

## Comparison of egg-yolk protein hydrolysate and soyabean protein hydrolysate in terms of nitrogen utilization

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Egg-yolk protein hydrolysate (YpP) is an alternative protein source in formulas for infants with intolerance to cow's milk or soyabean protein, or for patients with intestinal disorders. However, the nutritional value of YpP has never been investigated. YpP was prepared by enzymic hydrolysis of delipidated yolk protein, which led to an average peptide length of 2.6 residues. Three experiments were performed. In Expt 1, we compared the intestinal absorption rate of YpP and soyabean protein hydrolysate (SPp) in rats. YpP and SPp solutions were injected into the duodenum of anaesthetized rats and blood samples were taken from the portal vein at 7, 15, 30, 60, and 120 min. A higher amino acid concentration in the serum of the YpP group demonstrated that YpP was absorbed faster than SPp. In Expt 2, the effects of dietary YpP and SPp on body-weight gain, protein efficiency ratio (PER) and feed efficiency ratio (FER) were determined. At the end of the experiment, body weight had increased in both groups, while PER and FER were significantly higher in rats fed on YpP. In Expt 3, to investigate the effects of dietary YpP and SPp on N metabolism, we determined the biological value and net protein utilization. Yolk protein was the reference protein. Biological value and net protein utilization values were very similar between animals fed on yolk protein and YpP diets, and significantly higher than in rats fed on the SPp diet. The present findings demonstrate that there is no adverse effect of hydrolysis of yolk protein on N utilization, and that the nutritive value of YpP is similar to that of yolk protein and superior to that of SPp.

### Egg yolk: Soyabean protein: Nitrogen metabolism

The use of peptides in the form of protein hydrolysates has become indispensable in human clinical nutrition and infant formulas. The fast absorption of peptides (Matthews & Adibi, 1976; Grimble *et al.* 1986, 1987; Nakano *et al.* 1994a), their high N utilization rates (Funabiki *et al.* 1987) and their low allergenicity (American Academy of Pediatrics, Committee of Nutrition, 1983; ESPGAN, Committee of Nutrition, 1993) have made them an essential instrument for treatment of patients with intestinal disorders (Adibi *et al.* 1974; Silk *et al.* 1974) or with food-related immunogenic intolerance or allergy (Walker-Smith *et al.* 1989; Sampson *et al.* 1991). For these reasons, peptides from cow's milk proteins have been extensively studied and utilized over the years (Ohtomo, 1991).

Cow's-milk-based infant formulas cause food allergy in 0.3–7.5% of formula-fed infants in the United States (Bahna & Heiner, 1980). Soyabean-protein-based formulas are the most frequent alternative used to relieve allergic reactions in infants sensitized to cow's-milk protein

(American Academy of Pediatrics, Committee of Nutrition, 1983). However, soyabean-protein formula also causes adverse reactions in almost 50% of infants with cow's-milk allergies (Bishop *et al.* 1990). Because of their reduced allergenicity, casein- and whey-hydrolysate formulas have been introduced (American Academy of Pediatrics, Committee of Nutrition, 1989). Soyabean-protein peptides (SPp) have been investigated recently as a potential alternative for use in infant formulas (Zijlstra *et al.* 1995); however, in that study, differences in N utilization were not examined.

In spite of the superb quality of egg proteins (Food and Agriculture Organization/World Health Organization, 1985), they have never been considered as an alternative for use in human clinical nutrition or infant formulas due to the content of potential allergens in egg white (Hoffman, 1983) and the lipid content in egg yolk. Recently, a delipidated yolk protein has been developed (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results) as a potential

**Abbreviations:** FER, feed efficiency ratio; PER, protein efficiency ratio; SPp, soyabean-protein peptides; YP, yolk protein; YpP, yolk-protein peptides.

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high-quality protein source for infants with intolerance to cow's-milk- or soyabean-protein-based formulas.

Since studies comparing the effect of animal protein hydrolysates with plant protein hydrolysates on N utilization could not be found, we performed a nutritional evaluation of yolk-protein peptides (YPP) along with that of the original yolk protein (YP) and compared these with the nutritional value of a hydrolysate obtained from soyabean protein (SPp).

In the first section of the present study we evaluated the molecular mass distribution and the intestinal absorption rate of YPP compared with those of SPp. In the second section the growth of young rats fed on YPP-containing diets was investigated and compared with the growth of rats fed on SPp-containing diets. The protein efficiency ratio (PER) and feed efficiency ratio (FER) were determined. In the last section the effects of YPP on N metabolism in young rats were investigated and compared with those of the original protein (YP) and SPp. Biological value and net protein utilization were determined. The objective of this experiment was to ascertain whether or not the outstanding nutritional properties of YP (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results) were affected by the hydrolysis process to produce YPP.

## Materials and methods

### Original yolk protein

To prepare YP, hen's eggs were collected, broken, and yolks were separated from the albumen. Yolks were delipidated with ethanol under mild conditions. Then the YP fraction was filtered and dried. The general chemical composition of YP, the amino acid, vitamin and mineral contents will be described elsewhere (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results).

### Yolk-protein peptide

YPP was prepared by hydrolysis of YP by food-grade proteinases from *Bacillus* sp. YP was dissolved in water (200 g/l) and heat treated at 90° before enzyme digestion. Orientase (*EC* 3.4.21.62) (Hankyu Bioindustry, Osaka, Japan) and protease (*EC* 3.4.11.12) (Amano Seiyaku, Nagoya Japan) were used sequentially at pH 10 and 50°.

**Table 1.** Composition (g/kg) of the egg-yolk protein hydrolysate (YPP) and the soyabean protein hydrolysate (SPp) used in the present study

Component	YPP	SPp
Protein*	775	878
Carbohydrate†	125	26
Lipid‡	1	2
Ash§	64	58
Moisture	35	36

\* Kjeldahl method (Oser, 1965).

† Phenol-sulfuric acid method.

‡ Determined by difference (100 - (protein + carbohydrate + ash + moisture)).

§ Residue after ignition at 550°.

|| Measured by drying at 105° for 3 h.

**Table 2.** Essential amino acid composition of yolk protein (YP), yolk peptide (YPP) and soyabean peptide (SPp) (mg/g protein)

Amino acid	YP*	YPP	SPp†	Requirement at 2-5 years‡
Thr	48	54	37	34
Tyr	42	41	34	63
Phe	43	42	49	
Cys	20	15	13	25
Met	26	26	12	
Val	56	62	44	35
Ile	50	51	44	28
Leu	80	90	71	66
Lys	71	75	63	58
Trp	15	11	12	11
His	25	27	23	19
Score	100	100	100	

\* Mitsuya *et al.* (1998).

† Fuji Oil Co., Tokyo, Japan.

‡ Food and Agriculture Organization/World Health Organization (1985).

The hydrolysis reaction was stopped after 6 h by heating to 100° for 5 min. The soluble fraction was then filtered and spray-dried. The chemical composition of YPP (Table 1) and its essential amino acid content (Table 2) were determined by the methods in use in our laboratory (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results).

### Soyabean-protein peptide

SPp is a commercially available product developed by Fuji Oil Co. (Tokyo, Japan). The SPp used in the present study was purchased directly from the maker. The composition and essential amino acid content of SPp are shown in Tables 1 and 2 respectively. The molecular mass distributions of YP, YPP and SPp were estimated by gel permeation chromatography with u.v. detection at an optical density of 210 nm. The amino acid lengths of peptides in YPP and SPp were determined by the HCl hydrolysis method described by Nakano *et al.* (1994a).

### Diets

The compositions of the experimental diets were determined according to the methods of the Association of Official Analytical Chemists (1990) (Table 3). YPP, YP and SPp were used as the dietary N sources. Except for the control diet, all diets were isonitrogenous (1.6 g N/kg feed) and isoenergetic. Energy was supplied as  $\alpha$ -maize starch and soyabean oil. The protein content of the N sources was measured by the Kjeldahl method (Oser, 1965).

Similar levels of protein in all diets were obtained by adjusting the amounts of N source and  $\alpha$ -maize starch. Because YP contains 76 g lipid/kg (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results), in the YP diet the amount of soyabean oil was modified to yield a lipid content of the diet of 80 g/kg. Except for the N sources, all components of the diets were purchased from Oriental Yeast Co. (Tokyo, Japan).

**Table 3.** Composition of the experimental diets (g/kg diet)

	Yolk protein (YP)	Yolk peptide (YPp)	Soyabean peptide (SPp)	Control
Test substance*	127	154	145	0
Soyabean oil†	71	80	80	80
$\alpha$ -Maize starch‡	707	671	680	825
Mineral mixture§	35	35	35	35
Vitamin mixture§	10	10	10	10
Cellulose	50	50	50	50
Total	1000	1000	1000	1000

\* YP, YPp and SPp contained 126, 124 and 140 g N/kg respectively as evaluated by the Kjeldahl method (Oser, 1965).

† The amount of soyabean oil was adjusted to compensate for the 76 g lipid/kg in YP.

‡ The amount of  $\alpha$ -maize starch was adjusted to compensate for the different amounts of test substance.

§ Mineral and vitamin mixtures were prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1980).

### Animals

Wistar rats (3 weeks old, 41.3 g average body weight) were purchased from Nihon SLC Co. Ltd., Shizuoka, Japan. The animals were individually housed in stainless steel wire-bottomed suspended cages to limit coprophagy. Cages were kept in a room with controlled temperature (25°), a 12 h light period (from 07.00 to 19.00 hours), and controlled humidity (55%). Animal protocols followed the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985). For acclimatization, rats were given a standard diet (Oriental Yeast Co.) for 4 d.

### Analytical methods

**Nitrogen balance.** The N contents of pooled samples of faeces and urine were analysed by the Kjeldahl method (Oser, 1965). N balance was calculated by subtracting urinary and faecal N values from dietary N intake.

**Biological value and net protein utilization.** Biological value (BV) and net protein utilization (NPU) were calculated by the following equations (Hosoya, 1980):

$$BV = I - (F - Fo) - (U - Uo) / I - (F - Fo),$$

$$NPU = I - (F - Fo) - (U - Uo) / I,$$

where I is N intake, F is faecal N, U is urinary N, Fo is metabolic faecal N and Uo is endogenous urinary N.

**Serum constituents.** Serum constituents were analysed with commercially available kits (Wako Pure Chemical Co., Osaka, Japan).

### Surgical procedure

Rats were anaesthetized with an intraperitoneal injection of pentobarbital (1  $\mu$ l/g body weight). The abdomen was opened by a midline incision. The internal organs were moved until the portal vein was exposed. A needle (set at the end of a heparinized silicone tube) was then fixed in the portal vein. The internal organs were replaced in the peritoneal cavity. The solutions of YPp or SPp were administered by injection of 1 ml directly into the duodenum.

Blood samples were taken from the portal vein just before

the injection of the test substances and at 7, 15, 30, 60 and 120 min after the duodenal injection. Blood was centrifuged immediately and the free amino acid concentration in the portal blood serum was determined by the trinitrobenzene sulfonic acid method (Okuyama, 1973).

### Statistical analysis

Data were analysed by the method of Friedman (Ichihara, 1990) and by ANOVA.

## Experimental design

### Intestinal absorption

YPp, SPp and control groups each had three rats. Solutions of YPp and SPp with a N concentration of 8 g/l were prepared. The control solution contained no N source.

### Effect of YPp and SPp on protein efficiency ratio

Animals were divided into YPp and SPp dietary groups (nine rats in each group). The average body weights in YPp and SPp groups were 63.0 and 62.7 g respectively. The rats were given free access to experimental diets and water during the 28 d experimental period. Feed intake and weight gain were measured every day at 09.00 hours.

At the end of the experimental period, FER and PER were calculated using the following equations (Hosoya, 1980):

$$FER = \text{body-weight gain/feed intake},$$

$$PER = \text{body-weight gain/net protein intake}.$$

In addition, animals were anaesthetized with diethyl ether 12 h after food withdrawal and blood was taken from the inferior vena cava. Stomach, liver, large intestine, small intestines and caecum were excised immediately and weighed. Then the blood was centrifuged, serum separated and total protein, albumin, albumin:globulin ratio, blood urea N, creatinine, total cholesterol, triacylglycerols, phospholipids, and glucose values were determined.

### Effect of dietary N sources on N balance

Animals were divided into YPp, YP, SPp and control dietary groups (six rats in each group) and placed in metabolic cages. The average body weights were 63.3, 64.1, 63.8 and 63.9 g respectively. To obtain a protein-free diet for the control group, the N source was replaced by  $\alpha$ -maize starch (Table 3). This protein-free diet was used to determine the endogenous N losses in urine and faeces. Rats were given free access to experimental diets and water during a 7 d experimental period, 4 d for adaptation and 3 d for quantitative collection of faeces and urine.

Feed intake and weight gain were measured every day. Faeces and urine were collected separately during the last 3 d of the experimental period for determination of N excretion rate.

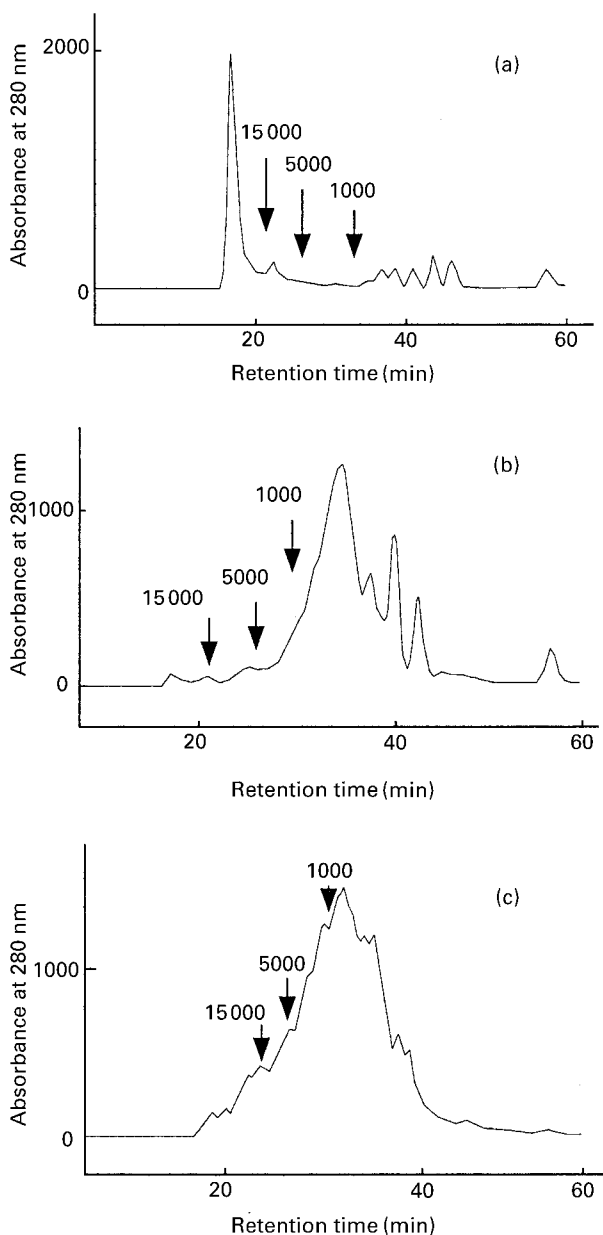
At the end of the experimental period, animals were anaesthetized with diethyl ether 12 h after food withdrawal and exsanguinated. After dissection, stomach, liver, large and small intestines and caecum were excised and weighed

to estimate the organ weight relative to the body weight. N balance was calculated on daily collection of food, faeces and urine.

## Results

Fig. 1 shows the gel permeation chromatography profiles of YP, YPp and SPp. The molecular mass distribution of YPp was lower than that of SPp.

The average chain lengths of YPp and SPp were 2.6 and 3.4 respectively. The general compositions of YPp and SPp are shown in Table 1. The amino acid score of both YPp and SPp was 100. The essential amino acid compositions of YP, YPp, and SPp are shown in Table 2.



**Fig. 1.** Gel filtration patterns of (a) egg-yolk protein, (b) egg-yolk peptide and (c) soyabean peptide. The arrows indicate the elution times of molecular mass markers.

## Intestinal absorption

The changes in total free amino acid concentration of plasma in rat portal vein are shown in Fig. 2. The serum free amino acid concentration of the YPp group at 7 min after the duodenal injection was higher than that of the SPp group. This demonstrates that YPp was absorbed faster than SPp.

## Protein efficiency ratio

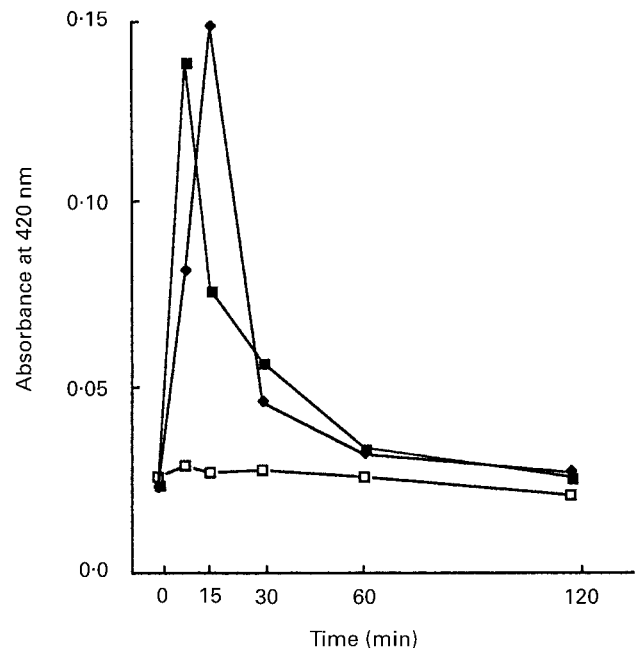
Fig. 3 shows the daily changes in mean body weight observed during the 28 d experimental period. Animals fed on the YPp diet showed a higher weight increase from the first day compared with the rats fed on the SPp diet. At the end of the experiment, the average final body weight in the YPp group was 185.0 g (total increase of 122.0 g), whereas the average final body weight in the SPp group was 151.9 g (total increase of 89.2 g) (Table 4).

Animals fed on the YPp diet showed mean values of feed intake (336.1 g) significantly higher than those for rats fed on the SPp diet (302.0 g) (Table 4). The PER value in the YPp group was 3.6, which was also significantly higher than the value of 3.0 in the SPp group (Table 4).

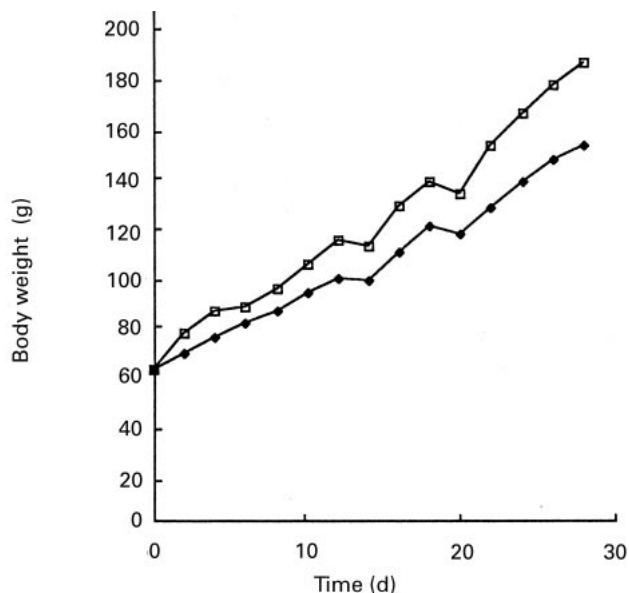
There were no significant differences between groups for weight of internal organs relative to body weight.

## Serum constituents

Regarding blood serum biochemical analysis, the concentrations of total protein and albumin in the YPp group were 71 and 41 g/l respectively, which were significantly higher



**Fig. 2.** Changes in total free amino acid concentration in plasma from the portal vein of rats given egg-yolk peptide (■), soyabean peptide (◆), or a N-free solution (control, □) by duodenal administration. For details of diets and procedures, see Tables 1 and 2 and pp. 478–479.



**Fig. 3.** Changes in the average body weight of rats fed on diets containing egg-yolk peptide (□) or soyabean peptide (◆) during the protein efficiency ratio test. For details of diets and procedures, see Tables 1–3 and pp. 478–480.

than 61 and 37 g/l respectively in the SPp group. The serum glucose concentration in animals fed on the YPp diet (4.5 mmol/l), was significantly higher than that in animals fed on the SPp diet (3.0 mmol/l) (Table 4). There were no significant differences between the groups in the serum levels of albumin: globulin, blood urea N, creatinine, total cholesterol, phospholipid or triacylglycerol.

#### Nitrogen balance test

Fig. 4 shows the changes in average body weight during the 7 d experimental period. Animals fed on YPp and YP diets showed similar increases in body weight (37.6 and 36.0 g respectively), and these were significantly higher than that of rats fed on SPp (20.2 g). The total body weight increase is shown in Table 5. Total feed intake values were similar in both YPp and YP groups (90.8 and 90.6 g respectively), and these were significantly higher than that for the SPp group (80.7 g) (Table 5). There was no significant difference in the FER between YPp and YP groups. These FER values were significantly higher than that for the SPp group (Table 5).

With regard to the weights of internal organs relative to body weight, no significant differences could be observed between the three groups for any of the internal organs.

The results relating to N balance are shown in Table 6. N intake during the last 3 d of the experimental period was significantly higher in both YPp and YP groups (662.8 and 664.0 mg respectively) than in the SPp group (617.6 mg). The amount of N excreted in faeces was significantly higher in both YPp and YP groups. On the other hand, the amount of N excreted in urine was significantly higher in the SPp group. The value for N balance in the YPp group was 481.3 mg/3 d, which was not significantly different from 490.0 mg/3 d obtained in the YP group. However, these

**Table 4.** Effects of dietary egg-yolk peptide (YPp) and soyabean peptide (SPp) on body-weight gain, feed intake, feed efficiency ratio, protein efficiency ratio (PER) and serum constituents of rats during a PER test†

(Mean values and standard deviations for nine rats per group)

	YPp		SPp	
	Mean	SD	Mean	SD
Initial body weight (g)	63.0	3.1	62.7	3.2
Final body weight (g)	185.0*	14.7	151.9	9.3
Body-weight gain (g)	121.9*	13.3	89.2	7.2
Feed intake (g)	336.1*	8.8	302.0	9.4
Feed efficiency ratio‡	0.4*	0.021	0.3	0.0
PER§	3.6*	0.20	3.0	0.2
Total protein (mg/l)	71*	3.9	61	4.0
Albumin (mg/l)	41*	2.3	37	2.0
Glucose (mmol/l)	4.5*	0.95	3.0	0.77

Mean values were significantly different from those for SPp, \* $P < 0.05$ .

† For details of diets and procedures, see Tables 1–3 and pp. 478–480.

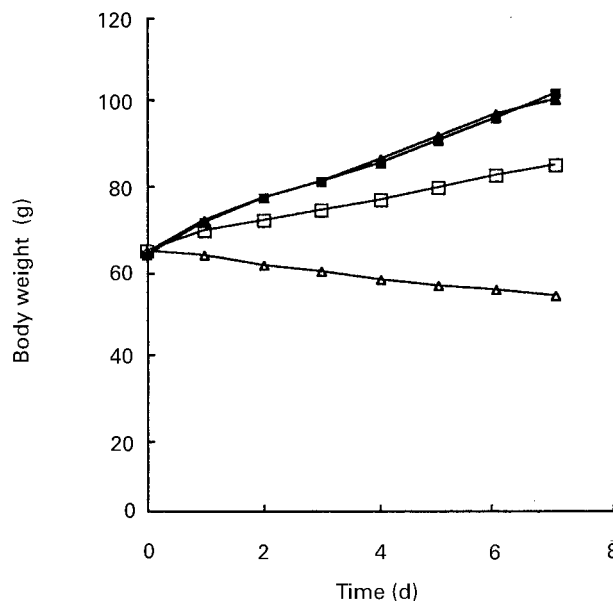
‡ Body-weight gain/feed intake.

§ Body-weight gain/protein intake.

values were significantly higher than 425.4 mg/3 d obtained in the SPp group. No significant difference was observed in the net protein utilization values among the three groups. Biological values of YPp and YP were also similar (94.4 and 95.2 % respectively) and significantly higher than that for SPp (89.0 %).

#### Discussion

Among the different functional properties of food materials, nutritional value is the most important, followed by organoleptic characteristics and biological functions (Yano,



**Fig. 4.** Changes in the average body weight of rats fed on a control diet (Δ), or on diets containing egg-yolk protein (▲), egg-yolk peptide (■) or soyabean peptide (□) during the N balance test. For details of diets and procedures, see Tables 1–3 and pp. 478–480.

**Table 5.** Effects of dietary egg-yolk protein (YP), egg-yolk peptide (YPp) and soyabean peptide (SPp) on body-weight gain, feed intake, feed efficiency ratio and carcass weight relative to body weight in rats during a nitrogen balance test†  
(Mean values and standard deviations for six rats per group)

	YP		YPp		SPp	
	Mean	SD	Mean	SD	Mean	SD
Initial body weight (g)	63.8	3.2	63.33	4.7	64.14	3.9
Final body weight (g)	99.83*	5.3	101.01*	3.9	74.37	5.21
Body-weight gain (g)	36.03*	2.6	37.67*	3.76	20.23	3.4
Feed intake (g)	90.6*	3.3	90.85*	3.1	80.73	3.7
Feed efficiency ratio‡	0.39*	0.02	0.41*	0.04	0.25	0.03
Eviscerated carcass (g)	80.01*	4.1	81.26*	3.7	65.04	4.3
Carcass weight/body weight (%)	80.15*	0.73	80.43*	0.9	77.08	0.91

Mean values were significantly different from those for the SPp group, \* $P < 0.05$ .

† For details of diets and procedures, see Tables 1–3 and pp. 478–480.

‡ Body-weight gain/feed intake.

1987). The present study investigated the effect of dietary YPp on N balance in young rats and compared it with the effect of dietary YP and SPp.

Absorption of intact dipeptides as well as tripeptides is well documented in human subjects (Adibi *et al.* 1975; Silk & Clark, 1975). It is well known that important amounts of unhydrolysed di- and tripeptides are removed from the intestinal lumen without luminal hydrolysis (Silk *et al.* 1974; Matthews & Adibi, 1976). Evidence suggests that the uptake of those unhydrolysed peptides is restricted to those containing two or three amino acid residues (Matthews & Payne, 1980; Silk, 1981). Absorption of tetrapeptides requires intestinal brush-border hydrolysis to dipeptides and tripeptides as a prerequisite for absorption (Burston *et al.* 1979). Marked differences in amino acid absorption can be observed even if the differences in peptide length are minimal (Grimble *et al.* 1987). These statements are in agreement with the results of the intestinal absorption section of the present study. YPp (2.6 average peptide length, mainly composed of di- and tripeptides) was absorbed faster than SPp (3.4 average peptide length, mainly composed of tri- and tetrapeptides).

Molecular mass is also a factor that influences extent of N absorption. N from low-molecular-mass hydrolysates was

absorbed to a significantly greater extent than N from high-molecular-mass hydrolysates (Grimble *et al.* 1986). This was also observed in the present study. YPp, with a lower molecular mass than SPp, was absorbed faster.

Some studies have suggested that a faster absorption of N produces a higher N utilization rate (Funabiki *et al.* 1987). This is in accordance with the results of the present study, where YPp, absorbed faster than SPp, produced a higher N utilization rate.

In the PER experiment the increases in food intake and body weight of animals fed on the YPp-containing diet resulted in a PER value of 3.63. This value is similar to the PER of the original YP (3.64) obtained in this laboratory (T Mitsuya, MA Gutierrez, H Yamaguchi, R Kobayashi, LR Juneja and M Kim, unpublished results) when comparing N efficiency of YP and casein. This demonstrates that the process of hydrolysis to produce YPp has no negative effect on the nutritional properties of YP. Protein sources with PER values of >3.5 are considered to be high-quality proteins (Hosoya, 1980).

Analysis of serum constituents showed significantly higher concentrations of total protein and albumin (71 and 41 mg/l respectively) in serum from rats in the YPp group, compared with those of the SPp group (61 and 37 mg/l

**Table 6.** Nitrogen balance in rats, biological value and net protein utilization of egg-yolk protein (YP), egg-yolk peptide (YPp) and soyabean peptide (SPp)†

(Mean values and standard deviations for six rats per group)

	YP		YPp		SPp	
	Mean	SD	Mean	SD	Mean	SD
N intake (mg/3 d)	664.0*	20.4	662.8*	20.3	617.3	21.4
Faecal N (mg/3 d)	48.36*	3.3	50.68*	3.7	29.5	4.5
Urinary N (mg/3 d)	125.7*	16.3	130.8*	11.6	162.7	18.9
N balance (mg/3 d)	490*	23.0	481.33*	26.9	425.4	16.1
Biological value‡	95.22*	2.49	94.34*	1.87	88.98	2.86
Net protein utilization (%)‡	91.1	1.6	90.6	1.7	88.2	3.1

Mean values were significantly different from those for SPp, \* $P < 0.05$ .

† For details of diets and procedures, see Tables 1–3 and pp. 478–480.

‡ Biological value and net protein utilization were calculated as described on p. 479, using the following values: metabolic faecal N = 24.5 (SD 6.2) mg/3 d; endogenous urinary N = 95.0 (SD 2.3) mg/3 d.

respectively). This shows that, in spite of the fact that YPp and SPp both exhibited amino acid scores of 100, YPp was more efficiently absorbed than SPp.

Regarding N intake, some studies have observed an improved N balance with an increment in the N intake (Kishi *et al.* 1978). This is in agreement with the results of the N balance test experiment of the present study, where the higher feed intake, with the corresponding higher N intake in both YP and YPp groups, produced a significantly higher increase in body weight and higher N balance than in the SPp group.

In this experiment, animals were offered feed *ad libitum*, and the difference in feed intake was significantly higher in the YP and YPp groups. Further experimentation with controlled pair-feeding to achieve similar N intakes is necessary.

Faecal N was significantly higher in the YPp and YP groups than in the SPp group. Since the intestinal absorption experiment showed that YPp was rapidly absorbed and the N balance test values were higher in the egg yolk groups, it is possible that the higher faecal content might not be related to reduced absorption. This finding is probably a consequence of the higher N intake in those groups. Nakano *et al.* (1994b), in an experiment with milk proteins, also found that whey protein and its hydrolysate showed higher nutritional value than casein, despite leading to a higher concentration of faecal N.

Our results showed that the concentrations of urine N were similar in the YPp and YP groups, and were significantly lower than in the SPp group, suggesting that the N absorbed from YPp and YP is more efficiently utilized.

Studies on urinary excretion of N from peptides could not be found. It is well known that the body mass increase in animals and human subjects is higher with intake of N from food of animal origin than from plant origin. High N loss through urine, as we observed with SPp, which is of plant origin, might partially explain that fact. The mechanism that produced a higher excretion of N through urine in the SPp group has yet to be investigated.

The higher nutritional value of YPp obtained in this study is not surprising since it is well known that egg proteins are of higher nutritional value than soyabean proteins.

Native protein and hydrolysis method were both found to influence absorptive profiles of protein hydrolysates (Keohane *et al.* 1985). The present findings, however, suggest that enzymic hydrolysis has no negative effect on the nutritional properties of YP.

Food allergy or food intolerance, especially in infants, is a great concern. Allergic reactions to infant formulas prepared with cow's-milk (Bahna & Heiner, 1980) or with soyabean protein (Bishop *et al.* 1990) are well documented. Enzymic hydrolysis of dietary protein might reduce antigenic epitopes (American Academy of Pediatrics, Committee on Nutrition, 1989; ESPGAN, Committee on Nutrition, 1993). However, allergic reactions against high-degree casein hydrolysates or whey-protein hydrolysate have also been reported (Businco *et al.* 1989; Sampson *et al.* 1991; Saylor & Bahna, 1991). Therefore, there is a need for hydrolysates from other dietary proteins. Allergens in egg are a major concern for infants with adverse reactions to foods. Most egg allergens are concentrated in the egg white

(Hoffman, 1983; Langeland, 1983), but the possibility of allergic reactions to egg yolk also exists (Langeland, 1983). However, in an evaluation by the radioimmune allergosorbent test, YPp showed a much reduced allergenicity against sera from patients with strong allergic reactions against egg yolk (results not shown). For clinical applications, reduced allergenicity of a protein hydrolysate cannot be dissociated from the nutritional efficiency. The YPp used in this study showed low allergenicity and kept the nutritional properties of the original protein.

YPp is a hydrolysed product of YP, which is a delipidated egg-yolk protein with almost no cholesterol. Therefore, it would be suitable for use by people with hypercholesterolaemia. Lactose has not been found in either YP or YPp, therefore, their use in patients with lactose intolerance is also feasible.

Chemically defined diets, with intact or pre-digested sources of N, have been used as specialized instruments of nutritional support for human patients for many years (Russell, 1975; Voitk, 1975; Freeman *et al.* 1976; Kaminski, 1976; Heymsfield *et al.* 1979). Due to its fast intestinal absorption, high nutritional value, low allergenicity and negligible cholesterol content, the potential use of YPp in full-term and premature infant nutrition, management of allergies and treatment of intestinal disorders is promising.

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