) and N in balance periods nos.* 2 *and* 3† *(days* 15–24 *of the experiment) for pigs given dietary treatments* 3-3, 1-2, 1-3 *and* 1-4. *Diet nos.* 1, 2, 3 *and* 4 *contained* 3·9, 14·8, 24·3 *and* 50·0 *g N/kg dry matter respectively*‡

(Mean values for six animals (treatment 3-3) and four animals (other treatments))

	Dietary treatments				SE of treatment means		Statistical significance of difference between treatments
	3-3	1-2	1-3	2-4	Treatment 3-3	Other treatments	
N balance							
N retention (g/d)	15·04	11·19	17·77	25·86	0·996	1·220	***
N retained ÷ digested N	0·49	0·62	0·58	0·41	0·022	0·027	***
Biological value §	0·64	0·85	0·72	0·49	0·020	0·025	***
Digestibility							
GE (apparent)	0·96	0·96	0·95	0·93	0·004	0·005	***
N (apparent)	0·93	0·90	0·93	0·95	0·006	0·007	**
(true)	0·97	0·98	0·98	0·96	0·006	0·008	NS

NS, not significant.
** $P < 0·01$, *** $P < 0·001$.
† For details of experimental procedures, see Table 5.
‡ For details of diets, see Tables 1–4.

§ BV $= \dfrac{\text{N intake} - (\text{faecal N} - \text{MFN}) - (\text{urinary N} - \text{EUN})}{\text{N intake} - (\text{faecal N} - \text{MFN})}$, where MFN is metabolic faecal N, EUN is the endogenous urinary N.

faecal N + MFN) ÷ N intake) showed the N from both diets to be equally effectively absorbed.

N utilization in balance periods nos. 2 and 3 (days 15–24) is shown in Table 7. There were no differences between results for the two balance periods, and the mean values of results for individual pigs are given. True digestibility of N remained unaffected by dietary treatment ($P > 0·05$). Pigs given dietary treatment 3-3 (33·2 g N/d) continued to retain 15·0 g N/d, with an apparent digestibility coefficient for N of 0·93 and a true digestibility coefficient for N of 0·97. The efficiency of N retention (N retained ÷ apparently digested N) was 0·49 and the BV was 0·64.

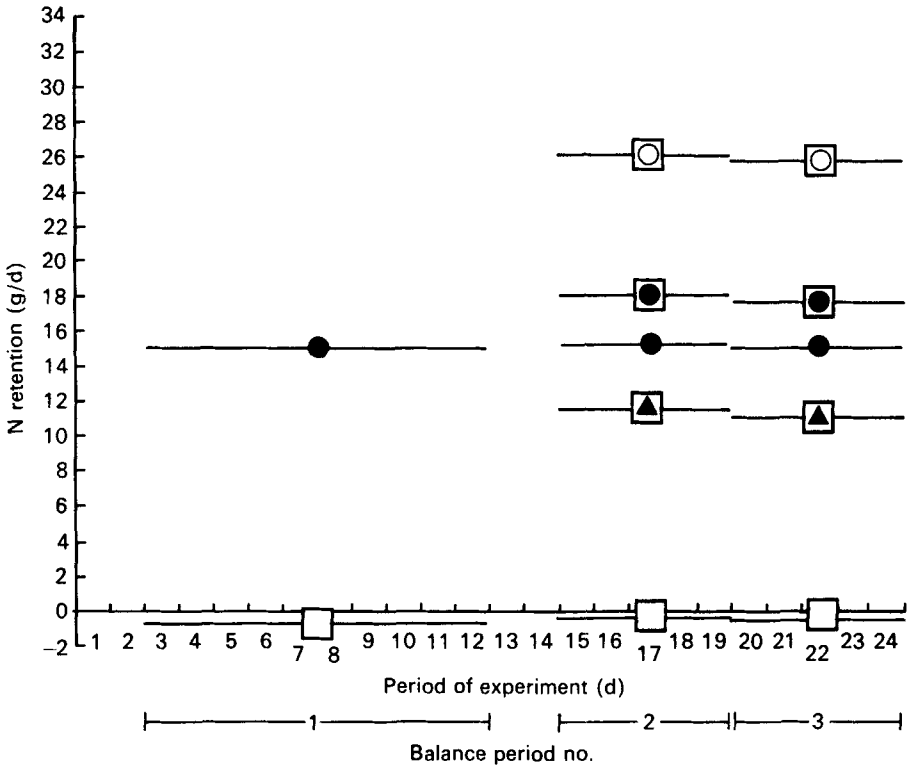

Fig. 1. Nitrogen retention (g/d) in balance periods nos. 1, 2 and 3 for pigs given dietary treatments 1-1 (□) (deprivation); 3-3 (●) (control); 1-2 (▲); 1-3 (◙), 1-4 (◎) (deprivation in balance period no. 1, followed by realimentation in balance periods nos. 2 and 3). Diet nos. 1, 2, 3 and 4 contained 3·9, 14·8, 24·3 and 50·0 g N/kg DM respectively. For details of diets and experimental procedures, see Tables 1–5 and p. 194.

Values for N retention by pigs throughout 34 d of N balance determinations are shown in Fig. 1.

The influence of N deprivation in pigs given diet no. 1 in balance period no. 1, on N balance in pigs given diet nos. 2, 3 and 4 in balance periods nos. 2 and 3 was to enhance the rate of N retention. Dietary treatment 1-3 effected a net increase of 2·73 g N/d in excess of that of dietary treatment 3-3 ($P < 0.05$). By comparison with diet treatment 3-3, the N from dietary treatment 1-3 was utilized with significantly greater efficiency (0·58 *v.* 0·49, $P < 0.05$) and had a higher BV (0·72 *v.* 0·64, $P < 0.05$) (see Table 7). Dietary treatment 1-2 had the highest value for efficiency of N utilization (0·62) and the BV of the N was 0·85. The level of N intake by pigs given diet no. 2 (20·0 g N/d) was below the requirement of the pig as evidenced by responses to diet no. 3; efficiencies found for diet no. 2 would therefore appear to have been representative of maximum values for the DMC N source. Pigs given dietary treatment 1-4 (67·4 g N/d) showed the highest rate of N retention (25·9 g N/d) but efficiency of utilization was markedly reduced.

N balances for two pigs each given dietary treatments 1-3 and 1-4 were continued for a further 10 d (balance periods nos. 4 and 5) to measure responses up to 22 d after deprivation. Enhanced retentions were still evident at average values of 18·7 and 29·2 g N/d for dietary treatments 1-3 and 1-4 respectively.

Table 8. *Metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) losses in balance periods nos.* 1, 2 *and* 3† *for pigs given dietary treatment* 1-1. *Diet no.* 1 *contained* 3·9 *g N/kg dry matter*‡

(Mean values for seventeen animals (balance period no. 1) and five animals (balance periods nos. 2 and 3))

	Balance period no.			SE of treatment means		Statistical significance of difference between treatments
	1	2	3	Balance period no. 1	Balance periods nos. 2 and 3	
No. of pigs	17	5	5			
MFN	1·84	1·27	1·23	0·073	0·135	***
EUN	3·86	3·76	4·17	0·127	0·235	NS

NS, not significant.
*** $P < 0.001$.
† For details of experimental procedures, see Table 5.
‡ For details of diets, see Tables 1–4.

DISCUSSION

The BV of 0·64 determined for DMC with diet no. 3 was similar to the value of 0·68 found by D'Mello *et al.* (1976), while the value of 0·85 determined with diet no. 2 exceeded the value of 0·79 of Schulz & Oslage (1976). It was evident that the potential efficiency of utilization of N from the DMC was high.

Values for MFN and EUN of 1·62 and 3·90 g/d respectively should be collated with those of 1·11 and 2·91 g/d measured by D'Mello *et al.* (1976) and 3·2 g/d for EUN measured by Lubaszewska, Pastuszewska & Kielanowski (1973) in pigs of similar weight. Lubaszewska *et al.* (1973) noted an increase in EUN with duration of protein deprivation. When expressed on a per unit food intake basis, Whiting & Bezeau (1957) found MFN to be 1·0 g/kg DM and Armstrong & Mitchell (1955) reported a value of 1·1 g/kg DM; the equivalent value for the present experiment was 1·2 g/kg DM. MFN was less and EUN tended to be greater in balance period no. 3 as compared to balance period no. 1 (Table 8) although the difference was not significant for EUN. The influence of the duration of deprivation on MFN and EUN was considered insufficient to merit use of individual values for each balance period, rather than the mean value, for determination of true digestibility and BV. The significantly higher MFN value measured in balance period no. 1 was quantitatively small, and may be attributable to 'carry-over' effects from the preliminary feeding period.

Comparison of dietary treatment 3-3 with dietary treatment 1-4 showed enhanced protein growth (N retention (g/d) 15·0 *v.* 25·9) during realimentation with ample protein; lost protein growth would be recouped in 12 d. Comparison of dietary treatment 3-3 with dietary treatment 1-3 demonstrates 'catch-up' protein growth in the strictest sense (N retention (g/d) 15·0 *v.* 17·8) together with simultaneously enhanced efficiency of protein utilization; there being no increase in dietary protein supply (efficiency of retention, 0·49 *v.* 0·58; BV, 0·64 *v.* 0·72). In this instance, lost protein growth would be recouped in 7 weeks.

The increase in efficiency was mediated through a reduction in the rate of urinary N loss. This could have resulted from a deceleration of protein catabolism or an increase in anabolic efficiency associated with protein turnover. Alternatively, there could have been an increase in the proportion of absorbed protein utilized. A decrease in catabolism, elicited by protein deprivation and sustained into the realimentation phase, is consistent with responses found in rats (Millward *et al.* 1976). The phenomenon is, however, probably

too slow-acting to be entirely responsible for the present result. Further, a reduction in the rate of protein turnover might have been expected to have caused a progressive reduction in EUN, but this did not occur; values for EUN suggested a response which was complete within the 2 d preliminary feeding period and more akin to a 'shutdown' of the urea cycle, an increase in the efficiency of anabolism and a reduction in the proportion of catabolized protein which was excreted. Das & Waterlow (1974) showed how the protein-deprived rat could in this way reduce N excretion by 75% in 30 h. Conversely, while the 'carry-over' effects of a decrease in catabolic rate are evident (Millward *et al.* 1976), it is not conceivable that 'shutdown' of urea synthesis would be maintained throughout the 20 or more d of realimentation feeding, for which period this experiment demonstrated enhanced BV.

The most plausible explanation for the enhanced BV with dietary treatment 1-3 would be an increase in the proportion of the constituents of the dietary protein utilizable by the pig. D'Mello *et al.* (1976) proposed some part of the nucleic acid fraction of DMC to be available to the animal. The availability of this fraction may be related to the extent of the animal's need, the latter being greater after protein deprivation. Of the N contained in DMC approximately 19% is nucleic acid-N. Subtraction of the appropriate proportion of nucleic acid-N from the various elements of the BV determination allows a calculation of BV on the assumption that none of the nucleic acid-N is utilized. Such calculations for dietary treatments 1-2 and 1-3 give BV values of 1·04 and 0·87 respectively, which indicates that either DMC protein is of quite exceptional quality or that some of the nucleic acid-N is usable by the pig.

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