Nutrition of Protein in Young Carp—I. Nutritive Value of Free Amino Acids

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In various kinds of animal, a mixture of free amino acids imitating protein has been shown to maintain their normal growth and used to decide the essential amino acids on eliminating each amino acid from it. Indispensable amino acids for the salmonoid fishes were thus established by HALVER *et al.*¹⁻⁴⁾

Recently we tried to keep young carp on the diets in which casein and gelatin were partly or wholly replaced by amino acids. The young carp devoured these diets, but showed a marked retardation of growth corresponding to the amount of free amino acids, clearly differing from the young rainbow trout.

The hydrolyzates of casein after treatment with trypsin could not replace the protein and modification of a binder in the amino acid test diet was also ineffective. In the course of the present study, we learned that DUPREE *et al.*⁵⁾ found a similar phenomenon on a channel catfish, *Ictalurus punctatus*, and succeeded in establishing a formula of amino acid test diet to sustain the normal growth. The success was reported to have been brought by adopting a low Mg^{++} and low SO_4^{--} salt mixture in place of mineral salt mixture USP XIV previously used. The amino acid test diet similar to that of DUPREE *et al.*, however, failed to maintain the growth of young carp, suggesting that nutrition of protein in young carp has a quite different aspect from those of many other animals.

The present paper deals with these results.

Experimental

Analytical methods. Amino acids were determined by the usual method using an automatic amino acid analyzer. Tryptophan was separately analyzed by the *p*-dimethylbenzaldehyde method⁶⁾.

Digestibility of nitrogen in the test diet was determined by the method using chromium oxide as an indicator⁷⁾. The serum protein was estimated by a refractometer and erythrocyte number by a hemocytometer (Thoma-Line) and Hayem's diluting solution. The hematocrit value was measured by centrifuging the blood in a heparinized capillary tube at 11,000 r.p.m. for 5 minutes.

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Feeding method. The feeding method was the same as those described in the previous papers on vitamins⁸⁾. The fish were kept preliminarily on Test diet A (see below) for 2 weeks. They were fed four times everyday. On weighing the experimental fish, the quantity of diet was adjusted every week at the level of approximately 3% of the total body weight on dry basis. The young carp were kept in plastic troughs of each 100 l capacity. The troughs were supplied at the rate of 1 l/min. with well water adjusted at 25°C and aerated sufficiently. The rainbow trout, *Salmo gairdneri irideus*, were reared similarly in running water at 15°C. The feeding experiments were carried out in the period from October 25, 1967 to July 11, 1969.

The test diets. The HALVER diet⁹⁾ in which corn oil was substituted by refined soybean oil was adopted as the original formula (Test diet A). Casein in Test diet A was substituted by a mixture of amino acids (Test diet B), basing on the analytical data shown in Table 1. Both casein and gelatin in Test diet A were similarly replaced by amino acids (Test diet C). The compositions of Test diet A, B, and C are shown in Table 2.

Table 1. Amino acid composition of casein and gelatin used (%).

Amino acids	Casein	Gelatin
Arginine	3.32%	6.22%
Histidine	2.51	0.42
Isoleucine	4.83	1.14
Leucine	8.48	2.45
Lysine	7.13	2.90
Methionine	3.92	0.56
Phenylalanine	4.84	1.49
Threonine	4.05	1.89
Tryptophan	1.60	0
Tyrosine	5.04	0.25
Valine	6.27	1.92
Glycine	1.75	19.13
Alanine	2.23	7.49
Aspartic acid	6.70	4.72
Cystine	0.51	0
Glutamic acid	23.91	8.15
Proline	10.89	10.80
Serine	4.38	2.84

Gelatin in Test diet B was substituted by dextrin in Test diet D. A part of both casein and gelatin (78:22) in Test diet E which was very similar to Test diet A was substituted by dextrin (Test diet F) or by the amino acid mixture and dextrin (Test diet G).

The amino acid test diet of HALVER¹⁾ for chinook salmon (Test diet H) was adopted after replacing carboxymethylcellulose (CMC) by 10% agar-agar (Test diet I) and that

Table 2. Amino acid composition of experimental diets.

Test diets	A	B	C
Groups No.	1, 3, and 5	2 and 4	6
L-Arginine.HCl		1.77	2.70
L-Histidine.HCl		1.26	1.26
L-Isoleucine		2.78	2.47
L-Leucine		4.25	4.43
L-Lysine. HCl		3.45	3.87
L-Methionine	1.0	2.39	2.94
L-Phenylalanine		2.40	2.54
L-Threonine		1.89	2.25
L-Tryptophan	0.5	1.13	1.25
L-Tyrosine		2.65	2.41
L-Valine		3.11	3.29
Glycine		0.88	4.34
L-Alanine		1.30	2.43
L-Aspartic acid		2.73	4.01
L-Cystine		0.17	0.24
L-Glutamic acid		9.97	12.71
L-Proline		5.18	7.10
L-Serine		2.65	2.57
Amino acid mixture	1.5	49.96	62.81
Casein	54	..	..
Gelatin	15	15	..
Soy-bean oil	9	9	9
Dextrin, white	8	12	10.2
Minerals	4	4	4
Cellulflour plus vitamins	8.5	8.5	9.0
Carboxymethylcellulose	..	1.5	..

of SHANKS *et al.*⁴⁾ for rainbow trout after replacing white dextrin by gelatinized wheat starch (Test diet J).

The binder, CMC, in Test diet A was replaced by 10% agar-agar (Test diet K). Casein in Test diet K was then replaced by the casein-hydrolyzates prepared as follows (Test diet L). One part of trypsin was added to 80 parts of casein in a 5% solution adjusted at pH 8.0 with 20% sodium hydroxide. After incubation at 25°C for 2 hours, the solution was heated at 90°C for 10 minutes and lyophilized. About 93% of nitrogen was digested in this treatment, and the hydrolyzates containing a very small amount of amino acids were obtained.

In Test diets M to O, the level of cellulose powder or agar-agar was elevated to control the passage of diet in the intestine. The mixed amino acids were based on the formula

Table 3. Ingredients of experimental diets.

Test diets	D	E	F	G	H	I	J	K	L	M	N	O
Groups No.	7-9	10	11	12	13	14	16,17	18	19	20	21	22
Casein	15	54.5	9.1	9.1	—	—	—	54	—	46.4	—	—
Gelatin	—	15.4	2.6	2.6	—	—	—	15	15	—	—	—
Amino acid mixture	49.96*	—	—	50**	70**	70**	46.4**	—	—	—	46.4**	46.4**
Casein-hydrolyzates	—	—	—	—	—	—	—	—	54	—	—	—
Dextrin, white	27	16.1	71.9	24.3	6	6	—	—	—	—	—	—
Gelatinized wheat starch	—	—	—	—	—	—	25.0	8	8	18	18	18
Soy-bean oil	9	4	4	4	7	7	7	9	9	9	9	9
L-Methionine	—	—	—	—	—	—	—	1	1	—	—	—
L-Tryptophan	—	—	—	—	—	—	—	0.5	0.5	—	—	—
Minerals	4	4	4	4	4	4	4	4	4	5	5	5
Celluloflour plus Vitamins	8.5	4	4.4	4	3	3	7.6	8.5	8.5	1	1	1
Carboxymethyl-cellulose	1.5	2	4	2	10	—	8	—	—	—	—	—
Cellulose flour	—	—	—	—	—	—	—	—	—	20	20	—
Agar-agar	—	—	—	—	—	10	—	10	10	10	10	20

* After Halver (1957), ** After Shanks *et al.* (1962).

of SHANKS *et al.* for rainbow trout⁴⁾. The compositions of Test diets D to O are shown in Table 3.

Finally, the test diet devised recently by DUPREE *et al.*⁵⁾ for channel catfish, *I. punctatus*, which consisted of amino acids 33, dextrin 20, cellulose powder 20, corn oil 11, CMC 10, vitamins in dextrose 4, and mixed minerals specially prepared 2, was adopted after replacing dextrin by gelatinized wheat starch, corn oil by soy-bean oil, and CMC by agar-agar, respectively (Test diet P).

Results

The amino acid compositions of casein and gelatin used are summarized in Table 1 and the results of feeding experiments in Table 4 and Fig. 1.

By repeated feeding experiments, it was confirmed that the mixtures of amino acids imitating casein and gelatin could not replace these proteins in maintaining the growth of young carp. On the other hand, the rainbow trout exhibited a normal or only slightly retarded growth on the amino acid test diets. The carp receiving the amino acid test diets showed a good appetite, took the diet as much as the casein-gelatin diet, but grew very slowly or not at all. When each ten fish in Groups 15 and 16 were pooled and analyzed at the end of experiment, the following values were obtained: moisture, 74.7 and 73.8%,

Table 4. Results of feeding experiments.

Diets	Groups No.	Fish	No. of fish	Feeding period in weeks	Average body weight g		Feed conversion %
					Start	Final	
A	1	Carp	30	10	4.0	21.5	86.3
B	2					5.9	16.8
A	3	Rainbow trout	30	10	8.3	29.0	62.9
B	4					22.3	45.1
A	5	Carp	40	4	6.1	14.1	106.7
C	6					5.9	—
D	7	Carp	30	6	5.3	4.6	—
	8					4.4	—
	9					5.0	—
E	10	Carp	30	4	3.2	7.8	101.5
F	11					4.1	24.5
G	12					4.3	30.0
H	13	Carp	40	3	6.7	6.1	—
I	14					6.6	—
A	15	Carp	30	8	4.5	30.5	91.9
J	16					8.9	15.7
J	17	Rainbow trout	50	4	1.1	2.0	51.5
K	18	Carp	40	4	4.6	10.2	94.8
L	19					5.6	24.0
M	20	Carp	40	4	4.6	10.0	85.9
N	21					4.6	—
O	22					4.6	—
P	23	Carp	40	4	5.4	5.4	—

crude protein, 17.0 and 16.0%, and crude fat, 7.1 and 7.0%, respectively. The serum protein was both 2.8%, erythrocyte numbers were quite normal, 973,000 and 1,191,000 per mm³, and hematocrit values were 40.2 and 36.9%, respectively. No abnormality was thus recognized on the fish receiving the amino acid test diet.

The digestibility of nitrogen was determined by feeding Test diets A and B to the larger carp weighing 300 g in average, and found to be 81.9% in the former and 90.9% in the latter. Retention time of diet in the intestine was measured both on the larger and smaller carp. The intestine of carp contained Test diet B within 1 to 2 hours after the last feeding, but Test diet A within 4 to 5 hours. The higher level of cellulose flour or agar-agar in Groups 20 to 22 elongated the retention time of amino acid test diet in the intestine comparably to that of Test diet B, but did not improve the growth of fish.

In Groups 18 and 19, the casein-hydrolyzates induced a very slight growth, as shown

Fig. 1. Increase in average body weight.

in Fig. 1. In the light of the fact that digestion and assimilation of protein proceed throughout in the alkaline media at pH 7.0–8.5 in the carp lacking the stomach, the effect of pH of the amino acid diet on the carp growth was examined. Test diet D showing pH 6.2 was adjusted to pH 6.6 and 8.6 with 10% sodium hydroxide in Groups 8 and 9, respectively. This treatment, however, was without effect.

Discussion

All the mixtures of amino acids, based on the formulae of HALVER¹¹ and SHANKS *et al.*⁴, failed to maintain the growth of young carp. This was also true for the hydrolyzates

of casein. This phenomenon may be not attributable to the inadequate composition of amino acids, since they supported well the growth of rainbow trout.

The retention time of Test diet B in the intestine was nearly a half of that of Test diet A, but 90.9% of nitrogen was absorbed. Although the retention time in the intestine was elongated by addition of a large amount of cellulose flour or agar-agar to the amino acid test diet, the growth of the experimental carp was not improved. The amino acid test diet similar to that devised for the channel catfish by DUPREE *et al.* was ineffective on the growth of experimental carp. This fact may suggest that the protein nutrition of carp is different from that of a channel catfish.

HANAOKA¹⁰ observed by feeding goldfish for 9 months that the supplementary effect of tryptophan, cystine, and lysine to gelatin or zein was not so significant. In various kinds of animal, such as the rat, mouse, chick, turkey, quail, pig, silk worm, and human being, the amino acid test diet has been reported to sustain their growth and health normally. The inavailability of amino acids in carp may be worth studying from the viewpoint of animal nutrition, too.

Summary

By repeated feeding experiments, amino acid test diets after the formulae both for salmonoid fishes and for channel catfish were found to be unable to sustain the growth of young carp.

The hydrolyzates of casein after treatment with trypsin could not replace casein itself. A higher level of cellulose flour or agar-agar was effective in controlling the retention

time of amino acid test diet in the intestine to the extent of that of the casein-gelatin test diet, but the growth of young carp was improved not at all. These results suggest that there is a specific aspect in the protein nutrition of young carp.

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