# Metabolism of Amino Acids in Aquatic Animals—II

The effect of an amino acid supplemented casein diet on the growth rate of carp

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#### Abstract

By feeding the casein diet supplemented with pure amino acid, it was confirmed that the nutritive values are different for different amino acid being supplemented to casein diet.

In the case of L-alanine, L-valine, L-tryptophane, L-methionine and L-proline, the growth rate became higher than the pure casein, but the addition of L-phenylalanine and serine to the casein diet did not improve its nutritive value in comparison with pure casein diet.

Aoe et al. 1) reported that a diet of casein hydrolyzates with trypsin, pepsin or pronase was unable to maintain the normal growth of young carp, whereas casein as well as hydrolyzates sustained the growth of young rainbow trout. Hashimoto et al. 2) reported that carp require ten amino acids for optimum growth which are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. It is not known why amino acid test diets can not sustain the growth of carp. Experiments using a protein diet supplemented with amino acids to improve the nutritive value have not been reported.

The present study was undertaken to determine the growth rate of carp and the relationship of growth rate to the retention time of the diet in their intestine using a casein diet supplemented with amino acids.

### Materials and Methods

1. Experimental fish and feeding methods: Healthy carp (about 5 cm length) were purchased from a local fish hatchery.

Twenty carp were placed in each tank and acclimated for a week. The fish in each tank were fed four times a day as follows: Tank 1 — casein diet, its composition is shown in **Table 1**: Tank 2 — amino acid test diet, its constituents are shown in **Table 2**: Tank 3 — casein diet supplemented with amino acid. The vitamin and mineral mixture was prepared according to HALVER et al. (3).

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Table 1. Composition of test diet for carp.

Composition	Content (gm)
Casein	50
Soybean oil	5
Cod liver oil	2
Dextrin	19
Cellulose powder+Vitamin	10
Mineral mixture	4
Carboxy methyl cellulose	10
Water	100

**Table 2.** Composition of amino acid mixture.

Constituents	Amount (%)
L-Arginine·HCl	7.0
L-Histidine·HCl·H2O	3.0
L-Isoleucine	6.0
L-Lecine	8.5
L-Lysine·HCl	7.0
L-Methionine	2.5
L-Phenylalanine	5.0
L-Threonine	4.0
L-Tryptophan	1.5
L-Tyrosine	5.5
L-Valine	6.0
Glycine	8.0
L-Alanine	6.0
L-Aspartic acid	8.0
L-Cystine	1.0
L-Glutamic acid	10.0
L-Proline	7.0
L-Serine	4.0

The amount of amino acid added to the casein test diet was twice that contained in the amino acid test diet, i.e.: per 100 g in dry matters, L-alanine, 6.0 g; L-valine, 6.0 g; L-tryptophane, 1.5 g; L-glutamic acid, 10.0 g; L-methionine, 2.5 g; L-proline, 7.0 g; L-serine, 4.0 g; L-phenylalanine, 5.0 g.

- 2. Feeding methods: An aquarium,  $30 \times 30 \times 100$  cm was prepared as follows: a filter aid was used, and during feeding the water was circulated, aerated a 400 ml per minute and kept at room temperature. The feeding experiment was carried out for four weeks, the fish were fed the experimental diet four times a day and were weighed once a week. The amount of experimental diet fed each day was about 10% of the subjects' weight.
- 3. Measurement of intestine retention time: Each test diet was mixed with sudan black as reported in the earlier experiments. (4). The amount used was 500 mg in 25 g of the diet (wet materials). The average retention time was calculated from the start of feeding to excretion of the digested matter.

## **Results and Discussion**

The results of the feeding experiment are shown in **Table 3**. From these results it is evident that nutritive values differ between the amino acid diet and the amino acid supplemented casein diet. In the case of alanine, the growth rate was  $130.86 \pm$ 

Diets	• .	ody Weight m) at end	Gain (gm)	Increment (%)	Rate of Growth
Casein	1.80±0.49	$3.41 \pm 0.71$	$1.61 \pm 0.22$	$93.41 \pm 9.86$	100
Amino acid mixture	$1.67 \pm 0.44$	$1.64 \pm 0.49$	$-0.03 \pm 0.05$	$-2.78 \pm 3.73$	$-2.47 \pm 3.66$
Casein+L-Alanine	$1.97 \pm 0.37$	$3.93 \pm 0.88$	$2.14 \pm 0.51$	$118.74 \pm 3.95$	$130.86\!\pm\!22.68$
Casein+L-Valine	$1.50 \pm 0.40$	$3.03 \pm 0.89$	$1.53 \pm 0.49$	$100.44 \pm 5.89$	$111.08 \pm 21.97$
Casein +L-Tryptophan	$1.68 \pm 0.40$	$3.32 \pm 0.88$	$1.64 \pm 0.48$	$96.28 \pm 5.65$	$106.48 \pm 21.07$
Casein $+$ L-Glutamic acid	$1.95 \pm 0.56$	$3.39 \pm 0.91$	$1.44 \pm 0.35$	$74.87 \pm 3.56$	81.57± 7.67
$egin{array}{c} { m Casein} \ + { m L-Methionine} \end{array}$	$1.84 \pm 0.50$	$3.77 \pm 1.01$	$1.93 \pm 0.51$	$105.12 \pm 0.85$	$115.18 \pm 15.31$
$egin{array}{c}  ext{Casein} \ + ext{L-Phenylalanine} \end{array}$	$3.49 \pm 0.59$	$6.15 \pm 1.32$	$2.66 \pm 0.73$	$74.82 \pm 8.27$	$83.35 \pm 20.63$
Casein+L-Proline	$1.72 \pm 0.32$	$3.56 \pm 0.81$	$1.78 \pm 0.49$	$101.71 \pm 9.57$	$113.04 \pm 26.21$
Casein+L-Serine	$1.62 \pm 0.33$	$2.86 \pm 0.58$	$1.24 \pm 0.25$	$76.58 \pm 0.17$	$83.97 \pm 11.65$

Table 3. Weight and Growth Rates.

22.68, higher than that with the pure casein diet where the growth is 100. When L-valine, L-tryptophane, L-methionine, and L-proline were used the growth rate was 111.08±21.98, 106.48±21.07, 117.18±15.31 and 113.04±6.21 respectively, the use of these also resulting in a more rapid growth rate when the pure casein diet was used. Thus it is confirmed that the addition of these amino acids to the casein diet therefore resulted in a marked improvement of the diet.

However, the addition of L-phenylalanine and serine to the case in diet did not improve the diets nutritive value to the same degree, the rates of growth being:  $81.57 \pm 7.67$ ,  $83.35 \pm 20.63$  and  $83.97 \pm 11.65$  respectively, compared to that of the pure case in diet which was 100.

Test diets	Average Retention Time (minutes)	Rate of Growth	
Casein	255±10	100	
Amino acid mixture	105	$-2.47 \pm 3.66$	
Casein + L-Alanine	300	$130.86 \pm 22.68$	
Casein+L-Valine	275	$111.86 \pm 21.97$	
Casein + L-Tryptophan	130	$106.48 \pm 21.07$	
Casein+L-Glutamic acid	342	$81.57 \pm 7.67$	
Casein+L-Methionine	270	$115.18 \pm 15.31$	
Casein+L-Phenylalanine	155	$83.35 \pm 20.63$	
Casein+L-Proline	105	$113.04 \pm 26.21$	
Casein+L-Serine	110	$83.97 \pm 11.65$	

Table 4. Comparison: Intestional Retention Period and Growth Rate.

From these results it can be concluded that the addition of some amino acids to the casein diet, can enhance the diets nutritive value, whereas the addition of other amino acids to the diet produce a negative growth rate.

In addition, it was found that there was no difference in intestional tract retention time using the casein diet or the amino acid supplemented casein diet (**Table 4**).

## References

- 1) H. Aoe, K. Ikeda and T. Saito: Bull. Jap. Soc. Sci. Fish., 40, 375-379, 1974.
- 2) T. Nose, S. Arai, D. L. Lee and Y. Hashimoto: ibid., 40, 903-908, 1974.
- 3) J. E. HALVER: J. Nutrition, 62, 225-243, 1957.
- 4) Y. Tanaka, S. Hokazono, T. Katayama, K. L. Simpson and C. O. Chichester: *This Bull.*, **26**, 39-43, 1977.