

Effect of dietary protein quality, feed restriction and short-term fasting on protein synthesis and turnover in tissues of the growing chicken

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The effect of dietary protein quality and quantity on fractional rates of protein synthesis (k_s) and degradation (k_d) in the skeletal muscle, liver, jejunum and skin of young growing chickens was studied. Chickens were either fasted overnight or were fed at frequent intervals, using continuous feeders, with equal amounts of a diet containing soya-bean meal as the sole protein source, unsupplemented, or supplemented with either lysine or methionine. Each of the three diets was provided at 2 or 0.9 × maintenance. On the higher intake, birds on the unsupplemented diet gained weight, lysine supplementation decreased and methionine supplementation increased body-weight gain (by –23% and +22% respectively). Birds fed at 0.9 × maintenance lost weight; supplementation with methionine or lysine did not influence this weight loss. None of the dietary regimens had significant effects on protein synthesis rates in any of the tissues, thus the mechanism whereby muscle mass increased in response to methionine supplementation appeared to be a decrease in the calculated rate of protein degradation. Similarly, on the 0.9 × maintenance diet the failure of the animals to grow appeared to be due to an increase in the rate of protein degradation rather than an effect on synthesis. Conversely, muscle k_s was decreased in fasted chickens previously fed on the unsupplemented diet at 2 × maintenance, and in birds which had received the 0.9 × maintenance diet fasting resulted in a similar reduction in protein synthesis in muscle; k_d in the liver and jejunum was also significantly decreased. The effect of fasting, unlike the effect of supplementation or restriction of the diet, appeared to be due to changes in the rate of protein synthesis.

Protein quality: Protein turnover: Feed restriction: chickens

Protein turnover rates are affected by many factors relating to the dietary and hormonal status of the animal (for recent review see Reeds & Davis, 1992). Millward *et al.* (1976) were the first to propose that, in the rat, whether a muscle grows or atrophies in response to short-term fasting or a change in hormonal status is dependent primarily on the rate of muscle protein synthesis and that the rate of protein degradation generally changes to a lesser extent, if at all; however, more severe treatments such as prolonged starvation and untreated diabetes elicit an 'emergency response' manifested by the loss of muscle ribosomes, a further fall in synthesis and a massive rise in muscle protein degradation (Millward *et al.* 1976).

In contrast, in young chickens an improvement in dietary quality had no effect on the rate of muscle protein synthesis (Maruyama *et al.* 1978), suggesting that the rate of protein degradation may be more sensitive to dietary manipulation than the rate of protein

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synthesis and contradicting the idea that protein degradation is relatively insensitive to any but the most severe treatments.

The present study was carried out to determine the extent to which the rates of protein synthesis and degradation in skeletal muscle, liver and jejunum of young chicks are affected by changes in dietary protein quality, changes in protein and energy intakes and short-term starvation.

MATERIALS AND METHODS

L-[2,6-³H]Phenylalanine was purchased from Du Pont de Nemours (NEN division, Dreiech, Germany). All materials used in the β -phenylethylamine assay were bought from Sigma Chemical Co. (Poole, Dorset).

Animals, diets and experimental procedure

Expt 1. Fast-growing White Rock male broilers (1-d-old) from a local hatchery (COBB Ltd., Alcalá de Henares, Madrid, Spain) were reared in conventional electrically heated starting batteries in a temperature-controlled room (25°) lighted 24 h/d. They were fed on a commercial starter diet *ad lib.* for 9 d. After 9 d, chickens were divided into three groups of fourteen birds of equal mean weight, 137.2 (SE 0.9) g and kept individually in metabolism cages. Three isoenergetic (13.1 MJ metabolizable energy (ME)/kg dry matter (DM)) and isonitrogenous (approximately 200 g crude protein/kg DM) semi-purified diets were fed at about 2 \times maintenance (M) level (1368 kJ ME/kg^{0.75}; Aguilera & Prieto, 1987) by continuous feeders for 9 d (a 3 d period of adaptation to the diets and an experimental period of 6 d). The use of continuous feeders was designed to produce a nutritional and metabolic steady-state. The diets (Table 1) contained soya-bean meal as the only protein source and were either unsupplemented (diet S) or supplemented with 20 g lysine (diet SL) or with 2 g methionine (diet SM)/kg diet; water was provided *ad lib.* Feed consumption and body weights were recorded daily. To eliminate the effect of differences in feed intake the ration for each animal was scaled to a component of body weight, on average, 102 and 40 g feed/kg body weight^{0.75} per day. At the beginning and end of the experimental period, i.e. on days 3 and 9, three chickens from each group were killed by decapitation, exsanguinated and then the biceps muscles, liver and jejunum were quickly dissected out, weighed, frozen in liquid N₂ and stored at -20° until analysed. Total carcass protein was also analysed by a Kjeldahl method and calculated as N \times 6.25.

On days 5 and 7, four chickens from each diet group were injected intraperitoneally with a single large dose of phenylalanine (150 mM; 10 ml/kg body weight) containing 1.85 MBq of L-[2-6-³H] phenylalanine/ml (Garlick *et al.* 1980). All animals were injected between 09.00 and 13.00 hours to minimize diurnal variation, and precisely 15 min after the injection the animals were killed, exsanguinated and the biceps muscle, the whole liver and a 50 mm section of jejunum (taken 200 mm below the gizzard/duodenal junction) were rapidly removed, weighed and frozen in liquid N₂. The specific radioactivity of the free phenylalanine in the homogenate pool and the protein-bound phenylalanine was determined after conversion to β -phenylethylamine (Garlick *et al.* 1980). This was measured fluorimetrically (Suzuki & Yagi, 1976) after extraction into 0.01 M-H₂SO₄, on a spectrophotofluorimeter (Shimadzu, Japan). Radioactivity in the same H₂SO₄ extract was measured on a 1500 Tri-Carb Scintillation Analyzer (Canberra Packard International S.A., Zurich, Switzerland) using Aquasol-2 scintillation fluid (Du Pont Biotechnology Systems NEN Research Products, Boston, MA, USA). The RNA content of the liver was determined using the orcinol method of Lin & Schjeide (1969) using purified yeast RNA (Sigma) as a standard. RNA in the other tissues was determined by the method of Munro & Fleck (1969) as modified by Ashford & Pain (1986). Protein content was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

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

	Diet		
	SL	S	SM
Ingredients			
Soya-bean meal	355.1	399.5	399.5
Maize oil	55.1	55.1	55.1
Mineral and vitamin premix	111.3	111.3	111.3
Lysine	20.0	—	—
Methionine	—	—	2.0
Maize starch	458.6	434.1	432.1
Chemical composition			
Dry matter (DM, g/kg)	930.3	924.4	929.9
Crude protein (N × 6.25, g/kg DM)	208.3	203.5	202.8
Gross energy (kJ/g DM)	18.42	18.41	18.45

Expt 2. In a second experiment of similar design, thirty-six broilers were fed on the same three semi-purified diets (S, SL and SM) but at $0.9 \times M$. Feed intake was, on average, 40 g feed/kg body weight^{0.75} per day. Four birds from each treatment were killed at the beginning and end of the 6 d experimental period (again following an acclimatization period of 3 d) for determination of the growth rate over this period. Protein synthesis was measured once only, on day 6, in the same three tissues as in Expt 1, and also in skin.

In both experiments a fourth group of four birds was allocated to diet S for 5 d and then fasted for 24 h before measurement of protein synthesis rates as described above.

Calculations and statistics

Rates of protein synthesis were calculated from the specific radioactivity (disintegrations/min per nmol) of the protein-bound phenylalanine of the tissues (S_b) and the free phenylalanine in the corresponding homogenate pool (S_a) using the formula $k_s = S_b/S_a \times 100/t$ where t is the incorporation time in days (Garlick *et al.* 1980). Fractional rates of degradation (k_d) in muscle and liver were calculated from the fractional rate of protein synthesis in those tissues and the fractional rate of growth of the whole animal over the 9 d experimental period. In Expt 2 there was a small net loss of body weight; in tissues from these birds, k_d was calculated as the sum of the fractional rates of protein synthesis and protein loss. The protein synthetic efficiency of RNA (g protein/g RNA per d), was calculated as $k_s/100 \times \text{g protein/g RNA}$ (Millward *et al.* 1975).

The data were subjected to a two-way analysis of variance based on a factorial design of treatments to assess the significance of main and interactive effects of diets (fasting, S, SL, SM) and level of feeding ($2.0 \times M$, $0.9 \times M$) by a general linear model (GLM) with a statistical package, SAS (Statistical Analysis Systems Institute Inc., 1985). Data exclusive to skin ($0.9 \times M$) were compared by one-way analysis of variance. Significant differences between means for each treatment were assessed using the Student Newman-Keuls' method at the 0.05 level (Anderson & McLean, 1974).

RESULTS

All birds fed at $2 \times M$ gained weight substantially but those on the lysine-supplemented diet grew less well (-23%) while those on the methionine-supplemented diet gained more weight ($+22\%$) than the birds on diet S (Table 2). The weight changes were associated with corresponding alterations in protein retention (Table 2). Growth rates were negative and

Table 2. *Body-weight gain, feed intake and protein retention rate in growing chickens fed on diets of different protein quality at 2.0 or 0.9 × maintenance (M)**

(Mean values and pooled standard errors of three observations for birds fed at 2 × M, and four observations for birds fed at 0.9 × M)

Diet	Body-wt gain (g/d)	Feed intake (g/d)	Protein retention rate (%)†
2.0 × M			
SL	11.9 ^a	28.2 ^a	49.9 ^a
S	15.5 ^b	29.4 ^a	56.0 ^b
SM	18.9 ^c	30.5 ^a	69.8 ^c
Pooled se	0.69	0.95	1.02
0.9 × M			
SL	-1.9 ^d	8.0 ^b	—
S	-2.7 ^d	8.0 ^b	—
SM	-2.1 ^d	8.0 ^b	—
Pooled se	0.31		

Diet S, soya-bean meal; diet SL, soya-bean meal supplemented with lysine; diet SM, soya-bean meal supplemented with methionine.

^{a, b, c, d} Mean values within a column bearing different superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 500–501.

† Protein retained × 100/protein intake.

Table 3. *Fractional rates of protein synthesis (k_s) and degradation (k_d) in tissues from growing chickens after 24 h fasting or after feeding soya-bean diets either unsupplemented (S) or supplemented with lysine (SL) or methionine (SM) at 2.0 or 0.9 × maintenance (M)**

(Mean values and pooled standard errors for four observations per dietary group for birds fed at 0.9 × M, and eight observations per dietary group for birds fed at 2.0 × M)

Tissue and feeding level	Treatment				
	Fasted	SL	S	SM	Pooled se
k_s (% per d)					
Muscle					
2.0 × M	9.7 ^{ax}	24.8 ^{bx}	27.5 ^{bx}	22.4 ^{bx}	2.38
0.9 × M	9.1 ^{ax}	19.3 ^{bx}	22.2 ^{bx}	24.1 ^{bx}	2.38
Liver					
2.0 × M	134.9 ^{ax}	96.0 ^{ax}	110.4 ^{ax}	100.3 ^{ax}	13.76
0.9 × M	51.4 ^{ay}	98.0 ^{bx}	117.2 ^{bx}	109.7 ^{bx}	13.76
Jejunum					
2.0 × M	71.7 ^{ax}	92.3 ^{ax}	76.9 ^{ax}	73.2 ^{ax}	7.07
0.9 × M	45.6 ^{ay}	76.4 ^{bx}	73.5 ^{bx}	68.2 ^{bx}	7.07
Skin					
0.9 × M	28.3 ^a	32.9 ^a	43.4 ^a	45.4 ^a	3.81
k_d (% per d)					
Muscle					
2.0 × M	—	16.5	16.2	8.7	
0.9 × M	—	20.7	24.2	25.7	
Liver					
2.0 × M	—	87.8	94.1	87.3	
0.9 × M	—	99.4	119.0	111.3	

All comparisons are within the same tissue. ^{a, b, c, d} Mean values within each level of feeding bearing different superscript letters were significantly different ($P < 0.05$); ^{x, y} Mean values between each level of feeding and within the same treatment column bearing different superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 500–501.

Table 4. *The RNA:protein ratio and the activity of RNA (g protein synthesised/g RNA per d; k_{RNA}) in tissues from growing chickens after 24 h fasting or after feeding on soya-bean diets either unsupplemented (S) or supplemented with lysine (SL) or methionine (SM) at 2.0 or 0.9 × maintenance (M)**

(Mean values and pooled standard errors for four observations per dietary group for birds fed at 0.9 × M, and eight observations per dietary group for birds fed at 2.0 × M)

Tissue and feeding level	Treatment				Pooled SE
	Fasted	SL	S	SM	
RNA:protein ($\mu\text{g}/\text{mg}$)					
Muscle					
2.0 × M	7.7 ^{ax}	13.4 ^{bx}	13.4 ^{bx}	14.8 ^{bx}	2.09
0.9 × M	17.3 ^{ay}	16.3 ^{ax}	21.7 ^{ax}	17.7 ^{ax}	2.09
Liver					
2.0 × M	81.1 ^{ax}	97.8 ^{ax}	84.0 ^{ax}	93.3 ^{ax}	5.09
0.9 × M	69.3 ^{ax}	73.8 ^{ay}	73.9 ^{ax}	70.3 ^{ay}	5.09
Jejunum					
2.0 × M	39.6 ^{ax}	49.6 ^{ax}	40.5 ^{ax}	47.1 ^{ax}	5.15
0.9 × M	45.7 ^{ax}	47.9 ^{ax}	55.7 ^{ax}	52.4 ^{ax}	5.15
Skin					
0.9 × M	15.9 ^a	14.1 ^a	16.1 ^a	15.6 ^a	1.76
RNA activity (g protein synthesis/g RNA per d)					
Muscle					
2.0 × M	10.6 ^{ax}	16.2 ^{abx}	19.5 ^{bx}	15.0 ^{abx}	2.23
0.9 × M	5.3 ^{ax}	11.8 ^{bx}	11.1 ^{bx}	13.8 ^{bx}	2.23
Liver					
2.0 × M	16.6 ^{ax}	10.1 ^{bx}	12.9 ^{abx}	11.2 ^{abx}	1.67
0.9 × M	7.3 ^{ay}	12.5 ^{bx}	15.2 ^{bx}	15.9 ^{bx}	1.67
Jejunum					
2.0 × M	18.2 ^{ax}	19.6 ^{ax}	19.3 ^{ax}	16.0 ^{ax}	2.06
0.9 × M	10.3 ^{ax}	16.2 ^{bx}	12.8 ^{abx}	13.3 ^{abx}	2.06
Skin					
0.9 × M	17.5 ^a	25.9 ^{ab}	27.5 ^{ab}	30.2 ^b	2.81

All comparisons are within the same tissue. ^{a, b, c, d} Mean values within each level of feeding bearing different superscript letters were significantly different ($P < 0.05$); ^{x, y} Mean values between each level of feeding and within the same treatment column bearing different superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Table 1 and pp. 500–501.

similar on the three diets for chicks fed at 0.9 × M. At each feeding level there was no significant difference in food intake between the three diets.

There were no significant differences in protein synthesis rates on days 5 and 7 in the first experiment, and therefore these data were pooled (Table 3). Fractional rates of protein synthesis (k_s) were unchanged in response to supplementation with methionine or lysine or to the reduction in feed intake from 2 × M to 0.9 × M. Ribosomal activity, i.e. g protein synthesised/g RNA per d (k_{RNA}), followed the same trend as k_s , i.e. there were no significant effects in response to supplementation or to restricted intake (Table 4).

No statistical difference was observed in the RNA:protein ratio between diets S, SL and SM at either intake (Table 4). However, when the two intakes were compared, the RNA:protein tended to be lower in the liver of birds fed at 0.9 × M; this effect attained significance only on diets SL and SM.

Effects of fasting appeared to be dependent on the previous dietary intake. Thus birds that were fasted following a period of restricted intake had significantly reduced rates of

protein synthesis in all tissues except skin when compared with birds fed on the same intake and not subsequently fasted. In birds which had been fed previously at $2 \times M$ a suppression in k_s in response to fasting was seen only in muscle (Table 3). Therefore when the two groups of fasted birds were compared, previous dietary intake appeared to have no effect on the rate of muscle protein synthesis (because muscle k_s was reduced by proportionately similar amounts in both fasted groups). However k_s of the liver and jejunum were significantly lower (by 64 and 38% respectively) when fasting followed restriction to $0.9 \times M$ than in the birds fed on a $2 \times M$ diet before fasting (Table 3). The k_{RNA} values showed a similar trend, being decreased by 52% in both muscle and liver and by 20% in the jejunum in birds fed at $0.9 \times M$ before the 24 h fast; but again, the reduced ribosomal activity in response to fasting of the $2 \times M$ fed birds was seen only in muscle. There was also a decrease in the RNA:protein ratio in muscle in response to fasting (Table 4); this effect however was seen only in the fasted birds previously fed at $2 \times M$.

Thus with the exception of a 24 h fast, changes in growth rate appeared to be achieved with little effect on rates of protein synthesis and the effect of amino acid supplementation on growth in the first experiment was apparently due mainly to a change in the fractional degradation rate (k_d) especially in muscle where a halving in the rate of degradation occurred on diet SM (Table 3). The cessation of growth when intake was reduced in Expt 2 (Table 2) was also accompanied by changes in the calculated k_d in muscle and liver (Table 3).

DISCUSSION

We have studied the effects of dietary changes and short-term starvation on protein synthesis and degradation in chickens. The dietary changes involved supplementation of a soya-bean-based diet with a limiting amino acid (methionine) or with an excess of a non-limiting amino acid (lysine) and the feeding of these three diets at two levels of intake.

Dietary-restricted birds were given protein and energy intakes of 1.5 g/d and 98 kJ ME/d respectively, therefore, according to the recommended intakes for growing chickens of similar age (3.0 g protein/d, 163 kJ ME/d; Scott *et al.* 1982), they were both protein and energy deficient and, as anticipated, showed slightly negative growth rates. Under these circumstances, supplementation with lysine or methionine had no effect on the growth rate whereas both the growth rate and feed efficiency increased in response to methionine supplementation and decreased in response to additional lysine in chickens fed at $2 \times M$.

Amino acid supplementation did not change the RNA:protein ratio in muscle, nor was there any significant effect of energy and protein restriction to $0.9 \times M$. These data are in agreement with the results of Maruyama *et al.* (1978) who found that protein and energy restriction did not alter the protein:RNA ratios in leg or breast muscle of 2-week-old White Leghorn chickens.

Protein synthesis

As might be expected, the rates of muscle protein synthesis observed here are generally higher than those reported for slower growing strains of chickens, e.g. 18–21% per d in 2-week-old White Leghorn chicks (Mauryama *et al.* 1978). Values of 9–11% per d in 43-d-old broilers (Bryan *et al.* 1983) are lower than the values quoted here, presumably due to the developmental fall in k_s that occurs with age. Fewer data are available on rates of protein synthesis in skin but the order of tissue k_s , liver > gut > jejunum > skin > muscle, is similar to that observed in previous studies with chicks (Pinchasov *et al.* 1988). In the present study the different dietary treatments gave skin:muscle k_s ratios ranging from 1.7–3.1. Very similar values have been found by others, e.g. a ratio of 2 in growing pigs (Sève *et al.* 1986) and 2–3 in neonate (Attaix *et al.* 1988) and growing (Lobley *et al.* 1992) lambs.

Effects of fasting

In common with mammalian studies (McNurlan *et al.* 1980) fractional rates of protein synthesis in the chicks were sensitive to feed deprivation. A 24 h fast reduced muscle k_s by 59–65% in birds that had been fed at $2 \times M$. Those that had been restricted to $0.9 \times M$ showed greater responses to the same duration of fasting; protein synthesis rates were decreased not only in muscle, but also in liver and jejunum (by about 59, 56 and 38% of the soya-bean-fed group respectively). These results suggest that splanchnic tissues from well-fed birds seem able to withstand a short period of starvation better than the same tissues of animals given an inadequate diet. As a major source of amino acids which can be mobilized rapidly to counteract nutritional deprivation (Garlick *et al.* 1988), it is perhaps to be expected that skeletal muscle responds rapidly to starvation regardless of previous dietary history.

Protein degradation

The calculation of k_d as the difference between the rates of protein synthesis and protein accretion or growth (Millward *et al.* 1975, 1976) is open to criticism (Garlick & Millward, 1972; Waterlow *et al.* 1978) on the grounds that the rate of protein synthesis measured over a 15 min period may not represent the mean rate over a 24 h period, whilst growth measured over a longer period may not be linear or proportional in all the tissues. Therefore, although the continuous feeding regimen adopted here should minimize diurnal variation, which in young rats has in any case been shown to be small (Reeds *et al.* 1986), the degradation rate cannot be considered as reliable a measure as the rates of growth and synthesis. Nevertheless, changes in the degradation rate may be an important determinant of growth, and in the response to changes in the quality and quantity of intake. Klasing & Calvert (1987) compared broiler and layer chicks and found that the increased rate of protein accretion in broilers was mainly due to a decreased rate of protein degradation. Similarly, in growing chicks fed on purified diets, Maruyama *et al.* (1978) found little change in the rate of protein synthesis in skeletal muscles, whereas the rate of degradation tended to be higher in response to deficient diets, whilst Muramatsu *et al.* (1985), using chicks subjected to protein starvation, showed that the fractional protein synthesis rate in breast muscle was reduced while the fractional degradation rate was increased. Interestingly, the increase in degradation was fully reversed and the decrease in synthesis was partially reversed by supplementation with only two amino acids, methionine and arginine. Our own observations confirm the importance of degradation rates in the control of growth since the birds which failed to grow on a $0.9 \times M$ diet had consistently higher degradation rates in both muscle and liver than the birds fed at $2 \times M$. In liver the difference in the rate of degradation between 0.9 and $2 \times M$ ranged from 13 to 27%. In muscle too, the lysine-supplemented and unsupplemented diets fed at $0.9 \times M$ resulted in degradation rates 25 and 49% higher respectively than on the corresponding diets fed at $2 \times M$, whilst the methionine-supplemented diet gave a degradation rate at $2 \times M$ that was only one third of that seen with the same diet at $0.9 \times M$. Thus, since feeding level and amino acid supplementation had no influence upon k_s , the increased growth rates obtained on methionine supplementation, the reduced growth induced with lysine and the cessation of growth on reduced intake all appeared to be due to changes in k_d .

There are circumstances in which the rat and the chicken appear to behave similarly, notably in the response of protein synthesis to overnight fasting. Similarly, reduced rates of protein synthesis were seen in the liver and jejunal mucosa of chickens fed on a protein-free diet for 9 d by Muramatsu *et al.* (1983), who noted that these changes were very similar to those seen in the liver and jejunal mucosa of rats fed on a protein-free diet for 8 d (McNurlan & Garlick, 1981). Thus chickens appear to behave similarly to rats in that

dietary protein is crucial for the maintenance of protein synthesis (McNurlan & Garlick, 1981). In other respects, however, the responses of chickens and rats may differ. Many of the definitive studies on the effect of diet have been performed in young, growing rats where effects of insulin (Garlick *et al.* 1983), glucocorticoids (Southorn *et al.* 1990) and fasting and refeeding (McNurlan *et al.* 1987) are principally on muscle protein synthesis. Similarly, restricted intake (Millward *et al.* 1976) and reduced dietary protein content (Jepson *et al.* 1988) resulted in a decrease in protein synthesis rates in rat muscle. The observations reported here, and in other experiments with young, growing chickens, that mild treatments such as the provision of a reduced intake or an amino acid imbalance may affect growth by changing protein degradation rates, suggest a difference between avian and mammalian species and contrast with the long-held belief (Millward *et al.* 1976) that mammalian muscle is a tissue which atrophies or grows principally as a result of changes in the rate of synthesis while protein degradation is unaffected or shows proportionately smaller changes.

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