# Influence of dietary non-protein energy intake on whole-body protein turnover in chicks

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1. Three experiments were conducted to investigate the influence of dietary energy intake on whole-body protein turnover in chicks.

2. In Expt 1 a semi-purified diet with various dietary metabolizable energy (ME) concentrations, 10·9, 12·6, 14·2 and 15·9 kJ/g, was fed *ad lib.* to young chicks. Whole-body fractional synthesis rate (FSR) was increased with each increment in dietary ME level from 12·6 to 15·9 kJ/g, and whole-body fractional degradation rate (FDR) showed a similar, though less sensitive, trend to that of FSR.

3. In Expts 2 and 3, chicks were given graded ME intakes of 84, 126, 167, 209 or 293 kJ/d with a fixed intake of dietary protein. FSR was increased when the energy intake was raised from 84 to 167 kJ/d, and above this level it was almost constant. Similar to the trend obtained with *ad lib.* feeding, the response of FDR to changes in dietary energy intake was less sensitive than that of FSR.

4. Total heat production was increased when dietary energy intake was increased from 84 to 167 kJ/d, and there was no further increase at 209 kJ/d. In contrast, the contribution of protein synthesis to total heat production was not affected by varying the dietary energy intake.

It has been reported that in man and mammals, protein turnover rates are affected by various nutritional factors, particularly dietary protein and energy intakes (Reeds & Fuller, 1983).

Changes in protein intake may cause alterations in protein turnover, leading to changes in growth and protein accretion. For example, elevation of whole-body protein synthesis by increasing dietary protein intake was reported in pigs (Reeds *et al.* 1981). In rat skeletal muscle and in the whole-body, both protein synthesis and degradation increased with increasing dietary protein level from 0 to 200 g/kg, with no further increase above this dietary protein level (Laurent *et al.* 1984). Although there has been a limited number of studies on whole-body protein turnover in avian species, results similar to those in mammals have been obtained (Muramatsu *et al.* 1987*b*).

As far as the effect of energy restriction on protein turnover is concerned, published results for man and mammals are in good agreement. Generally speaking, at a restricted energy intake, protein synthesis appears to be reduced, as found in rat mammary gland (Jansen & Hunsaker, 1986), skeletal muscle of man (Winterer *et al.* 1980), and in the whole body of pigs (Reeds *et al.* 1981) and man (Golden *et al.* 1977; Garlick *et al.* 1980*a*; Winterer *et al.* 1980). Protein degradation in skeletal muscle (Winterer *et al.* 1980) and whole body (Garlick *et al.* 1980*a*) of man seems unchanged by mild energy restriction.

In contrast, existing evidence for the effect of energy excess is conflicting. Motil *et al.* (1981) found in man that excess energy intake did not change whole-body protein synthesis. In rat liver and skeletal muscle, however, Glick *et al.* (1982) reported a reduction in protein synthesis by overfeeding, although in their study there was increased consumption of dietary protein as well as energy.

In avian species, there is little information in the literature on the relation between dietary energy intake and whole-body protein turnover. In the chick, a 50% energy

restriction decreased protein synthesis of leg muscle (Maruyama *et al.* 1978). Similarly, when young chicks were subjected to starvation, whole-body protein synthesis was reduced while protein degradation increased (Muramatsu *et al.* 1987*a*). The present study was conducted to investigate the influence of dietary non-protein energy intake on whole-body protein turnover in the chick.

## MATERIALS AND METHODS

Three experiments were conducted, and the same procedure was employed unless stated otherwise.

### *Expt 1*

Single comb White Leghorn male chicks (*n* 200) from a local hatchery (Hattori Yokei Ltd, Nagoya, Japan) were fed on a commercial chick mash (crude protein (nitrogen × 6·25) 215 g/kg, metabolizable energy (ME) 12·1 kJ/g; Marubeni Siryou Ltd, Tokyo, Japan) from hatching until 7 d of age in electrically heated brooders. In the morning, at 7 d of age, sixty-four birds with similar body-weights were selected, and were then distributed into four experimental groups of sixteen birds each with the body-weights as uniform as possible. Mean initial body-weight was 65·6 (SE 0·3) g. The birds were kept individually in metabolism cages and fed on an experimental diet and water *ad lib.* for the following 12 d. The compositions of the experimental diets with four different ME levels are presented in Table 1. The ME levels were set at 10·9, 12·6, 14·2 and 15·9 kJ/g and were adjusted by changing the proportion of maize starch, maize oil and non-digestible substances. Body-weight and food intake were recorded on alternate days. Ambient temperature was controlled at 29 ± 2° and continuous illumination was provided. On day 10 of the experimental period (17 d of age), ten birds were used for the measurement of whole-body protein synthesis, while on days 8 and 12, three birds per treatment were taken for the measurement of whole-body net protein growth rate. Whole-body fractional synthesis rate (FSR; %/d) of protein, expressed as the proportion of daily protein synthesis relative to whole-body protein mass, was measured using a large dose injection method (Garlick *et al.* 1980*b*) of L-[4-$^3$H]phenylalanine via a wing vein (10 ml/kg body-weight: 120 mM-phenylalanine, 40 $\mu$Ci/ml in physiological saline (8·5 g sodium chloride/l)). After 2 or 10 min the birds were killed by cervical dislocation, frozen in liquid nitrogen and stored at −20° until analysed. Whole-body fractional degradation rate (FDR; %/d) of protein was derived from the difference between FSR and the net protein growth rate. Details of the analysis of protein turnover have been described elsewhere (Muramatsu & Okumura, 1985). Carcass protein content was calculated as N × 6·25, which was determined by a Kjeldahl method.

### *Expt 2*

In order to eliminate the effect of the difference in food intake, and hence nutrient intake, the chicks were given fixed amounts of protein, vitamins, minerals and non-digestible substances, but non-protein energy was varied during an experimental period. The birds were kept as in Expt 1 until 7 d of age. In the morning, at 7 d of age, sixty-four birds were distributed into four experimental groups of sixteen birds each. Mean initial body-weight was 72·3 (SE 0·2) g. They were then fed on graded levels of energy for the subsequent 12 d. Daily intake levels are shown in Table 2. Daily energy intakes were set as 84, 126, 167 and 209 kJ/d, ranging from considerably deficient to adequate levels (Scott *et al.* 1982; National Research Council, 1984). All diets were force-fed at 09.00 and 17.00 hours as described by Muramatsu *et al.* (1987*b*). On day 10 (17 d of age), whole-body protein

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

| Calculated ME values (kJ/g)... | 10·9 | 12·6 | 14·2 | 15·9 |
|---|---|---|---|---|
| Maize starch | 210·2 | 319·8 | 343·6 | 330·6 |
| Maize oil | 15·0 | 15·0 | 50·0 | 100·0 |
| Cellulose | 131·9 | 72·3 | 43·5 | 26·5 |
| Aluminium silicate | 130·0 | 80·0 | 50·0 | 30·0 |
| Sucrose | 200·0 | | | |
| Methyl cellulose | 10·0 | | | |
| Casein (850 g CP/kg) | 220·0 | | | |
| L-Arginine hydrochloride | 5·3 | | | |
| L-Methionine | 1·1 | | | |
| Glycine | 7·1 | | | |
| Mineral mixture* | 64·9 | | | |
| Vitamin mixture* | 2·0 | | | |
| Choline chloride | 1·5 | | | |
| Inositol | 1·0 | | | |

ME, metabolizable energy; CP, crude protein (nitrogen × 6·25).
* Muramatsu & Okumura (1985).

Table 2. *Expts 2 and 3. Daily intake levels of ingredients (g/d)*

| | Expt 2 | | | | Expt 3 | |
|---|---|---|---|---|---|---|
| Calculated ME intake (kJ/d)... | 84 | 126 | 167 | 209 | 209 | 293 |
| Maize oil | 0·260 | 0·497 | 0·735 | 0·972 | 3·393 | 5·195 |
| Maize starch | 0·449 | 1·517 | 2·586 | 3·654 | 0·713 | 1·239 |
| Sucrose | 0·449 | 1·517 | 2·586 | 3·654 | 0·713 | 1·239 |
| Cellulose | 0·160 | | | | 0·480 | 0·480 |
| Aluminium silicate | 0·160 | | | | — | — |
| Methyl cellulose | 0·160 | | | | — | — |
| Casein (850 g CP/kg) | 3·275 | | | | | |
| L-Arginine hydrochloride | 0·085 | | | | | |
| L-Methionine | 0·018 | | | | | |
| Glycine | 0·114 | | | | | |
| Vitamin mixture* | 0·320 | | | | | |
| Mineral mixture* | 1·038 | | | | | |
| Choline chloride | 0·024 | | | | | |
| Inositol | 0·160 | | | | | |

ME, metabolizable energy; CP, crude protein (nitrogen × 6·25).
* Muramatsu & Okumura (1985).

synthesis was measured and the values for FSR, FDR and carcass crude protein were measured as in Expt 1. Carcass fat was extracted overnight (about 16 h) with diethyl ether using a Soxhlet apparatus. Total heat production was calculated by subtracting the retained energy in the whole body from ME intake.

For calculating the contribution of protein synthesis to total heat production, the energy cost of 3·56 kJ/g protein synthesized was assumed (Waterlow *et al.* 1978).

*Expt 3*

In Expt 3, the effect of excess energy intake on whole-body protein turnover was investigated. Chicks were maintained as in Expt 1 until 7 d of age. Mean initial body-weight

Table 3. *Expt 1. Effect of varying dietary metabolizable energy (ME) values on ME intake, protein intake, body-weight gain, protein retained, and whole-body protein synthesis and degradation in chicks*

(Mean values for six chicks)

| | | | | | Protein synthesis | | Protein degradation | |
|---|---|---|---|---|---|---|---|---|
| ME values (kJ/g) | ME intake (kJ/d) | Protein intake (g/10 d) | Body-wt gain (g/10 d) | Protein retained (g/10 d) | Fractional rate (%/d) | Absolute rate (g/d) | Fractional rate (%/d) | Absolute rate (g/d) |
| 10·9 | 175[a] | 32·1[a] | 69·8 | 12·7 | 26·3[a] | 6·37[ab] | 22·4[a] | 5·41 |
| 12·6 | 197[b] | 31·5[a] | 71·5 | 11·7 | 26·1[a] | 6·07[b] | 23·6[a] | 5·49 |
| 14·2 | 215[bc] | 30·3[ab] | 72·8 | 11·9 | 29·2[ab] | 6·84[ab] | 24·6[ab] | 5·74 |
| 15·9 | 224[c] | 28·2[b] | 72·7 | 10·9 | 32·9[b]† | 7·27[a]† | 28·7[b]† | 6·35† |
| SED | 8·6 | 1·3 | 5·2 | 0·8 | 1·8 | 0·41 | 2·3 | 0·48 |
| (df) | (20) | (20) | (20) | (20) | (19) | (19) | (19) | (19) |
| | | | | Analysis of variance | | | | |
| Linear | ** | ** | NS | NS | ** | * | * | NS |
| Quadratic | NS | NS | NS | NS | NS | NS | NS | NS |
| Cubic | NS | NS | NS | NS | NS | NS | NS | NS |

[a, b, c] Means within the same vertical column not sharing a common superscript letter were significantly different ($P < 0·05$).

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

* $P < 0·05$, ** $P < 0·01$.

† One missing value.

was 63·5 (SE 0·5) g. For the following 9 d, they were fed on fixed amounts of nutrients, with the exception of non-protein energy as shown in Table 2. Daily ME intakes were set as 209 and 293 kJ/d, being approximately 40% higher energy intake than the assumed voluntary intake. The two experimental diets were force-fed, as described for Expt 2. For the measurement of FSR, the labelled phenylalanine was injected on day 7 (14 d of age). On days 5 and 9, three birds per treatment were used for the determination of net protein growth rate and FDR. Chemical analyses used for determination of FSR, FDR and carcass crude protein were the same as those used in Expt 1.

Analysis of variance was done to assess the significance of the main effect of dietary energy level or energy intake with a statistical package, SAS (SAS Institute Inc., North Carolina), and Student's *t* test was used to evaluate the significance of difference between means (Snedecor & Cochran, 1980).

## RESULTS

### *Expt 1*

Values for body-weight gain, ME intake, protein intake, protein retained, and whole-body protein synthesis and degradation are shown in Table 3. Body-weight gain and protein retained during the experimental period were not significantly different for the ME values studied. Protein intake decreased in proportion to the increase in dietary ME values. There was no significant difference between the two lowest ME values, 10·6 and 12·6 kJ/g, for FSR and absolute synthesis rate (ASR; g/d), while an increase in these variables was observed with an increment in dietary ME values from 12·6 to 15·6 kJ/g. FDR showed a similar trend to that of FSR, but there was no significant difference in absolute degradation rate (ADR, g/d).

Table 4. *Expt 2. Effect of varying daily energy intakes ranging from deficient to adequate levels*† *on body-weight gain, protein retained, and whole-body protein synthesis and degradation in chicks*

(Mean values for six chicks)

| | | | Protein synthesis | | Protein degradation | |
|---|---|---|---|---|---|---|
| ME intake (kJ/d) | Body-wt gain (g/10 d) | Protein retained (g/10 d) | Fractional rate (%/d) | Absolute rate (g/d) | Fractional rate (%/d) | Absolute rate (g/d) |
| 84 | 8·5[a] | 2·5[a] | 20·5[a]‡ | 3·11[a]‡ | 22·8[a]‡ | 3·47[a]‡ |
| 126 | 39·0[b] | 8·2[b] | 26·5[b] | 5·54[b] | 24·6[a] | 5·15[b] |
| 167 | 54·0[c] | 9·9[c] | 32·3[c] | 7·28[c] | 29·3[b] | 6·62[c] |
| 209 | 60·5[d] | 10·5[c] | 32·2[c] | 7·49[c] | 29·0[b] | 6·74[c] |
| SED | 2·1 | 0·4 | 1·8 | 0·40 | 2·0 | 0·41 |
| (df) | (20) | (20) | (19) | (19) | (19) | (19) |
| | | | Analysis of variance | | | |
| Linear | ** | ** | ** | ** | ** | ** |
| Quadratic | ** | ** | * | ** | ** | * |
| Cubic | NS | ** | NS | NS | NS | NS |

[a, b, c, d] Means within the same vertical column not sharing a common superscript letter were significantly different ($P < 0·05$).

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

* $P < 0·05$, ** $P < 0·01$.

† Daily intake of nutrients other than non-protein energy was kept adequate according to the corresponding requirements recommended by the National Research Council (1984).

‡ One missing value.

*Expt 2*

Table 4 shows the values for body-weight gain, protein retained, and whole-body protein synthesis and degradation in chicks given ME intakes ranging from deficient to adequate levels. Body-weight gain increased with increasing daily ME intake. As daily ME intake was increased from 84 to 167 kJ/d, protein retention was improved, and above this level protein retention remained unchanged. The values for FSR and ASR increased as the daily ME intake rose from 84 to 167 kJ/d, and no further increase was found at the highest ME intake. Whole-body protein degradation showed a similar trend to that of protein synthesis.

The energy cost of whole-body protein synthesis on a per kg body-weight basis, total heat production and the contribution of whole-body protein synthesis to total heat production are shown in Table 5. The energy cost of whole-body protein synthesis per kg body-weight showed a similar trend to that of ASR which increased up to 167 kJ/d with no further increase at 209 kJ/d. Total heat production increased with increased dietary ME intake up to 167 kJ/d, whereas above this level the value remained constant. Varying dietary ME intakes had no significant effect on the contribution of whole-body protein synthesis to total heat production.

*Expt 3*

Table 6 shows the effect of increasing energy intake to approximately 40% of voluntary intake on body-weight gain, protein retained and whole-body protein turnover. Except for protein retained, which was significantly lower than that in the control group, no significant differences were found in any measurements.

Table 5. *Expt 2. Effect of varying daily energy intake, ranging from deficient to adequate levels,† on the energy cost of whole-body protein synthesis, total heat production and the contribution of whole-body protein synthesis to the total heat production*

(Mean values for six chicks)

| ME intake (kJ/d) | Energy cost of whole-body protein synthesis (A) (kJ/kg BW per d) | Total heat production (B) (kJ/kg BW per d) | Contribution of protein synthesis to heat production (B/A) (%) |
|---|---|---|---|
| 84 | 139[a]‡ | 983[a]‡ | 14·0[a]‡ |
| 126 | 178[b] | 1157[b] | 15·3[a] |
| 167 | 206[c] | 1370[c] | 15·0[a] |
| 209 | 199[c] | 1410[c] | 14·3[a] |
| SED | 10 | 34 | 1·0 |
| (df) | (19) | (19) | (19) |
| | Analysis of variance | | |
| Linear | ** | ** | NS |
| Quadratic | ** | * | NS |
| Cubic | NS | NS | NS |

[a, b, c] Means within the same vertical column not sharing a common superscript letter were significantly different ($P < 0·05$).

The energy cost of 3·56 kJ/g protein synthesis was assumed (Waterlow *et al.* 1978).

BW, body-weight; SED, standard error of difference; NS, not significant.

* $P < 0·05$, ** $P < 0·01$.

† Daily intake of nutrients other than non-protein energy was kept adequate according to the corresponding requirements recommended by the National Research Council (1984).

‡ One missing value.

## DISCUSSION

### *Examination of dietary non-protein energy intake*

The present study was done to clarify the relation between energy intake and whole-body protein turnover in the chick. In Expt 1, this was attempted by varying dietary ME values under an *ad lib.*-feeding regimen, and it was found that although ME intakes and whole-body protein turnover changed in concert, the extent of these changes were smaller than had been expected because of altered food intake. The changes in food intake were consistent with the well-known ability of chickens partially to adjust their total voluntary energy intake (Scott *et al.* 1982). Other nutrients besides non-protein energy varied because of intake differences; in consequence it was not possible to attribute changes in protein synthesis as due entirely to alterations in non-protein energy intake. The possible effect of changes in nutrient intake other than non-protein energy was, therefore, eliminated in Expts 2 and 3 by feeding a fixed amount of protein, minerals, vitamins and non-digestible substances to all treatment groups. The adequate total ME intake level was determined from the previous experiment for birds at 7–19 d of age (Muramatsu *et al.* 1987*b*), being 188 kJ/d, which was slightly higher than those suggested by the National Research Council (1984) and Scott *et al.* (1982).

### *Energy intake and protein turnover*

The present study has demonstrated that, in chicks, increasing the non-protein energy supply to provide levels ranging from deficient to adequate total ME intake resulted in co-

Table 6. *Expt 3. Effect of excess energy intake on body-weight gain, protein retained, and whole-body protein synthesis and degradation in chicks*

(Mean values for six chicks)

| Treatment | ME intake (kJ/d) | Body-wt gain (g/10 d) | Protein retained (g/10 d) | Protein synthesis: Fractional rate (%/d) | Protein synthesis: Absolute rate (g/d) | Protein degradation: Fractional rate (%/d) | Protein degradation: Absolute rate (g/d) |
|---|---|---|---|---|---|---|---|
| Control* | 209 | 63·8 | 10·4[a] | 25·8† | 4·77† | 22·8† | 3·78† |
| Excess | 293 | 59·0 | 9·3[b] | 27·4† | 4·85† | 20·4† | 3·84† |
| SED | — | 2·5 | 0·4 | 1·8 | 0·37 | 2·0 | 0·38 |
| (df) | — | (10) | (10) | (8) | (8) | (8) | (8) |

[a,b] Means within the same vertical column not sharing a common superscript letter were significantly different ($P < 0·05$).

* Daily intake of nutrients other than non-protein energy was kept adequate according to the corresponding requirements recommended by the National Research Council (1984).

† One missing value.

ordinated stimulation of both protein synthesis and degradation, the latter being less sensitive (Table 4). No further changes in whole-body protein turnover were observed with excess ME intake (Table 6). The results of energy restriction are in good agreement with the findings reported for leg muscle (Maruyama *et al.* 1978). Thus, as far as whole-body protein turnover in the chick is concerned, the responses to dietary energy intake from deficient to excess are analogous to those for dietary protein intake (Muramatsu *et al.* 1987*b*).

In general, the conclusions relating to energy restriction and excess energy drawn from the present study with chicks are the same as those suggested by studies in adult man and mammals (Golden *et al.* 1977; Garlick *et al.* 1980*a*; Winterer *et al.* 1980; Jansen & Hunsaker, 1986), though some differences were observed. Whole-body protein degradation (Garlick *et al.* 1980*a*) and skeletal-muscle protein degradation (Winterer *et al.* 1980) were unchanged in response to energy restriction in man. Besides the differences in the method used, and in the maturity of species studied and therefore factors associated with ageing such as hormonal sensitivity, the extent of energy deficiency would be an important factor which might account for the apparent disagreement, since the response of protein degradation to changes in nutrient intake is frequently less sensitive (Reeds *et al.* 1981). In addition, it should be borne in mind that protein degradation is determined indirectly from the difference between protein synthesis and net accretion, and is, therefore, more subject to error than that of synthesis.

There might be some complications in interpreting the present results, although they would not invalidate entirely the conclusion reached. Since the design of Expt 2 was based on the fixed daily food intake throughout the experimental period, the birds lost body protein during the last few days of the experimental period in the lowest intake group. Consequently, whole-body protein degradation became higher than whole-body protein synthesis in this group, leading to the incompatible results between protein retention over 10 d and whole-body protein turnover on the last day. Furthermore the dietary restrictions would probably have resulted in different lean compositions between the treatment groups, and this may in turn influence the rate of whole-body protein synthesis. Although the measurement of whole-body protein synthesis was not made at the last meal, this would

little affect the values for FSR and ASR. Y. Aoyagi, J. Okumura and T. Muramatsu (unpublished results) found in fasted chicks that after diet refeeding, the extent of enhanced whole-body protein synthesis at 2 and 6 h was significantly different, but only by 10%. Therefore, the maximum variation detectable in FSR due to the different time scale with respect to nutrient absorption should be less than 10%.

The differences between Expts 2 and 3 in FSR and ASR at the same ME intake, 209 kJ/d, might be ascribed to the changes in relative concentration of non-protein energy sources. In Expt 3 where the effect of excess energy intake was tested, more fat was used than in Expt 2 in order to facilitate the force-feeding procedure. According to findings in pigs (Reeds *et al.* 1981) as quoted by Garlick (1986), a dietary carbohydrate supplement has a larger stimulative effect on whole-body protein synthesis than that of a fat supplement. In addition, decreasing the dietary carbohydrate content might reduce plasma insulin concentration (Gill & Hart, 1979), which is an important factor in the regulation of protein synthesis (Garlick *et al.* 1983; Millward *et al.* 1983). Thus, the lower FSR and ASR found in Expt 3 compared with those in Expt 2 at the same ME intake level could possibly be brought about by the increased amounts of dietary fat relative to carbohydrates.

Reeds & Fuller (1983) have argued that changes in protein turnover rates associated with alterations in food intake may represent separate responses of body protein turnover to dietary protein and energy, and have suggested that the effects of dietary protein and energy on protein metabolism, including turnover, are additive rather than interactive. If this is also true for the chick, it follows that the responses to changes in intake of the whole diet can be predicted from the separate responses to protein and energy. This possibility remains to be investigated.

*Heat production and the contribution of protein synthesis*

As shown in Table 6, total heat production increased with increasing dietary ME intake; similar results were derived by Miller & Payne (1962). In the present study, the contribution of whole-body protein synthesis to total heat production was estimated to be about 14–15% (Expt 2), which was considerably lower than that of 20% reported by Muramatsu & Okumura (1985). This difference would be attributed to different feeding techniques: *ad lib.*-feeding in the former study and force-feeding in the present study. Alternatively, the lower estimate in the present study might be ascribable to the poorer growth and lower protein contents in the whole-body compared with those reported by Muramatsu & Okumura (1985). The poorer performance was brought about not only by the restricted feed intake owing to the force-feeding regimen but also by dietary protein source (casein in the present study *v.* isolated soya-bean protein in the previous study) despite the supplement of limiting-amino acids according to the requirements recommended by the National Research Council (1984). Judging from the fact that the present values for ASR obtained by adequate ME intake in Expt 2 were considerably lower than those found by Muramatsu & Okumura (1985) (7·3–7·5 g/d *v.* about 13 g/d) and that similar values for FSR were found at about 3 weeks of age, it appears that the protein content in the whole body gave the observed differences in ASR, and hence heat production associated with protein synthesis.

In order to obtain these estimates for the energy cost of protein synthesis, a factor of 3·56 kJ/g protein synthesis was used by assuming that 5 mol ATP would be required per mol peptide bond synthesized (Waterlow *et al.* 1978). However, the choice of the multiplicative factor depends on the allowance for the extra energy expenditure other than amino acid acylation and peptide bond formation. The suggested values from stoichiometry ranged from 3·0 to 7·3 kJ/g protein synthesis as summarized by Aoyagi *et al.* (1988). This may suggest that the present estimate of the contribution of protein synthesis to total heat production in chicks would fluctuate considerably, ranging from 12 to 31%.

The estimation of energy cost of protein synthesis by regression analysis has also been attempted, as in pigs (Reeds *et al.* 1980) and in chicks (Muramatsu & Okumura, 1985; Muramatsu *et al.* 1987*a*). The regression equation obtained in the present study was as follows:

$$HP = 649\ (SE\ 127) + 11{\cdot}6\ (SE\ 2{\cdot}5)\ PS + 16{\cdot}0\ (SE\ 5{\cdot}6)\ FD \quad (r\ 0{\cdot}95),$$

where HP, PS and FD are total heat production (kJ/kg body-weight per d), whole-body protein synthesis (g/kg body-weight per d) and fat deposition (g/kg body-weight per d) respectively. If a slope value of 11·6 kJ/g were used, the contribution of protein synthesis would account for 46–50% of total heat production in the present study. Uncertainty about the contribution of protein synthesis to heat production remains to be investigated in the future.

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