EFFECT OF SUPPLEMENTATION OF LIMITING AMINO ACIDS ON TYROSINE TOXICITY IN RATS FED WHEAT GLUTEN DIETS

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Summary The effect of supplementation of limiting amino acids on rats fed a 10 or 20% wheat gluten diet containing 5% tyrosine has been investigated. Growth-limiting amino acids in a 20% wheat gluten diet were lysine and threonine. When rats were fed a 20% wheat gluten diet containing excess tyrosine, the addition of 1.0% lysine·HCl or 1.0% lysine·HCl and 0.4% threonine completely prevented the development of tyrosine toxicity, and was accompanied by a lowering of free tyrosine concentrations in plasma, liver and muscle. In rats receiving a 10% wheat gluten diet, lysine, threonine and methionine were limiting for growth. The addition of 1.0% lysine·HCl to the 10% wheat gluten plus 5% tyrosine diet failed to alleviate the tyrosine toxicity, but the supplement of 1.0% lysine·HCl and 0.4% threonine could partially alleviate the toxicity. The combined addition of 1.0% lysine·HCl, 0.4% threonine and 0.5% methionine was most effective. The free tyrosine concentrations in plasma, liver and muscle were lowered greatly by the supplementation of three limiting amino acids, but amounts of free tyrosine and phenolic metabolites excreted in the urine did not decrease with addition of these limiting amino acids.

It has been shown in young rats fed a low protein diet containing excess tyrosine, that the growth is retarded and eye and paw lesions develop (1-6). Recently we found that the growth depression and appearance of pathological lesions which developed in rats fed a 10% casein diet containing 5% tyrosine were alleviated by the supplement of methionine and threonine, the first and second limiting amino acids respectively, in the diet (7,8).

On the other hand, it was also demonstrated in our laboratory (9) that the tyrosine toxicity produced by feeding an 11.6% wheat gluten diet containing 5% tyrosine (equivalent in N content to 10% casein) was not at all alleviated by the

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first limiting amino acid, lysine. The amounts of some amino acids such as threonine and methionine are also insufficient in the low wheat gluten diet (10,11), but the effect of these limiting amino acids in wheat gluten on tyrosine toxicity had not been investigated.

Therefore, the present study was performed in an attempt to elucidate the effect of those limiting amino acids in wheat gluten on tyrosine toxicity. Growth depression, free tyrosine concentration in plasma, liver and muscle, together with urinary excretion of tyrosine and its phenolic metabolites were determined.

EXPERIMENTAL

Animals and diets. Male weanling rats of the Wistar strain were fed a stock diet (25% casein) for 3 to 4 days prior to starting the experiments. Rats weighing 60 to 65 g were divided into two series and separated into several experimental groups of five animals each (Table 1).

In experiment I, 20% wheat gluten² was used in each group, and 5% L-tyrosine

Table 1. Composition of experimental diets.^a

Dietary groups	Wheat gluten	L-Tyrosine	L-Lysine·HCl	L-Threonine	L-Methionine	L-His-tidine·HCl	L-Trypto-phan
Experiment I				(%)			
20G	20	—	—	—	—	—	—
20G Lys	20	—	1.0	—	—	—	—
20G Lys Thr	20	—	1.0	0.4	—	—	—
20G Lys Thr Met	20	—	1.0	0.4	0.5	—	—
20G Lys Thr Met His Trp	20	—	1.0	0.4	0.5	0.3	0.1
20G Thr Met	20	—	—	0.4	0.5	—	—
20G5T	20	5	—	—	—	—	—
20G5T Lys	20	5	1.0	—	—	—	—
20G5T Lys Thr	20	5	1.0	0.4	—	—	—
20G5T Lys Thr Met	20	5	1.0	0.4	0.5	—	—
20G5T Lys Thr Met His Trp	20	5	1.0	0.4	0.5	0.3	0.1
20G5T Thr Met	20	5	—	0.4	0.5	—	—
Experiment II							
10G	10	—	—	—	—	—	—
10G Lys	10	—	1.0	—	—	—	—
10G Lys Thr	10	—	1.0	0.4	—	—	—
10G Lys Thr Met	10	—	1.0	0.4	0.5	—	—
10G5T	10	5	—	—	—	—	—
10G5T Lys	10	5	1.0	—	—	—	—
10G5T Lys Thr	10	5	1.0	0.4	—	—	—
10G5T Lys Thr Met	10	5	1.0	0.4	0.5	—	—

— ^a The other components of experimental diets have been described in the text.

² Hi-Pro, Shin-shin Food Industry Co., Ltd., Tokyo; the protein content on the basis of kjeldahl N × 5.70 was 71.0%.

was added to the last six groups. In addition, some growth-limiting amino acids of wheat gluten, *i.e.* L-lysine, L-threonine, L-methionine, L-histidine and L-tryptophan were added to some groups in various combinations.

In experiment II, 10% wheat gluten was used, and 5% L-tyrosine was added to the last four groups. L-Lysine, L-lysine and L-threonine, or L-lysine, L-threonine and L-methionine were added to some groups. The supplemental level of these amino acids was sufficient to fulfill the requirements of growing rats (10).

The composition of the 20% wheat gluten diet (in %) was as follows: wheat gluten 20.0, salt mixture³ (12) 5.0, vitamin mixture³ (12) 1.0, choline chloride³ 0.1, soybean oil 5.0, and α -corn starch³ 68.9. The diet also contained 0.07 ml of Chocola A⁴ per 100 g of diet. Variations in the amounts of added amino acids were made at the expense of α -starch. Food and water were provided *ad libitum*. During experimental periods all animals were individually housed in suspended screen-bottom cages and kept at a temperature of 24°C with a lighting period from 8:00 AM to 8:00 PM. Body weights were recorded daily, and food consumptions were determined on a dry material basis.

Analysis of urine. In experiment II, the urine was collected in 6 N HCl (20 ml) for each 2-day period, *i.e.* for days 2 and 3, 7 and 8, and 13 and 14, respectively. The collected urine was diluted with distilled water to a final volume of 250 ml. Aliquots of diluted urine were heated for one hour in a boiling water bath. Total phenols, *p*-hydroxyphenylpyruvic acid (pHPPA) and volatile phenols in acid-hydrolyzed urine were determined using the method of BERNHART and ZILLIKEN (13). It was confirmed that so called volatile phenols were not lost at all in these procedures. Homogentisic acid in the unhydrolyzed urine was determined by the micro-iodometric titration method of Neuburger (14).

Tyrosine determination. At the end of the experimental period, the rats were decapitated after a 6-hr fast and bled into heparinized test tubes. After centrifugation, samples of plasma were separated and deproteinized with an equal volume of 30% trichloroacetic acid and three volumes of water. Liver, which was perfused *in situ* with ice-cold 0.9% NaCl solution, and muscle of hind legs were homogenized in an equal volume of 30% trichloroacetic acid and three volumes of water. The deproteinized clear supernatants of plasma, liver and muscle were used for tyrosine determination. Free tyrosine concentration was determined by the method of Udenfriend and Cooper (15).

RESULTS

Weight gain and food intake

The supplement of 1.0% lysine·HCl and 0.4% threonine to the 20% wheat gluten diet (20G Lys Thr) resulted in the greatest weight gain, and the addition of

³ Tanabe Amino Acids Research Co., Osaka.

⁴ Vitamin A, 30,000 IU and vitamin D₂, 3,000 IU per ml. Eizai Co., Ltd., Tokyo.

Table 2. Effect of supplementation of limiting amino acids on growth and food intake of rats fed the 20% wheat gluten diets containing excess tyrosine (Experiment I).

Dietary groups	14 days			28 days			Development of lesions	
	Body weight gain (g)	Food intake (g)	Food efficiency* (%)	Body weight gain (g)	Food intake (g)	Food efficiency* (%)	Development of lesions	
							eye	paw
20G	20.3±1.3 ^b	132±1	16	34.9±1.6*	257±4	14	0/5	0/5
20G Lys	57.5±3.3**	156±4	37	122 ±4.8**	337±4	36	0/5	0/5
20G Lys Thr	64.5±2.7**	158±4	41	—	—	—	0/5	0/5
20G Lys Thr Met	63.1±1.5**	142±3	37	—	—	—	0/5	0/5
20G Lys Thr Met His Trp	65.2±1.2**	143±4	46	—	—	—	0/5	0/5
20G Thr Met	15.3±1.4***	120±6	13	38.5±2.4*	261±7	15	0/5	0/5
20G5T	13.6±1.4***	115±5	12	27.1±2.4***	231±8	12	2/5	2/5
20G5T Lys	53.6±1.2**	158±3	34	130 ±4.4**	376±10	35	0/5	0/5
20G5T Lys Thr	61.2±1.6**	168±6	37	—	—	—	0/5	0/5
20G5T Lys Thr Met	61.4±4.1**	144±3	43	—	—	—	0/5	0/5
20G5T Lys Thr Met His Trp	64.6±2.2**	140±3	46	—	—	—	0/5	0/5
20G5T Thr Met	10.5±1.2***	111±2	9	26.5±1.6***	236±4	11	2/5	2/5

^a (gain in body weight/weight of food eaten) × 100.^b Mean ± SE. Means not sharing a common number of asterisk are significantly different at $P < 0.05$.

For example, all the data having ** are not statistically significant, while those are significantly different from the data having * or/and. ***

1.0% lysine·HCl alone to the 20% wheat gluten diet (20G Lys) produced a weight gain of 95% of that found in the 20G Lys Thr group (Table 2). When rats were fed the 20% wheat gluten diet containing 5% tyrosine (20G5T) a growth depression was produced and two out of five animals suffered from pathological lesions of the eye and paw after three weeks. In rats receiving the 20G Lys diet including 5% tyrosine (20G5T Lys), pathological lesions were not observed and the growth depression was partially alleviated. When 0.4% threonine and 0.5% methionine were supplemented in the 20% wheat gluten diet without additional lysine (20G Thr Met), growth retardation was produced, and the inclusion of 5% tyrosine in the diet (20G5T Thr Met) caused eye and paw lesions in two out of five animals.

In rats given wheat gluten at the 10% level, the combined addition of 1.0% lysine·HCl, 0.4% threonine and 0.5% methionine (10G Lys Thr Met) was most effective in growth stimulation (Table 3). When excess tyrosine was included

Table 3. Effect of supplementation of limiting amino acids on growth and food intake of rats fed the 10% wheat gluten diets containing excess tyrosine (Experiment II).

Dietary groups	Body weight gain (g/14 days)	Food intake (g/14 days)	Food efficiency ^a (%)	Development of lesions	
				eye	paw
10G	8.0±1.0 ^b *	111±5	7.1	0/5	0/5
10G Lys	19.7±2.7***	130±8	14.9	0/5	0/5
10G Lys Thr	51.5±1.7****	158±4	32.7	0/5	0/5
10G Lys Thr Met	56.9±2.4****	160±6	35.7	0/5	0/5
10G5T	-4.9±0.8**	77±3	-4.8	5/5	5/5
10G5T Lys	1.0±0.9**	84±3	1.9	5/5	5/5
10G5T Lys Thr	23.8±3.9***	115±8	20.1	0/5	0/5
10G5T Lys Thr Met	46.0±4.7****	143±8	32.0	0/5	0/5

^a See Table 2.

^b Mean±SE. Means not sharing a common number of asterisk are significantly different at $P < 0.05$.

in the 10% gluten diet (10G5T), severe growth depression was caused and all animals suffered from eye and paw lesions. Addition of lysine·HCl alone to the diet (10G5T Lys) could alleviate neither growth retardation nor eye and paw lesions. When 1.0% lysine·HCl and 0.4% threonine were supplemented to 10G5T diet (10G5T Lys Thr), the growth depression due to excess tyrosine was partially alleviated and the pathological lesions diminished. When 0.5% methionine was added to the diet (10G5T Lys Thr Met), the growth was stimulated and the weight gain was about 92% of that found in the 10G Lys Thr Met group.

Ratio of weight gain to food intake correlated well with changes in body weight (Experiments I and II).

Tissue tyrosine concentrations

Table 4 shows the concentration of total phenols in plasma, and free tyrosine

concentrations in plasma, liver and muscle. When rats were fed the 20G5T diet, tyrosine concentrations were elevated very markedly in plasma, liver and muscle. Addition of lysine, the first limiting amino acid, to the diet effectively lowered free tyrosine levels of plasma, liver and muscle, although their values exceeded slightly that for the 20G group. The plasma tyrosine levels in animals fed the 20G5T Thr Met diet were not lowered; in fact the levels in liver and muscle exceeded even those in the 20G5T group.

Table 4. Effect of supplementation of limiting amino acids on total phenols and free tyrosine concentrations in plasma, liver and muscle of rats fed the 10% and 20% wheat gluten diets containing excess tyrosine.

Dietary groups	Total phenols	Free tyrosine		
	Plasma mg/100ml	Plasma mg/100ml	Liver mg/g	Muscle mg/g
Experiment I (28 days)				
20G	—	0.7±0.1 ^{a*}	0.07±0.01*	0.02±0.01*
20G5T	—	31.9±0.8***	0.13±0.03	0.50±0.02***
20G5T Lys	—	6.5±0.6**	0.10±0.01**	0.13±0.01**
20G5T Thr Met	—	24.4±7.1***	0.27±0.10	0.71±0.25***
Experiment II (14 days)				
10G	9.9±0.8*	1.0±0.1*	0.07±0.01*	0.01±0.002*
10G5T	118 ±20***	53.8±6.3***	0.26±0.06	0.30±0.09**
10G5T Lys	115 ±20***	45.6±9.4***	0.27±0.06***	0.61±0.16***
10G5T Lys Thr	125 ±15***	50.6±8.2***	0.34±0.06***	0.78±0.22***
10G5T Lys Thr Met	39 ±13**	6.8±1.8**	0.13±0.02**	0.14±0.05**

^a Mean±SE. Means not sharing a common number of asterisk are significantly different at $P < 0.05$.

Supplementing lysine, threonine and methionine in the 10G5T diet caused a lowering of the levels of total phenols in plasma, and of free tyrosine in plasma, liver and muscle, whereas the addition of lysine alone or lysine and threonine to the 10G5T diet was not only effective in lowering the levels of total phenols and free tyrosine in plasma but elevated free tyrosine levels in liver and muscle.

Urine analysis

A large increase in excreted amounts of total phenols, pHPPA and free tyrosine in urine was observed in rats fed the 10G5T diet (Table 5). When lysine and threonine or lysine, threonine and methionine were supplemented in the diet, the growth depression was alleviated as shown in Table 3. Urinary excretion of pHPPA and free tyrosine was not lowered and amounts of volatile phenols and homogentisic acid were higher than those in the 10G5T group.

DISCUSSION

In an early study (9) we observed that the toxic effect of tyrosine, which deve-

Table 5. Effect of supplementation of limiting amino acids on urinary excretion of total phenols, tyrosine metabolites and free tyrosine in rats fed the 10% wheat gluten diets containing excess tyrosine.

Days	Dietary groups	Total phenols	Volatile phenols (mg/2 days)	Total ketonic acids (pHPPA)	Homogentisic acid	Tyrosine
1-3	10G	21 ± 1 ^{a*}	4 ± 0.2 [*]	8 ± 0.5 [*]	1.5 ± 0.2 [*]	0.9 ± 0.1 [*]
	10G5T	223 ± 18 ^{**}	133 ± 14 ^{**}	51 ± 3 ^{**}	1.7 ± 0.1 [*]	13 ± 4 ^{**}
	10G5T Lys	242 ± 14 ^{**}	118 ± 12 ^{**}	48 ± 5 ^{**}	3.0 ± 0.3 ^{***}	16 ± 3 ^{**}
	10G5T Lys Thr	198 ± 23 ^{**}	131 ± 16 ^{**}	37 ± 4 ^{***}	1.4 ± 0.1 [*]	10 ± 2 ^{**}
	10G5T Lys Thr Met	247 ± 18 ^{**}	177 ± 2 ^{***}	38 ± 3 ^{***}	2.2 ± 0.6 ^{**}	10 ± 1 ^{**}
6-8	10G	18 ± 2 [*]	2 ± 1 [*]	10 ± 1 [*]	0.7 ± 0.1 [*]	1 ± 0.1 [*]
	10G5T	359 ± 20 ^{**}	94 ± 15 ^{**}	70 ± 6 ^{**}	0.7 ± 0.1 [*]	27 ± 1 ^{**}
	10G5T Lys	403 ± 21 ^{**}	120 ± 14 ^{**}	69 ± 5 ^{**}	1.2 ± 0.2 [*]	32 ± 3 ^{**}
	10G5T Lys Thr	438 ± 35 ^{**}	144 ± 14 ^{***}	66 ± 6 ^{**}	42.5 ± 28.1 ^{**}	25 ± 2 ^{**}
	10G5T Lys Thr Met	391 ± 52 ^{**}	169 ± 11 ^{****}	58 ± 6 ^{**}	75.1 ± 25.4 ^{***}	12 ± 1 ^{***}
12-14	10G	13 ± 1 [*]	3 ± 1 [*]	10 ± 1 [*]	0.8 ± 0.1 [*]	0.5 ± 0.02 [*]
	10G5T	232 ± 25 ^{**}	56 ± 4 ^{**}	69 ± 9 ^{**}	0.8 ± 0.1 [*]	20 ± 4 ^{**}
	10G5T Lys	301 ± 11 ^{***}	74 ± 3 ^{**}	75 ± 3 ^{**}	0.9 ± 0.1 [*]	23 ± 1 ^{**}
	10G5T Lys Thr	389 ± 60 ^{***}	203 ± 23 ^{***}	91 ± 11 ^{***}	49.2 ± 22.4 [*]	26 ± 3 ^{**}
	10G5T Lys Thr Met	445 ± 47 ^{***}	194 ± 2 ^{***}	101 ± 5 ^{****}	124 ± 9.0 ^{**}	27 ± 6 ^{**}

^a Mean ± SE. Means not sharing a common number of asterisk are significantly different at $P < 0.05$.

loped in rats fed a low wheat gluten diet containing excess tyrosine was not alleviated by addition of lysine, the first limiting amino acid. The present study confirmed that the growth retardation and the pathological lesions which developed in animals fed the 10% wheat gluten-high tyrosine diet were alleviated by the concomitant supplement of three limiting amino acids, lysine, threonine and methionine, but not by the addition of lysine alone. Furthermore, when 20% wheat gluten was used as the source of dietary protein, the tyrosine toxicity could be overcome by adding lysine alone or both lysine and threonine. The first and second limiting amino acids in this diet were lysine and threonine, respectively.

Another report (7) indicated that, when 10% casein was used as the protein source, the tyrosine toxicity was partially alleviated by the addition of methionine alone. In addition the growth retardation could be markedly reversed and the pathological lesions completely prevented by the supplement of both methionine and threonine which are limiting in this diet. Thus, the findings in the present and previous studies (7,8) demonstrate clearly that the addition of the limiting

amino acids to a low protein-high tyrosine diet has a beneficial effect in alleviating the toxicity.

The adverse effects resulting from amino acid additions can be classified by ELVEHJEM (16) and HARPER (17) into three categories: "toxicity," "antagonism" and "imbalance." Detrimental effects due to ingestion of diets containing excess amino acids have in general been included in amino acid "toxicity." However, it is suggested that in the case of tyrosine toxicity, the "toxicity" somewhat resembles a phenomenon of "imbalance," which is prevented by a small supplement of the limiting amino acid(s).

The present investigation has also demonstrated that the beneficial effect of the limiting amino acid supplements in reducing the toxicity in animals fed wheat gluten-high tyrosine diets was accompanied by a lowering of free tyrosine concentrations in plasma and tissues. This is in agreement with the results reported previously (8), in which the high levels of free tyrosine in plasma and tissues of animals fed the 10% casein diet containing excess tyrosine were lowered significantly by the addition of the limiting amino acids, methionine and threonine. ALAM *et al.* (2) have also shown that the supplement of threonine to a 6% casein diet containing excess tyrosine lowered the tyrosine concentration in plasma, liver, muscle and eye (3).

On the other hand, amounts of phenolic metabolites of tyrosine excreted in the urine of rats fed the low wheat gluten-high tyrosine diet supplemented with limiting amino acids, lysine, threonine and methionine, did not decrease significantly, as was the case in the results of a previous report (8) in which low casein-high tyrosine diet was supplemented by methionine and threonine. It would seem likely that the phenolic metabolite level in urine does not reflect the tyrosine toxicity.

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