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Effects of the amount and quality of dietary protein on nitrogen metabolism and heat production in growing pigs

BY M. F. FULLER, A. CADENHEAD, G. MOLLISON AND B. SEVE*

Department of Applied Nutrition, Rowett Research Institute, Bucksburn,
Aberdeen AB2 9SB

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1. Eight pigs with a mean weight of 48 kg were given, at a constant daily rate, diets of low (0·15) or high (0·30) protein content, very deficient in lysine, with or without a supplement of L-lysine (3·7 g/kg).

2. Measurements of nitrogen and energy metabolism were made in four successive 14 d periods in a Latin-square design.

3. The rate of protein accretion was substantially increased by increases in both protein and lysine supply, but the rate of heat production was not significantly changed.

4. The rate of fat deposition varied inversely with the rate of protein accretion, being reduced by both protein and lysine supplements.

5. The relation between heat production and protein accretion (allowing for a constant energy cost of fat deposition) suggested that heat production increased with additional protein accretion less when protein quality was improved than when more protein was given.

It was Kielanowski (1965) who first proposed, and who later elaborated (Kielanowski, 1966) the idea that the energy requirement of a growing animal can be considered to be the sum of three components: the energy requirements for maintenance, protein accretion (A) and fat deposition. Using multiple regression, he computed the association of metabolizable energy (ME) with A and fat deposition. The residual quantity was assumed to be the energy required for maintenance. In recent years, experiments in several species have been made to estimate the magnitude of these specific costs. As far as growing pigs are concerned, a survey of experimental evidence (Fowler *et al.* 1980; Agricultural Research Council, 1981) suggested that the ME required for A is, on average, 44 kJ/g and for fat deposition 54 kJ/g. By subtracting from these values the heats of combustion of body protein and fat (23·7 and 39·6 kJ/g; Franke & Weniger, 1958) the increases in heat production associated with protein and fat deposition are 20·3 and 14·4 kJ/g respectively. These estimates are based on statistical associations, rather than physiological causation. It is simply to say that when the rate of A is increased, energy expenditure increases in constant proportion: it is not to say that there is necessarily any direct causal link between the two.

By contrast, it has been estimated (Millward *et al.* 1976) that 4 mol ATP are required for each peptide bond formed and probably another 1 mol for additional associated energy expenditures in amino acid transport, RNA synthesis, etc. The synthesis of 5 mol ATP typically requires 400 kJ ME; assuming that the average molecular weight of amino acid residues is 116, this suggests that 3·45 kJ ME are expended for each 1 g protein synthesized. It might be thought that these estimates, 3·45 kJ/g for protein synthesis (S) and 20·3 kJ/g for A, could be reconciled if protein turnover exceeded A by a factor of 6, which indeed it commonly does (Waterlow *et al.* 1978). However, what is important is not the overall ratio, S:A but the marginal ratio, that is, the change in S associated with a change in A. In a previous paper (Reeds *et al.* 1980) we showed that when nitrogen

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* Present address: Station de recherches porcines, Saint-Gilles, 35590 L'hermitage, France.

retention was increased by giving more food the relation between S (g/d) and A (g/d) in 35 kg pigs was described by the equation

$$S = 230 + 2{\cdot}2\,A.$$

The large intercept term reflects the fact that, even when there was no net accretion of protein, 230 g protein were synthesized daily. The energy expended in synthesizing this quantity of protein would be included in the maintenance term. The marginal ratio, $S{:}A$ was 2·2 and on this basis if the energy requirement for S was the only determinant of the energy requirement for A, the increase in heat production (H) associated with increased A would be 2·2 × 3·5, i.e. 7·7 not 20·3 kJ/g.

In most of the studies so far made of this subject, increases in A have been associated with increases in either protein or energy intake or both and it is possible that alterations of other components of metabolism induced by these changes may have been statistically associated with the observed changes in A. We now report the results of experiments in which A was approximately doubled with virtually no change in energy or protein intake, by doubling the supply of the most limiting amino acid in the diet. It was not technically feasible to make all measurements on the same animals, so two experiments were made with two sets of similar animals. Measurements of N metabolism and H were made in the first experiment, described in the present paper. Measurements of protein turnover are reported in the following paper (Fuller *et al.* 1987*b*). A preliminary report of some of these results has been given (Fuller *et al.* 1987*a*).

MATERIALS AND METHODS

Animals

Eight female pigs, Large White × (Large White × Landrace) were used. They were selected when they weighed approximately 25 kg and were confined in metabolism cages during measurement periods and for 4 d beforehand. The room was maintained at 20°.

Design

The experiment was arranged as two 4 × 4 Latin squares, one running 1 week behind the other. Each period lasted for 2 weeks, during which the pig was given the appropriate diet. To allow adequate time for adaptation to the new diet, measurements were made in the 2nd week of each period, so that measurements were being made on pigs in the two Latin squares in alternate weeks.

Diets and feeding

Four diets were used (Table 1). Diet 3 was the basic high-protein diet (HP) from which all the others were formed. It included a mixture of ingredients with lysine-deficient proteins, barley, sunflower-seed meal, sesame-seed meal and hydrolyzed feather meal. To form diet 1 (LP), diet 3 was diluted with a mixture of maize starch, sucrose, cellulose and maize oil, thus maintaining the same amino acid profile in each diet. Each of these diets was then supplemented with 4·7 g L-lysine hydrochloride/kg diet to give diets 2 (LP+) and 4 (HP+). One batch of each diet was used throughout. The diets were given in two daily meals at the rate of 120 g/kg body-weight $(W)^{0.75}$ per d, where W is the live weight the pigs were expected to reach at the middle of the period of measurement.

Table 1. *Composition of the diets*

Diet . . .	1 (LP-)	2 (LP+)	3 (HP-)	4 (HP+)
Ingredients (g/kg)				
Ground barley	248	248	496	496
Sesame-seed meal	92	92	184	184
Sunflower-seed meal	75	75	150	150
Hydrolysed feather meal	68·5	68·5	137	137
Maize starch	410	410	0	0
Sucrose	30	30	0	0
Cellulose	25	25	0	0
Maize oil	10	10	0	0
Dicalcium phosphate	35	35	30	30
Sodium chloride	3·8	3·8	3·8	3·8
Vitamins and minerals	2·6§	2·6§	2·5‡	2·5‡
L-lysine hydrochloride	0	4·7	0	4·7
Analysis				
Dry matter (g/kg)	888	887	889	889
Nitrogen × 6·25 (g/kg)	151	156	302	307
Heat of combustion (kJ/g DM)	19·10	—	18·77	—
Total lysine†	3·7	7·4	7·4	11·1

DM, dry matter.

† Calculated from amino acid analyses of ingredients.

‡ Pigvite no. 2 (Norvite Ltd, Kennethmont, Aberdeenshire), supplying (/kg diet): retinol 3·64 mg, cholecalciferol 50 µg, α-tocopherol 6·7 µg, phytylmenaquinone 2 mg, riboflavin 5 mg, pantothenic acid 10 mg, nicotinic acid 20 mg, cyanocobalamin 12 µg, iron 100 mg, zinc 100 mg, manganese 40 mg, copper 15 mg, iodide 2 mg, cobalt 0·5 mg, selenium 0·15 mg.

§ Pigvite no. 2 plus pyridoxine 20 mg, pantothenic acid (additional) 5 mg, magnesium 210 mg.

Measurements

On the 1st day of each 2-week period the pigs were weighed and their rations for the period were calculated by adding 3·5 kg to their weight. On the 6th day bladder catheters were introduced and on the 7th day complete collections of faeces and urine were begun. In each period two successive 24 h measurements of energy exchange were made in two of the closed-circuit respiration chambers described by Wainman & Blaxter (1969). The chambers were maintained throughout at 20° (actual mean 19·6°). For two pigs in each four these measurements were on days 11 and 12 and for the other two pigs on days 13 and 14 of each period. H (kJ) was calculated from oxygen consumption (litres), carbon dioxide production (litres) and urinary N excretion (g) using the equation of Hoffman (1958) which was designed specifically for pigs, and which, converted to kJ, is:

$$H = 5{\cdot}23\ CO_2 + 16{\cdot}07\ O_2 - 122{\cdot}9\ N.$$

Urine was collected directly into 250 ml 10 M-sulphuric acid; faeces were removed daily and transferred to acid.

Statistical analysis

Results were analysed using GENSTAT. The weights of the animals at the times of measurement were calculated by linear interpolation between weights taken every 2 weeks.

Table 2. *Nitrogen and energy metabolism in pigs given high- (HP) and low- (LP) protein diets deficient in lysine, with (+) or without (−) lysine supplementation*†

Diet . . .	1 LP−	2 LP+	3 HP−	4 HP+	SED Protein	SED Lysine	SED Lysine × protein
Body-wt (W ; kg)	45·3	48·3	48·0	49·7	0·85**	0·85**	1·20
W gain (kg/d)	0·54	0·86	0·75	0·90	0·030***	0·030***	0·040**
Apparent digestibility of energy	0·87	0·86	0·79	0·78	0·006***	0·006	0·008
Apparent digestibility of nitrogen	0·81	0·81	0·80	0·80	0·009	0·009	0·013
Metabolizable energy (MJ/kg $W^{0.62}$ per d)	2·402	2·363	2·147	2·075	0·0355***	0·0355	0·0502
N digested (g/kg $W^{0.62}$ per d)	3·34	3·68	6·08	6·41	0·114***	0·114**	0·161
Heat production (MJ/kg $W^{0.62}$ per d)	1·172	1·201	1·188	1·166	0·0122	0·0122	0·0172*
Energy retained (MJ/kg $W^{0.62}$ per d)	1·239	1·170	0·969	0·918	0·0278***	0·0278**	0·0394
N retained (g/kg $W^{0.62}$ per d)	0·95	1·97	1·74	2·43	0·127***	0·127***	0·180

SED, standard error of difference.

* $P < 0.1$, ** $P < 0.05$, *** $P < 0.001$.

† For details see Table 1 and p. 278.

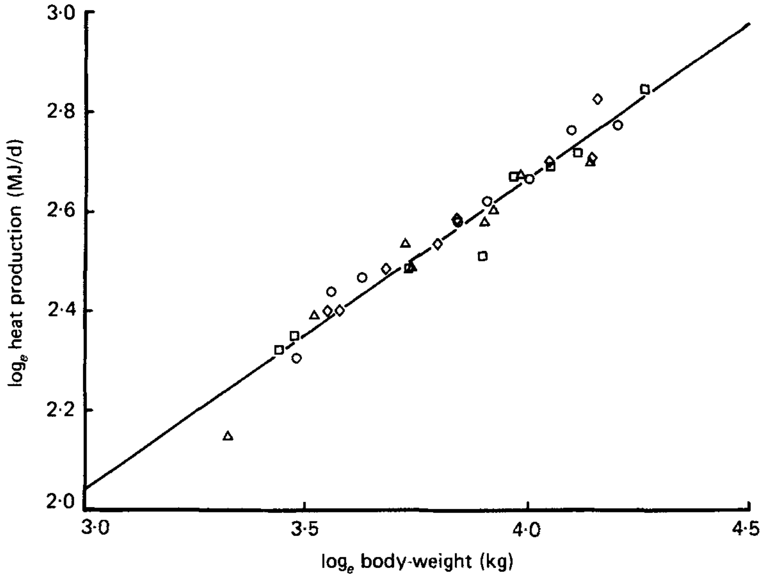

Fig. 1. The relation between $\log_e$ heat production (H) and $\log_e$ body-weight (W). Each point represents the mean of two consecutive 24 h measurements of the H of the same animal. No adjustment has been made for treatement differences. (△), low-protein (LP) diet without lysine supplementation; (◇), LP with lysine supplementation; (○), high-protein (HP) diet without lysine supplementation; (□), HP diet with lysine supplementation. For details of diets, see Table 1 and p. 278. The equation was:

$$\log_e H = 0{\cdot}172\ (\text{SE } 0{\cdot}0959) + 0{\cdot}623\ (\text{SE } 0{\cdot}0250)\ \log_e \mathrm{W}\ (r\ 0{\cdot}977).$$

RESULTS AND DISCUSSION

There were no missing observations: all the pigs ate well and there were only three minor food refusals.

Body-weights and weight gain

The rates of weight gain are given in Table 2 and show the very large effect of lysine supplementation. The slight differences in weights between treatment probably arose because, for the purpose of rationing the animals, the same increase in body-weight from day 1 to the midpoint of the period was assumed, whereas the weight gains were in fact greatly affected by treatment.

Expression of results

Because of the increase in body-weight during the experiment it was necessary to adjust results in successive periods for differences in body-weight. Examination of the results suggested that linear covariance would be inappropriate and the results were then expressed per kg $\mathrm{W}^{0{\cdot}75}$. However, these scaled values also changed significantly with body-weight and accordingly an alternative basis was sought. The overall relation between H (kJ/d) and body-weight (kg) (Fig. 1) was described by the equation

$$H = 1188\ \mathrm{W}^{0{\cdot}62}.$$

The correlation coefficient was 0·977. Although the exponent 0·62 is substantially lower than 0·75, it accords with exponents found in previous work with growing pigs: 0·57 (Breirem, 1939), 0·57 (Fuller & Boyne, 1972); 0·55 (Fuller *et al.* 1980). Analysis of published

Table 3. *Rates of energy retention as protein and as fat and estimates of the heat production which was associated with fat deposition (13·9 kJ/g) and that which was not in pigs given high- (HP) and low- (LP) protein diets deficient in lysine, with (+) or without (−) lysine supplementation*†

(All values are expressed as kJ/kg body-weight$^{0.62}$ per d)

Diet . . .	1 LP−	2 LP+	3 HP−	4 HP+
Energy retained as protein	140	291	257	360
Energy retained as fat	1101	881	702	549
Heat production :				
Associated with fat deposition	387	310	247	193
Not associated with fat deposition	785	891	941	973

† For details of diets, see Table 1 and p. 278.

values (Agricultural Research Council, 1981; Brown & Mount, 1982) strongly supports the contention that, although 0·75 is the appropriate exponent for comparing adults of different species, the appropriate exponent for comparing different sizes of growing pigs is close to 0·6. Accordingly, the results of these experiments have been expressed per kg $W^{0.62}$. The residual coefficient of variation of H from analysis of the two Latin squares was 0·027 of which 0·023 was the variation between consecutive 24-h measurements. Individual values for the major variables are given in the Appendix.

N metabolism

In Table 2 are given the mean values for each diet of N apparently digested, apparent N digestibility and N retention. The N of all diets was apparently equally well digested, 0·80–0·81, and supplementation with lysine did not alter its digestibility. This is discussed further in the following paper (Fuller *et al.* 1987*b*).

The rate of N retention was significantly increased by the addition of lysine to both the LP and HP diets. The increase was much greater with the LP diet, although the interaction was not significant.

Energy metabolism

The digestibility of the energy of the LP diets was significantly higher than that of the HP diets. The LP diets included a large proportion of highly digestible carbohydrates. Accordingly, the daily ME was significantly greater for the pigs on the LP diets.

Differences in H with diet were small and there was no significant effect of increasing either protein or lysine concentration. There was a tendency for the addition of lysine to the LP diet to increase H, whereas with the HP diet, H tended to be reduced; this interaction was almost significant ($P < 0.06$).

General discussion

The main question which this experiment sought to answer was how much does H increase with A when there is no change in energy intake? Had addition of lysine provoked no changes other than in N retention and H, it would have been possible to draw some direct inferences about the relation between the two. In fact, when lysine was added to the diets there was not only an increase in N retention but a concomitant reduction in fat deposition. This can be seen in Table 3, where the energy retention is divided into that retained as protein (N retention × 148·1 kJ/g) and that retained as fat (the remainder). With diet 1, fat accounted for 0·89 of the retained energy, whereas with diet 4 the proportion was only 0·60.

There is a second slight complication, which arises from differences in the composition of the substrates available for ATP and fat formation. It has been calculated (for example, see Schiemann, 1963) that the energy of the carbon skeletons of amino acids are utilized for ATP formation with only 0·85 of the efficiency of carbohydrates. From the urinary N excretion the contribution of amino acids to total ME (other than that of the protein accreted) can be calculated. For diets 1–4, this proportion was 0·16, 0·12, 0·34 and 0·34 respectively. Compared with all-carbohydrate diets, therefore, the relative efficiency of utilization of the energy in the four diets was calculated to be 0·98, 0·98, 0·95 and 0·95 respectively. This means that rather more heat would have been generated in the metabolism (to ATP and fat) of the energy in diets 3 and 4 than in diets 1 and 2.

To calculate the increases in H associated with the increases in A, it is necessary to make allowance for the simultaneous change in the rate of fat deposition. The ME required for the deposition of 1 g fat from (predominantly) carbohydrate has been estimated in respiration experiments with mature pigs to be 53·5 kJ (Schiemann *et al.* 1961). Numerous other estimates have given substantially the same value (for review, see Agricultural Research Council, 1981).

An overall regression was calculated of the H not associated with fat deposition (H_n; kJ/kg $W^{0.62}$ per d) on the rate of A (g/kg $W^{0.62}$ per d). This (adjusted for the effects of animals and periods) gave the relation:

$$H_n = 668\ (\text{SE } 33{\cdot}1) + 16{\cdot}6\ A\ (\text{SE } 2{\cdot}03)\ (n\ 32).$$

The constant term 668 is an estimate of the H not associated with either fat deposition or A. It is rather lower than the energy requirement for maintenance (in the sense of energy equilibrium) of 741 kJ/kg $W^{0.62}$ per d (i.e. the Agricultural Research Council (1981) estimate of 719 kJ/kg $W^{0.63}$ per d converted to the slightly different exponent at the mean body-weight of these pigs). This discrepancy is to be expected as a result of the considerable rate of N retention associated with energy equilibrium in growing pigs which has been observed in our own (Fuller *et al.* 1976; Reeds *et al.* 1980) and others' experiments (Close *et al.* 1978).

The regression coefficient 16·6 suggests that H was increased by 16·6 kJ/g increase in A. However, this overall regression is not appropriate because the changes in H_n with change in A were quite different according to whether lysine or protein was used to increase A. Addition of lysine increased H_n by 16·6 kJ/g increase in A with the LP diet and by 7·4 kJ/g with the HP diet. With addition of protein on the other hand, H_n increased by 31·6 kJ (without lysine) and 28·5 kJ (with lysine)/g increase in A. It seems clear that to increase A by giving more dietary protein is energetically more expensive than by improving the quality of the protein. The ratio, H_n:A from all the treatments was 19·4, an estimate not significantly different from the value of 20·3 suggested (Fowler *et al.* 1980; Agricultural Research Council, 1981) as representative of a number of experiments with different protocols which involved changes in energy intake, protein intake or both. The present results suggest that the observed associations do not arise from a general increase in metabolism consequent on the ingestion of increased amounts of energy or protein but are specifically associated with the processes of protein metabolism. Our examination of some of these processes in the same dietary circumstances is described in the following paper (Fuller *et al.* 1987*b*).

The authors wish to thank Miss R. A. Fordyce for chemical analysis, Mr J. S. Smith for help with the measurements of energy exchange and Dr A. C. Brewer for statistical analysis.

Appendix. *Individual observations of the key variables in each period for pigs given high- (HP) and low- (LP) protein diets deficient in lysine, with (+) or without (−) lysine supplementation*

Pig no.	Period	Diet†	Mean wt (kg)	ME (MJ/d)	Heat production (MJ/d)	Energy retention (MJ/d)	Nitrogen digested (g/d)	Urinary N (g/d)	N retained (g/d)
1	1	HP−	35·21	18·06	11·20	6·86	51·05	37·88	13·17
2	1	HP+	32·33	16·34	10·30	6·05	50·91	31·06	19·85
3	1	LP−	27·80	16·60	8·50	8·10	22·58	18·32	4·26
4	1	LP+	35·81	20·476	10·96	9·51	31·65	13·58	18·08
5	1	LP+	34·80	20·76	10·95	9·81	32·08	14·68	17·39
6	1	HP−	32·50	17·92	9·80	8·12	50·58	39·16	11·42
7	1	LP−	33·81	20·59	10·82	9·77	28·01	20·73	7·28
8	1	HP+	31·24	15·45	10·05	5·40	45·28	23·51	21·76
1	2	LP−	41·51	24·00	12·48	11·52	32·56	24·67	7·89
2	2	LP+	39·74	23·63	11·90	11·74	36·30	15·81	20·49
3	2	HP−	46·71	23·59	12·88	10·72	67·18	51·78	15·40
4	2	HP+	49·34	20·49	12·04	8·44	62·91	46·28	16·63
5	2	LP−	42·11	24·60	11·89	12·71	34·50	24·67	9·83
6	2	HP+	41·80	21·65	11·76	9·89	65·94	36·94	29·00
7	2	LP+	44·66	22·76	12·46	10·30	34·20	23·34	10·86
8	2	HP−	37·70	18·74	11·58	7·16	51·14	37·00	14·14
1	3	HP+	52·91	26·84	14·13	12·72	83·02	48·62	34·40
2	3	LP−	49·64	27·16	13·06	14·10	37·54	29·28	8·26
3	3	LP+	57·34	30·22	14·78	15·44	47·75	22·31	25·44
4	3	HP−	54·79	27·62	14·01	13·61	78·84	57·92	20·92
5	3	HP−	49·89	24·85	13·41	11·44	68·80	49·26	19·54
6	3	LP−	50·69	29·74	13·35	16·39	42·04	25·23	16·82
7	3	HP+	57·61	26·65	14·40	12·26	83·44	53·44	30·01
8	3	LP+	46·60	26·51	13·10	13·40	41·46	18·53	22·93
1	4	LP+	64·00	33·80	16·71	17·09	54·08	23·00	31·08
2	4	HP−	60·34	29·74	15·52	14·22	84·85	51·28	33·57
3	4	HP+	71·40	34·00	16·74	17·27	108·46	71·55	36·90
4	4	LP−	63·03	33·26	14·71	18·54	47·54	31·52	16·03
5	4	HP+	61·17	27·93	14·78	13·15	86·48	56·08	30·40
6	4	LP+	63·11	32·47	14·83	17·64	51·57	21·54	30·04
7	4	HP−	67·13	30·56	15·59	14·98	89·97	59·93	30·04
8	4	LP−	53·79	30·31	14·31	16·01	43·03	30·32	12·70

ME, Metabolizable energy.

† For details, see Table 1 and p. 278.

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