Influence of the gut microflora on protein synthesis in tissues and in the whole body of chicks

Tatsuo MURAMATSU, Osamu TAKASU, Mitsuhiro FURUSE, Iwao TASAKI and Jun-ichi OKUMURA
Laboratory of Animal Nutrition, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

1. The influence of the gut microflora on protein synthesis in individual tissues and in the whole body of young chicks was investigated by the large-dose injection of [^3H]phenylalanine. 2. Growth of germ-free chicks was significantly better than that of conventional controls. Wet weights of liver, spleen, duodenum, jejunum + ileum and caeca were heavier in conventional birds than in germ-free counterparts. 3. Fractional rates of protein synthesis were higher in jejunum + ileum and whole body of conventional birds than in those of germ-free birds. Amounts of protein synthesized were larger in liver, jejunum + ileum and caeca in the presence of the gut microflora. 4. When tissues were classified into gut + liver and the remainder of the carcass, in the presence of the gut microflora an enhanced protein synthesis in fractional and absolute rate was found in the gut + liver, which is in direct contact or in close association with micro-organisms, whereas virtually no effect of the gut micro-organisms was detected in the remainder of the carcass. 5. The contribution of protein synthesis of gut + liver to that of the whole body was larger in conventional chicks than in germ-free birds, whereas the reverse was true for the remainder of the carcass.

INTRODUCTION

There have been indications that the gut microflora may modify the protein metabolism in the whole body of chickens. For example, germ-free chicks grew faster than their conventional controls (Coates *et al.*, 1963). The better growth of the germ-free bird could be explained by a more efficient use of dietary protein, since protein accretion is the most important component of chick growth. Protein utilization in the conventional state may be decreased to a certain extent by partial utilization of dietary protein as an energy source, probably because of a higher energy requirement of conventional birds compared with that of germ-free counterparts (Furuse & Yokota, 1985).

In tissues such as gastrointestinal tract and liver, which are in close association with the microflora, possible changes in protein metabolism by the activity of micro-organisms would also be expected to occur. Gordon & Bruckner-Kardoss (1961) reported thicker gut wall in conventional birds than in germ-free birds. Migration rates of gut epithelial cells in conventional chicks were faster than in germ-free counterparts (Cook & Bird, 1973; Rolls *et al.*, 1978). The liver of conventional chicks was sometimes, though not always, heavier (Reyniers *et al.*, 1960), possibly because of the need for detoxification of ammonia (Visek, 1974) and amines (Cheeseman & Fuller, 1966) produced by microbial activities in the digestive tract.

Since protein growth in individual tissues and in the whole body is altered through changes in protein synthesis or degradation, or both, it is of considerable importance to study protein turnover in both conventional and germ-free states for better understanding of the impact on host protein metabolism of the presence of gut micro-organisms. So far, however, only a few reports are available. Protein synthesis in jejunal mucosa and liver was higher, though not significantly, in conventional chicks than in germ-free counterparts (Muramatsu *et al.*, 1983), whereas little effect of the gut microflora was

noticed in the breast-muscle protein-turnover rates (Muramatsu *et al.*, 1985).

The present study was undertaken to investigate more extensively the effect of the gut microflora on protein synthesis in individual tissues and in the whole body of young chicks.

MATERIALS AND METHODS

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 for hatching in clean incubators. The details for the method of producing germ-free birds were described elsewhere (Yokota *et al.*, 1984). The hatched chicks were reared individually in metabolism cages until 23 days of age in either germ-free or conventional environments. During the 23-day experimental period they received no food for the first 2 days after hatching, and thereafter were allowed free access to water and an experimental diet. The experimental diet was based on a practical chick mash fortified with vitamins in order to compensate for possible losses during the irradiation procedure for food sterilization (Coates *et al.*, 1969). The composition of the practical diet is as follows (per kg of diet): chick mash (Nihon Nosan Co., Yokohama, Japan), 941.7 g; cellulose, 34.3 g; vitamin mixture, 8 g; vitamin A, 6800 i.u.; vitamin E, 10 i.u. The vitamin mixture contained (per kg of diet): calcium pantothenate, 60 mg; riboflavin, 24 mg; pyridoxine hydrochloride, 16 mg; nicotinic acid, 160 mg; folic acid, 6 mg; biotin, 800 μg ; vitamin B₁₂, 80 μg ; thiamin hydrochloride, 12 mg; vitamin D₃, 640 ICU; vitamin K₃, 2 mg; and glucose, 7.716 g. Calculated values for crude protein and metabolizable energy were 19.0% and 12.1 kJ/g respectively. The ambient temperature was maintained at $30 \pm 1^\circ\text{C}$ throughout the experimental period, and light was provided continuously for 24 h per day.

At 23 days of age, fractional rate of protein synthesis in individual tissues was measured by the large-dose injection method of Garlick *et al.* (1980), which depends on the analysis of specific radioactivity. The method involved the enzymic conversion of phenylalanine into phenethylamine, followed by radioactivity counting and fluorimetric determination. Radioactive L-[4-³H]-phenylalanine was combined with unlabelled phenylalanine to give 40 μ Ci and 120 μ mol/ml of 0.15 M-NaCl and injected into a wing vein at a dose of 1 ml/100 g body wt. Details were given elsewhere (Muramatsu & Okumura, 1985; Muramatsu *et al.*, 1985). At 2 or 10 min after the injection of [4-³H]phenylalanine, four and six birds respectively in germ-free and conventional environments were killed by neck dislocation. The abdominal cavity was opened, and tissues, including liver, spleen, duodenum, pancreas, jejunum + ileum, caeca and bursa of Fabricius, were removed quickly, and placed in ice-cold 0.15 M-NaCl. The gastrointestinal tract was cut longitudinally, and its contents were washed gently with ice-cold 0.15 M-NaCl. The tissues were blotted, weighed, frozen by plunging into liquid N₂, and stored at -20 °C until analysis. The viscera-free carcass was similarly treated.

The frozen viscera-free carcass was minced with a meat grinder, which was previously cooled with solid CO₂, and frozen again with liquid N₂. This mincing procedure was repeated three times to get homogeneous samples.

Tissue protein was determined by the procedure of Lowry *et al.* (1951), except for the viscera-free carcass protein, which was estimated by N content ($\times 6.25$), determined by a Kjeldahl method. This was necessary because some proteins, such as those in feathers, were insoluble in 0.3 M-NaOH. RNA content in tissues was analysed by a modified Schmidt-Thannhauser method as described by Munro & Fleck (1969), and determined spectrophotometrically by an orcinol reaction, the colour of which was read at 660 nm. The values for protein content, RNA content and protein synthesis in the whole body were calculated as the sum of those in individual tissues and the viscera-free carcass. The derivation of the fractional rate of protein synthesis from more than one tissue was made by calculating the sum of protein synthesized divided by the sum of protein content.

Results were presented as means $\pm$ S.E.M., and statistical significance was assessed by Student's *t* test.

RESULTS

The values for body-weight gain, feed intake and feed efficiency ($100 \times \text{g weight gain/g feed intake; \%}$) of germ-free and conventional birds from 2 to 23 days of age are given in Table 1. As might be expected, germ-free birds grew significantly better than their conventional controls, but little difference was observed in feed intake and feed efficiency between the germ-free and conventional states.

Table 2 gives the values for wet weight, contents of protein and RNA and the RNA/protein ratio in individual tissues and in the whole body of germ-free and conventional chicks, together with the values in gut + liver and in the remainder of the carcass. All the values were expressed per 100 g body wt., to adjust for differences in body weight between the birds in the germ-free and conventional environments. The values for wet weight of liver, spleen, duodenum, jejunum + ileum, caeca

Table 1. Body-weight gain, feed intake and feed efficiency of germ-free and conventional chicks

The birds were reared from 2 to 23 days of age. The values are means $\pm$ S.E.M. for ten birds. Statistical significance was assessed by using Student's *t* test: NS, not significant ($P > 0.05$); ** $P < 0.01$.

	Germ-free	Conventional
Body-weight gain (g/21 days)	145 $\pm$ 4	131 $\pm$ 2**
Feed intake (g/21 days)	339 $\pm$ 12	319 $\pm$ 10 NS
Feed efficiency (g gain/g feed intake, %)	44 $\pm$ 1	41 $\pm$ 1 NS

and gut + liver were significantly larger in conventional birds than in germ-free counterparts, whereas the reverse was true for the remainder of the carcass. The protein contents in liver, caeca and gut + liver of conventional birds were significantly higher than those of germ-free birds. The RNA contents were higher in duodenum, caeca and gut + liver of conventional birds than those of germ-free counterparts. The presence of the gut microflora little affected the RNA/protein ratio in individual tissues and in the whole body of chicks, except for that in caeca, where a significantly higher ratio was found in conventional than in germ-free birds.

Protein synthesis expressed as fractional rate, and amounts synthesized per 100 g body wt. or per g of RNA, are shown in Table 3, with protein synthesized in individual tissues as a proportion of that in the whole body. Generally speaking, the conventional tissues which are in close association with the gut microflora, such as liver, duodenum, jejunum + ileum and caeca, showed higher, though not always significantly, fractional rates of protein synthesis than the germ-free counterparts. Significant differences in fractional rates of protein synthesis were detected in jejunum + ileum, total gut + liver and in the whole body, being higher in conventional than in germ-free chicks. There was also a higher fractional rate in spleen, but the difference was not significant. Little difference was detected in fractional rates in bursa of Fabricius, pancreas and remainder of the carcass. Surprisingly, it was noticed that fractional synthesis rate in pancreas was remarkably faster than those in any other tissues, even twice as fast as that in liver.

Amounts of protein synthesized per 100 g body wt. in individual tissues tended to be higher in the conventional than in the germ-free states on most occasions, and significant differences were detected in liver, jejunum + ileum, caeca and gut + liver. When protein synthesis was compared on a per-g-of-RNA basis, a significantly higher value for jejunum + ileum was found in conventional birds compared with germ-free counterparts.

In gut + liver, the contribution of individual tissues to the whole-body protein synthesis was significantly higher in the conventional (26%) than in the germ-free (21%) state, whereas the reverse was true for the remainder of the carcass. The contribution of caeca protein synthesis in conventional chicks was almost twice as much as that in germ-free birds.

Table 2. Relative weights, protein content, RNA content and the RNA/protein ratio in individual tissues and whole body of germ-free (GF) and conventional (CV) chicks

Values are means $\pm$ S.E.M. for ten birds, except for six for the RNA/protein ratio, at 23 days of age. 'Gut+liver' is the sum of duodenum, jejunum+ileum, caeca and liver. Statistical significance was assessed by Student's *t* test: NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$.

		Weight (g/100 g body wt.)	Protein (mg/100 g body wt.)	RNA (mg/100 g body wt.)	RNA/protein (mg/g)
Liver	GF	2.6 $\pm$ 0.06	478 $\pm$ 17	23 $\pm$ 2	48 $\pm$ 2
	CV	2.9 $\pm$ 0.04**	548 $\pm$ 12**	25 $\pm$ 1 NS	45 $\pm$ 3 NS
Spleen	GF	0.12 $\pm$ 0.01	16 $\pm$ 1	0.9 $\pm$ 0.1	58 $\pm$ 2
	CV	0.15 $\pm$ 0.01*	19 $\pm$ 2 NS	1.2 $\pm$ 0.1 NS	57 $\pm$ 2 NS
Bursa of Fabricius	GF	0.60 $\pm$ 0.03	86 $\pm$ 5	6.2 $\pm$ 0.4	72 $\pm$ 3
	CV	0.68 $\pm$ 0.04 NS	88 $\pm$ 11 NS	5.4 $\pm$ 0.4 NS	70 $\pm$ 4 NS
Duodenum	GF	1.2 $\pm$ 0.04	180 $\pm$ 20	11 $\pm$ 1	64 $\pm$ 3
	CV	1.5 $\pm$ 0.06**	226 $\pm$ 8 NS	16 $\pm$ 1*	68 $\pm$ 3 NS
Jejunum + ileum	GF	2.3 $\pm$ 0.08	492 $\pm$ 35	21 $\pm$ 2	42 $\pm$ 1
	CV	3.4 $\pm$ 0.11**	592 $\pm$ 47 NS	24 $\pm$ 2 NS	41 $\pm$ 1 NS
Caeca	GF	0.31 $\pm$ 0.02	38 $\pm$ 1	1.8 $\pm$ 0.1	46 $\pm$ 1
	CV	0.63 $\pm$ 0.01**	80 $\pm$ 5**	4.6 $\pm$ 0.5**	57 $\pm$ 4*
Pancreas	GF	0.41 $\pm$ 0.01	84 $\pm$ 3	3.5 $\pm$ 0.3	42 $\pm$ 4
	CV	0.43 $\pm$ 0.02 NS	85 $\pm$ 3 NS	4.1 $\pm$ 0.4 NS	48 $\pm$ 4 NS
Whole body	GF	100.00	19842 $\pm$ 638	570 $\pm$ 32	29 $\pm$ 1
	CV	100.00	18954 $\pm$ 412 NS	543 $\pm$ 9 NS	29 $\pm$ 1 NS
Gut + liver	GF	6.48 $\pm$ 0.19	1189 $\pm$ 58	57 $\pm$ 3	48 $\pm$ 1
	CV	8.53 $\pm$ 0.25**	1447 $\pm$ 55**	69 $\pm$ 3*	48 $\pm$ 2 NS
Remainder of carcass	GF	93.5 $\pm$ 0.2	18653 $\pm$ 664	513 $\pm$ 34	28 $\pm$ 2
	CV	91.5 $\pm$ 0.2**	17508 $\pm$ 404 NS	474 $\pm$ 8 NS	27 $\pm$ 1 NS

DISCUSSION

It was previously found that the presence of the gut microflora enhanced, though not significantly, protein synthesis in the chick liver or jejunal mucosa (Muramatsu *et al.*, 1983), which is in close association with micro-organisms in conventional life. In contrast, little or no effect was found in breast muscle, which is remote from the bacterial activity (Muramatsu *et al.*, 1985). Thus, the closer the tissue to microbial association, the more the stimulation of protein synthesis would occur. In the present study, this was examined more extensively in individual tissues and in the whole body of young chicks, and essentially the same conclusion was reached: protein synthesis in fractional and absolute rates was enhanced in gut + liver of conventional birds compared with that of germ-free counterparts, whereas little difference was observed in the remainder of the carcass, which comprised primarily skeletal muscle and other visceral organs.

The reasons for the enhancement of protein synthesis by bacterial association in the conventional state were unknown. However, it may probably be that bacterial products generated in the gut might have a stimulating effect on protein synthesis in tissues which are in direct contact or in close association with the gut micro-organisms. In the digestive tract of animals, short-chain fatty acids, which were mainly the products of microbial activity (Annison *et al.*, 1968), were found to stimulate

epithelial-cell division (Sakata & Yajima, 1984). Cholic acid produced by hydrolysing enzymes of bacterial origin was also known to increase turnover of intestinal-mucosal cells (Ranken *et al.*, 1971). In addition, considerable amounts of ammonia (Vissek, 1974) and amines (Cheeseman & Fuller, 1966) were produced by the gut microflora. All these compounds could possibly be responsible for enhanced protein synthesis in gut + liver in the presence of the gut micro-organisms. The possibility remains to be clarified.

The increased protein synthesis in the gut and liver resulted in an elevated fractional rate of protein synthesis in the whole body of conventional birds compared with their germ-free counterparts (Table 3). An analogous increment in whole-body protein synthesis by pathogenic bacterial association was found by Pomposelli *et al.* (1985) in rats, although the extent of the enhancement in absolute synthesis rates seemed to be quite different between that caused by the pathogenic bacteria in their study (20%) and by the burden of ordinary gut microflora in the present study (5%).

Unlike in rats and mice, caecal enlargement in the germ-free state (Gordon, 1968) was not observed in chicks. In general, wet weight and protein and RNA contents in the gut + liver were higher in the presence of the gut micro-organisms (Table 2). These results in chickens were in good agreement with those of Reyniers *et al.* (1960) and of Furuse & Yokota (1984). Increased protein growth in these tissues appeared to be brought

Table 3. Protein synthesis in individual tissues and in the whole body of germ-free (GF) and conventional (CV) chicks

Value are means $\pm$ S.E.M. for six birds at 23 days of age; †one missing value. 'Gut+liver' is the sum of duodenum, jejunum+ileum, caeca and liver. Statistical significance was assessed by Student's *t* test: NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$.

		Fractional synthesis rate (%/day)	Protein synthesized		
			(g/day per g of RNA)	(g/day per 100 g body wt.)	(% of total)
Liver	GF	67 $\pm$ 3	14 $\pm$ 1	318 $\pm$ 17	9.8 $\pm$ 0.5
	CV	73 $\pm$ 5 NS	17 $\pm$ 1 NS	399 $\pm$ 20**	11.0 $\pm$ 0.8 NS
Spleen	GF	41 $\pm$ 2	7 $\pm$ 0.4	7 $\pm$ 0.7	0.2 $\pm$ 0.02
	CV	46 $\pm$ 2 NS	8 $\pm$ 0.4 NS	9 $\pm$ 0.9 NS	0.2 $\pm$ 0.03 NS
Bursa of Fabricius	GF	71 $\pm$ 3	10 $\pm$ 0.7	61 $\pm$ 4	1.8 $\pm$ 0.1
	CV	67 $\pm$ 5 NS	11 $\pm$ 0.8 NS	58 $\pm$ 5 NS	1.6 $\pm$ 0.2 NS
Duodenum	GF	71 $\pm$ 3	11 $\pm$ 0.8	128 $\pm$ 14	3.8 $\pm$ 0.5
	CV	77 $\pm$ 8 NS	12 $\pm$ 1.4 NS	175 $\pm$ 19 NS	4.5 $\pm$ 0.4 NS
Jejunum + ileum	GF	49 $\pm$ 2	12 $\pm$ 0.2	243 $\pm$ 21	7.2 $\pm$ 0.7
	CV	58 $\pm$ 2**	14 $\pm$ 0.5**	347 $\pm$ 32*	9.2 $\pm$ 0.9 NS
Caeca	GF	60 $\pm$ 2	13 $\pm$ 0.4	23 $\pm$ 1	0.7 $\pm$ 0.04
	CV	63 $\pm$ 5 NS	11 $\pm$ 1.2 NS	50 $\pm$ 4**	1.3 $\pm$ 0.07**
Pancreas	GF	178 $\pm$ 12	44 $\pm$ 5.1	149 $\pm$ 12	4.4 $\pm$ 0.4
	CV	172 $\pm$ 12 NS	37 $\pm$ 2.5 NS	146 $\pm$ 11 NS	3.9 $\pm$ 0.3 NS
Whole body	GF	17 $\pm$ 0.5	6 $\pm$ 0.3	3393 $\pm$ 69	100
	CV	19 $\pm$ 0.3*	7 $\pm$ 0.2 NS	3575 $\pm$ 116 NS	100
Gut + liver	GF	60 $\pm$ 1.5	12 $\pm$ 0.4	711 $\pm$ 50	21.0 $\pm$ 1.6
	CV	67 $\pm$ 2.6*	14 $\pm$ 0.8 NS	972 $\pm$ 56**	26.0 $\pm$ 1.4*
Remainder of carcass	GF	14 $\pm$ 0.4	5 $\pm$ 0.3	2682 $\pm$ 88	79.0 $\pm$ 1.6
	CV	15 $\pm$ 0.4 NS†	6 $\pm$ 0.3 NS†	2648 $\pm$ 121 NS†	74.0 $\pm$ 1.4*†

about by enhanced protein synthesis in the conventional state (Table 3). However, the increased fractional rates of protein synthesis in gut+liver did not necessarily result in better protein growth in the whole bird, since these tissues contributed only 6–8% of total body protein, and since the net growth was merely the reflection of differences between synthesis and degradation. Taken together with better body-weight gain in germ-free birds than in conventional controls (Table 1) and increased fractional rates of whole-body protein synthesis, it was likely that whole-body protein degradation in chicks could be enhanced by the association of micro-organisms, so that the increased protein synthesis was completely offset, or even poorer growth was brought about by the presence of the gut microflora.

In view of the energy cost of protein synthesis, faster protein turnover may result in inferior over-all energy utilization. Muramatsu & Okumura (1985) and Muramatsu *et al.* (1987) suggested that in the fed state the energy cost of whole-body protein synthesis accounted for about 17–20% of the total heat production of chicks at a similar age. It follows, therefore, that from the difference found in the amount of whole-body protein synthesis between the conventional and germ-free states, the energy wasted owing to faster turnover rates in the conventional state could only account for less than 1% of total heat production. This is too small to account for low efficiency of energy utilization in the conventional state, since Furuse & Yokota (1985) found that energy

utilization was about 20% higher in germ-free chicks than in conventional controls. Thus factors other than increased protein turnover, such as the difference in the extraction of dietary energy, may primarily be responsible for the poorer energy utilization in the conventional chicks.

As a well-known characteristic of germ-free chickens, serum IgG is considerably lower than that of conventional birds (Wostmann, 1968; Sato *et al.*, 1986). Since IgG was considered to be synthesized mainly in spleen and bone marrow (Keily & Abramoff, 1969), protein synthesis in spleen was expected to be higher in conventional than in germ-free birds. However, a non-significant increase in synthesis rates of total spleen protein was observed in the presence of the gut microflora. This may suggest that in chickens the synthesis of IgG contributes little to the total protein synthesis in the spleen. Talal (1966) also failed to detect any differences in protein synthesis in spleens between conventional and germ-free rats. Whether or not the synthesis of IgG is suppressed in the spleen of germ-free chicks remains to be studied.

To our knowledge, the present study was the first to report protein synthesis *in vivo* in various tissues of young chicks. Surprisingly, pancreas showed a very high fractional synthesis rate. This was attributable to a remarkably high protein synthesis rate per unit of RNA. Assuming that the mean transit time of ribosomes on mRNAs is not very different among the tissues tested, the

number of translatable mRNA molecules in pancreas might be larger than that in any other tissues. As the measurement of protein synthesis was done within 10 min by the method of Garlick *et al.* (1980), not only the pancreas protein itself, but also secreted proteins, may have been measured. Therefore the rapid rate of protein synthesis in pancreas may probably be related to its physiological function, such as the need for quick synthesis and secretion of digestive enzymes in order to facilitate digestion processes.

Financial support was provided by a grant-in-aid (No. 61760241) for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- Annison, E. F., Hill, K. J. & Kenworthy, R. (1968) *Br. J. Nutr.* **22**, 207–216
- Cheeseman, G. C. & Fuller, R. (1966) *J. Appl. Bacteriol.* **29**, 596–606
- Coates, M. E., Fuller, R., Harrison, G. F., Lev, M. & Suffolk, S. F. (1963) *Br. J. Nutr.* **17**, 141–150
- Coates, M. E., Ford, J. E., Gregory, M. E. & Thompson, S. Y. (1969) *Lab. Anim.* **3**, 39–49
- Cook, R. H. & Bird, F. H. (1973) *Poult. Sci.* **52**, 2276–2280
- Furuse, M. & Yokota, H. (1984) *Br. Poult. Sci.* **25**, 429–439
- Furuse, M. & Yokota, H. (1985) *Br. Poult. Sci.* **26**, 97–104
- Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719–723
- Gordon, H. A. (1968) in *The Germ-Free Animal in Research* (Coates, M. E., Gordon, H. A. & Wostmann, B. S., eds.), pp. 127–150, Academic Press, New York
- Gordon, H. A. & Bruckner-Kardoss, E. (1961) *Acta Anat.* **44**, 210–225
- Keily, S. D. & Abramoff, P. (1969) *J. Immunol.* **102**, 1058–1063
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Munro, H. N. & Fleck, A. (1969) in *Mammalian Protein Metabolism* (Munro, H. N., ed.), vol. 3, pp. 443–525, Academic Press, New York and London
- Muramatsu, T. & Okumura, J. (1985) *J. Nutr.* **115**, 483–490
- Muramatsu, T., Coates, M. E., Hewitt, D., Salter, D. N. & Garlick, P. J. (1983) *Br. J. Nutr.* **49**, 453–462
- Muramatsu, T., Salter, D. N. & Coates, M. E. (1985) *Br. J. Nutr.* **54**, 131–145
- Muramatsu, T., Aoyagi, Y., Okumura, J. & Tasaki, I. (1987) *Br. J. Nutr.* **57**, 269–277
- Pomposelli, J. J., Palombo, J. D., Hamawy, K. J., Bristrian, B. R., Blackburn, G. L. & Moldawer, L. L. (1985) *Biochem. J.* **226**, 37–42
- Ranken, R., Wilson, R. & Bralmer, P. M. (1971) *Proc. Soc. Exp. Biol. Med.* **138**, 270–272
- Reyniers, J. A., Wagner, H., Luckey, T. D. & Gordon, H. A. (1960) in *Lobund Reports* (Ervin, R. F., Gordon, H. A. & Wagner, M., eds.), no. 3, pp. 7–171, University of Notre Dame Press, Indiana
- Rolls, B. A., Turvey, A. & Coates, M. E. (1978) *Br. J. Nutr.* **39**, 91–98
- Sakata, T. & Yajima, T. (1984) *Q. J. Exp. Physiol.* **69**, 639–648
- Sato, K., Nagai, H. & Kai, O. (1986) *Jpn. Poult. Sci.* **23**, 91–96 (in Japanese)
- Talal, N. (1966) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **25**, 439
- Visek, W. J. (1974) *J. Agric. Food Chem.* **22**, 174–184
- Wostmann, B. S. (1968) in *The Germ-Free Animal in Research* (Coates, M. E., Gordon, H. A. & Wostmann, B. S., eds.), pp. 192–209, Academic Press, New York
- Yokota, H., Furuse, M., Okumura, J. & Tasaki, I. (1984) *Jpn. J. Zootech. Sci.* **55**, 600–603

Received 3 March 1987/11 May 1987; accepted 21 May 1987