Modification of energy metabolism by the presence of the gut microflora in the chicken

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Whether the association with gut microflora modifies the energy metabolism of chickens was investigated by varying the metabolizable energy consumption level from zero to above the maintenance requirement in the germ-free and conventional states. Single comb White Leghorn chicks were either fasted for 3 d (Expt 1), or fed for 6 d at a fixed daily meal intake of 2, 5 or 8 g/d (Expt 2), or 5, 10 or 15 g/d (Expt 3). Changes in carcass energy deposition and heat production indicated that when no dietary energy was available the presence of the gut microflora could benefit the birds by reducing energy losses, whereas when dietary energy was supplied the efficiency of energy utilization was reduced by the presence of the gut microflora. It was concluded, therefore, that the heavy burden of the gut microflora modifies energy metabolism by exerting a buffering or a counter-productive action on the energy utilization of the chicken.

Gut microflora: Energy utilization: Chickens: Carcass energy deposition: Heat production

The presence of normal gut microflora may affect the metabolism of host birds in various ways. In protein metabolism, for example, little or no effect on overall protein utilization was produced by the presence of the gut microflora (Salter & Coates, 1971). When birds were subjected to protein starvation, however, the excretion of endogenous N was lowered in the conventional (CV) state compared with the germ-free (GF) state (Salter *et al.* 1974; Okumura *et al.* 1978). A more pronounced difference was found in protein synthesis of tissues that are in direct contact or close association with the gut micro-organisms. Protein synthesis in the gut mucosa and the lower gut was increased by the presence of the gut microflora (Muramatsu *et al.* 1983, 1987, 1988*b*, 1993), whereas little effect was found in skeletal muscle (Muramatsu *et al.* 1985).

In the past, the impact of the gut microflora on the energy metabolism of host birds has not been extensively studied. Hegde *et al.* (1982) have reported that dietary metabolizable energy (ME) values are marginally but significantly increased by the presence of the gut microflora, suggesting that chickens can utilize extra amounts of dietary energy extracted through the action of gut bacteria. Indeed, it has been shown that when chickens are fed on a low-energy, high-fibre diet, the energy extracted from dietary-fibre digestion by bacterial action can be efficiently utilized for growth of the host bird (Muramatsu *et al.* 1991).

The gut microflora can modify energy conversion and utilization in the chicken not only at a digestion step but also at an internal metabolism step. Furuse & Yokota (1984) have suggested, from the results of multiple regression, that the association with the gut bacteria increases the maintenance energy requirement of the chicken. On the other hand, fasting heat production is decreased by the association with the gut microflora (Muramatsu *et al.*

* For reprints.

1988*a*). It appears, therefore, that the presence of the gut microflora may play either a buffering or a counter-productive action in energy utilization of birds, i.e. saving the heat loss when no energy supply is available, but lowering the energy utilization when normal growth is maintained.

The above hypothesis of the counter-productive effect, however, has not been substantiated because no attempt has ever been made in the CV and GF states to quantify energy utilization and ME intake level with a wide range from zero to an amount above the maintenance requirement in chickens. The present study was conducted, therefore, to investigate the relationship between energy utilization of the chicken and the association with the gut microflora by varying the level of ME intake.

MATERIALS AND METHODS

Animals and diets

The birds were cared for under Guidelines for Animal Experimentation, laid down by the Committee of Experimental Animal Care, Nagoya University, Japan.

Fertilized eggs from single-comb White Leghorn hens and cocks, which were supplied from Gifu Poultry Experimental Station, Gifu, Japan, and kept in our poultry house, were incubated in a clean incubator. The details of the method for producing GF birds have been described elsewhere (Yokota *et al.* 1984). The hatched chicks were reared in pairs in metabolism cages, and were given free access to a commercial diet (Chick 15; Marubeni Shiryou Co. Ltd, Tokyo, Japan), which was fortified with vitamins to compensate for the possible loss due to ^{60}Co irradiation sterilization (Coates *et al.* 1963), until 9 d of age in either GF or CV states. At this stage, twenty-two, sixty, and eighteen birds with similar body weights from both GF and CV groups were selected in Expts 1, 2, and 3, respectively. In Expts 2 and 3 the birds were then distributed into three experimental groups of twenty and six birds respectively, in each gut environment. The average body weights of GF and CV birds were (mean (SEM)): Expt 1, 71·4 (1·6) and 67·7 (1·8) g; Expt 2, 75·1 (0·6) and 75·3 (1·5) g; and Expt 3, 74·6 (2·1) and 72·3 (1·6) g respectively.

In Expt 1 the birds were fed on a semi-purified experimental diet for the following 3 d and subsequently fasted for 3 d, while in Expts 2 and 3 the birds were given the same semi-purified experimental diet at a fixed amount of 2, 5 or 8 g/d (Expt 2), and 5, 10 or 15 g/d (Expt 3), for the following 6 d. The composition of the semi-purified experimental diet is given in Table 1. In all experiments, the chicks were allowed free access to water. The ambient temperature was maintained at $28 \pm 2°$ throughout the experimental period, and light was provided continuously for 24 h/d. During the experimental period, body weight and food consumption were recorded at 3 d intervals.

At the beginning of the fasting period (12 d of age) in Expt 1, and at the middle of the feeding period (12 d of age) in Expts 2 and 3, half the birds assigned to each dietary treatment in both CV and GF states were killed by neck dislocation to determine initial carcass composition. Subsequently, at the end of the experimental period (15 d of age) the remaining birds were killed similarly by neck dislocation, and the carcass samples were stored at $-20°$ until analysis. In all experiments the droppings were collected in 300 ml 0·05 M-HCl for the last 3 d from 12 to 15 d of age to determine the ME value of the experimental diet.

Chemical analysis

The preparation of the carcass and excreta samples has been described elsewhere in detail (Muramatsu & Okumura, 1985). The N contents of the diet, excreta and carcass were determined by a Kjeldahl method, and carcass protein was calculated as N × 6·25. Carcass

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

Ingredients	
Isolated soya-bean protein*	226·0
Maize starch	281·5
Sucrose	200·0
Cellulose	100·0
Maize oil	30·0
Vitamin mixture†	8·0
Mineral mixture†	64·9
Choline chloride	1·5
Inositol	1·0
Aluminium silicate	78·8
Glycine	4·2
L-Methionine	2·9
L-Threonine	1·2
Calculated value:	
Crude protein (g/kg)	190·0
Metabolizable energy (mJ/kg)	12·1

* Crude protein content 840 g/kg.
† As described previously (Muramatsu *et al.* 1987).

fat was determined gravimetrically after extraction with diethyl ether. Retained energy in the carcass and heat production were calculated as described previously (Muramatsu *et al.* 1988*a*). The values for changes in carcass fat, protein and energy, and those for heat production were expressed on a unit body weight basis to eliminate a possible effect of differences in body weight between the two gut environments. Dietary ME value was calculated after correction for retained N as described by Hill & Anderson (1958).

Statistical analysis and calculation

Statistical treatment was done by a one-way analysis of variance (Expt 1) and a 2×3 factorial analysis of variance (Expts 2 and 3) with the General Linear Model procedures of SAS (Statistical Analysis Systems Institute Inc., 1985) to assess the significance of the main effects of gut environment, and feeding level, and the interaction. For comparison of means of main effects, and of individual treatments, a protected last significant difference method was used (Snedecor & Cochran, 1980). The significance of differences between individual treatment means within the same gut environment or within the same feeding level was not tested unless a significant interaction was detected. For the pooled data from Expts 1, 2 and 3, linear regression of carcass energy deposition or heat production on ME intake was done, and the slopes and intercepts between the CV and GF states were compared (Snedecor & Cochran, 1980).

RESULTS

The values for body weight, carcass fat and protein contents, and the deposition of carcass fat and protein over the 3 d fasting period are given in Table 2. The initial body weight before fasting was significantly higher in the GF birds than in the CV controls, while after 3 d fasting no significant difference was detected. After fasting for 3 d the carcass fat concentration was significantly higher in the GF chicks than in the CV counterparts, whereas no difference was found in the carcass protein concentration. Carcass fat deposition over the 3 d fasting period showed a significantly larger loss in the GF state than in the CV state with no difference in carcass protein deposition.

Table 2. *Expt 1. Body weight (BW), carcass fat and protein concentrations, and fat and protein depositions after fasting for 3 d in germ-free (GF) and conventional (CV) chicks*†

Gut environment...	CV	GF	Pooled SE (20 df)	Statistical significance of effect
n...	11	11		
BW before fasting (g)	83·6	90·1	2·5	*
BW after fasting (g)	63·9	71·6	3·8	NS
Carcass fat (g/kg)	30	46	3	**
Carcass protein (g/kg)	187	185	2	NS
Fat deposition (g/kg BW per d)	−14	−18	1	**
Protein deposition (g/kg BW per d)	−10	−11	1	NS

Significance level: NS, not significant; *, $P < 0·05$; **, $P < 0·01$.
† For details of birds and procedures, see pp. 710–711.

Table 3. *Expt 2. Effect of restricted feeding on body weight (g), carcass fat and protein concentrations (g/kg), fat and protein depositions (g/kg body wt per d), and dietary metabolizable energy (ME) value (kJ/g) in germ-free (GF) and conventional (CV) chicks*‡

Gut environment (E)	Food intake level (FI) (g/d)	*n*	Body wt	Carcass		Deposition of		ME value
				Fat	Protein	Fat	Protein	
CV	2	10	62·1	37·6	182	−7·1	−5·3	8·7
	5	10	77·2	44·9	180	−6·9	1·2	11·6
	8	9	92·9	52·2	178	−5·0	4·9	12·1
Group mean			77·4	45·0	180	−6·3	0·2	10·8
GF	2	10	65·1	42·1	189	−7·6	−7·0	8·3
	5	9	80·6	55·3	188	−8·5	−1·2	11·0
	8	8	95·7	50·8	185	−4·0	4·6	11·6
Group mean			80·5	49·4	187**	−6·7	−1·2*	10·3**
SE (50 df) for:								
Group means			1·1	1·7	1·1	0·5	0·4	0·12
Individual means			2·0	3·0	2·0	0·9	0·7	0·21

Source	df	Analysis of variance Mean square					
E	1	131	2·71	7·02††	0·02	0·28†	2·89††
FI	2	8633††	7·68††	0·81	0·52†	4·87††	54·57††
E × FI	2	1	1·51	0·02	0·07	0·05	0·07
Residual	50	36	0·82	0·36	0·08	0·05	0·40

Mean value was significantly different from that for CV chicks: * $P < 0·05$, ** $P < 0·01$.
†, $P < 0·05$; ††, $P < 0·01$.
‡ For details of diets and procedures, see Table 1 and pp. 710–711.

Table 3 gives the values for final body weight, carcass fat and protein concentrations, carcass fat and protein depositions, and dietary ME in Expt 2. There was a tendency for higher body weight in the GF chickens than in the CV controls, but this was not significant. The final carcass protein concentration was significantly lower in the CV than in the GF chicks, but the carcass fat concentration was not significantly different between the two gut

Table 4. *Expt 3. Effect of moderately restricted feeding on body weight (g), carcass fat and protein concentrations (g/kg), fat and protein depositions (g/kg body wt per d), and dietary metabolizable energy (ME) value (kJ/g) in germ-free (GF) and conventional (CV) chicks*‡

Gut environment (E)	Food intake level (FI) (g/d)	*n*	Body wt	Carcass		Deposition of		ME value
				Fat	Protein	Fat	Protein	
CV	5	3	75·3	30·3	182	−5·4	1·1	12·2
	10	3	105·0	49·5	178	2·8	4·9	12·3
	15	3	119·0	55·0	170	1·1	6·2	12·5
Group mean			99·8	45·0	177	−0·5	4·1	12·4
GF	5	3	85·0	34·4	186	−8·8	2·0	11·3
	10	3	106·3	48·1	183	−1·5	6·3	11·7
	15	3	124·0	70·0	175	1·0	9·8	11·7
Group mean			105·1	50·9	181	−3·1	6·0	11·5**
SE (12 df) for:								
Group means			1·8	3·0	2·2	1·0	0·6	0·11
Individual means			3·1	5·3	3·7	1·7	1·1	0·20
Source	df	Analysis of variance Mean square						
E	1	128	1·57	0·99	0·30	0·17	3·02††	
FI	2	5129††	13·66††	3·89†	2·53††	1·25††	0·47	
E × FI	2	52	1·04	0·02	0·16	0·03	0·05	
Residual	50	28	0·83	0·42	0·09	0·04	0·12	

Mean value was significantly different for CV chicks: ** $P < 0·01$.
†, $P < 0·05$; ††, $P < 0·01$.
‡ For details of diets and procedures, see Table 1 and pp. 710–711.

environments. Carcass protein deposition was significantly lower in the GF birds than in the CV controls, whereas no difference was detected in carcass fat deposition. Dietary ME values were significantly higher in the CV than in the GF state.

The values for final body weight, carcass fat and protein concentrations, carcass fat and protein depositions, and dietary ME in Expt 3 are given in Table 4. The final body weight tended to be higher in the GF birds than in the CV controls, but this was not significant. Concentrations and deposition of carcass fat and protein were not significantly different between the two gut environments. Dietary ME values were significantly higher in the CV state than those in the GF state.

Using the pooled data from all the experiments, regression lines of energy deposition or heat production on ME intake were compared between the CV and GF states. The regression lines with calculated equations in the CV and GF states are presented in Fig. 1 together with the analysis of variance for the comparison in Table 5. The comparison showed that in both energy deposition and heat production the elevation term was significant, indicating a significant difference in the intercepts between the CV and GF states. In addition the parallelism term was significant, indicating a significant difference in the slopes between the two gut environments. For energy deposition the intercept was lower in the GF than in the CV states, whereas the reverse was true for the slopes. The regression lines for heat production showed the opposite trend to carcass energy deposition; the

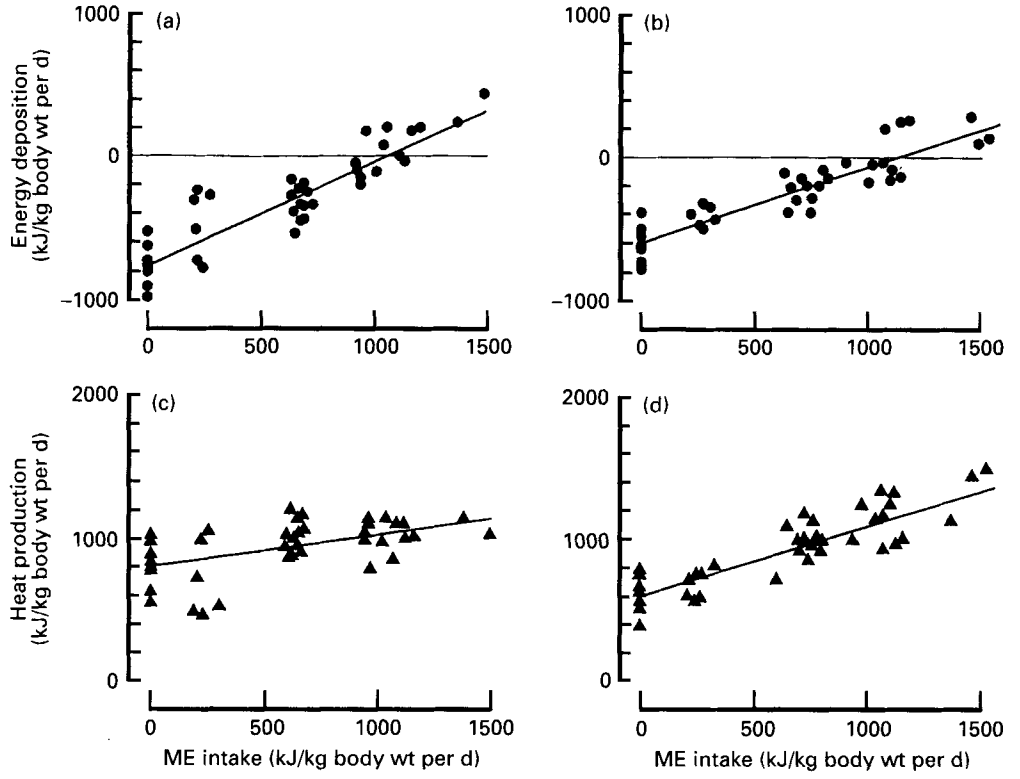

Fig. 1. The relationship between energy deposition (a, b) or heat production (c, d) and metabolizable energy (ME) intake levels varying from zero to above the maintenance requirement in the chicken reared in a germ-free (a, c) or conventional (b, d) state. For regression equations, see footnote to Table 5.

Table 5. *Analysis of variance for regression of heat production and carcass energy deposition on metabolizable energy (ME) intake when chicks were reared in germ-free (GF) and conventional (CV) environments, and subjected to fasting or restricted feeding*†

Output variable... Source	df	Energy deposition Mean square	Heat production Mean square
ME intake	1	77351***	25441***
Elevation between gut environments	1	2133***	1941***
Parallelism between gut environments	1	1986***	2016***
Residual	84	157	156

***, $P < 0{\cdot}001$.

† Results from experiments 1, 2, and 3 were pooled. For details, see pp. 710–711.

The regression equations obtained for carcass energy deposition or heat production (kJ/kg body weight per d) on ME intake (kJ/kg body weight per d) were:

Energy deposition (GF) $= -766{\cdot}9 + 0{\cdot}729 \times$ ME intake (r 0·92, $P < 0{\cdot}001$)

Energy deposition (CV) $= -605{\cdot}8‡ + 0{\cdot}523‡ \times$ ME intake (r 0·92, $P < 0{\cdot}001$)

SE for coefficient 43·7 0·058

comparison

Heat production (GF) $= 766{\cdot}0 + 0{\cdot}265 \times$ ME intake (r 0·66, $P < 0{\cdot}001$)

Heat production (CV) $= 612{\cdot}3‡ + 0{\cdot}473‡ \times$ ME intake (r 0·90, $P < 0{\cdot}001$)

SE for coefficient 43·5 0·058

comparison

‡ Significantly different from the corresponding GF value; $P < 0{\cdot}001$.

intercept was lower in the CV than in the GF state, and the slope was higher in the CV birds than in their GF counterparts.

DISCUSSION

The results of the present study indicate that carcass energy deposition and heat production in relation to varying ME consumption levels, from zero to above the maintenance requirement, are affected by the association with the normal gut microflora in the chicken. In the fasting state, heat production, calculated as energy loss from the carcass, was lower in the CV birds than in their GF counterparts as indicated by changes in the intercepts of the regression lines on ME intake levels. This confirmed our previous findings of lower fasting heat production (Muramatsu *et al.* 1988*a*), and was in line with the lower body temperature in the CV chickens (Harrison & Hewitt, 1978; Muramatsu *et al.* 1988*a*). It follows, therefore, that the presence of the gut microflora may benefit the host birds by saving energy loss from the body when no dietary energy supply is available. On the other hand, the efficiency of dietary energy utilization as indicated by changes in slopes of the regression lines on ME intake levels was lowered by the burden of the gut micro-organisms. This is also in line with the implication from previous findings (Furuse & Yokota, 1984). Thus, the present results clearly substantiate the hypothesis of the buffering or counter-productive action of the gut microflora on energy metabolism in the chicken.

If the counter-productive action of the gut microflora on the energy metabolism of host birds does really exist, then a question may arise as to how it can be attained. At present, the exact mechanism is not known, but the action might be explained at different steps. First, dietary ME values were significantly increased by the presence of the gut bacteria (Tables 3 and 4). The difference in the dietary ME values between the two gut environments would be largely accounted for by the difference in the digestibility of energy sources, predominantly dietary fibre (Muramatsu *et al.* 1991). Accordingly, the modifications of the host energy utilization by the gut microflora might occur at the digestion step where the most likely products of carbohydrate (including dietary fibre) degradation would be monosaccharides with a relatively small amount of volatile fatty acids, which would serve as a poor source of metabolic fuel (Bolton & Dewar, 1965; Yoshida *et al.* 1970; Baker, 1977; Furuse & Okumura, 1989). The production of volatile fatty acids in the gut may account, at least in part, for the higher ME value but lower efficiency for net energy deposition in the chicken harbouring the gut microflora. Second, the association with the gut microflora might cause increases in energy cost by modifying the rate of energy-consuming reactions such as protein turnover within the chicken body. This is certainly true for the gastrointestinal tract in the chicken when the gut micro-organisms are present (Muramatsu *et al.* 1983, 1987, 1988*b*, 1993). The energy cost of protein synthesis would account for approximately 20 to 30% of the total heat production in the chicken (Muramatsu & Okumura, 1985). By assuming that the energy cost of protein synthesis *in vivo* is 5·4 kJ/g (Aoyagi *et al.* 1989), at least a few percent of increased heat production, i.e. energy waste, in the CV state is expected. As protein degradation also requires energy at about a quarter of that needed for protein synthesis (Harris & Lobley, 1991), the extra waste of energy derived from body protein degradation in the CV state should be added to the above energy waste due to protein synthesis.

In mammalian species, modification of energy metabolism by the presence of the gut microflora has been suggested from studies with antibiotics. Supplementing a diet with an antibiotic, and thereby suppressing the microbial activity in the diet, increased dietary energy utilization in rats (Eggum & Chwalibog, 1983). Yen *et al.* (1985) also demonstrated that fasting heat production was reduced by feeding an antibiotic to the pig. Although the results with the pig (Yen *et al.* 1985) are in contrast to our findings in the chicken, the

contradiction might be attributable to species differences. Alternatively, it may mean that the antibiotic used in the pig study could have affected the fasting heat production not only by suppressing the activity of the gut microflora in the digestive tract but also by directly inhibiting the metabolic rate *per se*.

In the present study, further partition of energy utilization, i.e. partial efficiency of protein and fat energy depositions, was not compared between the two gut environments. When the heat production or ME intake were regressed on protein and fat energy depositions, partial efficiency coefficients could be obtained for the CV and GF chickens. In theory, this method should be able to clarify the issue of which form of energy deposition (protein or fat) is more susceptible to the association with the gut microflora. However, it was shown that there was a high correlation between protein and fat energy depositions ($r = 0{\cdot}698$, $P < 0{\cdot}0001$) in the present study. This was attributable to the experimental design used which caused changes in protein and fat depositions in the same direction by varying feed intake, and hence ME intake level. The existence of this high multicollinearity interfered with the accurate estimation of partial efficiency of energy utilization for each component. Consequently, if a more detailed analysis were to be carried out using a multiple regression, protein and fat energy depositions should be manipulated separately and independently. This could be achieved by systematically varying levels of dietary protein and ME intakes at the same time.

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