

Effect of Delayed Supplementation of Wheat Gluten with Lysine and Threonine on its Capacity to Promote Growth in the Weanling Rat

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ABSTRACT When the food intake of rats was restricted, lysine supplementation of a wheat gluten diet was equally effective in supporting growth whether it was incorporated into the diet or given by gavage 6 hours after feeding. When food intake was unrestricted, incorporation of lysine into the diet proved to be the more effective mode of administration. If the second limiting amino acid, threonine, was also supplied, with consequent more rapid growth, incorporation of lysine into the diet became more effective even if feeding was restricted. The growth response to threonine supplementation of a wheat gluten-lysine diet was found to be independent of the time of supplementation.

Berg and Rose (1), using a tryptophan deficient diet, were the first to show that amino acid supplementation of a diet lacking an amino acid may be relatively ineffective if the amino acid is not administered approximately simultaneously with the diet. Since that time the results with tryptophan have been confirmed and the same principle has been found to apply to diets deficient in lysine and methionine (2). It has been generally accepted that this principle applies to all incomplete amino acid mixtures or proteins, i.e., supplementation with any amino acid is relatively ineffective unless the supplement is ingested at approximately the same time as the deficient amino acid mixture or protein. Furthermore, it has been assumed that this is so because the animal's body is unable to store excess amino acids but catabolizes them immediately. More recently, Yang et al. (3) reported that lysine, when administered by intubation as much as 16 hours after feeding a wheat flour-based diet, was equally as effective in increasing growth rate and food efficiency as when it was incorporated into the diet. These experiments, however, were carried out under conditions of restricted food intake.

Reported herein are the results of studies designed to determine the relative effectiveness of supplementation of diets defi-

cient in lysine or threonine by incorporation of the limiting amino acid into the diet, or by administering it apart from the bulk of the diet by gavage under conditions of restricted and unrestricted food intake.

EXPERIMENTAL AND RESULTS

Male weanling rats of the Holtzman strain weighing 60 to 70 g were housed individually in wire-bottom cages in quarters maintained at constant temperature and humidity. Ten animals were used on each dietary regimen and all were allowed free access to water.

The protein of the diets was supplied by wheat gluten of which lysine and threonine are the first and second limiting amino acids, respectively. The basal diet contained 1.6% nitrogen and had the following percentage composition: wheat gluten,¹ 17; salt mixture, (4) 4; cellulose,² 5; corn oil,³ 8; glucose, 66 and a complete vitamin addendum.⁴

Received for publication June 14, 1963.

¹ Vicrum, Huron Milling Company, Huron, Michigan.

² Cellu Flour, Chicago Dietetic Supply House, Chicago.

³ Mazola, Corn Products Company, Argo, Illinois.

⁴ Micronutrients/100 g of food: (in milligrams) thiamine·HCl, 1.0; riboflavin, 2.0; pyridoxine·HCl, 1.0; Ca pantothenate, 10.0; niacinamide, 10.0; inositol, 5.0; choline, 100.0; *p*-aminobenzoic acid, 30.0; biotin, 0.05; folic acid, 0.2; α -tocopherol, 14.2; menadione, 14.2; vitamin B₁₂ triturate (0.1% triturate with mannitol), 10.0. In units: ergocalciferol, 300; vitamin A palmitate, 1600.

The experimental diets are shown in table 1. All additions were made at the expense of glucose. In the first experiments, rats of groups 1 to 3 were allowed free access to diets A (wheat gluten), B (wheat gluten + 0.3% L-lysine·HCl), and E (wheat gluten + 0.6% lysine + 0.45% DL-threonine), respectively. Food consumption was measured daily for animals receiving diets B and E. Groups 4 and 5 were allowed to consume diets A and C

TABLE 1
Composition of experimental diets

Diet	
A	Basal
B	Basal + 0.3% L-lysine·HCl
C	Basal + 0.6% L-lysine·HCl
D	Basal + 0.45% DL-threonine
E	Basal + 0.6% L-lysine·HCl + 0.45% DL-threonine

TABLE 2
Effect of feeding rats lysine- and threonine-deficient diets with the amino acid supplements either mixed in the diet or administered apart from the diet

Group no.	Dietary treatment	28-Day wt gain	Body length	Wt of fat body	Fat body
		g	cm	mg	% body wt
1	Diet A, (wheat gluten) ad libitum	31.6 ± 1.5 ¹	15.90 ± 0.17	501 ± 36	0.58
2	Diet B, (wheat gluten + 0.3% L-lysine·HCl) ad libitum	85.6 ± 6.2	18.66 ± 0.14	1359 ± 52	0.90
3	Diet E, (wheat gluten + 0.6% L-lysine·HCl + 0.45% DL-threonine) ad libitum	146.5 ± 5.4	20.13 ± 0.15	1918 ± 108	0.91
4	Diet A, (wheat gluten) ad libitum + L-lysine·HCl by gavage	65.7 ± 3.7	17.67 ± 0.24	899 ± 35	0.72
5	Diet C, (wheat gluten + 0.6% L-lysine·HCl) ad libitum + DL-threonine by gavage	138.4 ± 7.2	20.01 ± 0.18	1722 ± 123	0.85
6	Diet A, (wheat gluten), 10 g/day	13.6 ± 1.5			
7	Diet B, (wheat gluten + 0.3% L-lysine·HCl), 10 g/day	48.9 ± 1.9	17.8 ± 0.08	870 ± 35	0.76
8	Diet E, (wheat gluten + 0.6% L-lysine·HCl + 0.45% DL-threonine), 10 g/day	67.8 ± 1.7	18.3 ± 0.09	710 ± 57	0.59
9	Diet A, (wheat gluten), 10 g/day + 30 mg L-lysine·HCl by gavage	44.5 ± 1.8	17.4 ± 0.10	790 ± 52	0.72
10	Diet C, (wheat gluten + 0.6% L-lysine·HCl), 10 g/day + 45 mg DL-threonine by gavage	68.0 ± 1.9	17.9 ± 0.10	820 ± 45	0.62
11	Diet E, (wheat gluten + 0.6% L-lysine·HCl + 0.45% DL-threonine) ad libitum	126.3 ± 3.5	19.2 ± 0.19	1440 ± 100	0.75
12	Diet D, (wheat gluten + 0.45% DL-threonine) ad libitum + L-lysine·HCl by gavage	79.2 ± 3.9	18.0 ± 0.21	1010 ± 72	0.70
13	Diet E, (wheat gluten + 0.6% L-lysine·HCl + 0.45% DL-threonine), 10 g/day	65.0 ± 1.8	17.1 ± 0.17	870 ± 39	0.67
14	Diet D, (wheat gluten + 0.45% DL-threonine), 10 g/day + 60 mg L-lysine·HCl by gavage	47.7 ± 1.8	16.7 ± 0.11	660 ± 25	0.59

¹ S.E.

(wheat gluten + 0.6% lysine·HCl) ad libitum and in addition each was given by stomach tube in a single dose 6 hours after feeding, the quantity of L-lysine·HCl or DL-threonine calculated to have been consumed on the previous day by the animals receiving diets B and E, respectively.

The rats comprising groups 6 to 10 were each restricted to 10 g of food daily as follows: group 6, diet A; group 7, diet B; group 8, diet E; group 9, diet A + 30 mg L-lysine·HCl by stomach tube 6 hours after feeding; and group 10, diet C + 45 mg DL-threonine by stomach tube 6 hours after feeding.

The animals were weighed at 5-day intervals and were killed after 28 days, when body length was measured and the epididymal fat bodies were weighed. The results shown in table 2 are the averages of at least 2 experimental groups.

The listed averages of body weight gains, body lengths and weights of epididymal fat bodies of rats of group 7 are not significantly different from the corresponding values for animals of group 9. This observation corroborates that of Yang et al. (3) that the time of supplementation of a wheat protein based diet with its first limiting amino acid, lysine, is not critical when the diet is restricted. The averages of body weight gains, body lengths and weights of fat bodies of animals of group 4, however, are all significantly less than the corresponding values for animals of group 2 ($P < 0.01$). This result demonstrates clearly that for maximal growth when food is unrestricted the time of supplementation is critical. Unexpectedly, the growth response due to the second limiting amino acid, threonine, was independent of the time of supplementation whether feeding was restricted or ad libitum. (The listed averages of the values for groups 3 and 5 and for groups 8 and 10 are not significantly different.)

It has been demonstrated that total body fat is proportional to the weight of the epididymal fat body (5). The weights of these organs and the body length measurements indicate that the observed differences in weight were not due solely to differences in amount of fat deposited.

To test the possibility that time of lysine supplementation might be critical even

with restricted food intake if the protein were of a higher quality and the animals, therefore, growing more rapidly, the following experiment was performed. The animals of group 11 were allowed unrestricted access to diet E. The food consumed was measured daily. Group 12 was allowed to consume diet D (basal + 0.45% DL-threonine) ad libitum and in addition each animal was given by stomach tube in a single dose the quantity of L-lysine·HCl calculated to have been consumed on the previous day by the animals receiving diet E. The rats in group 13 and 14 were each restricted to 10 g of diet E and D, respectively. In addition, group 14 received by stomach tube 6 hours after feeding 60 mg of L-lysine·HCl. The 28-day average weight gains of rats of duplicate experiments are also recorded in table 2. The listed averages of body weight gains, body lengths and weights of fat bodies of animals of group 13 are all significantly greater than the corresponding values for animals of group 14 ($P < 0.02$), and they show that rats even with a restricted food intake will utilize lysine more efficiently when it is incorporated into the diet than when given by stomach tube in a single daily dose if the quality of the protein is sufficiently high.

DISCUSSION

It has been adequately demonstrated that the lysine requirement for maintenance is much lower than that for growth (6, 7). This fact may help to explain why time of administration of lysine to rats ingesting a lysine-deficient diet, deficient also in a second amino acid, is not critical, when food is restricted and why, when the second limiting amino acid is also supplied with consequent more rapid growth it becomes so.

The results obtained in the present study with threonine supplementation make untenable the hypothesis that for efficient utilization all essential amino acids must be administered approximately simultaneously. These results also raise the question of the storage of threonine in the animal body. It is a matter of interest whether threonine is unique in this respect or whether one or more of those amino acids yet untested — histidine, leucine,

phenylalanine and valine — may also be well utilized by the rat when fed at a different time than the remainder of the diet. The report by Spolter and Harper (8) that injected isoleucine is much less effective than injected lysine in supporting growth of rats suggests that delayed supplementation with isoleucine would be relatively ineffective.

LITERATURE CITED

1. Berg, C. P., and W. C. Rose 1929 Tryptophan and growth. I. Growth upon a tryptophan deficient basal diet supplemented at varying intervals by the separate feeding of tryptophan. *J. Biol. Chem.*, 82: 479.
2. Geiger, E. 1947 Experiments with delayed supplementation of incomplete amino acid mixtures. *J. Nutrition*, 34: 97.
3. Yang, S. P., H. E. Clark and G. E. Vail 1961 Effects of varied levels and a single daily supplement of lysine on the nutritional improvement of wheat flour proteins. *J. Nutrition*, 75: 241.
4. Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart 1941 Choline in the nutrition of chicks. *J. Biol. Chem.*, 138: 459.
5. Stoerk, H. C., and C. C. Porter 1950 Prevention of loss of body fat by cortisone. *Proc. Soc. Exp. Biol. Med.*, 74: 65.
6. Mitchell, H. H. 1947 Protein utilization by the adult rat: The lysine requirement. *Arch. Biochem.*, 12: 293.
7. Henry, K. M., and S. K. Kon 1952 The biological value of the proteins (nitrogen) of dried skim milk: Effects of the addition of certain amino acids, of age of rat, and of level of protein intake. *J. Dairy Res.*, 19: 216.
8. Spolter, P. D., and A. E. Harper 1961 Utilization of injected and orally administered amino acids by the rat. *Proc. Soc. Exp. Biol. Med.*, 106: 184.