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Enhanced whole-body protein synthesis by methionine and arginine supplementation in protein-starved chicks*

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1. The effect of supplementing with methionine alone or in combination with arginine on whole-body protein synthesis and degradation was studied in protein-starved chicks, fed on a protein-free (PF) diet, by a massive-dose injection of L-[4-^{3}H]phenylalanine.

2. Methionine or methionine and arginine (MA) supplementation reduced body-weight loss and improved N balance compared with unsupplemented controls.

3. Whole-body protein synthesis was significantly increased both in terms of fractional rate and absolute amounts by methionine and MA addition, whereas the fractional degradation rate was unchanged.

4. No significant difference was found between methionine and MA supplementation except for energy balance of the birds.

5. It was concluded that the N-sparing effect of methionine or MA when added to a PF diet was primarily brought about by enhanced whole-body protein synthesis.

The nitrogen-sparing effect of methionine, as shown by reduced body-weight loss and improved N balance when added to a protein-free (PF) diet, was first indicated in dogs (Miller, 1944), and later found in rats (Brush *et al.* 1947) and pigs (Lubaszewska *et al.* 1973). A similar N-sparing effect was also found in chickens by supplementing a PF diet with methionine as the only source of N (Okumura & Muramatsu, 1978). This effect of methionine was fortified by simultaneous addition of arginine but not by any of the other amino acids (Muramatsu & Okumura, 1979*a*). Among the nitrogenous compounds in urine of protein-depleted chickens, only uric acid excretion was reduced by methionine and arginine (MA) addition (Muramatsu & Okumura, 1979*b*). Furthermore, Muramatsu & Okumura (1979*c*) found that among individual tissues skeletal muscle showed greatest alleviation of N loss on supplementation with MA, almost entirely accounting for the total N spared in the whole body (Muramatsu & Okumura, 1979*c*). The addition of MA did not influence the energy balance of the birds subjected to protein starvation when an equal amount of dietary energy was given (Muramatsu & Okumura, 1980).

Although a series of experiments was conducted in the chicken, as stated previously, the mechanism by which the N-sparing effect of MA is brought about has not been clarified. There was an indication, however, from the work of Muramatsu & Okumura (1979*c*) that modified protein-turnover rates, i.e. enhanced protein synthesis or reduced protein degradation, or both, particularly in muscle, would be responsible for bringing about the N-sparing effect of MA.

Decreased protein catabolism and possibly increased protein synthesis in rat liver and muscle were suggested when small amounts of amino acids were added to a PF diet (Yokogoshi *et al.* 1974, 1976; Yokogoshi & Yoshida, 1979). However, hard evidence on this point in chickens was not available until more recent studies which showed that MA supplementation increased protein synthesis in jejunal mucosa and possibly in liver (Muramatsu *et al.* 1983) and, to a considerable extent, in breast muscle (Muramatsu *et al.*

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1985). If this were the case, an elevation of protein synthesis might also occur at the whole-body level by MA addition, resulting in an improved re-utilization of endogenously formed amino acids that originate from body protein degradation. In fact, the existing evidence seems to support the possible diversion of whole-body amino acid flux in favour of protein synthesis resulting in N conservation, since amino acid oxidation was drastically reduced by MA supplementation in protein-depleted birds (Muramatsu *et al.* 1985). No direct measurement, however, has been attempted to test the possibility of changes in whole-body protein turnover by MA supplementation.

In the present study, therefore, whole-body protein turnover rate was measured in vivo to study the effect of MA supplementation in protein-starved chicks. The results showed an enhancement by the addition of methionine alone and in combination with arginine, both in protein synthesis and degradation rates (the former to a greater extent), thereby bringing about the N-sparing effect.

MATERIALS AND METHODS

Single-comb White Leghorn male chicks (1-d-old) were raised in groups on a practical chick mash until 14 d of age, distributed into three experimental groups of nine birds so that the average body-weight was as even as possible, and thereafter kept individually in metabolism cages for 10 d. Ambient temperature was controlled at $29 \pm 2°$ and light was provided continuously. During the experimental period, the birds were allowed free access to experimental diets and water. Food consumption and body-weight were recorded on alternate days. For the last 2 d, droppings were collected in 150 ml 0·05 M-hydrochloric acid so that N balance of the birds could be measured. The composition of the PF diet, having a calculated metabolizable energy value of 14·5 kJ/g, was as follows (g/kg): maize starch 671·6, sucrose 200, maize oil 30, cellulose 30, mineral mixture (Muramatsu & Okumura, 1985) 64·9, vitamin mixture in glucose (Muramatsu & Okumura, 1980) 2·0, choline chloride 1·5. The addition of L-methionine (5 g/kg) alone or in combination with L-arginine hydrochloride (2 g/kg) was done at the expense of maize starch.

On the last day, whole-body protein synthesis rate was determined in vivo by the method of Garlick *et al.* (1980), depending on the measurement of radioactivity in free and protein-bound phenylalanine of the whole carcass after injection of a massive dose of labelled phenylalanine. Radioactive L-[4-^{3}H]phenylalanine (23·5 Ci/mmol, Amersham International Ltd, Amersham) was used, and the isotope was combined with unlabelled L-phenylalanine to give 40 μCi and 120 μmol/ml physiological saline (8·5 g sodium chloride/l) and injected into a wing vein at a dose of 10 ml/kg body-weight. At 2 and 10 min after the injection, four and five birds respectively were killed by neck dislocation and their abdominal cavities were opened quickly. The whole carcass was frozen as soon as possible by plunging into liquid N_2 and was stored at $-20°$ until analysis.

Calculation of the fractional synthesis rate (FSR) was done using the formula described by McNurlan *et al.* (1979). The fractional growth rate (FGR) was estimated by retained protein from N balance values for 2 d before the injection and the amount of the whole-body protein at day 10. The fractional degradation rate (FDR) was then calculated as the difference between FSR and FGR.

The frozen carcass was minced with a meat grinder, which was previously cooled with solid carbon dioxide, and was frozen again with liquid N_2. This mincing procedure was repeated three times to get homogenous samples of the whole carcass. Carcass protein was calculated as N × 6·25, which was determined by a Kjeldahl method. Carcass RNA content was determined by a modified Schmidt–Thannhauser method as described by Munro & Fleck (1969). The specific radioactivity of free and protein-bound phenylalanine was

determined by the method of Garlick *et al.* (1980). Carcass fat was extracted overnight with diethyl ether using a Soxhlet apparatus and determined gravimetrically. Retained energy was calculated by using the values 39·12 and 23·68 kJ/g for fat and protein retained respectively (Davidson *et al.* 1957). The amino acid flux (Q) was estimated from the following equation:

$$Q = I + D = S + O,$$

where I, D, S and O are protein intake, protein degradation, protein synthesis and amino acid oxidation respectively (Waterlow *et al.* 1978). According to this model, the ratio, protein synthesis:flux (S/Q) should represent or be close to the re-utilization rate of the amino acid, phenylalanine in the present study, since I contributes only a small portion to the flux under the present condition of protein starvation and since it is assumed that no distinction is made in the free amino acid pool between amino acids originating from food and those originating from body protein degradation.

Analysis of variance was carried out to assess the significance of the effect of amino acid supplementation, and Student's t test was used to assess the significance of differences between means (Snedecor & Cochran, 1980).

RESULTS

Table 1 gives the values for body-weight change, food intake, N excretion, N balance and energy balance. Body-weight loss was significantly reduced by supplementing with methionine alone, and simultaneous addition of arginine further, but not significantly, reduced body loss of the birds subjected to protein starvation. Food intake was increased by supplementing with methionine alone or in combination with arginine compared with unsupplemented controls. Significant reduction in N excretion (and thereby improved N balance) by methionine supplementation was found, but no further significant change was detected by the addition of MA. Methionine supplementation improved energy balance of the protein-starved birds fed on the PF diet, and concomitant addition of MA had a still higher value than methionine alone.

The values for FSR, FGR and FDR of whole-body protein are shown in Table 2. The FSR was significantly enhanced by methionine or MA addition compared with unsupplemented birds. The response of FGR was in parallel with FSR, i.e. increased by methionine or MA addition, whereas the enhancement of FDR by methionine or MA supplementation was not statistically significant.

Whole-body protein turnover is expressed as absolute rates in Table 3 together with the amino acid flux and the ratio, synthesis:flux. Both absolute synthesis and degradation rates were significantly lower in the PF group than in the methionine and MA groups. The same was true for the flux value. The ratio, whole-body protein synthesis:flux, which could be considered as the re-utilization rate of endogenously-formed amino acids, ranged from 0·82 to 0·90, indicating a very high efficiency of amino acids recycling under the present condition of protein depletion, and this value was significantly increased by methionine or MA supplementation. However, no difference was found between birds fed on the methionine- and MA-supplemented diets.

As an indicator of protein synthesis, total RNA content, the ratio, RNA:protein and amounts of protein synthesis per unit RNA in the whole-body are presented in Table 4. It was shown that RNA content and the ratio, RNA:protein were not affected by supplementation with methionine alone or MA, whereas the amount of protein synthesis per unit RNA was significantly higher in the methionine and MA groups than in the PF group.

Table 1. *Effect of supplementation with methionine alone or in combination with arginine (MA) on body-weight change, food intake, nitrogen excretion, N balance and energy balance in chicks fed on a protein-free (PF) diet*

(Mean values for five birds except for the PF group being four)

Diet	Body-wt change (g/10 d)	Food intake (g/10 d)	N excretion (mg/2 d)	N balance (mg/2 d)	Energy balance (kJ/10 g)
Protein-free (PF)	$-31{\cdot}5^{c}$	$66{\cdot}5^{c}$	109^{a}	-99^{c}	-181^{c}
PF + methionine	$-20{\cdot}6^{d}$	$102{\cdot}6^{d}$	83^{b}	-71^{d}	60^{d}
PF + MA	$-18{\cdot}4^{d}$	$113{\cdot}2^{d}$	84^{b}	-60^{d}	118^{e}
SEM (11 df)	1·5	5·9	6	5	10

Initial body-weight of chicks was 117 g for all groups.

[a, b, c, d, e] Means within the same column not sharing a common superscript letter were significantly different: [a, b] $P < 0{\cdot}05$, [c, d, e] $P < 0{\cdot}01$.

Table 2. *Effect of supplementation with methionine alone or in combination with arginine (MA) on fractional turnover rates of protein in the whole-body of chicks fed on a protein-free (PF) diet*

(Mean values for five birds except for the PF group being four)

	Fractional rate		
Diet	Synthesis (%/d)	Growth (%/d)	Degradation (%/d)
Protein-free	$11{\cdot}0^{b}$	$-2{\cdot}2^{b}$	$13{\cdot}2^{a}$
PF + methionine	$13{\cdot}5^{c}$	$-1{\cdot}4^{c}$	$14{\cdot}8^{a}$
PF + MA	$13{\cdot}6^{c}$	$-1{\cdot}1^{c}$	$14{\cdot}6^{a}$
SEM (11 df)	0·6	0·1	0·7

[a, b, c] Means within the same column not sharing a common superscript letter were significantly different: [b, c] $P < 0{\cdot}01$.

Table 3. *Effect of supplementation with methionine alone or in combination with arginine (MA) on amounts of protein synthesized and degraded, amino acid flux and the ratio, synthesis : flux in the whole body of chicks fed on a protein-free (PF) diet*

(Mean values for five birds except for the PF group being four)

	Absolute rate			
Diet	Synthesis (*S*) (g/d)	Degradation (g/d)	Flux (*Q*) (g/d)	*S*:*Q*
Protein-free	$1{\cdot}57^{a}$	$1{\cdot}88^{a}$	$1{\cdot}91^{a}$	$0{\cdot}82^{a}$
PF + methionine	$2{\cdot}29^{b}$	$2{\cdot}52^{b}$	$2{\cdot}55^{b}$	$0{\cdot}89^{b}$
PF + MA	$2{\cdot}42^{b}$	$2{\cdot}61^{b}$	$2{\cdot}68^{b}$	$0{\cdot}90^{b}$
SEM (11 df)	0·09	0·11	0·13	0·005

[a, b] Means within the same column not sharing a common superscript letter were significantly different: $P < 0{\cdot}01$.

Table 4. *Effect of supplementation with methionine alone or in combination with arginine (MA) on RNA content, the ratio, RNA:protein and amounts of protein synthesis per unit RNA in the whole body of chicks fed on a protein-free (PF) diet*

(Mean values for five birds except for the PF group being four)

Diet	RNA (mg)	RNA:protein (mg/g)	Protein synthesized:RNA (g/g per d)
Protein-free	242[a]	17·0[a]	6·5[a]
PF+methionine	252[a]	15·0[a]	8·9[b]
PF+MA	280[a]	15·7[a]	8·8[b]
SEM (11 df)	14	0·8	0·6

[a, b] Means within the same column and sharing a common superscript letter were significantly different: $P < 0·05$.

DISCUSSION

It was previously suggested that supplementation with MA might modify protein turnover rates of protein-starved chickens especially in muscle, thereby bringing about the N-sparing effect (Muramatsu & Okumura, 1979*c*). In jejunal mucosa and possibly in liver, protein synthesis was increased by MA addition compared with unsupplemented controls (Muramatsu *et al.* 1983). McNurlan & Garlick (1980) emphasized a major contribution of liver and gastrointestinal tract to whole-body protein synthesis in rats. Garlick *et al.* (1975) have also suggested that in rats only 19% of whole-body protein synthesis would be accounted for by skeletal muscle. However, in protein-starved chicks, the contribution of skeletal muscle to the amount of N spared in the whole body by MA supplementation was high, accounting for approximately 80% (Muramatsu & Okumura, 1979*c*). Accordingly, the enhanced protein synthesis found in skeletal muscle by MA addition (Muramatsu *et al.* 1985) should be reflected in increased protein synthesis at the whole-body level. Indeed, the results from Tables 2 and 3 clearly indicate that protein synthesis in terms of both fractional rates and absolute amounts were significantly elevated by the addition of methionine alone or in combination with arginine. Although the amounts of whole-body protein degradation increased on the supplemented diets, FDR values were not significantly affected. This was somewhat different from the decreased protein catabolism reported in muscle and liver of rats by supplementing a PF diet with small amounts of amino acids (Yokogoshi *et al.* 1974, 1976), but the results obtained by isotope disappearance from tissues could be difficult to interpret due to considerable recycling of isotope. Problems associated with the measurement of protein turnover have been discussed elsewhere in detail (Waterlow *et al.* 1978). It was concluded, therefore, that enhanced whole-body protein synthesis would be primarily, if not completely, responsible for the resultant reduced N excretion, and hence N spared by methionine or MA supplementation in the whole body of protein-starved chicks.

The results in Table 3 also show an increase in protein synthesis:flux, suggesting an improved re-utilization rate of endogenously-formed amino acids originating from body protein degradation since the contribution of dietary amino acids under the conditions of the present study was negligible in comparison with the total flux. This is in agreement with the reduced oxidation of a labelled amino acid in vivo following MA addition in protein-deprived birds (Muramatsu *et al.* 1985). The reason for improvement in the re-utilization remains unclear. Muramatsu & Okumura (1979*c*) found that feather proteins, of which cyst(e)ine and arginine contents are relatively higher than those in other tissues (Mitchell, 1959), were still growing in protein-deprived birds. Consequently, during protein

starvation, the bird might require more sulphur-containing amino acids and arginine than other amino acids. This increased requirement for supporting feather growth could be met at the expense of tissue protein degradation, skeletal muscle probably being the major source (Muramatsu & Okumura, 1979*c*). Although there may be some other unknown reasons for the high needs of S-containing amino acids and arginine, dietary supply of these amino acids would improve the amino acid pattern from the endogenous source, becoming more suitable for re-synthesis of body proteins including feather proteins, which could in turn reduce wasteful N in other unusable amino acids. Thus, the supplementation with methionine alone or in combination with arginine would spare not only the N in these amino acids but also N in other amino acids that were formed endogenously. The reduced N may most probably be accounted for by reduction in uric acid-N excretion, as found by Muramatsu & Okumura (1979*b*).

Laurent *et al.* (1978) suggested that both the ratio, RNA:protein and the amount of protein synthesis per unit RNA would be important determinants of FSR. This led us to measure RNA content and its relation to protein or protein synthesized per unit RNA in the whole body. The results in Table 4 show that no significant difference was found in either RNA content or RNA:protein. In contrast, the amount of protein synthesized per unit RNA in the whole body was significantly accelerated by supplementing with methionine alone or in combination with arginine as found in skeletal muscle (Muramatsu *et al.* 1985). Therefore, in the present situation, enhanced efficiency of amino acid translation as suggested by the increased amount of protein synthesis per unit RNA appears to be important in bringing about an increased FSR on methionine or MA supplementation. The present finding was in line with improved hepatic polyribosome aggregation which was found in rats by supplementing a PF diet with small amounts of amino acids (Yokogoshi & Yoshida, 1979).

The energy balance of birds fed on a PF diet was improved by methionine addition, and still further by MA supplementation (Table 1). This improvement would most probably be attained by increased food consumption since it was found that when the birds were given an equal amount of dietary energy supply, addition of MA did not improve energy retention of protein-starved birds (Muramatsu & Okumura, 1980). When birds were allowed free access to the diet, increased food consumption was frequently observed by supplementing a PF diet with MA (Muramatsu & Okumura, 1979*a–c*).

In the present study, the effect of supplementing with methionine alone was compared with the simultaneous addition of MA. However, no significant difference was found in any measurements made except for energy balance, although in general the combined addition of methionine and arginine tended to give better performance than did methionine alone. This was probably due to the fact that the supplemented level of methionine chosen in the present experiment, 5 g/kg, was so high that it might have masked a marginal fortifying effect of arginine which was found with 3 g methionine/kg (Muramatsu & Okumura, 1979*a*). The role of arginine in the N-sparing effect remains to be studied.

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