The Importance of Food Combination on Nutritional Efficiency and Liver Fat Contents in Rats

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Introduction

Rice is one of the most important food for Asian people, not only as calorie source, but also as protein source. The first limiting amino acid of rice protein is lysine, with its Protein Score 78. It has been considered theoretically that if rice is supplemented with lysine, its nutritive value will increase. But, it was made clear by Pecora & Hundley[2] in 1951 that effect of lysine supplementation on nutritional value of rice protein was little, in case of investigation for rats by using rice as the only protein source, and addition of lysine plus threonine improved it remarkably. This problem has then become an important subject of study in Japan as well. A lot of experiments about amino acids supplementation to rice have been repeated by many researchers,[1-3,13-16,18-20,22,23] and similar result has been obtained at the feeding test under relatively lower protein level (5–8%). On the other hand, the useful feeding test, using similar amino acids mixture in spite of rice protein, was performed by Sakurai & Miyazaki[24] and Yoshida & Ashida.[2-5] In these studies, it was observed that the addition of lysine alone improved the nutritional value of this amino acids mixture diet, but the further addition of threonine showed ineffective.

Since then, poor physiological availability of threonine in rice protein has long been in question.

Utility of rice protein

We tried to clarify this problem from the digestion point of view. At first, we experimented on artificial digestion of rice glutelin, which is the major protein of polished rice, by many kinds of protease in the mammal alimentary canal in order, and fractionated by many biochemical methods. About 24% of amino acids and 40% of threonine originated from glutelin sample were contained in the peptide fraction, its molecular weight was more than 500. Five kinds of peptide, those molecular weight was 1500–2000 and consisted of 12–15 amino acid residues, were found out in this peptide fraction (Fig. 1).[17] In those peptides, two kinds of peptides were more quantitatively than other peptides, and the existence of threonine in the neighborhood of N-terminal of these 2 peptides was confirmed (Fig. 2).

Later, it was found that the remaining quantity of these indigestible peptides was affected by a slight difference of pH value, within opt.-pH range, when pepsin reacts to them as the first step of artificial digestion. Optimum pH of pepsin lies normally between 1.5 and 2.0. But we observed on the chromatogram the marked decline of peptides containing threonine, when pepsin reacts rice protein at lower limit of its opt.-pH.[5] Fig. 1 shows the chromatogram of peptides from

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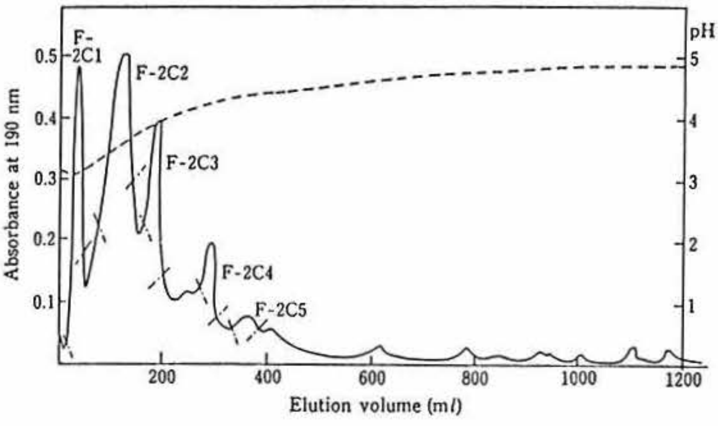

Fig. 1. Chromatogram of cation exchange resin column chromatography of F-2 H fraction
Resin: Aminex AG 50 W-X 2 (200–325 mesh), 0.8×100 cm.
Solvent: Pyridine-acetic acid buffer, pH 3 to pH 5 (gradient).
Detection: One hundredth volume of fractions dried and (diluted with water) was determined by UV absorption at 190 nm.
Elution speed: 49.8 ml/hr.

F-2C1: (NH_2)-Gly-Glu-Asp-Asp-
F-2C2: (NH_2)-Gly-Thr-Asp-Asp-Leu-
F-2C3: (NH_2)-Asp-Thr-Asp-Leu-

Fig. 2. Amino acid sequence of indigestible peptides from rice glutelin

digested solution, when pepsin reacts to rice protein at pH 2, followed by the artificial digestion by pancreatin, trypsin and erepsin. Peak 2 (F-2C2) and peak 3 (F-2C3) were peptides containing threonine. Fig. 3 shows the chromatogram when rice protein was digested at lower pH by pepsin and others in the same method as above. Decline of peak 2 and peak 3 was noticeable in comparison of Fig. 3 and Fig. 1.

Partially digested rice powder sample was prepared by pepsin reaction for 48 hr under strictly controlled condition of pH to 1.5, and then it was neutralized and spray-dried. On this prepared sample and nontreated rice powder as control, the feeding test on rats was experimented and compared. When lysine, the first limiting amino acid, was supplemented, significant difference of protein efficiency ratio (PER) was recognized between the two groups. PER was significantly high at the treated rice diet group. Thus we figured out difference of pH value in stomach at the time of rice intake may be a factor of controlling the utility of rice protein. On this

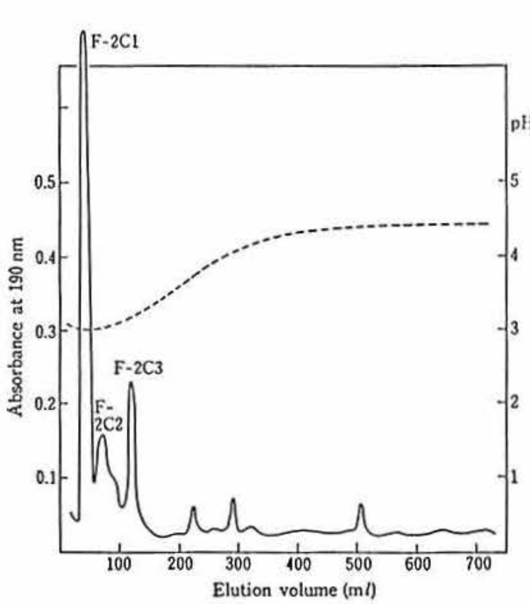

Fig. 3. Chromatogram of cation exchange resin column chromatography of F-2H fraction reacted with pepsin at lower pH.

Table 1. Protein efficiency ratio for 3 weeks period (2–4 weeks)[1)]

Groups[3)]	Initial weight(g)	Growth(g)	Food intake(g)	Protein intake(g)	PER[2)]
L-1t	56.8	107.7±7.7	369±25	23.3±1.6	4.62±0.10
L-1	57.3	40.8±4.0	200±22	12.2±1.4	3.34±0.14
L	57.4	29.3±6.5	215±31	12.6±1.8	2.32±0.18
K-1t	56.8	97.8±7.2	366±16	23.1±1.0	4.23±0.14
K-1	57.3	37.6±7.2	213±27	13.0±1.6	2.86±0.21
K	57.7	33.9±2.0	240±10	14.1±0.6	2.40±0.18
R-1t	56.8	86.0±8.2	332±19	21.0±1.2	4.09±0.15
R-1	57.4	38.8±3.8	216±10	13.2±0.6	2.93±0.21
R	57.3	34.8±8.9	248±38	14.6±2.2	2.37±0.29

1) Means ± SD of 6 rats.
2) Significantly different between 2 groups at $^{***}p<0.005$, $^{****}p\ll0.005$.
3) R: 90% rice, K: 90% rice+3% konnyaku mannan, L: 90% rice+4.5% soy lecithin, -1: +0.3% L-lysine-HCl, -1t: +0.3% L-lysine-HCl+0.2% L-threonine.

hypothesis, we made further experiments in pursuit of possibility of increasing nutrition efficiency of rice protein by applying various food combination.[6)]

Several diets were prepared. Control diet (R), consisting of 90% rice powder, 4% salts mixture, 1% fiber and rice starch, Lecithin diet (L) and Konnyaku diet (K), consisting of supplementation with 4.5% soy-lecithin or 3% konnyaku mannan respectively, besides these (R), (L), (K) basic diets, diets which were fortified further with 0.3% lysine-HCl or lysine plus 0.2% threonine were prepared, which made the total of 9 varieties in diet. Protein level of diets was 5.88% in basic diets. Kneaded diets with addition of vitamin mixture solution of controlled quantity and water were given to male rats, weighing 57 g, for 5 weeks ad libitum, to evaluate PER. The animals experimented were Wister-Imamichi strain rats, which were obtained by self-breeding. Table 1 shows protein intake and PER during the middle 3 weeks. No significant differences were recognized in food consumption and PER among the 3 basic groups. However, in the fortified diets with lysine, PER of lecithin group was evidently higher than the others at the level of 1%.[7)] After the feeding test, rats of each group were made fasted for 14 hr. Then fed to their full with the basic diets in a limited time. They seemed to fulfill their stomachs in between 5 and 10 min. After 1 hr, we removed the diet vessels and set this time to point 0. Rats were momentarily killed in a certain interval of time, by way of the cerebral dislocation method, stomach was taken out from each rat, and pH of the content in pylorus A and fundus (at the bottom of stomach) B.C. were measured by using a needle-electrode. The figures showed significant difference depending on which part of stomach pH was examined. In the rats which were fed with the konnyaku diet, pH of the content was 1.6 at pylorus, whereas in fundus, pH was 7 after 3 hr and 5.5 after 5 hr. As concerns pH of content in pylorus, slight differences were seen in each individual and also depending on presence or nonpresence of amino acids fortification. Yet in general, pH 2.6–3.0 on control groups, 1.9–2.3 on lecithin groups and as low as 1.6 on konnyaku groups were recorded.[8)] These data suggested that secretion of acid in stomach was promoted by supplementation of a small quantity of konnyaku mannan. Furthermore, our data suggested that not just pH but also protease activity in stomach affect the utility of rice protein. At that time, it was made clear by Furihata[4)] that there exist five other minor pepsinogen other than primary pepsinogen, which has 90% activity of total protease in rat stomach.

Table 2. Composition of diets[1)]

Components	Groups							
	E	RE	RSE	S	RS	R	R-1	R-1t
Rice	—	45	36	—	45	90	90	90(%)
Soybean	—	—	4.86	16.2	8.1	—	—	—
Egg yolk	18.4	9.2	5.52	—	—	—	—	—
L-Lysine-HCl	—	—	—	—	—	—	0.3	0.3
L-Threonine	—	—	—	—	—	—	—	0.2
Rice starch	76.6	40.8	48.62	78.8	41.9	5	4.7	4.5
Mineral mixture[2)]	4	4	4	4	4	4	4	4
Fiber	1	1	1	1	1	1	1	1
Protein level	5.95	5.94	5.94	5.95	5.93	5.92	6.16	6.36

1) Diets were supplemeted with vitamin mixture solution.
2) Johnson salts were used.

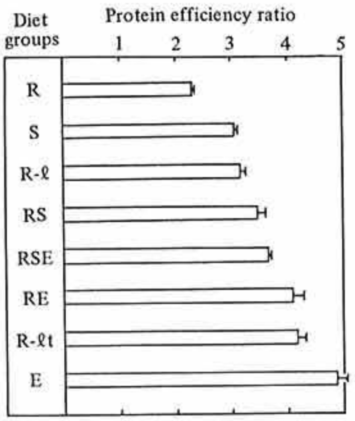

Fig. 4. Protein efficiency ratio for a period of 5 weeks Means ± SD of 6 rats

That is to say, the peptides containing threonine which remained indigested by primary pepsin, seem to be influenced by the other minor pepsin and utilized in vivo. Furthermore, supplementation of soy-lecithin not only promoted secretion of acid but also activated of these minor enzymes in the stomach. It is very interesting to realize that biological condition of stomach can be influenced by a small difference of food composition, and thereby utility of rice protein can be increased.[6)]

Next, we made experiments on PER, carcass constitution and pH of content in stomach when rice was fed together with soybean and egg, which are the food stuffs most common to Japanese people. We prepared 8 different diets with 6% protein level as shown in Table 2. These included diets, containing independently rice powder (R), whole soybean powder (S), egg yolk powder (E), diets containing rice and soybean (RS) or rice and egg yolk (RE) in 1 : 1 ratio by protein level, and rice-soybean-egg yolk (RSE) in 4 : 3 : 3 ratio, and fortified diets to (R) with amino acids. Using male rats, we performed feeding tests for a period of 5 weeks. PER are shown in Fig. 4. In the case of the diets rice or soybean alone, PER was as low as 2.33 for rice and 3.08 for soybean respectively. But, PER of the combined diets of rice with soybean was much higher indicating 3.48. This difference was significant at the level of 0.5%. Furthermore, PER of RS diet group was significantly higher than rice plus lysine group at the level of 2.5%. Therefore, it is satisfactory to consider that the improvement of PER in RS diet will result in not only mutual supplementation of limiting amino acids, but also the development of utility of rice protein itself, which is brought about by changing physiological conditions in stomach. Therefore, the nutritional efficiency ratio of rice in the actual diets appeared to be higher than the one estimated by rice alone.[9)]

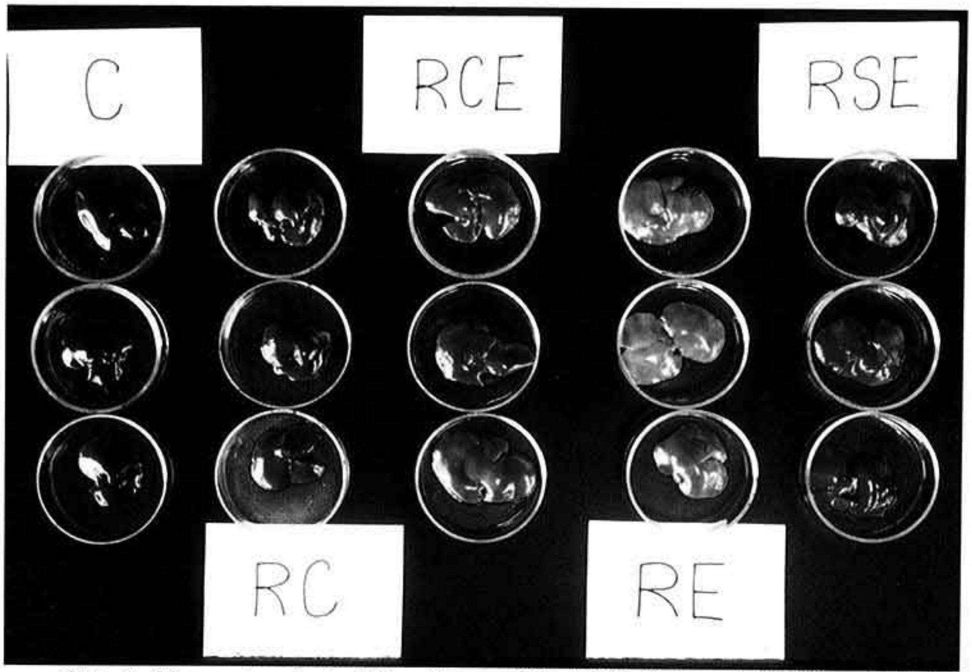

Plate 1 Liver color of rats fed various combined diets for a period of 3 weeks
C: Chick pea, E: Egg yolk, S: Soybean, R: Rice

Fat contents in rat liver

After the feeding test, the liver was removed and the carcass and liver were freeze-dried separately. The liver was analyzed for its crude fat content by the Soxhlet extraction method using ether. The carcass was pulverised and the crude fat content was determined by the same method. As it can be seen in Fig. 5, liver fat of the rats fed on rice and soybean diets was 13–20% level per dry matter, and it is considered normal. On the other hand, abnormal fatty liver containing 45% fat was observed in the male rats fed on the combined diet with rice and egg yolk at lower protein level. It was significantly higher than that caused by the diet of egg yolk alone (38%, $p<0.5\%$).[5] These facts were hard to be explained by the existing knowledge. But, it was observed that the addition of whole soybean flour to the combined diet with rice and egg yolk seems to produce the control effect against fatty liver because it gave liver fat of 22%. You can clearly see fatty liver of rats brought about by eating the combined diet with rice and egg yolk, and control effect by soybean in Plate 1. The liver of rat of on the combined diet was very soft like as "Tofu" or "Soy curd", making it difficult to handle, and appeared pale and unhealthy. But, the supplementation of whole soybean to diet recovers the healthy liver color. Chick pea is a popular legume in India, Pakistan, Bangladesh, Spain, Mexico and so on. This legume has the same effect as soybean to con-

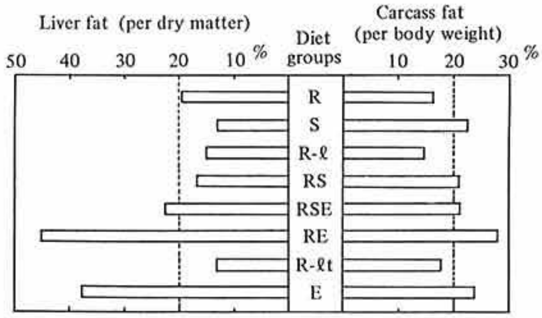

Fig. 5. Crude fat content in liver and carcass of rats fed various diets for a period of 5 weeks

Table 3. Proportion of liver fat[1)]

Groups	Liver fat (per dry matter)	Dry matter in liver	Raw liver / Whole carcass
RE ♂	43.8±5.3	38.8±2.7	3.52±0.35
RE ♀	27.4±5.3	33.8±2.1	4.06±0.33
RSE ♂	22.8±1.9	31.9±1.1	3.23±0.45
RE & RS ♂	22.4±0.8	31.6±1.0	3.33±0.18
RSE ♀	19.8±2.2	31.1±1.0	3.54±0.18

1) Means ± SD of 6 rats. Significantly different between 2 groups at $^{**}p<0.01$, $^{***}p<0.005$, $^{****}p\ll0.005$.

trol the accumulation of liver fat.[11)] While it is true that the egg is one of the most important protein supplying food, we must pay the close attention that the soybean in the combined diets carries out the buffer action against the deposition of liver fat.

Carcass fat content of the rats fed on combined diet with rice and egg yolk was especially high (27.8% per body weight), while the addition of soybean to this diet decreased the carcass fat content to 21.3%.

When rats were given the same diet, the degree of dietary influence was different between male and female. Accumulation of liver fat produced by the combined diet with rice and egg yolk were 44% for males, and 27% for females. The males seem to produce the fatty liver much more easily (Table 3).

When we tried to exchange the diets, RS and RE, day by day, during 5 weeks (RS & RE), liver fat content of male rats was the same as in RSE diet. But, the carcass fat content of the male rats fed on RS & RE diet was significantly higher in statistics than those on RSE diet. This suggests importance of an additional intake of soybean in one meal a day in avoiding the deposition of carcass fat.[12)]

Protein level of diets is another important factor contributing to the accumulation of liver fat. We prepared 9 different diets using rice, soybean, and egg yolk as protein source. Protein levels for the diets were 6%, 11.3%, and 16.4% respectively. Using 9 weeks old male and female rats, we performed feeding tests for 3 weeks to determine the fat content of liver and carcass of rats. As for the effect of protein level, liver fat content of male rats was high at 6% protein level, but low at 11%, and the fat content increased at 16% protein level like as at 6% protein level. It was recognized that at around 11% protein level, the deposition of liver fat was less than both higher and lower protein levels. So it was suggested that there is the optimum protein level to avoid deposition of liver fat. In the case of female rats, the fall of deposition of liver fat at around 11% was not observed, and at 16% level, liver fat content became significantly higher. In comparison between male and female at 16% protein level, there was no significant difference.[10)]

Since old times, the combination of rice and beans has been like an intimate friend. This relationship can be explained from the point of view on both the increase in nutritional value and the control effect against fat deposition.

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