Effect of dietary protein quality on energy metabolism in growing chickens

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A slaughter experiment was carried out to study the effect of dietary protein quality on maintenance energy requirements and energy costs for protein accretion and fat deposition in fast-growing broiler-type male chickens. Three isonitrogenous (200 g crude protein/kg DM) and isoenergetic (14 kJ metabolizable energy (ME)/g DM) semipurified diets based on soyabean meal unsupplemented (diet S) or supplemented with 20 g L-lysine/kg (diet SL) or 2 g DL-methionine/kg (diet SM), in order to promote a decrease or an increase in growth rate respectively, were selected and given at four feeding levels (*ad lib.* or restricted to 40, 28 and 18 g DM/d, on average) to 10-d-old fast-growing male broiler-type chicks for 2 weeks. Both the efficiency with which ME was used to support growth (k_g) and the maintenance requirements (ME_m) significantly decreased inversely to the biological value of the dietary protein (k_g = 0·660, 0·600 and 0·572; ME_m = 597, 522 and 464 kJ/kg $W^{0.75}$ per d, for diets SL, S and SM respectively). The partial efficiencies of use of ME for protein accretion (k_p) or fat deposition (k_f) were also inversely related, the former increasing with the quality of the protein offered. An alternative procedure was used to try to overcome the statistical problems inherent in the partition of ME between fat and protein.

Protein quality: Energy costs: Maintenance requirements: Chickens

Protein deposition depends to a great extent on amino acid supply and therefore on the quantity and the quality (biological value, BV) of dietary protein. While much work has been published on the effect of the concentration of protein in the diet on the utilization of dietary energy, very little has been reported (Fuller *et al.* 1987 *a, b*) on the extent to which the utilization of dietary energy, and specifically the relative costs of protein and fat deposition, are modified by dietary protein quality. In a previous study (Aguilera & Prieto, 1987 *a*) we showed that growing rats given several sources of dietary protein which differed widely in their BV presented marked differences in N retention and subsequently in level of production and relative rates of protein and fat deposition. In these experiments there were differences in the efficiency of utilization of metabolizable energy (ME) for growth, while no differences in energy requirements for maintenance (ME_m) attributable to dietary protein quality could be detected. The experimental design adopted was, however, unable to provide information on the effect of dietary protein quality on the partial efficiencies of ME utilization for protein (k_p) or fat (k_f) deposition. These experiments also involved changes in energy and/or protein intake. In this respect it has been reported that the intakes of both protein and non-protein energy (Fuller *et al.* 1987 *a–c*) are associated with changes in protein accretion. We have now studied the effect of changing the rate of protein accretion without alteration in energy or protein intake, through supplementation of a basal diet based on soyabean with the limiting amino acid (methionine) or with an excess

of a non-limiting amino acid (lysine). Increases in N retention have been associated with higher rates of both protein synthesis and breakdown in the whole body (Reeds *et al.* 1980). This may have an associated energy penalty and affect differences in the energy costs of protein deposition as dietary quality varies. In this respect, we also showed (Nieto *et al.* 1994) that in muscle of growing chickens an amino acid imbalance affected growth by changing protein degradation rates, without effect on protein synthesis. Although extrapolation of these results to the whole-body situation may be misleading, the hypothesis is that changes in the rate of protein degradation, assuming a constant rate of protein synthesis, through alterations in the BV of dietary protein, may influence the energetic costs of protein deposition. The aim of the present study was to provide additional information as to whether or not dietary protein quality has an influence on the utilization of dietary energy in growing animals. Apart from other reasons (economic; quality and quantity of previous work on responses to amino acids), the broiler type chick was used mainly because genetic selection has yielded an animal with high rates of protein and weight gain, an ideal circumstance for the present investigation. Preliminary aspects of these studies have been reported elsewhere (Nieto *et al.* 1994).

MATERIALS AND METHODS
Animals, diets and experimental design
White Rock male broilers (1 d old) were raised in conventional electrically heated chick battery brooders in a fully lit, controlled-temperature room (30°) and given a commercial starter diet for 10 d. Then they were divided into three groups, each of approximately forty birds, of similar body weight (mean live weight 136 (SE 1·6) g), and individually housed in metabolism cages with a wire floor. After an adaptation period of 5 d to the diet studied, for an experimental period of 9 d three isonitrogenous (200 g crude protein/kg DM) and isoenergetic (14 kJ ME/g DM) semipurified diets were offered, based on soyabean meal deficient in methionine, either unsupplemented (S), supplemented with 2 g DL-methionine/ kg (SM) or supplemented with 20 g L-lysine/kg (SL) (Table 1) at four feeding levels (*ad lib.* or restricted to 40, 28 and 18 g DM/d, on average). The birds had continuous access to water and the cages were arranged randomly. The environmental temperature was decreased gradually from 30° for 1-d-old chickens to 22° at 4 weeks of age. The birds were kept under a daily lighting schedule of 14 h light and 10 h dark. Feed intake, corrected for spillage, was recorded. A partial collection of excreta (25 % of the total) was made daily and stored at $-20°$ before freeze drying. Cr_2O_3 (10 g/kg) was included in the diets as a Cr_2O_3–starch–water bread and used as a marker to estimate total energy losses in excreta. Care was taken to remove feathers from the droppings tray. On days 16 (initial groups, mean live weight 172 (SE 3·3) g) and 25 (final groups, mean live weight 340 (SE 9·4) g) of age a sample of birds was slaughtered for body composition determination; the content of the digestive tract was rapidly removed and the whole birds were minced and stored at $-20°$ until analysis.

Calculation of metabolizable energy, energy retention and partition
The DM content of feed and excreta was determined by standard procedures (Association of Official Analytical Chemists, 1975) and total N by a Kjeldahl procedure using mineralization (Block Digestor Selecta S-509), distillation units (Büchi Laboratoriums Technik AG, Flawil, Switzerland) and titration units (Metrom AG, Herisau, Switzerland). Gross energy (GE) was measured in an adiabatic bomb calorimeter (Gallenkamp Autobomb CBA 305, Loughborough, Leics). Samples were freeze-dried in a polyethylene

Table 1. *Composition of the experimental diets (g/kg)*

	Diet		
	S (control)	SL	SM
Ingredients			
Soyabean meal*	402·0	360·0	402·0
Maize oil	55·1	55·6	55·1
Mineral and vitamin premix	111·3	111·3	111·3
L-Lysine	—	20·0	—
DL-Methionine	—	—	2·0
Maize starch†	431·6	453·6	429·6
Chemical composition			
Dry matter (DM) (g/kg)	924	930	930
Crude protein (N × 6·25; g/kg DM)	203·8	201·3	202·6
Ether extract‡	68·6	68·3	68·6
Crude fibre‡	15·3	13·9	15·3
N-free extractives‡	554·4	562·3	554·4
Metabolizable energy (kJ/g DM)	14·7	14·5	14·6

* Chemical composition (g/kg DM): crude protein, 532·6; ether extract, 26·2; crude fibre, 38·8; total minerals, 71·6.

† Ether extract: 9·4 g/kg DM.

‡ Calculated from the nutrient composition of ingredients (g/kg DM).

sheet of known energy value and their GE values were obtained by difference. Apparent ME intake (MEI) was determined from the GE content of the feed minus faecal and urinary energy (EE). Apparent metabolizability (q) was calculated as the ratio MEI:GE. Total N and energy retained (RE) were determined by the slaughter technique. RE was partitioned as energy retained as protein (REP; N retention × 6·25) and energy retained as fat (REF; the remainder). The values of 23·8 and 38·7 kJ/g were used for the energy contents of protein and fat respectively.

To adjust for the differences in body weight between birds the results were scaled per kg body weight $(W)^{0.75}$. Measurements of energy balance of broiler chickens given the experimental diets at the four feeding levels considered were used to relate ME intake to RE, both expressed as kJ/kg $W^{0.75}$ per d, using the linear regression:

$$RE/kg\ W^{0.75} = a_1 + b_1 \times MEI/kg\ W^{0.75}\ \text{per}\ d + e_i. \tag{1}$$

This equation predicts ME_m as the intercept on the x axis, and the regression coefficient (b_1) provides an estimate of the efficiency of utilization of ME above maintenance; a_1 is RE when intake is zero; e_i is a random residual with null mean and variance σ^2.

Multiple regression equations (Kielanowski, 1965) were used with MEI as dependent variable and REP and REF as independent variables. The model used was:

$$MEI/kg\ W^{0.75} = a_2 + b_2\ REP/kg\ W^{0.75} + c_2\ REF/kg\ W^{0.75} + e_i, \tag{2}$$

where a_2 is a regression intercept interpreted as the maintenance requirements; b_2 and c_2 represent the energy costs and their reciprocals, $1/b_2\ (k_p)$ and $1/c_2\ (k_f)$, represent estimates of partial efficiencies of ME utilization for protein and fat deposition respectively; and e_i is a random residual.

To try to avoid the problems of co-linearity inherent in the multiple regression procedure, regressions on principal components were also used (Bernier *et al.* 1987).

Statistical analysis

Results were evaluated by a factorial analysis involving the three diets at either all four or the three restricted levels of intake (to equalize feeding). The statistical significance of differences between means were assessed by Tukey's or Bonferroni's *t* test for paired or non-paired groups respectively. The data were also treated using linear and multiple regression analyses when appropriate. Regression on principal components was also used.

RESULTS

Feed intake, growth performance and utilization of N are presented in Table 2. Factorial analysis 3 (diet) × 4 (level of feeding) of the data showed that DM intake and N intake, both expressed per kg $W^{0.75}$ per d, remained unchanged; however, daily body gain and N retained were significantly different ($P < 0.05$) between treatments, increasing as the quality of dietary protein improved (SL < S < SM). Similar trends were observed in the factorial analysis 3 (diet) × 3 (levels of restricted intake) but significant differences ($P < 0.05$) appeared only between diets SL and SM.

Results of energy deposition are shown in Table 3. By previous experience and calculation it was expected that within each diet level (including *ad lib.*) the birds would have similar energy and N intakes but MEI for diet SM was found to be significantly ($P < 0.05$) higher (1318 kJ/kg $W^{0.75}$ per d; Table 3) than that for diets SL and S, which did not differ from each other (1240 and 1249 kJ/kg $W^{0.75}$ per d). This was due to differences in the metabolizability of energy (q), which was significantly ($P < 0.05$) higher for diet SM (0.801) than for diets SL (0.782) and S (0.785). Consequently, in order to avoid the confounding effects of differences in MEI between treatments, the factorial analysis 3 (diet) × 3 (only restricted levels of feeding) was also made. Overall, total RE was significantly higher ($P < 0.05$) for diet SM (424 kJ/kg $W^{0.75}$ per d) than for diets SL and S (390 and 383 kJ/kg $W^{0.75}$ per d respectively). REP tended to increase with the BV of protein (SL < S < SM) although the effect was only significant ($P < 0.05$) for SM. No significant differences in heat production or REF between treatments were found.

Table 4 summarizes the solutions to equation (1). There was a trend for the efficiencies of utilization of ME for production (k_g) to be negatively affected by dietary protein quality (SL < S < SM). In order to estimate the standard deviation of the intercept (ME_m), MEI was also regressed *v.* RE and the range of ME estimates were non-overlapping at 645–715, 564–644 and 483–561 kJ/kg $W^{0.75}$ per d for diets SL, S and SM respectively.

The solutions to equation (2) are shown in Table 5. Our results indicate that k_p values increase and k_f values decrease on increasing the BV of dietary protein. No significant differences between treatments were found for ME_m. Also, multiple regression equations of ME above maintenance (ME_g, calculated as $MEI - ME_m$) *v.* REP and REF were made. The results obtained (k_p: 0.402, 0.438 and 0.538; k_f: 1.28, 0.885 and 0.618, for diets SL, S and SM respectively) were similar to those presented in Table 5.

Regression *v.* principal components, a biased regression technique, was also applied to the experimental data. Estimates of ME_m were 628, 569 and 519 kJ/kg $W^{0.75}$ per d, for diets SL, S and SM respectively. Partial efficiencies of protein (k_p) and fat (k_f) deposition were 0.500, 0.513 and 0.575, and 0.971, 0.813 and 0.649, for diets SL, S and SM respectively.

As an alternative procedure, the energy costs of growth were recalculated assuming a constant theoretical value for k_f (0.8). Accordingly, in each case the MEI ascribed to fat deposition (REF/k_f) was subtracted from the total MEI and the residual MEI (for maintenance and protein deposition, ME_{m+p}) was regressed *v.* REP. The estimates of k_p obtained were 0.366, 0.383 and 0.426 for diets SL, S and SM respectively. There were no

Table 2. *Feed intake, daily weight gain and nitrogen balance in broilers given diets of different protein quality**

(Mean values for the factor diet of the factorial analysis 3×4 (or 3×3 in parentheses))

	Diet			
	SL	S	SM	Pooled SE
DM intake (g/kg $W^{0.75}$ per d)	85.7[a]	86.1[a]	88.8[a]	1.01
	(82.2[a])	(81.7[a])	(81.5[a])	(0.54)
Body-wt gain (g/d)	15.6[a]	18.7[b]	21.6[c]	0.46
	(13.8[a])	(16.3[b])	(16.7[b])	(0.201)
NI (g/kg $W^{0.75}$ per d)	2.79[a]	2.81[a]	2.89[a]	0.076
	(2.70[a])	(2.70[a])	(2.71[a])	(0.018)
NR (g/kg $W^{0.75}$ per d)	1.20[a]	1.32[b]	1.60[c]	0.027
	(1.12[a])	(1.19[a])	(1.40[b])	(0.026)
NR/NI (%)	42.3[a]	45.9[b]	54.0[c]	0.86
	(40.6[a])	(43.5[a])	(50.6[b])	(1.01)

NI, nitrogen intake; NR, nitrogen retention.

[a,b,c] Mean values within a row bearing unlike superscript letters were significantly different, $P < 0.05$.

* For details of procedures, see Table 1 and pp. 164–166.

Table 3. *Energy deposition in broilers given diets of different protein quality**

(Mean values, expressed as kJ/kg $W^{0.75}$ per d, for the factor diet of the factorial analyses 3×4 and 3×3)

Diet ...	All 4 intake levels				3 restricted intake levels			
	SL	S	SM	Pooled SE	SL	S	SM	Pooled SE
GEI	1581[a]	1582[a]	1639[a]	18.7	1516[a]	1497[a]	1498[a]	10.1
EE	341[a]	333[a]	321[a]	7.4	332[a]	319[ab]	299[b]	7.3
MEI	1240[a]	1249[a]	1318[b]	14.8	1184[a]	1178[a]	1199[a]	9.8
q	0.782[a]	0.785[a]	0.801[b]	0.036	0.778[a]	0.782[ab]	0.797[b]	0.0458
RE	425[a]	433[a]	489[b]	12.6	389[a]	383[a]	424[b]	8.7
HP	814[a]	815[a]	829[a]	9.3	795[a]	795[a]	774[a]	9.5
REP	180[a]	199[b]	241[c]	5.7	167[a]	178[a]	208[b]	3.8
REF	248[a]	234[a]	249[a]	11.3	224[a]	205[a]	216[a]	8.7

GEI, gross energy intake; EE, faecal and urinary energy; MEI, metabolizable energy intake; q, metabolizability of energy; RE, energy retained; HP, heat production (MEI−RE); REP, energy retained as protein (N × 6.25 × 23.8); REF, energy retained as fat (RE−REP).

[a,b,c] Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 164–166.

significant differences between treatments, but k_p tended to increase with the BV of protein (SL < S < SM).

DISCUSSION

There is evidence suggesting that a severe amino acid imbalance has a primary effect on feed intake which, in turn, can affect growth rate and carcass composition (Solberg *et al.* 1971; Tasaki *et al.* 1976; Okumura & Mori, 1979; Summers & Leeson, 1985). Amino acid

Table 4. *The relationship between metabolizable energy intake (MEI, kJ/kg $W^{0.75}$ per d) and energy retained (RE, kJ/kg $W^{0.75}$ per d) in broilers fed on diets of different protein quality**

Diet	Linear regression equations	r	RSD	n
SL	RE = −395 + 0·660 MEI (SE 48) (SE 0·0375)	0·936	50·7	46
S	RE = −314 + 0·600 MEI (SE 44) (SE 0·0340)	0·944	52·9	40
SM	RE = −266 + 0·572 MEI (SE 36) (SE 0·0267)	0·966	44·6	35

RSD, residual standard deviation
* For details of diets, see Table 1.

Table 5. *Multiple regression equations of metabolizable energy intake (MEI) v. energy retained as protein (REP) and fat (REF) in broilers fed on diets of different protein quality**

(All values are expressed as kJ/kg $W^{0.75}$ per d)

Diet	Multiple regression equations	k_p†	k_f†	R^2	RSD	n
SL	MEI = 603 + 2·47 REP + 0·79 REF (SE 35) (SE 0·289) (SE 0·149)	0·405	1·27	0·908	61·2	46
S	MEI = 559 + 2·14 REP + 1·11 REF (SE 39) (SE 0·229) (SE 0·144)	0·467	0·901	0·909	75·4	40
SM	MEI = 519 + 1·73 REP + 1·55 REF (SE 40) (SE 0·199) (SE 0·175)	0·578	0·645	0·929	76·1	35

RSD, residual standard deviation.
* For details of diets, see Table 1.
† Efficiency of energy utilization for protein (k_p) and fat (k_f) deposition.

excesses can also result in impaired growth performance (Snetsinger & Scott, 1961; Griminger & Fisher, 1968; Katz & Baker, 1975; Han & Baker, 1993). It is clear, therefore, that studies on amino acid supplementation require careful matching of intakes so that the effects of protein quality and quantity are not confounded.

In our experiment the control diet (S) was moderately deficient in methionine, and provided an adequate concentration of lysine. The supplementation with sufficient DL-methionine (2 g/kg diet SM) or with an excess of L-lysine (20 g/kg diet SL) resulted in significant ($P < 0.05$) improvements or decreases respectively, in body gain, N retention and REP, while DM intake and N intake remained unchanged.

In nutritional studies the net efficiency of dietary ME utilization for growth (k_g) is closely related to the composition of body gain, i.e. to the ratio protein:fat retained. The deposition of fat is a more efficient process than that of protein, e.g. theoretical efficiencies with which the carbohydrates, lipids and proteins are employed in synthesis of body fat are 0·80, 0·96 and 0·66 respectively, and experimental values close to these have been observed. However, experimental values for protein deposition are usually much lower than theoretical due to the high cost of protein turnover (Blaxter, 1989). Indeed, published findings show that k_g decreases as protein:fat increases (Zausch et al. 1961; Zausch, 1969),

an observation repeated in the current study (see also Table 6). The lower value found for diet SM may be related to the extra costs associated with protein deposition.

It is now well established (McCracken *et al.* 1980; Close *et al.* 1983) that at similar rates of energy intake protein accretion is higher and fat deposition lower as dietary protein concentration is increased to an optimum and also that at similar rates of protein intake, the lower the protein: energy relationship the greater the protein accretion (Close *et al.* 1983). Under such circumstances the greater the protein accretion the less total energy is retained, due again to the energy penalty associated with elevated protein turnover in response to changes in total protein supply.

With differences in protein quality rather than quantity the situation is less clear. In growing pigs offered a diet supplemented with lysine, the first limiting amino acid, Fuller *et al.* (1987*a–c*) found that the rate of heat production was not significantly changed, whereas the rate of protein accretion increased. The rate of fat deposition was inversely related to that of protein accretion. In these experiments the feed intake was not strictly controlled but improved daily gain and N retention ($P < 0.05$) were accompanied by increased total energy retention ($P < 0.05$). Meanwhile supplementation with extra protein increased N retention but increased heat production. These changes in protein quality or quantity involved different metabolic responses. It was also found that increases in the rate of protein accretion resulting from lysine supplementation were brought about primarily by a reduction in body protein breakdown. This is believed to involve less energy than equivalent changes in protein synthesis (Lobley, 1988). Maruyama *et al.* (1978) also showed that in the chick an improvement in dietary protein quality had no effect on the rate of muscle protein synthesis despite increased gain, suggesting that the rate of protein degradation was reduced. Similar observations have been made for muscle from chickens receiving a similar improved diet quality to the current study (Nieto *et al.* 1994).

An alternative energy saving might involve less uric acid synthesis. In birds the excretion of excess amino acid N which is not used for protein synthesis takes place mainly as uric acid. The energy cost of this process is difficult to assess but the value of 1·4 MJ ME/mol uric acid synthesized (Buttery & Boorman, 1976) is usually preferred. Solberg *et al.* (1971) showed that in the chick a diet marginally deficient in methionine resulted in increased uric acid production. A similar observation was made by Thomas *et al.* (1969) for the laying hen given a lysine-deficient diet. Our results support this as the mean rates of N excretion were 1·59, 1·49 and 1·29 g/kg $W^{0.75}$ per d, for diets SL, S and SM respectively. The calculated extra costs would only account for $+0.4$ and -0.4 kJ ME for diets SL and SM respectively, insufficient to account for the energy differences.

If, therefore, it is assumed that the increased protein accretion is achieved through a reduced protein degradation and that this does not incur extra heat production, then the question is raised whether the resultant improved energetic efficiency occurs in the 'maintenance' and/or 'growth' component. This is difficult to resolve because the regression approaches involved have strong co-linearity, i.e. values for ME_m and k_g (or the partial efficiencies k_p and k_r) are not independent and small changes in one component have an equal and opposite effect on the other. This means that such analyses must be treated with caution. Nevertheless, the current results indicate that improving diet quality may reduce both ME_m and k_g. This again would support the hypothesis of a reduced protein degradation which should have consequences above and below N equilibrium (protein balance).

In summary, the hypothesis of the present study was that a change in dietary protein quality, attained by adding either a limiting amino acid (DL-methionine) or a surplus of a non-limiting amino acid (L-lysine) to an otherwise adequate diet, would affect either the energy requirement for maintenance or the partition of ME for protein accretion or fat

Table 6. *Estimates of energy requirements for maintenance (ME_m) and the efficiency of utilization of the metabolizable energy (ME) by chickens*

Type and sex	Age (weeks)	Weight (kg)	Type of diet	ME_m (kJ/kg $W^{0.75}$ per d)	Efficiency of ME utilization*			Reference
					k_g	k_p	k_f	
Broiler ♂	1–3	0·08–0·30	purified	356	57·6	—	—	Nijkamp et al. (1973)
Vedette INRA ♂	1–3	—	semi-purified	692–784	56·6–70·2	49	82	Guillaume (1976)
Broiler ♂	1–3	0·17	semi-purified	462	51·6–55·1	—	—	Sibbald & Wolynetz (1986)
Vedette INRA ♂	1–4	—	semi-purified	480–660	58–77	—	—	Guillaume et al. (1980)
Hubbard ♂	2–4	0·25–0·70	cereal +soyabean	540	58·7–61·1	—	—	De Groote (1968)
White Plymouth Rock ♂	3–4	0·36	cereal + fat	306	—	51	78	Petersen (1970)
Leghorn ♀	3–4	0·12–0·72	semi-purified	577–639	—	—	—	Johnson & Crownover (1976)
Broiler ♂	4–5	0·76–1·06	commercial	684	—	—	—	Aguilera & Prieto (1987b)
Broiler ♀	4–5	0·56–0·81	semi-purified	653–754	74–78	47–57	102–103	MacLeod (1990)
Broiler ♂	2–4	0·15–0·55	semi-purified	466–597	57·2–66·0	40·5–57·8	64·5–127·0	Present paper

* Efficiency of ME for growth (k_g), protein deposition (k_p) and fat deposition (k_f).

deposition. This hypothesis was substantiated by the present findings, as improvement in dietary protein quality affected not only the efficiency of ME utilization for growth (k_g) but also the ME_m requirements and the partition of the same amount of retained energy between protein and fat.

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