On Japanese Foods

Report 2

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I. On Rice (continued)

4. Amino acid composition of rice protein

The great majority of farm-workers and other people of the lower classes in Japan take as much rice as ever not only for its calorific value but also as the main source of protein, their every-day diet comprising only in a small proportion such protein rich food as fish, meat or pork. Rice is therefore all-important in an over-populated country like Japan where the land is mountainous and not arable in most parts and the climate is wet and rainy. According to Watanabe *et al*[11]. of Tokyo, the calorie obtainable from per unit area of farmland is greatest for sweet-potatoe, and next so for rice. As protein source obtainable from the same area rice stands fifth on the list and is far below soy-beans and pea-nuts. Sweet-potatoe are not storable for long for their quick decomposition and admit of no easy conveyance. The soy-bean and pea-nut are low in their yield per unit area of land and their calorific yields are 1/3 and 7/10 respectively, of that of rice. It is believed that a high incidence of gastro-intestinal diseases among the Japanese results from too much rice they eat. The protein content of rice, though highly nutritive as K. Thomas[20] pointed out in 1909, constitutes no more than 6.5–7.5% of polished (white) rice. This accounts for the fact that the rice-eating Japanese have had to meet the body requisite for essential amino acids by taking rice too much for their digestive power.

Under these circumstances it is of special importance in Japan that the exact composition of the amino acids in rice protein be determined and, if possible, the nutritive value of rice protein increased. In the reports hitherto presented on this subject the amino acid composition of rice protein are described as having been determined mostly by microbiological assay.

In our laboratory S. Kimura[11] isolated whole proteins from polished rice in the form of its mixture with a small amount of fibre; the starch in a dilute paste obtained by boiling rice flour was removed by glucamylase digestion and by subsequent dialysis. The crystalline glucamylase used was prepared from Rhizopus Delemar and donated to us generously enough by Dr. J. Fukumoto of the Osaka Municipal University.

The protein so isolated was hydrolysed twice with 6-N HCl kept boiling, first, for 24 hours and next, for 72 hours. Ordinary amino acids were estimated by Moore and Stein's[12] column chromatography using Dowex 5×12 as adsorption medium, proline by Chinard's[13], and tryptophan by Eckert's[14] method. The amino acid composition of the zero hour's hydrolysate was computed from the results of the 24 and 72 hours' hydrolysis, as described by Schroeder and Kay[15].

Tab. 12. Amino acid composition of non-glutinous rice protein (S. Kimura[11])

Amino acid	%	Amino acid	%	Amino acid	%
Asp	9.78	Cys	—	His	2.29
Thr	4.17	Val	6.57	Lys	4.92
Ser	4.82	Met	1.90	Arg	7.98
Glu	16.56	Ileu	5.29	Try	1.29
Pro	4.81	Leu	9.10	NH_3	1.20
Gly	5.00	Tyr	6.08		
Ala	5.65	Phe	4.91	Total	102.33

A. Sakka[16] isolated protein from glutinous and non-glutinous (ordinary) rice both polished, and also from the embryo of the latter, by the use of dilute NaOH, the hydrolysis was carried out as the above experiment of S. Kimura. Ordinary amino

Tab. 13. Amino acid composition of rice protein (A. Sakka[16])

Amino acid	Non-glutinous rice polished	Glutinous rice polished	Embryo of non-glutinous rice
Asp	11.47	9.51	9.68
Thr	3.53	4.41	3.83
Ser	6.38	6.76	4.87
Glu	17.78	16.99	14.54
Pro	5.45	5.09	5.78
Gly	4.51	4.58	4.82
Ala	6.00	5.73	5.89
Cys	2.04	1.96	1.28
Val	6.00	6.37	4.88
Met	1.30	1.07	2.57
Ileu	4.26	4.49	4.67
Leu	8.96	8.98	8.96
Tyr	6.49	5.24	4.50
Phe	6.50	6.03	5.87
His	2.44	2.74	3.85
Lys	4.04	3.82	6.08
Arg	10.68	9.75	13.07
Try	1.81	1.63	2.06
NH_3	1.97	1.73	1.18
Total	109.80	105.25	108.38

Abbreviations used in this article are: Asp: aspartic acid; Thr: threonine; Ser: serine; Glu: glutamic acid; Pro: proline; Gly: glycine; Ala: alanine; Cys: cystine; Val: valine; Met: methionine; Ileu: isoleucine; Leu: leucine; Tyr: tyrosine; Phe: phenylalanine; His: histidine; Lys: lysine; Arg: arginine; Try: tryptophan.

acids were determined also as above but using Dowex $50W \times 8$, tryptophan by a slight modification of Spies' method,[17] methionine by computation from total S and cystine S, of which the former by Micro-carius, the latter by hydrazinolysis according to Kuratomi *et al.*[18] The contents of amino acids in zero hour's hydrolysate were calculated by the method mentioned above. The results reached are given in **Table 13.**

The amino acid content in 1 g N of protein was determined and compared with that of reference protein described by FAO. As shown in **Table 14,** the first limiting amino acid is neither lysine nor tryptophan; it is invariably S amino acids. The protein score (FAO) of nonglutinous and glutinous rice and that of the embryo of the former were 77, 70 and 93 respectively.

Tab. 14. Protein score of rice protein

	Thr	Met	Cys	Total S amino ac.	Ileu	Leu	Tyr	Phe	Lys	Try	Val
Reference protein	180	144	126	270	270	306	180	180	270	90	270
Non-glutinous rice (mg/N, g)	223	81	128	209	281	564	399	430	264	112	397
Protein score				77							
Glutinous rice	307	67	123	190	293	585	363	393	267	104	414
Protein score				70							
Embryo of non-glutinous rice	294	164	88	252	290	556	287	366	389	134	303
Protein score				93							

In her second experiment Sakka[19] isolated with an adequate solvent each protein fraction from the perisperm and embryo of non-glutinous rice. The quantitative ratio in which these fractions were isolated is shown in **Table 15.**

Tab. 15. Ratio of 4 protein fractions of rice (%)

	Embryo of non-glutinous rice	Perisperm of polished non-glutinous rice
Prolamine	7.88	1.76
Albumin	21.46	15.47
Globulin	15.28	13.36
Glutelin	55.47	69.40

Each fraction of the protein was hydrolysed and amino acid composition was estimated as above and shown in **Table 16.**

5. Nutritive value of rice protein

(a) Biological value.

Tab. 16. Amino acid composition of each fraction of rice protein (A. Sakka[19])

Amino acid	Perisperm of non-glutinous rice polished				Embryo of non-glutinous rice			
	Albumin	Globulin	Prolamine	Glutelin	Albumin	Globulin	Prolamine	Glutelin
Asp	7.80	7.91	11.06	9.47	6.96	6.90	17.13	4.46
Thr	3.95	1.99	3.19	4.02	7.05	2.21	3.14	3.91
Ser	4.83	7.82	6.02	6.15	4.23	4.71	3.85	5.17
Glu	10.47	16.60	17.57	19.66	7.78	11.80	23.51	2.45
Pro	4.59	7.08	6.06	7.94	3.68	4.65	4.92	2.66
Gly	6.17	7.85	4.59	4.45	5.65	5.54	8.93	5.07
Ala	6.50	7.11	6.42	6.90	6.72	6.14	8.91	3.86
Cys	2.27	1.62	0.86	2.18	7.99	7.81	3.86	6.50
Val	6.19	6.14	5.51	4.98	5.46	3.45	2.60	6.48
Met	1.24	0.59	1.05	0.59	2.13	0.89	1.10	0.70
Ileu	4.52	2.81	4.53	4.59	3.99	3.05	1.31	4.39
Leu	9.35	9.66	11.01	8.61	6.86	5.41	2.44	8.10
Tyr	4.55	4.45	8.74	7.98	6.71	4.36	0.60	2.74
Phe	5.56	0.73	6.86	0.93	2.77	4.86	1.63	4.40
His	1.50	2.00	2.33	2.49	3.28	7.60	3.37	3.93
Lys	3.97	2.33	0.56	3.26	9.59	6.61	6.64	5.73
Arg	14.04	16.90	6.00	7.27	19.47	18.73	6.31	25.81
Try	1.05	0.85	0.56	1.12	1.23	1.19	0.51	1.52
NH_3	2.51	1.32	2.99	1.83	1.17	1.35	2.60	1.06
Total	101.06	105.76	105.91	104.42	112.72	107.26	103.36	98.94

The biological value of rice protein has been determined by K. Thomas[20] and many other workers. M. Yamamoto[21] of our laboratory estimated by Barnes' method [22,23] the comparative nutritive value both for growth and maintenance and computed the biological value by the following formula:

$$\text{Biol. val.} = \frac{13.6 \times \sqrt[3]{a^2} + b}{c} \times 100$$

where 13.6 is the averaged minimum N quantity of whole egg in mg requisite per body surface (cm^2) for maintenance of body protein in mice not allowed to increase in weight;

a: denotes the mean body weight of mouse during the experiment;

b: increase of body N in mg; and

c: real absorbed N in mg.

The results are shown in **Table 17.** The biological value obtained appears rather low, however the term biological value described by Thomas concerns principally to the nutritive value for maintenance of body protein, whereas that defined by

Barnes is effected relatively strong by the nutritive value for growth of the animal examined.

Tab. 17. Biological value of rice protein

Protein from	Comparative nutritive value		Biological value (%)		
	For growth	For maintenance	Biological value	Parts participate to growth	Parts participate to maintenance
Whole egg	100	100	99		
Non-glutinous rice	44	83	42	40	60
Glutinous rice	42	82	41	45	55

(b) The comparison of the nutritive value of rice protein and mixed protein of good quality.

M. Hayashi[24] compared the nutritive value of rice protein diet and that of a mixed diet of rice and animal protein by the effect on growth of rat. The diet used was of two kinds as follows:

Basal diet: The flour of polished rice, added with a vitamin-(1%) and salt mix-

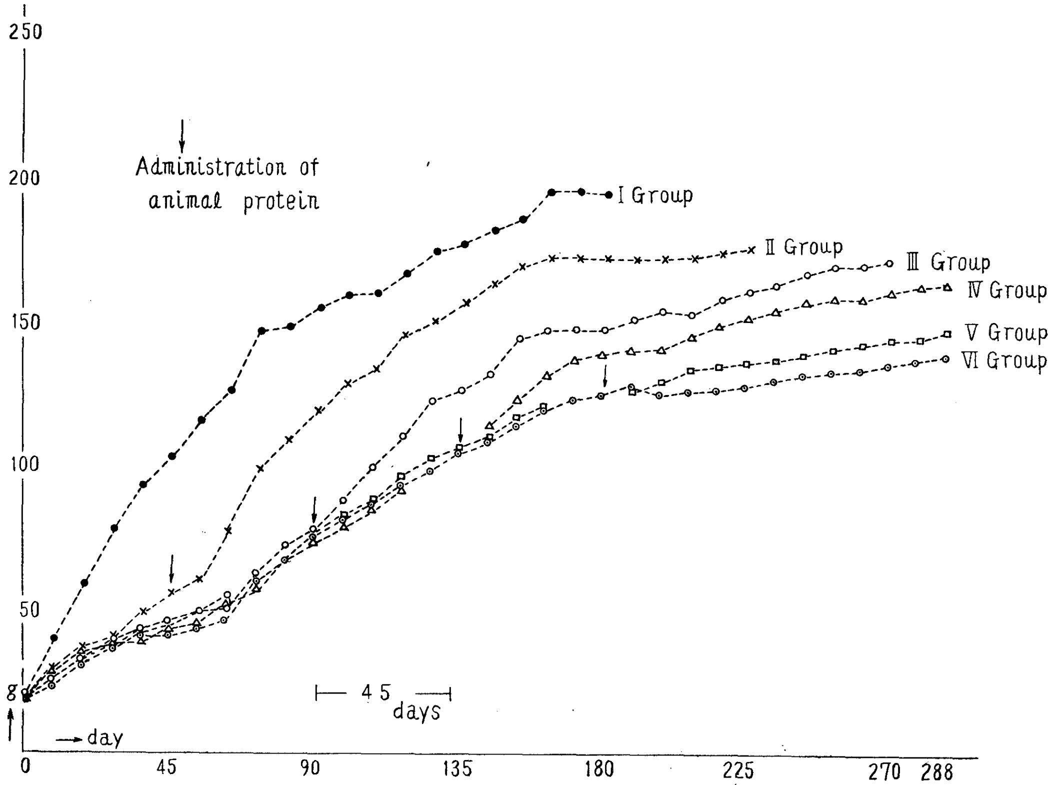

Fig. 1. Growth curve of male rat

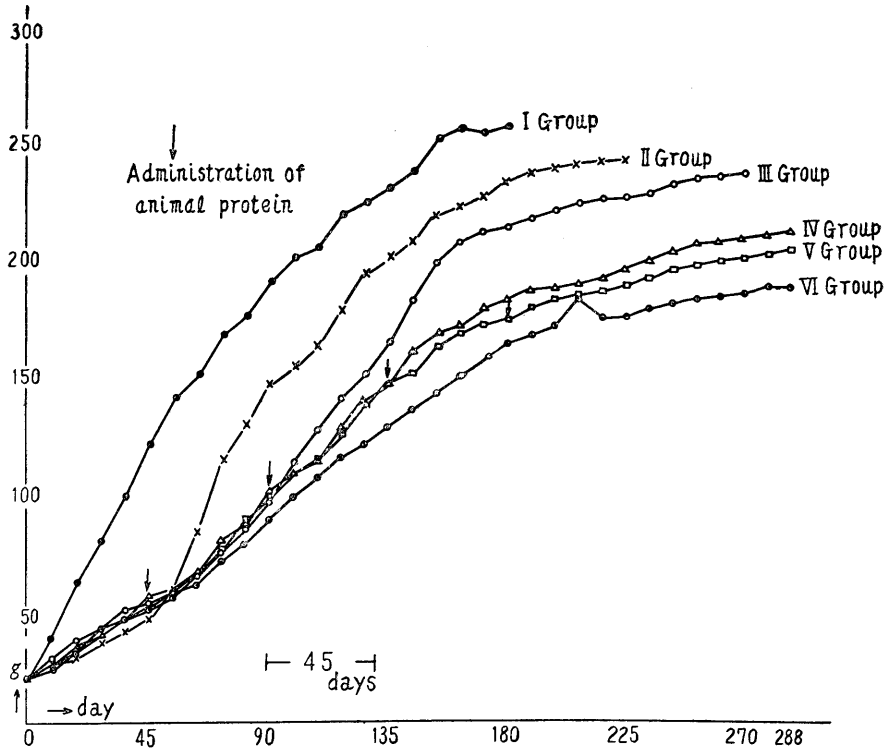

Fig. 2. Growth curve of female rat

ture (McCollum) (4%). Its protein contents were 6.23% (Diet B)

Animal protein diet: Diet B was mixed with defatted dried milk (15%) and its contents of total protein were 10% (Diet A)

Rats used were just 25 days old and ablactated, born on the same day; these were divided into 6 groups, each except Group VI consisting of two males and two females, and the same uterine rats were distributed as equall as possible for the 6 groups. The feeding on Diet A was started at an interval of 45 days for each group. From the 1st day onwards Group I was fed on Diet A and the other 5 groups were on Diet B. From the 45th day onwards Group II was kept on Diet A, from the 90th day onwards Group III on the same diet, and so on. But Group VI was maintained exclusively on Diet B till the end of the experiment. The effect of each diet on the growth of the rats is shown diagrammatically in **Figs. 1.** and **2.**

The male rats fed exclusively on Diet B (Group VI) weighed only 189g on the 291st day of the feeding, showing the low nutritive value of rice protein, whereas those kept on Diet A from the day of weaning weighed 257g on the 180th day; of the rats fed on Diet A, those (in Group II) so fed from the 45th day after ablactation onwards weighed 243g on the 225th day; those (in Group III) so fed from the

90th day 237g on the 270th; those (in Group IV) 214g on the 291st; and those (in Group V) 207g on the 291st; indicating that the growth promoting effect of a protein diet of good quality depends largely on the period of life when the feeding on it is started. The increase of body weight of male rats averaged as follows: (**Tab. 18**).

Tab. 18. The increase of body weight of male rat by the feeding on rice protein (Diet B) in comparison with that of rat fed on a mixed protein of good quality (Diet A).

Group	The particular day of post ablactation period when the feeding on Diet A was started	Duration of feeding on		Increase of body weight
		Diet B	Diet A	
		days	days	g
I	0	0	180	237
II	45 th	45	180	184
III	90 th	90	180	139
IV	135 th	135	156	65
V	180 th	180	111	31
VI	0	291	0	166

(c) The nutritive value of some cereals. Nutritive value of some cereals taken much by the Japanese was tested on rat growth by M. Hayashi[25] and found to be in the descending order as follows:

Soy-bean > buck-wheat > rice > barley > wheat

This conclusion was reached not only by feeding with each native cereal powdered but also with each cereal flour which protein contents were made 6.56% by adding dextrin in case of necessity.

(d) Increase in the nutritive value of rice protein.

i) The supplementary effect of amino acids on the nutritive value of rice protein reported by some groups of American researchers[26-29] was reexamined by Y. Okuda *et al.*[30] and confirmed as is in the following **Table 19** shown:

Tab. 19. Supplementary effect of amino acid on the nutritive value of rice protein (18. III—7. IV, 1958)

Group	Food composition in 100 parts						Feeding experiment				
							Rat		Body weight of rat (g, M±δ)		
	Rice flour	L-Lys	DL-Thr	L-Try	Salt mixt.	Vitam. mixt.	♂	♀	Initial	Final	Increase/week
I	95.0	0	0	0	4	1	3	1	30.9±2.1	49.0±1.4	6.0±0.5
II	94.0	1.0	0	0	4	1	1	2	36.5±1.9	70.8±1.6	11.5±0.7
III	93.8	0	1.2	0	4	1	2	3	34.9±6.1	57.2±8.5	7.4±1.2
IV	92.8	1.0	1.2	0	4	1	3	2	31.7±1.6	82.0±2.7	16.8±0.8
V	92.7	1.0	1.2	0.1	4	1	4	1	35.3±5.5	87.3±8.0	17.3±1.0

He found moreover the addition of tryptophan besides the both amino acids: L-lysine and DL-threonine, failed to improve the nutritive value of rice protein in any significant degree.

ii) M. Hayashi[24] examined the supplementary effect of other cereal proteins on the nutritive value of rice. The quantitative ratio in which rice and the cereals, each pulverized, were to mix was determined according to the dietary habit of the Japanese. The mixed foodstuffs were each added with vitamin- (1%) and salt mixture (McCollum) (4%), and given as food *ad libitum*. The result of this experimental investigation is given in **Table 20.** The rice was nearly doubled in its nutritive value when it was taken along with rice, barley, wheat, soy-bean, buck-wheat in the ratios 6:1.5:0.5:1:1 respectively.

Tab. 20. Supplementary effect of other cereals on the nutritive value of rice protein (I)

Group	Food composition in 100 parts*							Body weight of rat**(mean)g				
	Rice	Barley	Wheat	Buck-wheat	Soy-bean	Dex-trin	Total protein	Rat ♂	Rat ♀	Initial	Final	Increase /week
I	100	0	0	0	0	0	6.56	4	4	33.0	78.3	9.6
II	80	20	0	0	0	0	7.01	3	3	32.3	84.4	11.1
III	75	18.3	5.2	0	0	1.5	7.01	3	3	31.7	90.1	12.4
IV	70	20	0	10	0	0	7.58	3	3	32.4	101.1	14.6
V	65	19.0	5.0	9.7	0	1.3	7.58	2	4	32.4	106.7	15.8
VI	70	20	0	0	10	0	10.33	3	4	32.3	112.3	17.0
VII	65	19	5.0	0	9.9	11.0	10.33	3	3	31.3	122.0	19.2
VIII	60	15	5.0	10.0	10.0	0	10.93	4	4	32.3	129.2	20.5

* Each group food was added with vitamin-(1%) and salt mixture (4%).
** The duration of feeding was 33 days.

iii) The supplementary effect of soy bean protein along with wheat on the nutritive value of rice was compared with that of both amino acids lysine and threonine by Y. Okuda *et al.*[30] The soy-bean protein was added to rice in its fortified form with methionine and leucine according to A. Murakami,[31,32] who had noticed previously a remarkable effect of the amino acids on the nutritive value of soy-bean protein, as will be described later in the next report. For the more accurate evaluation of the nutritive value of each food were measured, in addition of the growth rate, the weight, the total N, the moisture, the lipids contents and the xanthine oxidase activity (XOA) (by Litwack's method[33]) of the liver. The result reached is shown in **Table 21.**

iv) Another comparative examination of different protein mixtures of a diet containing 10% total protein, a mixture of rice (3 parts) and wheat (1 part), added with a small amount of sardine meat (a sample of cheap fish in Japan) and a varying quantity of soy-bean protein, with or without amino acids added to it, was car-

Tab. 21. Supplementary effect of other cereals and amino acids on the nutritive value of rice protein (2. V—27. VI, 1960)

A. Composition of food in 100 parts

Group	Flour of		Crude protein of		L-Lys	DL-Thr	DL-Met	L-Leu	Rape-oil	Vitam. mixt.	Salt mixt.	Total protein
	Rice	Wheat	Rice	Soy-bean								
I	90	0	5. 23	0	0	0	0	0	5	1	4	10
II	90	0	5. 23	0	0. 01	0. 012	0	0	5	1	4	10
III	67. 5	22. 5	0	3. 36	0	0	0. 27	0. 07	5	1	4	10
IV	90	0	0. 5	0	0	0	0	0	5	1	4	6. 5

B. Feeding experiment

Group	Rat		Body weight of rat, (g, M±δ)			Liver analysis (M±δ)			
	♂	♀	Initial	Final	Increase per week	Weight (g)	Total N (%)	Lipids (%)	XOA (0_2 μl/hr/g)
I	2	3	38. 3±9. 4	191. 9±28. 7	19. 2±2. 9	8. 5±1. 2	2. 33±0. 11	6. 19±1. 17	120. 0±74. 2
II	3	3	39. 1±7. 4	204. 4±23. 4	20. 6±3. 1	8. 6±1. 4	2. 35±0. 14	5. 78±0. 91	121. 1±91. 0
III	3	3	38. 7±9. 5	204. 0±26. 2	20. 6±2. 4	8. 0±1. 4	2. 32±0. 24	6. 29±1. 54	145. 9±96. 1
IV	2	3	33. 5±4. 2	151. 9±12. 2	14. 8±1. 9	6. 5±0. 4	2. 12±0. 18	7. 78±1. 06	78. 4±49. 1

ried out by Y. Okuda *et al.*[30] with a view to improve Japanese diet. The results obtained are indicated in **Table 22.** In the table 1.58 and 2.58g soy-bean protein is equivalent to about 4 and 6g dried beans and 1.2 and 3.3g sardine meat to about 3 and 4g of fresh sardine.

As is presented in Tab. 22, there can exist no significant difference of nutritive value among 7 different diets for Groups I–VII, however the diet for Groups II, IV and VI seems somewhat better in nutrition than that for the other groups. It would be conceivable that fortification with amino acid may have no remarkable effect on the nutritive value of the food given to Groups I–VII, since the diet for each group contained animal protein and moreover the total protein contents of each food amounted to 10% of the entire food. It seems very profitable for the people of the lower classes in Japan that the addition of soy-bean protein to the rice-wheat diet can reduce the amount of sardine meat to be added to 1/10 from 1/3 of the total protein without lowering the nutritive value of the food.

SUMMARY OF THE STUDIES ON RICE

(1) How the stepwise polishing of rice affects the original calorific value and chemical constituents—fat (oil), proteins, carbohydrate, fibre, ash and vitamin B_1—of the cereal was examined from the dietetic point of view.

(2) The proportions in which rice polished in different degree was absorbed in

Tab. 22. Supplementary effect of other cereals, amino acids and fish protein on the nutritive value of rice protein (14. VI–16. VIII, 1960)

A. Composition of food in 100 parts

Group	Flour of		Protein of			L-Lys	DL-Thr	DL-Met	L-Leu	Rape-oil	Salt mixt.	Vitam. mixt.	Dextrin	Total protein
	Rice	Wheat	Soy-b.	Rice	Sardine									
I	64.5	21.5	0	0.36	3.3	0	0	0	0	5	4	1	0.34	10
II	64.5	21.5	1.58	0	2.0	0	0	0	0	5	4	1	0.42	10
III	64.5	21.5	1.58	0	2.0	0	0	0.13	0.03	5	4	1	0.26	10
IV	64.5	21.5	2.58	0	1.0	0	0	0	0	5	4	1	0.42	10
V	64.5	21.5	2.58	0	1.0	0	0	0.21	0.05	5	4	1	0.16	10
VI	64.5	21.5	2.58	0	1.0	0.2	0.2	0	0	5	4	1	0.02	10
VII	63.9	21.3	2.58	0.103	1.0	0.2	0.2	0.21	0.05	5	4	1	0.46	10

B. Feeding experiment

Group	Rat ♂	Body weight of rat, (g, M±δ)			Liver analysis (M±δ)				
		Initial	Final	Increase per week	Weight (g)	Total-N (%)	Moisture (%)	Lipids (%)	XOA (O_2 μl/hr/g)
I	3	31.3±4.2	164.7± 7.8	15.0±0.5	6.17±0.18	2.38±0.18	69.3± 1.2	6.53±1.16	123.4± 76.8
II	5	45.7±5.3	181.6±15.9	15.3±1.7	6.27±0.44	2.69±0.14	61.5± 8.8	5.85±0.38	171.5± 96.4
III	5	45.3±4.4	182.5±14.6	15.5±2.0	5.98±0.64	2.79±0.10	69.3± 1.8	6.02±0.42	151.8±113.4
IV	5	45.2±4.4	177.5± 5.5	14.9±1.1	6.59±0.44	2.80±0.08	69.5± 0.5	6.25±0.64	154.1± 98.9
V	4	45.7±5.6	184.2±15.9	15.6±1.5	5.78±0.64	2.74±0.11	64.9±11.0	7.07±1.61	158.6± 87.5
VI	5	48.2±7.0	181.6± 7.2	15.1±1.3	5.90±0.35	2.67±0.12	69.9± 0.9	6.07±0.46	230.1±128.1
VII	5	46.0±4.0	187.2±12.9	15.8±1.2	5.87±0.13	2.87±0.09	70.1± 7.7	5.96±1.06	143.5± 67.0

the human body were estimated thrice over first in 6, next in 14, and finally in 3 humans; 1–2% polished rice was found to have significantly higher calorific value than that polished in any other degree.

(3) 38 kinds of rice grown in 10 different districts of South-East Asia were analyzed.

(4) The adaptation of a whole rice diet was recognized to be a matter of habit.

(5) The real absorption ratio of the three main constituents of fully polished rice was found to average as follows ($M \pm \delta$): protein 84.14 ± 1.88; fat 83.31 ± 1.00; carbohydrate $91.59 \pm 0.24\%$.

(6) The amino acid composition of the following proteins was determined by column chromatography and its certain other amino acid ingredient by other appropriate methods:

Whole protein of non-glutinous rice polished;
4 Protein fractions of the perisperm of non-glutinous rice polished;
4 Protein fractions of the embryo of non-glutinous rice:
Whole protein of glutinous rice polished.

(7) The first limiting amino acid of rice protein was found to be S amino acids and the protein score computed and found to be 77, 70 and 97 for non-glutinous rice, glutinous rice and for embryo of the former respectively.

(8) The comparative nutritive value and the biological value of rice protein was measured according to Barnes.

(9) A repeated attempt was made to supplement rice protein by the use of amino acids, other cereal proteins and fish meal and the result discussed in many respects.

(to be continued)

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