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Effect of Excessive Basic Amino Acids in Supplemented Diet on the Weight Gain and Blood Urea Nitrogen Concentration of Mice

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염기성 아미노산의 과잉 투여가 흰쥐의 체중 및 혈액 중의 Urea Nitrogen 농도에 미치는 영향

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염기성 아미노산 (lysine, histidine, arginine)의 과잉투여가 흰쥐의 체중 및 혈액중의 urea nitrogen 농도에 미치는 영향을 조사하였다. 10% casein 먹이와 염기성 아미노산이 각각 5%씩 첨가된 먹이로 28일간 사육한 결과는 아래와 같다.

1. 염기성 아미노산이 과잉 투여된 群은 對照群(10% casein diet)에 비하여 성장율과 사료 섭취율이 감소되었으며 L-histidine HCl 添加群의 성장이 가장 저조했다.
2. 혈액중의 urea nitrogen 의 농도는 염기성 아미노산이 과잉 투여된 群이 對照群에 비하여 모두 높았으며 L-arginine 添加群이 가장 높았다.
3. 혈액중의 urea nitrogen 농도는 사료에 포함된 질소의 양과 관계가 있으며 성장량과는 무관한 것으로 나타났다.

Introduction

The addition of proper amounts of protein was reported to promote animal growth (Edozien and Switzer, 1978). Some previously reported data (Earle *et al*, 1942 ; Roth and Allison, 1949 ; Eagle, 1955 ; Naber *et al*, 1956 ; Baron, 1958 ; Cohen *et al*, 1958 ; Benevenga and Harper, 1967 ; Smith, 1968 ; Muramatsu *et al*, 1971 ; Benevenga, 1974 ; Rhee *et al*, 1978 ; Lee *et al*, 1979) have shown that the addition of excessive single amino acids to a low casein diet produced a growth depression, reduction of food intake, tissue damages of various organs and pathological lesions in animals.

Methionine was used very often to study the effect of excess amounts in young animals and was found to be the most toxic amino acid (Earle *et al*, 1942 ; Roth and Allison, 1949 ; Baron, 1958 ; Cohen *et al*, 1958 ; Benevenga and Harper, 1967 ; Benevenga, 1974 ; Lee *et al*, 1979). It was postulated that the toxic product was intermediate in the degradation of methionine (Roth and Allison, 1949). Tryptophan was considered to be the second toxic amino acid (Roth and Allison, 1949).

Several investigators studied on the alleviation effects of toxicity by supplementing some of the individual amino acids or combined ones (Roth and Allison, 1949 ; Benevenga and Harper, 1967 ; Smith, 1968).

The investigation reported in this paper is designed to explore the effects of addition of excesses of the three basic amino acids, lysine, histidine and arginine to the 10% casein diet to determine the growth depression and concentration of blood urea nitrogen.

Materials and Methods

1. The growth test of mice

Male weanling mice of the I. C. R. strain were used in this experiment. The initial body weights of mice were 20±1 *g* and were kept in individual suspended cages with screen bottoms.

In the first 5 days, the all young animals were fed basal diet ; after that they were separated into 4 groups, whose average weight differed by less than one gram. The composition of the basal diet was given in Table 1.

Table 1. Composition of basal diet

Constituent	%
corn starch[1]	80
casein[2]	10
soy bean oil[3]	5
salt mixture[4]	4
vitamin mixture[5]	1

1. Hayashi Pure Chemical Industries LTD, Osaka, Japan
2. Junsei Chemical Co., LTD, Tokyo, Japan
3. Samyang Food Industrial Co., LTD, Seoul, Korea
4. Supplying(*g/kg* diet) $CaCl_2$, 20.12 ; $MgCO_3$, 1.0 ; $MgSO_4 \cdot 7H_20$, 0.64 ; NaCl, 4.44 ; KCl, 4.456 ; KH_2PO_4, 8.48 ; $FeSO_4 \cdot 7H_2O$, 0.8;KI, 0.0032 ; $MnSO_4 \cdot nH_2O$, 0.014 ; NaF, 0.004 ; $AlK_2(SO_4)_4$, 0.0284 ; $CuSO_4 \cdot 5H_2O$, 0.036
5. Supplying (*mg/kg* diet) vitamin A(5,000 *IU/g*) 2.8523 ; vitamin D_2(400 *IU/g*), 0.0173 ; thiamin·HCl, 26.964 ; riboflavin, 26.964 ; ascorbic acid, 656.2942 ; nicotinic acid amide, 213.1897 ; pyridoxin·HCl, 5.0702 ; Ca-pantothenic acid, 42.6379 ; folic acid, 0.057 ; cyanocobalamine, 0.023 ; vitamin E, 25.9302

The experiments were carried on in 4 groups of 5 mice each. The groups were the control group and the groups supplemented with the 5% individual basic amino acids (lysine, histidine and arginine); all test animals were fed for 28 days.

In making the diet for the three groups supplemented with the additional amino acids, the same percentage was drawn out from corn starch. Amino acids added to the diet were L-forms. The experimental diet of each group are given in Table 2.

Table 2. Composition of experimental diet (%)

Constituent	Control group	Lysine group	Histidine group	Arginine group
corn starch	80	75	75	75
casein	10	10	10	10
soy bean oil	5	5	5	5
salt mixture	4	4	4	4
vitamin mixture	1	1	1	1
L-lysine·HCl[1]	0	5	0	0
L-histidine·HCl[2]	0	0	5	0
L-arginine[3]	0	0	0	5

1. Kanto Chemical Co., INC, Tokyo, Japan
2. Kanto Chemical Co., INC, Tokyo, Japan
3. Merck

Diets were mixed well into dry form and given ad libitum with fresh water. Animals were weighed at two days intervals in the morning just before being fed daily ration.

2. Blood urea nitrogen

Twenty eight experimental days later, the animals were killed and the amounts of the blood urea nitrogen by the Urease method (Berthelot reaction : Lee, 1970) were estimated. In brief the procedure was as follows : From the killed animals, whole blood was kept in indoors for 30 min and centrifuged (2,000 rpm, 10 min). Three tubes (test, standard, blank) were prepared, and each tube contained 0.2 *ml* of urease buffer. In the test tube was : 0.02 *ml* of serum;

in the standard tube, 0.02 *ml* of standard solution. In the blank tube 0.02 *ml* of distilled water was added, and incubated in a water bath (37°C) for 15 min. Then 1 *ml* of phenol color reagent and alkali hypochlorate was added to each. After that they were reincubated in the same condition. Eight *ml* of distilled water was then added and shaken well. In the blank tube an optical density of 670 *mμ* was read.

Results and Discussion

1. The growth test of mice

The effects of excessive feeding of three basic amino acids in a 10 % casein diet to growing mice are presented in Table 3. The data are ranked in increasing order of the growth depression.

The growth of mice was most effective in the control group. Decreasing orders of growth were L-arginine, L-lysine•HCl and L-histidine•HCl group, the latter showing the most remarkable depression. It was depressed by 33.73 % as compared with the control group. Fig. 1 showed the growth curves of mice fed experimental diets for 4 weeks.

The curves were drawn at the maximum state of around 3 weeks showing sigmoid shapes. Food intake of the control group was 96.24 *g* for 28 days experimental period, while 5 % L-arginine group was 95. 13 *g* ; 5 % L-lysine•HCl, 94.27 *g* and 5 % L-histidine•HCl, 82.10 *g*. The

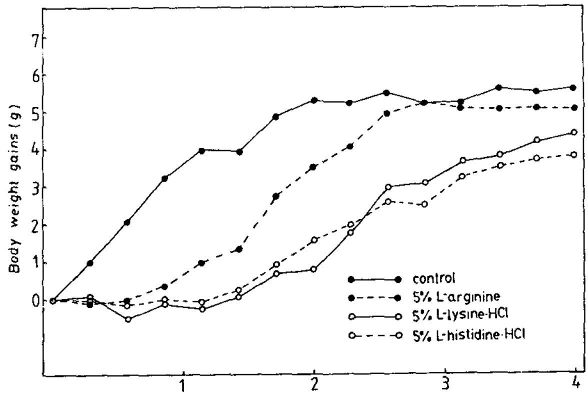

Fig. 1. Body weight of mice fed experimental diets for 4 weeks.

most remarkable depression was shown in the 5 % L-histidine•HCl supplemented group and the degree of depression appeared 14.69 % as compared with the control group.

Food intake and food efficiency were in the same pattern of decreasing order as the growth depression. These results (growth depression and food intake) indicate that the depression was due primary to food intake.

Sauberlich (1961) studied that toxicity of 19 amino acids including many DL-forms of them. He demonstrated that, when rats were fed a 6 % casein diet containing 5 % of individual amino acids, all amino acids studied except L-alanine produced growth depression to varying degrees. The data reported by Muramatsu *et al* (1971) also proved that the grades of toxicities of 5 % individual L-amino acids supplemented to the

Table 3. Effect of excessive level of individual amino acids on the weight gain, food intake and food efficiency of mice fed a 10% casein diet for 4 weeks*

Addition to basal (10%casein) diet	Initial body weight (*g*)	Final body weight (*g*)	Weight gain		Food intake		Food efficiency
			(*g*)	(%)	(*g*)	(%)	
none (control)	19.21±0.68	25.14±0.58	5.93±0.36	100	96.24±1.44	100	6.15±0.89
5% L-arginine	21.15±0.76	26.40±1.00	5.25±0.71	88.53	95.13±5.13	98.85	5.66±0.71
5% L-histidine•HCl	21.40±0.34	25.82±0.18	4.43±0.47	74.70	94.27±3.47	97.95	4.55±0.58
5% L-lysine•HCl	20.16±0.29	24.09±0.32	3.93±0.37	66.27	82.10±3.40	85.31	4.46±0.32

* : Results were expressed as mean±s.e. and food efficiencies were calculated in the formula of : gain in body weight/weight of food eaten × 100.

10% casein diet, all produced growth depression to varying degress.

Similar interelationships were observed in our experiments in the depression order of 5 % L-arginine, 5 % L-lysine·HCl and 5 % L-histidine·HCl supplemented groups to the 10 % casein diet compared with them.

All three groups supplemented with the basic amino acids showed a greater growth reduction rate than a food intake reduction rate. Smith (1968) showed that L-arginine (0.5-3.5 %) supplemented groups produced a greater food intake depression than weight gain depression while a L-lysine (0.4 %) and L-histidine (1.6 %) supplemented group produced a greater weight gain depression than food intake depression in chicks. There is a discrepancy between Smith's work and our study. No explanation for this discrepancy is available at this time. Arginine can be synthesized by rats, but not at a sufficiently rapid rate to meet the demands for maximum growth ; so its classification as essential or non-essential is purely a matter of definition (Rose *et al*, 1948).

Several investigators have demonstrated the effects of diets supplemented with excess arginine. Previously Rogers and Harper (1965) demonstrated that the addition of arginine (1 % arginine·HCl) to the amino acids diet showed weight gain of 1*g*/day in average during the two week period. Tews (1981) concluded that the growth depressions caused by arginine (4.5 %) were consistantly associated with large decreases in brain lysine concentrations and large decreases in tissue arginine and ornithine concentrations. The addition of 5 % L-arginine to the 10 % casein diet resulted in the slight growth depression and reduction of food intake (Muramatsu *et al*, 1971).

Lysine was widely used to elucidate the effects of weight gain and food intake alone or combined with some other amino acids added in casein diet. The investigation reported earlier showed that the supplementation of lysine to the diet significantly effected growth gain (Rosenberg and Culik, 1957 ; Lee *et al*, 1976 ; Lee *et al*, 1977). Lee *et al* (1977). demonstrated that a group on rice diet supplemented with 0.25 % L-lysine·HCl gained the highest weight.

The group with food supplemented with 0.5 % to 0.10 % of L-lysine·HCl showed substantial improvements in growth rate and in the efficiency of food utilization accompanied by a decrease in the amounts of fat and by an increase in the amounts of protein deposited in the liver (Rosenberg and Culik, 1957). The L-lysine·HCl supplemented group was effected in growth gain by exceedingly increased fat in the liver and a decrease of free threonine level in the plasma (Lee *et al*, 1976). The two papers about the contents of fat in the liver and the feeding of a L-lysine supplemented diet showed the discrepancy. In the present study, no attemps were made to define the contents of fat in the liver when added the L-lysine to the diet. However, the discrepancies in these studies of various investigators may be due to the different diet, experimental environments, strains or the complex relationships among them.

The requirment of histidine in the rat by Rose *et al* (1948) is 0.4 % of the diet. Rama Rao *et al* (1959) demonstrated the minimum requirements for histidine by the weanling rats for maximum growth was 0.21 % of the diet. Miyazaki *et al* (1961) demonstrated the effects of feeding 2 to 3 % excess individual essential amino acid on the growth depression of rats fed a low casein diet. They have found that DL-methionine, DL-tryptophan, L-phenylalanine and L-histidine caused greater growth depression than the other essential amino acids. Muramatsu *et al* (1971) also elucidated that L-histidine was the most severely depressing amino acid in the second group based on the three groups they examined. Thus the ratio of total protein in the diet, the ratio of amino acids in intact protein and the ratios of individual amino acids in mixture can all affect food intake (Mercer *et al*, 1981). The efficiencies are different according to individual amino acids. Eventually depressed food intake caused depressed growth gain.

2. Blood urea nitrogen

The observed values of blood urea nitrogen concentrations from the control, L-lysine·HCl, L-histidine·HCl and L-arginine groups are listed in Table 4 and graphed in Fig. 2 for easy comparison.

Table 4. Comparison of blood urea nitrogen concentration by feeding excess basic amino acid supplemented

(unit: *mg*/100 *ml*)

Addition to basal diet	Blood urea nitrogen
None (control)	8.44
5 % L-lysine·HCl	11.58
5 % L-histidine·HCl	14.12
5 %L-arginine	19.28

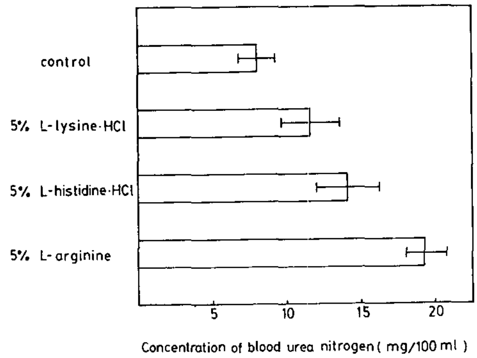

Fig. 2. Effects of the excessive basic amino acid supplemented diet on the concentration of blood urea nitrogen.

These findings are arranged by each group in increasing order of blood urea nitrogen concentration, not in increasing order of growth depression. Based on the present study the concentration of blood urea nitrogen was highest in 5 % L-arginine supplemented group and lowest in the control group.

This order differed greatly with the data on the animal growth patterns. Lysine, histidine and arginine contain 2, 3, and 4 nitrogen elements, respectively. Therefore our investigations suggest agrement with the hypothesis that the concentrations of blood urea nitrogen were due to the amounts of nitrogen contained in the diet, and not due to the growth gain. There has been a lack of agreement in the literature concerning the concentration of blood urea nitrogen in mice fed the low casein diet supplemented with basic amino acids. In reference, normal values of blood urea nitrogen in human has been 10-18 *mg/dl* of blood (Lee, 1970).

Summary

The effects of casein diet supplemented with excessive levels of three basic amino acids - lysine, histidine and arginine - were studied in male, weanling, I. C. R. strain mice.

The relationship between weight gain and concentration of blood urea nitrogen was also discussed with the followg results:

1. The groups supplemented with basic amino acids were demonstrated depressed growth and food intake than the control (10 % casein diet) group, 5 % L-histidine·HCl supplemented group being most depressed.
2. The concentration of blood urea nitrogen in the basic amino acid supplemented groups were higher than the control group. The value of L-arginine supplemented group was highest.
3. The concentration of blood urea nitrogen was related to the amounts of nitrogen contained in the diet not related to the growth gain.

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