EFFECT OF LYSINE AND LYSINE PLUS THREONINE SUPPLEMENTS TO RICE AND WHEAT PROTEIN\(^1,2\)

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The effect of lysine or lysine and threonine as supplements to rice and wheat diets at three different protein levels (5.5, 11.0 and 15.0%) was studied in growing rats with initial body weights of 68 g in experiment I and 55 g in experiment II. The following results were obtained: At the 5.5% protein level addition of lysine alone to the wheat diet had no effect on the growth of the rats, but there was significant effect of lysine plus threonine. At the same level of protein, addition of lysine increased the growth of rats receiving the rice diet, and the effect of threonine with lysine was more significant. At the 15% protein level, addition of lysine had a significant effect on the growth of rats receiving both rice and wheat diets, but no effect of threonine with the wheat+lysine or rice+lysine diet was observed. With 11% protein level, addition of threonine to the lysine-supplemented rice diet had little effect on the growth of rats, whereas it caused a marked improvement in the growth of rats on the wheat diet. It was also observed that the body weight gain or changes in body water correlated well with the lysine intake of rats receiving nonsupplemented as well as those with supplemented rice and wheat diets.

It is well known that lysine and threonine are, respectively, the first and second limiting amino acids in cereal proteins. Numerous papers on the effect of lysine and threonine supplements to wheat and rice diets have already been reported. Most of these studies have been conducted at protein levels of 10–13% in the wheat diet\(^{(1, 2)}\) and of 5–6% in the rice diet\(^{(3, 4)}\), since the diets were prepared, respectively, from wheat flour or bread backed with wheat flour and rice meal with fat, minerals, and vitamin mixture added.

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Since the nutritional response of proteins varies depending on the protein level of the diet, the effect of lysine and threonine supplements to the cereal protein would differ with protein levels in the diet.

Therefore, protein concentrates were used to investigate the effect of lysine alone and lysine plus threonine as supplements to the rice or wheat proteins at three levels of protein, 5.5, 11.0, and 15.0%, in growing rats, following our previous experiment(5) in which three protein levels, 5.5, 11.0, and 19.0%, of the rice or wheat diets were compared.

EXPERIMENTAL

Animals and Diets. Male rats of the Donryu strain were used. The average initial body weight was 68 g in experiment I and 55 g in experiment II. Animals were fed the rice and wheat diets ad libitum for 20 days in experiment I and for 14 days in experiment II. Each group consisted of 6–8 rats. During the experimental period, the food intake and the body weight were measured about every other day. Compositions of the rice and wheat diets for the 9 groups fed each protein are shown in Tables 1 and 2. The amounts of lysine and threonine supplied represented the difference between the content of these amino acids in the wheat diet and the ideal level according to MIYAZAKI's pattern(6) investigated with growing rats. Glutamic acid was supplied to the nonsupplemental groups and the lysine groups to make the three groups at each protein level isonitrogenous.

The concentrated rice protein was prepared(5, 7) from rice meal treated with crystalline or liquid protease-free α-amylase. The crystalline α-amylase was purchased from Seikagaku Kogyo, Tokyo. The liquid protease-free α-amylase was donated by Ueda Kagaku, Osaka. Glutene was kindly donated by Shinshin Shokuryo, Tokyo.

Experiment I. At each protein level (5.5, 11.0, and 15.0%) of the rice and wheat diets, the nonsupplemented, lysine, and lysine plus threonine groups were compared with respect to food intake and body weight gain for 20 days. The protein efficiency ratio (PER) in each group was calculated. Within each group at the same protein level significant differences were found between the nonsupplemented and lysine groups and between the lysine and lysine plus threonine groups.

Experiment II. In the rice and wheat diets containing 11.0% protein, the nonsupplemented, lysine, and lysine plus threonine groups were compared as described in experiment I. In addition, at the end of this experiment, the animals were killed by anesthesia with ether. The contents of the gut were removed completely, and carcasses were frozen and kept until analysis.

Analytical procedures. The frozen carcasses (only in experiment II) were chopped into slices, put into a weighed beaker, and dried in an oven at a temperature of 100±5°C for several days to obtain the body water content. The dried samples were ground and used for other experimental purposes.
EFFECT OF AMINO ACIDS ADDED TO CEREAL

Table 1. Composition of rice diet (%).

| Constituents          | 5.5  | 11.0 | 15.0 | Group    |
|-----------------------|------|------|------|----------|
|                       | I    | II   | III  | IV      | V       | VI     | VII    | VIII   | IX      |
| Rice                  | 88.9 | 63.4 | 43.9 |         |         |        |        |        |         |
| Starch                | —    | —    | —    |         |         |        |        |        |         |
| Conc. rice\(^a\)      | —    | 25.5 | 45.0 |         |         |        |        |        |         |
| Corn Oil              | 5.0  | 5.0  | 5.0  |         |         |        |        |        |         |
| Salt mix.             | 4.0  | 4.0  | 4.0  |         |         |        |        |        |         |
| Vitamin mix.          | 2.0  | 2.0  | 2.0  |         |         |        |        |        |         |
| Choline-HCl           | 0.1  | 0.1  | 0.1  |         |         |        |        |        |         |
| L-Lysine-HCl          | —    | 0.24 | 0.48 | 0.66    | 0.66    |        |        |        |         |
| L-Threonine           | —    | 0.12 | 0.24 | 0.33    |         |        |        |        |         |
| L-Glu. A.             | 0.54 | 0.15 | 1.05 | 1.46    | 0.40    |        |        |        |         |
| Total lysine (%)      | 0.19 | 0.38 | 0.37 | 0.50    | 1.03    | 1.03   |        |        |         |
| Total threonine (%)   | 0.21 | 0.33 | 0.65 | 0.56    | 0.56    | 0.89   |        |        |         |

\(^a\) Protein: 27.0\%  
Chocola A: 0.05 ml/100 g diet were added to supply V.A 1500 I.U. and V.D 150 I.U.

Table 2. Composition of wheat diet (%).

| Constituents          | 5.5  | 11.0 | 15.0 | Group    |
|-----------------------|------|------|------|----------|
|                       | I    | II   | III  | IV      | V       | VI     | VII    | VIII   | IX      |
| Wheat                 | 42.6 | 85.9 | 82.5 |         |         |        |        |        |         |
| Starch                | 46.3 | 3.0  | —    |         |         |        |        |        |         |
| Gluten\(^a\)          | —    | —    | 6.4  |         |         |        |        |        |         |
| Corn oil              | 5.0  | 5.0  | 5.0  |         |         |        |        |        |         |
| Salt mix.             | 4.0  | 4.0  | 4.0  |         |         |        |        |        |         |
| Vitamin mix.          | 2.0  | 2.0  | 2.0  |         |         |        |        |        |         |
| Choline-HCl           | 0.1  | 0.1  | 0.1  |         |         |        |        |        |         |
| L-Lysine-HCl          | —    | 0.24 | 0.48 | 0.66    | 0.66    |        |        |        |         |
| L-Threonine           | —    | 0.12 | 0.24 | 0.33    |         |        |        |        |         |
| L-Glu. A.             | 0.54 | 0.15 | 1.05 | 1.46    | 0.40    |        |        |        |         |
| Total lysine (%)      | 0.12 | 0.31 | 0.25 | 0.34    | 0.87    | 0.87   |        |        |         |
| Total threonine (%)   | 0.15 | 0.27 | 0.31 | 0.42    | 0.42    | 0.75   |        |        |         |

\(^a\) Protein: 69.4\%  
Chocola A: 0.05 ml/100 g diet were added to supply V.A 1500 I.U. and V.D 150 I.U.

RESULTS

Body weight changes and food intake

Body weight changes and food intake of rats fed the unsupplemented and supplemented rice and wheat diets at the 5.5, 11.0, and 15.0\% protein levels in experiment I are shown in Figs. 1, 2, and 3, respectively. The results of experi-
Fig. 1. Body weight changes and food intake (5.5% Prot. and Suppl. AAs).

Fig. 2. Body weight changes and food intake (11% Prot. and Suppl. AAs).
Fig. 3. Body weight changes and food intake (15% Prot. and Suppl. AAs).

Fig. 4. Body weight changes and food intake (11% Prot. and Suppl. AAs).
In the rice diet, some effect of lysine supplement on body weight gain was observed at 5.5% \((P<0.05)\), 11.0% \((P<0.05\) in Expt. I and \(P<0.005\) in Expt. II) and 15.0% \((P<0.01)\) protein levels. In the wheat diet some effect of lysine supplement on the body weight gain was observed at the 11.0% \((P<0.01\) in Expt. I and \(P<0.005\) in Expt. II) and 15.0% \((P<0.01)\) protein levels, but not at the 5.5% level.

Some effect of threonine added to the rice+lysine diet on body weight gain was observed at the 5.5% protein level \((P<0.01\) in Expt. I), but there was little effect at the 11.0% protein level \((P<0.05\) only in Expt. II). On the other hand, supplemental threonine in the wheat+lysine diet affected body weight gain at the 5.5 and 11.0% protein levels \((P<0.01\) and \(P<0.01\), respectively, in Expt. I, \(P<0.005\) in Expt. II).

**Protein efficiency ratio**

The values found in experiment I for protein efficiency ratio (PER) of the rice and wheat diets at all protein levels, and the same diets with lysine or lysine and threonine supplementation are shown in Table 3. PER obtained in experiment II in the rice and wheat diets are shown in Table 4. From Table 3, improvement of PER in the rice diet with 5.5% protein by addition of 0.24% lysine-

| Diet   | Protein % | Supplement of | PER       | Sig.     |
|--------|-----------|---------------|-----------|----------|
|        |           | Lysine-HCl % | Threonine % |         |
| Rice   | 5.5       |              |           |          |
|        | 0.24      |              |           |          |
|        | 0.24      | 0.12         |           |          |
| Rice   | 11.0      |              |           |          |
|        | 0.48      |              |           |          |
|        | 0.48      | 0.24         |           |          |
| Rice   | 15.0      |              |           |          |
|        | 0.66      |              |           |          |
|        | 0.66      | 0.33         |           |          |
| Wheat  | 5.5       |              |           |          |
|        | 0.24      |              |           |          |
|        | 0.24      | 0.12         |           |          |
| Wheat  | 11.0      |              |           |          |
|        | 0.48      |              |           |          |
|        | 0.48      | 0.24         |           |          |
| Wheat  | 15.0      |              |           |          |
|        | 0.66      |              |           |          |
|        | 0.66      | 0.33         |           |          |

Average initial body weight of rats was 68 g. Fed for 20 days.
EFFECT OF AMINO ACIDS ADDED TO CEREAL

HCl ($P < 0.01$) is obvious. Addition of 0.12\% threonine to the rice+lysine diet further improved PER ($P < 0.01$). At the 11.0\% level of rice protein, supplement of 0.48\% lysine-HCl improved PER ($P < 0.05$ in Expt. I as shown in Table 3 and $P < 0.005$ in Expt. II as shown in Table 4), and an additional supply of 0.24\% threonine increased PER further ($P < 0.01$ in Expt. I and $P < 0.05$ in Expt. II).

Table 4. PER of rice and wheat diet (Expt. II).

| Diet  | Protein % | Supplement of | PER       | Sig.     |
|-------|-----------|---------------|-----------|----------|
|       |           | Lysine-HCl %  | Threonine % |         |
| Rice  | 11.0      | —             | —         | 2.25±0.21 | $P < 0.005$ |
|       | 11.0      | 0.48          | —         | 3.07±0.28 | $P < 0.05$    |
|       | 11.0      | 0.48          | 0.24      | 3.58±0.18 |             |
| Wheat | 11.0      | —             | —         | 0.73±0.24 | $P < 0.005$ |
|       | 11.0      | 0.48          | —         | 1.90±0.32 | $P < 0.005$ |
|       | 11.0      | 0.48          | 0.24      | 2.63±0.39 |             |

Average initial body weight was 55 g. Fed for 14 days.

On the wheat diet at the 5.5\% protein level, the rats failed to grow, and no improvement was observed with the addition of lysine, but PER was significantly improved ($P < 0.01$) with the addition of 0.24\% lysine-HCl together with 0.12\% threonine. At the 11.0\% protein level the lysine supplement had a significant effect on PER ($P < 0.01$ in Expt. I, $P < 0.005$ in Expt. II), and on addition of threonine to the wheat+lysine diet improvement of PER was also significant ($P < 0.01$ in Expt. I, $P < 0.005$ in Expt. II).

Relationship between lysine intake and body weight gain or changes in body water

Figure 5 shows the relationship between lysine intake and body weight gain of individual rats for nonsupplemented groups at all protein levels and includes data obtained in the previous experiment(5) at 5.5, 11.0, and 19.0\% protein levels. It shows that the body weight gain correlated well with the lysine intake with $r = 0.962$. Correlation between lysine intake and body weight gain of individual rats in experiment I was also observed with $r = 0.910$ for the 5.5 and 11.0\% protein levels, including groups with supplemental lysine plus threonine, and 15\% protein of rice and wheat diets (except the latter supplemented groups). The relationship between lysine intake and changes in body water for all rats in experiment II and for rats receiving only the lysine plus threonine supplied diets and basal rice and wheat diets was calculated, since changes in body water and body weight gain in the experiment correlated with $r = 0.922$ and are also cited in literature(8, 9). As seen in Fig. 6 for individual rats on the rice and wheat diets and the supplemented diets with lysine plus threonine, correlation between the lysine intake and changes in body water could be observed with $r = 0.938$, which was higher than that calculated for all rats including the supplemented lysine diets ($r = 0.909$).
Fig. 5. Changes in body weight and lysine intake (non-supplemental groups).

Fig. 6. Changes in body weight and lysine intake (Exp. II).
DISCUSSION

In our previous studies comparing nutritional qualities of rice and wheat protein(5), we observed that differences in nutritional value of rice and wheat protein are greater than those mentioned in the literature. The present experiments were designed to observe effects of lysine alone or lysine and threonine as supplements to the rice and wheat proteins at three different protein levels. At the low protein level, 5.5%, the effect of threonine supplement on the growth of rats fed the rice+lysine or wheat+lysine diet was prominent. However, the effect of threonine supplement was also observed on a casein diet with a 5.5% protein level in our previous study(10). Therefore, the effect of threonine on the low protein diet is not only to improve the cereal protein quality, but also generally to promote the growth of rats on low protein diets. On the other hand, the effect of threonine supplement on growth of rats fed the wheat+lysine diet with 11.0% protein was statistically significant, this was not the case for the rice+lysine diet at the same protein level.

Although the threonine supplement did not improve the growth of rats receiving the 11.0% rice diet, it did have an influence on the PER. This effect was greater in experiment I, where food intake in the threonine-lysine group was relatively low, than in experiment II ($P<0.01$ and $P<0.05$, respectively). From the results of the two experiments it may be concluded that the supplement of threonine had a greater effect on the wheat+lysine diet than on the rice+lysine diet when both were fed at the 11% protein level.

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REFERENCES

1) Light, R. F. and Frey, C. N., Cereal Chem., 20, 645 (1943).
2) Rosenberg, H. R. and Rohdenberg, E. L., Arch. Biochem. Biophys., 37, 461 (1952).
3) Pecora, L. J. and Hundley, J. M., J. Nutr., 44, 101 (1951).
4) Rosenberg, H. R., Culk, R., and Eckert, R. E., J. Nutr., 69, 217 (1959).
5) Murata, K., Yamamoto, K., Ikeda, K., and Tanaka, T., Eiyo to Shokuryo (in Japanese), 24, 355 (1971).
6) Miyazaki, M., Amino Acid Research, 25, 89 (1965).
7) Murata, K., Amino Acid Research, 52, 3 (1971).
8) Hegsted, D. M., Raymond, N., and Worcester, J., J. Agr. Food Chem., 16, 190 (1968).
9) Said, A. K. and Hegsted, D. M., J. Nutr., 99, 474 (1970).
10) Murata, K., Miyagawa, K., Ikehata, H., and Otsuka, K., Eiyo to Shokuryo (in Japanese), 12, 159 (1959).