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Contribution of whole-body protein synthesis to basal metabolism in layer and broiler chickens*

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1. The effect of starvation on whole-body protein synthesis and on the contribution of protein synthesis to basal metabolic rate was investigated in young chickens (Expt 1). Strain differences between layer and broiler chickens in whole-body protein synthesis and degradation rates were examined when the birds were starved (Expt 2).

2. In Expt 1, 15-d-old White Leghorn male chickens were used, while in Expt 2 Hubbard (broiler) and White Leghorn (layer) male chickens at 14 d of age were used. They were starved for 4 d, and heat production was determined by carcass analysis after 2 and 4 d of starvation. Whole-body protein synthesis rates were measured on 0, 2 and 4 d of starvation (Expt 1), and on 0 and 4 d of starvation (Expt 2).

3. The results showed that starving reduced whole-body protein synthesis in terms of fractional synthesis rate and the amount synthesized. Whole-body protein degradation was increased by starvation both in terms of fractional synthesis rate and the amount degraded on a per kg body-weight basis.

4. Reduced fractional synthesis rate of protein in the whole body was accounted for by reductions in both protein synthesis per unit RNA and RNA:protein ratio.

5. In the fed state, whole-body protein synthesis and degradation rates, whether expressed as fractional rates or amounts per unit body-weight, tended to be higher in layer than in broiler chickens. In the starved state, the difference in the rate of protein synthesis between the two strains virtually disappeared, while the degradation rates were higher in layer than in broiler birds.

6. Based on the assumed value of 3·56 kJ/g protein synthesized (Waterlow *et al.* 1978), the heat associated with whole-body protein synthesis in the starved state was calculated to range from 14 to 17% of the basal metabolic rate with no strain difference between layer and broiler chickens.

The efficiency of metabolizable energy utilization in an animal is determined by the amount of heat production. It is important, therefore, to know the factors comprising heat production such as dietary-induced thermogenesis, in order to attain improved productivity in animal agriculture. To understand the significance of these factors, considerable efforts have been devoted during the last decade to evaluating the contribution of various metabolic reactions to the total heat production of the animal.

Classically, heat production may be subdivided into two major components: one is concerned with maintenance, and the other is involved in production, i.e. the heat associated with the retention of protein and fat. According to this classification, starving heat production, frequently referred to as basal metabolic rate, would be a fixed component in the total heat production of an animal. From a functional point of view, however, a different partition may be applicable. The total heat production might be divided into three main components which originate from synthesis and degradation of body components, active transport of nutrients and metabolites through membranes, and physical activities.

Among metabolic processes related to synthesis and degradation of body components, protein synthesis would be one of the expensive steps in terms of energy consumption. Webster (1981) suggested that the energy cost of protein synthesis might account for 20–25% of the total heat production in animals at rest. Indeed, Muramatsu & Okumura (1985) found that in young chickens, 20–28% of the total heat production in animals was accounted for by protein synthesis using an estimate of 3·56 kJ/g protein synthesized

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(Waterlow *et al.* 1978). However, the energy cost of this process could be higher than this (Waterlow *et al.* 1978; Muramatsu & Okumura, 1985). In both fed and starved states the heat directly associated with protein turnover, especially with synthesis, would account for a significant part of the total heat production. This was demonstrated in children (Kien *et al.* 1978). However, in starved chickens, whether the contribution of protein synthesis to the total heat production compared with fed controls is increased or decreased remains unknown. The present study was conducted, therefore, to investigate the effect of starvation on whole-body protein turnover and on the contribution of protein synthesis to the total heat production in young chickens of layer and broiler strains.

MATERIALS AND METHODS

Expt 1

The experiment was done to investigate the effect of starvation on whole-body protein synthesis and on the contribution of the heat generated by protein synthesis to the total starving heat production. White Leghorn male chickens (1-d-old) were used. They were fed on a commercial chick mash diet (crude protein (nitrogen $\times$ 6·25; CP) 190 g/kg, 12·1 kJ ME/g; Nihon Nosan Co. Ltd, Yokohama) *ad lib.* until 15 d of age, and thereafter subjected to a 4 d starvation period. During the starvation period, the birds were reared in groups of ten in brooders and allowed free access to water. Light was provided continuously and ambient temperature was controlled at $30 \pm 2°$. At 0, 2 and 4 d of starvation, ten birds were taken for measurement of whole-body protein synthesis.

Expt 2

Strain differences in response to starvation were examined in Expt 2 using 1-d-old White Leghorn (layer) and Hubbard (broiler) male chickens. Until 14 d of age, these birds were fed on the following diet (g/kg): commercial chick mash 888, isolated soya-bean protein (CP 840 g/kg; Fujipro Co. Ltd, Osaka) 68, DL-methionine 2, maize oil 42. Calculated values for CP and metabolizable energy were 230 g/kg and 13·4 kJ/g respectively. Subsequently, they were starved for 4 d as in Expt 1. During the starvation period the birds were kept individually in metabolism cages, since in Expt 1 coprophagy was suspected when the birds were starved and kept in groups. At 0 and 4 d of starvation, ten birds were taken for the measurement of whole-body protein synthesis. In addition, 2 d before and 2 d after the day of the measurement of whole-body protein synthesis, three birds were taken for the determination of changes in body protein content, from which fractional growth rate of whole-body protein was derived using the regression on time. Fractional degradation rate of whole-body protein was calculated as the difference between the synthesis and growth rates.

Fractional protein synthesis rate in the whole body, which is the protein synthesized daily relative to the total protein mass, was measured by the method of Garlick *et al.* (1980), depending on the measurement of radioactivity in free and protein-bound phenylalanine of the whole carcass after injection of a massive dose of labelled phenylalanine. At 2 and 10 min after the injection, four and six birds respectively were killed by neck dislocation and their abdominal cavities were opened quickly. The whole carcass including feathers was frozen as soon as possible by plunging into liquid N_2 and was stored at $-20°$ until analysis. The frozen carcass was minced with a meat grinder, which was previously cooled with solid carbon dioxide, and was frozen again with liquid N_2. This mincing procedure was repeated three times to get homogenous samples of the whole carcass. The method has been described elsewhere in more detail (Muramatsu & Okumura, 1985; Muramatsu *et al.* 1986). For the measurement of RNA in carcasses, a modified Schmidt–Thannhauser method (Munro &

Fleck, 1969) was used. Carcass crude protein was determined by a Kjeldahl method. Carcass fat was extracted overnight (about 16 h) with diethyl ether using a Soxhlet apparatus, and determined gravimetrically. The starving heat production at 2 and 4 d in Expt 1 and at 4 d in Expt 2 was estimated by calculating the mean heat production between 0–2 d and 2–4 d from the corresponding losses of body protein and fat, multiplying by 18·2 and 39·1 kJ/g loss respectively, followed by an adjustment for body-weight of the bird at 2 and 4 d by assuming that the heat produced per unit body-weight was the same throughout the 2 d measurement period from days 0–2 or days 2–4. In the above calculation it was assumed that all the N in urine originating from the catabolism and oxidation of body protein, having a combustion energy of 23·7 kJ/g, was excreted as uric acid-N, which has a combustion energy of 34·4 kJ/g N. Thus,

$$\text{heat production (kJ/2 d)} = (39{\cdot}1 \times \text{fat loss (g/2 d)} + (23{\cdot}7 - 5{\cdot}5) \times \text{protein loss (g/2 d)}) \times 0{\cdot}9,$$

where the factor of 0·9 was included since the above calculation ignored the energy loss in faeces during starvation. The comparison of starving heat production calculated only from fat and protein losses and by actual measurement of energy contents in carcass and excreta showed that the latter was consistently lower than the former by a factor of 0·897 (T. Muramatsu, S. Nakajima, I. Tasaki and J. Okumura, unpublished results). The amounts of the heat generated from whole-body protein synthesis was calculated by multiplying by 3·56 kJ/g protein synthesized, according to Waterlow *et al.* (1978).

Analysis of variance was carried out to assess the significance of the effect of starvation (Expts 1 and 2), strain (Expt 2) and the interaction of starvation × strain (Expt 2), and the protected least significant difference method was used to test the significance between means (Snedecor & Cochran, 1980). The comparison between strain means at each day of starvation was made only when a significant interaction between starvation × strain was detected.

RESULTS

The values for body-weight and whole-body protein synthesis as fractional rates and absolute amounts in Expt 1 are shown in Table 1. The body-weight of the birds decreased continuously as starvation was prolonged. Similarly, but more remarkably, whole-body protein synthesis was reduced by starvation both in fractional rates and in absolute amounts, the extent being larger for days 0–2 than for days 2–4 of the starvation period.

Table 2 gives the values for cumulative losses of protein and fat, starving heat production and the heat due to whole-body protein synthesis, both the absolute value and relative to the total starving heat production in Expt 1. Losses of protein and fat increased as starvation was prolonged, whereas starving heat production decreased from days 2 to 4 of starvation. The heat due to whole-body protein synthesis decreased significantly as starvation was prolonged, as did its contribution to total starving heat production, from 21 to 17%.

Differences in body-weight and whole-body protein turnover rates in layer and broiler chickens are given in Table 3 (Expt 2). Broiler birds naturally grew faster than the layer birds as shown by the larger body-weight at day 0. The reduction in body-weight for the entire starvation period was 73 g for broiler and 45 g for layer birds. When expressed as a proportion of the day 0 value, layer birds lost body-weight slightly faster than broiler chickens. Since day 0 body-weight was quite different for the two strains, the amounts of protein synthesized or degraded were expressed as g/kg body-weight per d. The fractional synthesis rate and the amount of protein synthesized in the whole-body were reduced by starvation for 4 d in both strains. Although no significant strain difference was found in

Table 1. *Effect of starvation of body-weight and whole-body protein synthesis in layer chickens*

(Mean values for six birds)

Period of starvation (d)	Body-wt (g)	Whole-body protein synthesis	
		%/d	g/d
0	140[a]	37·3[a]	9·4[a]
2	120[b]	19·2[b]†	4·5[b]†
4	106[c]	12·5[c]†	†2·7[c]†
SED	1	1·1	0·3

SED standard error of difference.
[a,b,c] Mean values within a column not sharing a common superscript letter are significantly different: $P < 0·01$.
† One missing value.

Table 2. *Body protein and fat losses, starving heat production and the contribution of whole body protein synthesis to the total starving heat production in chickens subjected to starvation for 4 d*

(Mean values for six birds; the energy cost of 3·56 kJ/g protein synthesized was assumed (Waterlow *et al.* 1978))

Period of starvation (d)	Cumulative losses of:		Starving heat production (A) (kJ/d)	Heat due to whole-body protein synthesis (B)	
	Protein (g)	Fat (g)		kJ/d	B:A
2	1·75	3·83	74·3	15·9†	0·212†
4	2·76**	5·83**	56·0**	9·7**†	0·173*†
SED	0·31	0·14	2·1	1·1	0·016

SED, standard error of difference.
Mean values are significantly different from those at day 2: * $P < 0·05$, ** $P < 0·01$.
† One missing value.

the fractional synthesis rate, in the fed state (day 0), broiler birds tended to have a lower fractional synthesis rate than did their layer counterparts and the marginal difference disappeared completely at day 4. When expressed as the amount synthesized per kg body-weight the strain × starvation interaction was significant, indicating that at day 0 layer birds had a higher value than did broiler chickens, while the difference virtually disappeared after 4 d starvation. In contrast to synthesis rate, whole-body protein degradation in terms of fractional rates and absolute amounts was significantly increased by starvation for 4 d in both strains. The fractional degradation rate was always higher in layer than in broiler chickens and the difference became larger after 4 d starvation as indicated by a significant strain × starvation interaction. As for the fractional degradation rates, amounts of protein degraded per kg body-weight were consistently higher in layer than in broiler chickens.

From Expt 2, losses of protein and fat, starving heat production, the heat due to whole-body protein synthesis and its contribution to the total starving heat production, are given in Table 4. Amounts of whole-body protein synthesized and degraded are expressed

Table 3. *Effect of starvation on body-weight and whole-body protein turnover rates in broiler (BR) and layer (LA) chickens*

(Mean values for six birds)

Period of starvation (d)	Type of bird	Body-wt (g)	Whole-body protein synthesis		Whole-body protein degradation	
			%/d	g/kg body-wt per d	%/d	g/kg body-wt per d
0	BR	295	20·4	31·1	10·2	15·6
4		222	14·8†	24·5†	17·7†	29·3†
Group mean		261	17·9	28·1	14·0	22·0
0	LA	133**	22·2	37·0**	12·4*	20·6
4		88**	14·7	25·3 NS	21·3**	36·7
Group mean		110**	18·5 NS	31·2*	16·8**	28·6**
SED between:						
Any two means		3	0·7	1·2	0·7	1·1
Group means		4	1·0	1·7	0·9	1·5

NS, not significant; SED, standard error of difference.

Means values showing the effects of starvation within both BR and LA groups are significantly different ($P < 0·01$) for all measurements.

Mean values for LA birds are significantly different from corresponding values for BR birds: * $P < 0·05$, ** $P < 0·01$.

† One missing value.

Table 4. *Body protein and fat losses, starving heat production and the contribution of whole-body protein synthesis to the total starving heat production in broiler (BR) and layer (LA) chickens subjected to starvation for 4 d*

(Mean values for five (broiler) and six (layer) birds; the energy cost of 3·56 kJ/g protein synthesized was assumed (Waterlow *et al.* 1978))

Type of bird	Loss of:		Starving heat production (A) (kJ/kg body-wt per d)	Heat due to whole-body protein synthesis (B)	
	Protein	Fat		kJ/kg body-wt per d	B:A
	(g/kg body-wt per 2 d)				
BR	6·3†	26·0†	555†	87·3†	0·155†
LA	18·2**	24·1**	655**	90·1	0·138
SED	3·4	0·7	23	6·6	0·013

SED, standard error of difference.

Mean body-weights (g) of the birds at day 2 of starvation were: BR, 253; LA, 101; and at day 4: BR, 222; LA, 88.

Means values are significantly different from those for BR birds: ** $P < 0·01$.

† One missing value.

per kg body-weight because the difference in body-weight was very large (broiler birds were about three times heavier than their layer counterparts) and because basal metabolic rates of young layer and broiler chickens weighing up to 500 g have been shown to be proportional to body-weight rather than to the power of 0·75 (Kuenzel & Kuenzel, 1977). After 4 d starvation, layer birds showed a higher loss of protein and a slightly but

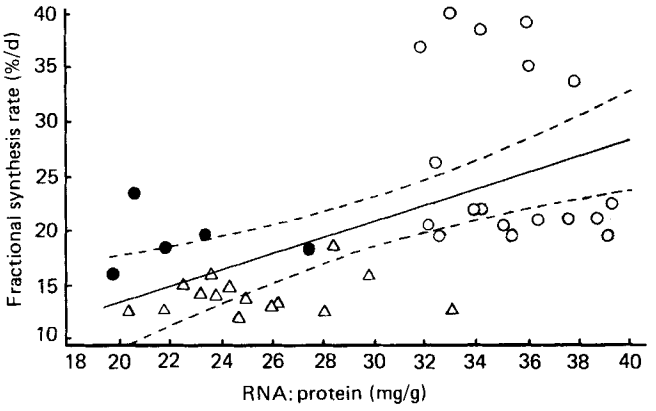

Fig. 1. The relation between fractional synthesis rate of protein and RNA: protein in the whole body of chickens subjected to starvation for 4 d. (– –), 95% confidence intervals of the regression line obtained. ○, 0 d starvation; ●, 2 d starvation; △, 4 d starvation; r 0·55 was significant ($P < 0{\cdot}01$).

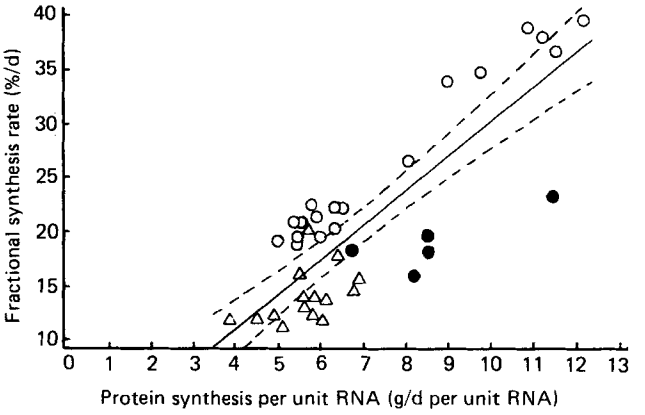

Fig. 2. The relation between fractional synthesis rate of protein and the amount of protein synthesized per unit RNA in the whole body of chickens subjected to starvation for 4 d. (– –), 95% confidence intervals of the regression line obtained. ○, 0 d starvation; ●, 2 d starvation; △, 4 d starvation; r 0·84 was significant ($P < 0{\cdot}001$).

significantly lower loss of fat than did broiler chickens, expressed on a per kg body-weight basis. The starving heat production per kg body-weight was significantly lower in broiler than in layer chickens, whereas the heat due to whole-body protein synthesis and its contribution to the total starving heat production were not significantly affected by the difference in strain.

Figs. 1 and 2 show the relations between fractional synthesis rate and RNA: protein ratio or protein synthesis per unit RNA based on the pooled results of Expts 1 and 2. For both indices, significant correlations with fractional synthesis rate (r 0·55 for RNA: protein ratio and r 0·84 for protein synthesis per unit RNA) were found, suggesting that starvation caused the reduction in both indices which in turn resulted in reduced fractional synthesis rate.

DISCUSSION

Starving heat production, frequently referred to as basal metabolic rate, has been treated as if it were a fixed component in the total heat production in a particular animal at a particular age. However, it is actually made up of various sources of heat generated by many metabolic reactions necessary to the life of an animal. Kien *et al.* (1978) demonstrated that a significant proportion of basal energy expenditure was directly associated with energy needs for whole-body protein turnover, especially for protein synthesis. The present study attempted to evaluate the contribution of the energy cost of protein synthesis to the total starving heat production in young chickens.

The starving heat production in chickens was assessed in the present study by carcass analysis with the assumption that all the N originating from oxidation of body proteins was excreted in the form of uric acid-N. This is, of course, an over-simplification, but no serious error appeared to be caused in the present estimate of starving heat production since the value at day 4 of starvation agreed well with that reported by Okumura *et al.* (1973). In Expt 1, the values for heat production at day 2 of starvation were significantly higher than those at day 4 (Table 2). This was probably due to the fact that the former would include additional heat produced from food remaining in the gastrointestinal tract. In general, weight and carcass components are likely to be lost exponentially so that the rate of loss will always be higher in the first period of measurement than in the second. Consequently, it was considered that the values at day 4 of starvation may be designated as the basal metabolic rate of chickens in the present study. In Expt 2, therefore, only the heat production at day 4 of starvation was compared for broiler and layer birds.

The contribution of energy expenditure associated with whole-body protein synthesis to the basal metabolic rate was estimated to be 17% for layer chickens in Expt 1, and 16% for broiler and 14% for layer chickens in Expt 2. On the whole, the contribution of whole-body protein synthesis to the total heat production seemed to be lower in the starved state (14–17% in the present study) than in the fed state (20% in birds of similar age; Muramatsu & Okumura, 1985). Since protein synthesis is an energetically expensive process, starvation might cause a more serious effect on protein synthesis than on other metabolic events from which heat is produced.

The difference found in the values for layer birds between Expts 1 and 2 might reflect the difference in the method of rearing, i.e. in groups (Expt 1) or individually (Expt 2) during the starvation period. The starved birds reared in groups seemed to have more opportunity for coprophagy than did individually reared chickens due to the difference in the structure of rearing units (brooders for groups in Expt 1 and metabolism cages for individuals in Expt 2). The difference in the method of rearing might also explain to some extent the different effect of the starvation period on the contribution of protein synthesis to total heat production. If coprophagy occurred during starvation, the reduction in carcass protein and fat should become smaller, resulting in low estimates of total starving heat production and hence an apparently large contribution of protein synthesis to the basal metabolic rate.

No strain difference between broiler and layer chickens was observed in the proportion of heat produced by protein synthesis. However, a lower basal metabolism in broiler birds than in layer birds, shown by the starving heat production at day 4 in Table 4, implied a more efficient utilization of dietary energy by the broilers. The lower basal metabolic rate in broilers than in layer chickens was in good agreement with the finding of Kuenzel & Kuenzel (1977).

The values calculated for the contribution of protein synthesis in all the above estimates were based on the assumption that 5 mol ATP would be consumed/mol peptide bond synthesized, leading to an energy cost of 3·56 kJ/g protein synthesized (Waterlow *et al.*

1978). However, the calculated contribution could be varied by using different factors for the energy cost (kJ/g protein synthesized): 3 (Buttery & Boorman, 1976), 4·5–5 (Webster, 1981) and 7·3 (Whittemore & Fawcett, 1976). The difference depends on the extent to which additional energy other than amino acid acylation and peptide-bond formation is taken into account. In the present study the problem of estimating the exact energy cost of protein synthesis was further explored by using a regression of heat production on protein synthesized from the pooled values from Expts 1 and 2. The regression equation obtained was:

$$\text{heat production (kJ/d)} = 43{\cdot}1\ (\text{SE}\ 5{\cdot}8) + 6{\cdot}2\ (\text{SE}\ 1{\cdot}8) \times \text{protein synthesized (g/d)} + 46{\cdot}5\ (\text{SE}\ 5{\cdot}4) \times \text{type of birds (broiler 1, layer 0)}\ (R\ 0{\cdot}97,\ P < 0{\cdot}001).$$

The slope value of 6·2 kJ/g protein synthesized was considerably higher than the value of 3·56 kJ quoted by Waterlow *et al.* (1978), but lower than 7·3 kJ/g protein synthesized suggested theoretically by Whittemore & Fawcett (1976) and lower than 13·0 kJ/g protein synthesized actually obtained in fed chickens by the same regression equation (Muramatsu & Okumura, 1985). It appears, therefore, that as far as the regression approach is concerned, the starved state would give better estimates of the energy cost of protein synthesis than the fed state, probably because of less physical activity and other metabolic reactions, the associated heat of which could affect the estimate of the slope value. If the slope value of 6·2 kJ/g protein synthesized were the true energy cost, 25–30% of the total starving heat production would be accounted for by the heat due to whole-body protein synthesis in young chickens. Whatever the true energy cost of protein synthesis may be, a significant proportion of basal energy expenditure was certainly accounted for by whole-body protein synthesis in chickens subjected to starvation.

As might be expected, starvation produced a reduction in protein synthesis in the whole body of chickens both in terms of fractional synthesis rate and amounts synthesized (Tables 1 and 3). After starvation, a reduced protein synthesis was also reported in muscle (Millward *et al.* 1976), liver and jejunal mucosa (McNurlan *et al.* 1979) of rats. Laurent *et al.* (1978) suggested that the fractional synthesis rate was a function of both RNA:protein and protein synthesis per unit RNA. Therefore, correlations between the fractional synthesis rate and these determinants were calculated to investigate which was the more important under the conditions in the present study (Figs. 1 and 2). In contrast to refeeding, where only protein synthesis per unit RNA was closely related to the change in fractional synthesis rate (Y. Aoyagi, T. Muramatsu, J. Okumura and I. Tasaki, unpublished results), highly significant correlation coefficients detected between fractional synthesis rate and RNA:protein or protein synthesis per unit RNA implied that these determinants might both be important in the regulation of changes in fractional synthesis rate when birds were starved. This was further confirmed when a multiple regression of fractional synthesis rate on these determinants was calculated:

$$\text{fractional synthesis rate (\%/d)} = -21{\cdot}67\ (\text{SE}\ 1{\cdot}19) + 3{\cdot}09\ (\text{SE}\ 0{\cdot}09) \times \text{protein synthesis per unit RNA} + 0{\cdot}70\ (\text{SE}\ 0{\cdot}03) \times \text{RNA: protein}\ (R\ 0{\cdot}99,\ P < 0{\cdot}001),$$

indicating that although the change in one unit of protein synthetic efficiency of RNA had a larger effect on fractional synthesis rate than that of RNA:protein, both determinants were important and the variation of fractional synthesis rate could be explained almost completely by both protein synthesis per unit RNA and RNA:protein.

The comparison between strains revealed that in the fed state, whole-body protein turnover rates, whether expressed as fractional synthesis rate or amounts per unit

body-weight, tended to be higher in layer chickens than in broiler chickens (Table 3). However, the growth rate of whole-body protein was estimated over a 4 d period whereas the synthesis rate was measured almost instantaneously within 10 min after isotope administration. Accordingly, an error arising from the difference in measurement periods might be inherently included in the degradation rate calculated from the difference between the synthesis and growth rates.

In skeletal muscle, Kang *et al.* (1985) argued that the faster growth of broiler chickens was predominantly attributable to a lower degradation rate rather than a higher synthesis rate. In the whole body, however, reduction in both protein synthesis and degradation in the fed state was detected in broiler birds compared with layer chickens in the present study. Since the growth rate is merely a reflection of the difference between synthesis and degradation rates, faster growth is not necessarily accompanied by faster protein turnover rates. For example, Bates & Millward (1981) reported that in rats of a fast-growing strain, both fractional synthesis and degradation rates in skeletal muscle were lower than those in slow growing rats for the age (23–330 d) examined. The slower the whole-body protein turnover, the lower the energy cost for this process. Thus, together with a lower basal metabolic rate, the tendency towards a slow turnover rate of body protein in broiler chickens would favour an efficient utilization of dietary energy compared with layer chickens. This conclusion, however, was reached only with broiler and layer birds at the same age. Therefore, broiler and layer birds having the same body-weight would have to be used to examine further the possibility of slower protein turnover in the whole body of broiler chickens.

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