

Effect of dietary protein and energy intakes on whole-body protein turnover and its contribution to heat production in chicks

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A factorial 3 × 3 experiment was conducted with chicks to investigate the effect of manipulating crude protein (N × 6.25) intake (CPI) and metabolizable energy intake (MEI) simultaneously, in the range low to high (including adequate) levels with regard to the respective requirements, on whole-body protein turnover and its contribution to total heat production. The fractional rate of whole-body protein synthesis was increased curvilinearly by increasing MEI or CPI from low to high levels. In terms of absolute rates whole-body protein synthesis was enhanced by increasing MEI from low to adequate levels, the effect being greater at adequate and high CPI than at low CPI. The effect of varying CPI and MEI on whole-body protein degradation was similar, but less sensitive, to that on whole-body protein synthesis. Increasing MEI from low to high levels elevated total heat production at all CPI levels. There were no interactive effects of varying CPI and MEI on the contribution of whole-body protein synthesis to total heat production, and in general the contribution increased with increasing CPI and decreased with increasing MEI. The contribution of whole-body protein synthesis to total heat production fell within a small range from 11.2 to 16.5%.

Chick: Whole-body protein turnover: Heat production

In the last decade the nutritional regulation of whole-body protein turnover, particularly by varying dietary protein and energy intakes, has been extensively investigated in domestic animals and fowls, and research findings on this subject have been accumulating rapidly.

In the rat, for example, whole-body protein synthesis was increased by increasing dietary protein intake from low to adequate levels, and above this level no further increment was found (Laurent *et al.* 1984). Also in the chick, varying the dietary protein intake produced a similar response in whole-body protein synthesis and degradation (Muramatsu *et al.* 1987*b*; Kita *et al.* 1989*a*). There have been several studies in which the response of whole-body protein synthesis to energy intake has been investigated at a low or adequate level in mammalian species: whole-body protein synthesis increased with increasing dietary energy intake in humans (Garlick *et al.* 1980*a*; Winterer *et al.* 1980), pigs (Reeds *et al.* 1981) and steers (Lobley *et al.* 1987). However, the effect of dietary energy at a high intake level relative to the requirement is poorly understood. According to the findings for chicks (Kita *et al.* 1989*b*), both whole-body protein synthesis and degradation were little affected by increasing energy intake from adequate to high levels.

Despite the rapid accumulation of information, limited studies are available where the effect of both dietary protein and energy intake was investigated systematically. Reeds & Fuller (1983) suggested from their study in pigs that dietary protein and non-protein energy influenced whole-body protein synthesis, probably through different mechanisms because the responses were additive rather than interactive. In avian species there are few reports

available. The current status of available information on this topic was reviewed recently (Muramatsu, 1990).

The present study was conducted to investigate systematically the concomitant effect of both dietary protein and energy intakes on whole-body protein turnover in young chicks. In addition, changes in the contribution of whole-body protein synthesis to total heat production was examined.

MATERIALS AND METHODS

Single-comb White Leghorn male chicks (200 birds) from a local hatchery (Hattori Yokei Ltd, Nagoya, Japan) were maintained on a commercial chick mash diet (crude protein ($N \times 6.25$) 215 g/kg, metabolizable energy 12.1 kJ/g; Marubeni Siryu Ltd, Tokyo, Japan) from hatching until 7 d of age in electrically heated brooders. At this stage, eighty-one birds of similar body weights were selected, and distributed into nine experimental groups of nine birds so that mean group body weight, 71.4 (SE 0.3) g, was as uniform as possible. The birds were kept individually in metabolism cages and force-fed on an experimental diet, as described previously (Muramatsu *et al.* 1987*b*), with free access to water during the following 10 d. Table 1 shows dietary intake levels for each ingredient for the nine experimental groups arranged as a 3×3 factorial design during the experimental period. Three levels of both daily crude protein intake (CPI) and daily metabolizable energy intake (MEI) were designated as low, adequate and high with regard to the respective requirements for the chick at this age according to Scott *et al.* (1982) and the National Research Council (1984). In the present study the amount of meal had to be kept within a range which could be force-fed easily and at the same time the desired CPI and MEI could be achieved. The proportion of non-protein energy sources was set at 4:4:2 (by wt) for maize starch, sucrose and maize oil respectively at low and adequate MEI, and at 3:3:4 (by wt) at high MEI. Extreme excess of dietary fat content may affect whole-body protein turnover as discussed previously (Kita *et al.* 1989*b*), but the change in the proportion of fat in the present study was expected only to cause small effects. Continuous illumination was provided and ambient temperature was controlled at $29 \pm 2^\circ$.

On day 10 (17 d of age) the birds were used for the measurement of whole-body protein synthesis. Fractional synthesis rate (FSR; %/d) of whole-body protein was measured using a large-dose injection of L-[4- 3 H]phenylalanine (Garlick *et al.* 1980*b*). At 2 and 10 min after the isotope injection four and five birds respectively in each treatment were killed by cervical dislocation. The value for FSR over the 10 d period was calculated by taking a mean value at 7 and 17 d of age. For this purpose whole-body protein synthesis at 7 d was determined by running two preliminary experiments, giving FSR of 37.9 (SE 0.9) %/d (n 5) and 37.4 (SE 3.0) %/d (n 5). Using a mean value of 37.7 %/d at the beginning of the experiment (7 d of age), mean FSR over the 10 d experimental period for individual birds were recalculated and used for computing the contribution to total heat production. The whole-body fractional degradation rate (FDR; %/d) of protein was calculated from the difference between FSR and net protein growth rate derived from carcass N content at 7 and 17 d of age. Whole-body absolute synthesis rate (ASR; g/d) and whole-body absolute degradation rate (ADR; g/d) of protein at 7 and 17 d of age were calculated as FSR and FDR multiplied by the amount of protein present at the corresponding time-points. Details of analytical procedures have been described elsewhere (Muramatsu & Okumura, 1985). Protein contents in the whole body were calculated as $N \times 6.25$, and N content was determined by a Kjeldahl method. Carcass fat in the whole body was extracted overnight (about 16 h) with diethyl ether using a Soxhlet apparatus, and was determined gravimetrically.

Table 1. *Dietary intake levels of ingredients during the experimental period (g/10 d)*

CPI (g/d)....	Low 1.5			Adequate 3.0			High 4.5		
	Low	Adequate	High	Low	Adequate	High	Low	Adequate	High
MEI (kJ/d)....	126	188	251	126	188	251	126	188	251
Maize starch	19.33	31.99	27.39	13.19	25.85	23.62	7.05	19.71	19.86
Sucrose	19.33	31.99	27.39	13.19	25.85	23.62	7.05	19.71	19.86
Maize oil	9.66	16.00	36.52	6.59	12.93	31.50	3.53	9.86	26.48
Casein (850 g CP/kg)	—	15.26	—	—	32.91	—	—	50.56	—
L-arginine hydrochloride					0.795				
L-methionine					1.065				
Glycine					0.165				
Vitamin mixture*					0.300				
Mineral mixture†					9.740				
Choline chloride					0.230				
Inositol					0.150				
Cellulose					4.500				

CPI, crude protein ($N \times 6.25$) intake; MEI, metabolizable energy intake.

* The vitamin mixture supplied (mg/kg diet): calcium pantothenate 15, riboflavin 6, pyridoxine hydrochloride 4, nicotinic acid 40, pteroylmonoglutamic acid 1.5, biotin 200 μg , cyanocobalamin 20 μg , thiamine hydrochloride 3, retinyl acetate 1 mg, cholecalciferol 5 μg , tocopheryl acetate 10 mg, menadione 500 μg .

† The mineral mixture supplied (g/kg diet): calcium phosphate (dibasic) 20.7, calcium carbonate 14.8, potassium dihydrogenphosphate 10.0, potassium chloride 3.0, sodium chloride 6.0, magnesium sulphate 3.0, ferric sulphate 500 mg, manganese sulphate 350 mg, potassium iodide 2.6 mg, copper sulphate 40 mg, zinc oxide 62 mg, cobalt chloride 1.7 mg, sodium molybdate 8.3 mg, sodium selenite 400 μg .

The values for total heat production over the 10 d experimental period were derived by subtracting the retained energy in the whole body from MEI. The contribution of whole-body protein synthesis to 10 d total heat production was calculated using a factor of 3.56 kJ/g protein synthesized according to Waterlow *et al.* (1978).

Although eighty-one birds were used in the present study, all measurement values were derived from five birds killed 10 min after the isotope injection in each dietary treatment and the remaining four birds killed 2 min after the injection were used to give the value for mean specific radioactivity of free phenylalanine over the 10 min period. A two-way analysis of variance based on a factorial design of treatments was done to assess the significance of main and interactive effects of CPI and MEI by a general linear model (GLM) with a statistical package, SAS (SAS Institute Inc., North Carolina, USA). Significance of difference between means for each CPI treatment was assessed by Student's *t* test (Snedecor & Cochran, 1980) only when the interactive effect was significant.

RESULTS

The values for body weight gain, protein deposition, fat deposition, FSR, FDR, ASR and ADR are summarized in Table 2. Increasing CPI from low to adequate levels increased body weight gain, but no increment was observed when CPI was increased from adequate to high levels. Body weight gain increased significantly with increasing MEI. Protein deposition increased with an increasing MEI from low to adequate levels at the low and adequate CPI levels, but this effect was not significant at the high CPI level. At all CPI levels fat deposition increased with increasing MEI except that there was no significant difference between adequate and high MEI levels at the low CPI level. FSR was increased

Table 2. Effects of varying dietary crude protein (CPI) and metabolizable energy (MEI) intakes* ranging from low to high levels on body weight gain, protein deposition, fat deposition, and fractional and absolute rates of protein synthesis and degradation in the whole body of chicks

CPI (g/d)	MEI (kJ/d)	Body wt gain (g/d)	Fractional rate†			Absolute rate		
			Protein deposition (g/d)	Fat deposition (g/d)	Protein synthesis (%/d)	Protein degradation (%/d)	Protein synthesis (g/d)	Protein degradation (g/d)
1.5	126	1.4	0.37 ^a	0.05 ^a	27.5†	24.6†	3.7 ^a †	3.3
	188	3.2	0.61 ^b	0.77 ^b	28.6	24.4	4.2 ^b	3.5
	251	3.7	0.52 ^{ab}	0.99 ^b	28.9	25.2	4.1 ^{ab}	3.5
3.0	126	3.4	0.78 ^a	-0.18 ^a	28.9†	23.8†	4.4 ^a †	3.7 ^a
	188	6.4	1.26 ^b	0.39 ^b	31.6	24.4	5.6 ^b	4.3 ^b
	251	6.8	1.24 ^b	1.08 ^c	32.3	25.3	5.8 ^b	4.5 ^b
4.5	126	3.6	0.82 ^a	-0.08 ^a	28.7	23.3	4.4 ^a	3.6 ^a
	188	5.4	0.95 ^a	0.72 ^b	32.0†	26.2†	5.3 ^b †	4.3 ^b
	251	7.0	1.15 ^b	1.49 ^c	33.2	26.6	5.8 ^c	4.7 ^c
Pooled SEM for individual means		0.30	0.05	0.08	0.65	0.72	0.15	0.13
df		36	36	36	33	33	33	33
CPI								
Linear	2	0.001	0.001	0.001	0.001	0.325	0.001	0.001
Deviation	1	0.001	0.001	0.099	0.001	0.336	0.001	0.001
MEI	1	0.001	0.001	0.001	0.014	0.329	0.001	0.001
Linear	2	0.001	0.001	0.001	0.001	0.014	0.001	0.001
Deviation	1	0.001	0.001	0.001	0.001	0.003	0.001	0.001
CPI × MEI	4	0.003	0.001	0.206	0.079	0.698	0.004	0.046
Linear × linear	1	0.072	0.002	0.001	0.194	0.260	0.009	0.049
Linear × deviation	1	0.317	0.077	0.001	0.020	0.076	0.002	0.004
Deviation × linear	1	0.317	0.016	0.079	0.571	0.730	0.702	0.736
Deviation × deviation	1	0.046	0.021	0.102	0.724	0.183	0.108	0.374
					0.812	0.645	0.257	0.726

Statistical significance: $P =$

a, b, c Mean values within the same CPI level with unlike superscript letters were significantly different ($P < 0.05$).

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

† Fractional rate of protein synthesis was the mean of the final (17 d of age) and initial (7 d of age) values. Initial fractional rate of protein synthesis of 37.7%/d was determined preliminary.

‡ One missing value.

Table 3. *Effects of varying dietary crude protein (CPI) and metabolizable energy (MEI) intakes* ranging from low to high levels on energy cost of whole-body protein synthesis, total heat production and the contribution of whole-body protein synthesis to total heat production in chicks*

(Mean values for five chicks per treatment)

CPI (g/d)	MEI (kJ/d)	Energy cost of whole-body protein synthesis (A)† (kJ/100 g body wt per d)	Total heat production (B) (kJ/100 g body wt per d)	Contribution of whole-body protein synthesis to total heat production ((A/B) × 100) (%)	
1.5	126	16.8‡	136.8	12.5‡	
	188	17.1	147.8	11.7	
	251	16.3	152.1‡	11.2‡	
3.0	126	17.9 ^a ‡	124.2	14.5‡	
	188	19.2 ^b	135.6	14.5	
	251	19.2 ^b	148.7	13.0	
4.5	126	17.7 ^a	111.6	15.9	
	188	18.8 ^b ‡	119.0	16.5‡	
	251	19.2 ^b	162.5	11.9	
Pooled SEM for individual means		0.39	7.2	0.84	
df		33	35	32	
Statistical significance: <i>P</i> =					
		df			
CPI		2	0.001	0.059	0.001
Linear		1	0.001	0.023	0.001
Deviation		1	0.001	0.633	0.160
MEI		2	0.001	0.001	0.002
Linear		1	0.001	0.001	0.002
Deviation		1	0.004	0.028	0.091
CPI × MEI		4	0.009	0.067	0.154
Linear × linear		1	0.002	0.022	0.106
Linear × deviation		1	0.702	0.093	0.066
Deviation × linear		1	0.108	0.475	0.432
Deviation × deviation		1	0.257	0.545	0.677

^{a, b} Mean values within the same CPI level with unlike superscript letters were significantly different (*P* < 0.05).

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

† Energy cost of whole-body protein synthesis was derived from the calculation of the absolute rate of protein synthesis multiplied by the factor of 3.56 kJ/g protein synthesis (Waterlow *et al.* 1978).

‡ One missing value.

curvilinearly by increasing MEI or CPI from low to high levels. Varying CPI had no effect on FDR but increments in MEI tended to elevate FDR. ASR was enhanced by increasing MEI from low to adequate levels, the effect being greater at adequate and high CPI than at low CPI. Increased MEI elevated ADR, and the effect of varying MEI was greater at the two higher CPI levels.

Table 3 shows the energy cost of whole-body protein synthesis, total heat production and the contribution of whole-body protein synthesis to total heat production. The effect of varying CPI and MEI levels on the energy cost of protein synthesis was similar to that on ASR. Total heat production was increased with increasing MEI. There were no interactive

effects of varying CPI and MEI levels on the contribution of whole-body protein synthesis to total heat production. As shown in the analysis of variance of Table 3, in general, the increment in CPI from low to adequate levels increased the contribution of whole-body protein synthesis to total heat production, while the increase in MEI from adequate to high levels decreased the contribution.

DISCUSSION

The present study was done to investigate systematically the concomitant effect of dietary protein and energy intakes on whole-body protein turnover and the contribution of whole-body protein synthesis to total heat production in chicks. In the present study CPI and MEI were set at low, adequate and high levels based on the voluntary food consumption observed in a previous study (Muramatsu *et al.* 1987*b*). For growing Leghorn-type chicks at 14 d of age recommended requirements for CPI and MEI by Scott *et al.* (1982) are 3.0 g/d and 163 kJ/d respectively. According to the National Research Council (1984) the corresponding values are 2.3 g/d and 156 kJ/d. By taking into account the age of the chicks, 17 d old, therefore, it was considered that CPI and MEI levels denoted as adequate in the present study would meet the requirements recommended generally.

Whole-body protein turnover

As shown in Table 2, at adequate MEI levels FSR and ASR represented a quadratic response to an increase in CPI, reaching a plateau at the adequate CPI level. This curvilinear response of whole-body protein synthesis to varying CPI was in good agreement with the previous results in chicks (Muramatsu *et al.* 1987*b*) and in some mammalian species (Motil *et al.* 1981; Reeds *et al.* 1981; Laurent *et al.* 1984). Similarly, at the adequate CPI level FSR and ASR increased with an increase in MEI but the rate of increase diminished at higher MEI levels. Under the condition of energy restriction the same conclusion was reached in humans (Garlick *et al.* 1980*a*), in pigs (Reeds *et al.* 1981) and in chicks (Kita *et al.* 1989*b*). Glick *et al.* (1982) reported that when rats were fed at 1.35 times voluntary CPI and MEI liver protein synthesis decreased but muscle protein synthesis did not change in comparison with those in control rats. In the present study, at high CPI and MEI levels corresponding to 1.5 times the respective adequate levels, whole-body protein synthesis was not significantly different from that at the adequate CPI and MEI levels. This suggests that the response of whole-body protein synthesis to CPI and MEI in chicks was analogous to that of muscle protein synthesis in rats.

In chicks the response of whole-body protein degradation to varying CPI was found to be less sensitive than that of whole-body protein synthesis (Muramatsu *et al.* 1987*b*). This was in good agreement with the results obtained by varying CPI, as shown in Table 2. In skeletal muscle the restriction of both CPI and MEI by 50% tended to decrease protein degradation in chicks (Maruyama *et al.* 1978) and in rats (Millward *et al.* 1976). These findings on muscle protein degradation were also in excellent agreement with the results for whole-body protein degradation in the present study when the value for FDR at adequate CPI with adequate MEI was compared with that at low CPI with low MEI (Table 2).

Although Reeds & Fuller (1983) suggested that changes in both CPI and MEI had an additive effect the present results do not provide clear support for this suggestion. Rather, the detected response appeared to be interactive as shown by the analysis of variance in Table 2. Thus, judging from the results, a general conclusion would be that the effect of changing CPI on whole-body protein turnover is critically affected by the level of MEI, and *vice versa*. For example, only a marginal and non-significant effect of increasing MEI from low to adequate levels on FSR was found at the low CPI level, whereas at adequate and high

CPI levels FSR was clearly increased by the same treatment. Therefore, the impact of major dietary components, CPI and MEI, on whole-body protein turnover should be understood in terms of both the starting levels for these dietary variables and their varying extents.

The contribution of whole-body protein synthesis to total heat production

There is a wide range of values for the energy cost of protein synthesis (from 2.8 to 24.2 kJ/g protein synthesis) in the literature, as summarized by Aoyagi *et al.* (1988). This variability is due mainly to the fact that small values represent estimates of minimal stoichiometric cost whereas large values include heat production, some of which is statistically associated with protein deposition during growth above the minimal cost.

Even with a stoichiometric approach the contribution of whole-body protein synthesis to total heat production varies by 2-fold, ranging from 3.0 to 7.3 kJ/g protein synthesized depending on the assumption employed (Aoyagi *et al.* 1988). In the present study a factor of 3.56 kJ/g (Waterlow *et al.* 1978) was used, and it was shown that the contribution of whole-body protein synthesis to total heat production ranged from 11.2 to 16.5%.

These values agree closely with the range of 10–14% found in mammalian species (Garlick, 1986) but are lower than the estimate of 20–21% of Muramatsu & Okumura (1985) for fully-fed chicks using the same factor for energy cost. It is possible that the lower value in the present study may be related to poorer growth in the present study than that in the study of Muramatsu & Okumura (1985), or it could be due to the difference in feeding techniques, i.e. force-feeding in the present study and *ad lib.*-feeding in the previous study.

It has been recognized that the proportion of energy expenditure due to whole-body protein synthesis can be affected by changing nutritional states. Assuming the energy cost of protein synthesis to be 3.56 kJ/g protein synthesized, the contribution due to whole-body protein synthesis in young pigs ranged between 13 and 21% of total heat production depending on dietary conditions used (Reeds *et al.* 1982). In young chicks starvation decreased the contribution of whole-body protein synthesis to total heat production (Muramatsu *et al.* 1987*a*), and varying MEI from deficient at marginally excessive levels relative to the recommended requirement did not influence the contribution significantly (Kita *et al.* 1989*b*).

In the present study the effect of varying CPI and MEI on the contribution of whole-body protein synthesis to total heat production was investigated systematically, and significant linear terms of both CPI and MEI were observed (Table 3). The regression equation for the contribution in response to varying CPI and MEI was as follows: contribution = 14.3 (SE 1.4) + 0.047 (SE 0.012) CPI – 0.019 (SE 0.006) MEI (r 0.63), where 'contribution', CPI and MEI are the contribution of whole-body protein synthesis to total heat production (%), daily crude protein intake (kJ/d) and daily metabolizable energy intake (kJ/d), respectively. Although it was reported previously that varying MEI had no significant effect on the contribution (Kita *et al.* 1989*b*), in the present study the contribution decreased with increasing MEI. The discrepancy might be due to the difference in CPI levels used, only one intake level in the previous study and three levels (low, adequate and high with respect to the requirement) in the present study. Therefore, it is considered that a decrease in contribution of protein synthesis to total heat production as MEI increased, especially at high CPI level, was a real metabolic consequence brought about by manipulation of energy intake, although its nutritional and physiological significance remains unclear.

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