# 魚類の消化酵素に関する研究 II.

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# Studies on Digestive Enzymes of Fishes—II. Effect of Dietary Change on the Activities of Digestive Enzymes in Carp Intestine

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In order to investigate the responses of digestive enzymes to dietary changes, young carp were fed diets with different contents of protein and carbohydrate for 75 days. Fish meal was used as the protein source and potato starch as the carbohydrate source. Maltase, amylase and protease activities of intestine showed adaptation to the dietary change within a week, and these three enzymes showed generally higher activities in the groups given diets with 40–60% starch throughout the experimental period. Low growth rate was noticed only in the high starch diet group (90 % starch) and a clear difference was not seen among the other three groups (20, 40 and 60% starch diets).

When four kinds of carbohydrate (maltose, sucrose, lactose and starch) were used as carbohydrate sources, maltase and amylase activities in intestine after 10 days feeding were high in carp fed the diets containing starch or lactose.

Intestinal protease activity of young carp clearly showed adaptation to the fish meal content in the diet when the starch content in the diet was kept constant. Growth rate increased in accordance with the increase of fish meal content in the diet.

In mammals, adaptation of various enzymes to the composition of diet has been investigated[1–3]. But there are very few studies about it in fishes[4].

In the previous paper[5], carbohydrase activities were determined in the digestive organs of several fishes. The present study was undertaken to investigate the adaptation of digestive enzymes in carp intestine to the diet by changing the relative contents of protein and carbohydrate; adaptation of carbohydrases to the various carbohydrate sources was also investigated.

## Materials and Methods

**Samples.** Young carp, *Cyprinus carpio*, weighing 7.5–30 g in body weight were supplied from a culturing pond near Maizuru, and breeded in the experimental aquarium ($32\times62\times41$ cm$^3$) maintained at the temperature of 22°C by circulating water with aeration.

**Diets.** White fish meal was used as the protein source and potato starch as the carbohydrate source. Premixed powder for carp purchased from KOHKIN Chemical Co., Ltd. was used as the vitamin source, and McCollum's salt mixture No. 185 was used as the mineral source. Diets equivalent to 3–5% of body weight of carp were given twice

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a day. All carp had a good appetite throughout the experimental period. For 7 days prior to the start of experiments commercial pellets for carp were given.

**Preparation of enzyme solution.** Five carps from each group were killed 2 hours after feeding in the morning. Intestine from each carps was rinsed with distilled water after discarding the contents. The tissue was homogenized in an adequate quantity of distilled water by a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000×g for 20 minutes. The supernatant was diluted to an adequate volume with distilled water and used as enzyme solution. Protein-N in the crude enzyme solution was determined by the biuret method.

**Assay of carbohydrase activities.** Activities of maltase and amylase were assayed by determining the liberated glucose with the Tauber-Kleiner's method with some modifications developed by the authors[5]. Assay conditions and composition of reaction mixture were as described in the previous paper[5]. The specific activity was expressed as μg of glucose liberated/mg of protein-N/hr.

**Assay of protease activity.** Activity of trypsin-like enzyme was assayed by determining the liberated tyrosine with the Folin's method. Milk casein was employed as substrate for the enzymic assay. Reaction was conducted at pH 9.5 and 37°C. The specific activity was expressed as μg of tyrosine liberated/mg of protein-N/hr.

## Results and Discussion

### Experiment I. Effect of relative contents of protein and carbohydrate in diet on the activities of digestive enzymes.

Carp averaging 30 g in body weight were divided into 4 groups. After being fed commercial pellets for 7 days, each group (40 carps) was fed different experimental diets for a definite period that followed. Compositions of the experimental diets are shown in Table 1.

**Table 1.** Compositions of experimental diets (Expt. I).

| Composition \ Group | A | B | C | D |
|---|---|---|---|---|
| Fish meal | 90 g | 60 g | 40 g | 10 g |
| Potato starch | 10 | 40 | 60 | 90 |
| Salts* | 1.5 | 1.5 | 1.5 | 1.5 |
| Vitamins** | 2 | 2 | 2 | 2 |

* McCollum's salt mixture No. 185.

** "Kohkin premix" powder (Kohkin Chemical Co., Ltd.). The compositions of "Kohkin premix" are as follows: Vitamins in amount per 100 g, A 50000 I.U., $B_1$ 50 mg, $B_2$ 100 mg, $B_6$ 50 mg, C 100 mg, D 10000 I.U., E 500 I.U., H 2 mg, $K_3$ 10 mg, Ca-Pantothenate 200 mg, Inositol 500 mg, Folic acid 10 mg, Nicotinamide 300 mg, Choline chloride 5000 mg, *p*-Aminobenzoic acid 300 mg.

Maltase activity of carp intestine, as shown in Fig. 1, increased in accordance with the increasing content of starch in the diet during 5 days of feeding. After 43 days, the activity was generally high in groups B and C. After 69 days feeding, all groups were fed C-diet (60% starch) for the purpose of estimating adaptation to the new diet, and then fed for 6 days. The activity of maltase after 75 days did not show any significant difference among these four groups. The activity of amylase, as shown in Fig. 2, showed almost similar patterns to that of maltase.

From the results, the adaptation of carbohydrase activities to the diet composition seems to appear in less than a week.

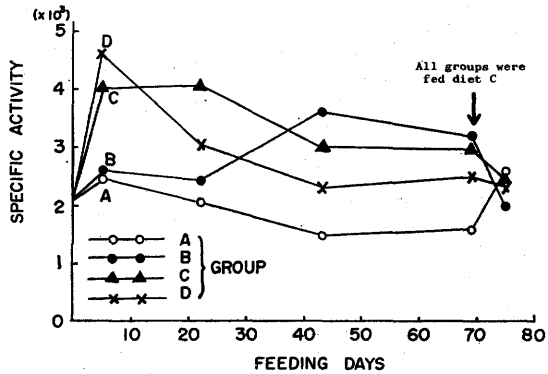


**Fig. 1.** Effect of diet variation on maltase activity in young carp intestine.

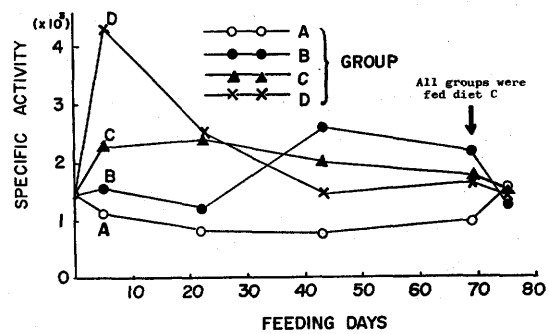


**Fig. 2.** Effect of diet variation on amylase activity in young carp intestine.

**Table 2.** Effect of diet variation on the protease activity* in carp intestine.

| Days \ Group | A | B | C | D |
|---|---|---|---|---|
| 0 | 4552 | | | |
| 5 | 4767 | 2538 | 3337 | 927 |
| 22 | 2637 | 2224 | 2859 | 1923 |
| 43 | 1942 | 2023 | 1090 | 129 |
| 69 | 2395 | 2912 | 1943 | 1096 |

* $\mu$g of tyrosine liberated/mg of protein-N/hr.

**Table 3.** Ratio of the protease activity to the amylase activity in carp intestine.

| Days \ Group | A | B | C | D |
|---|---|---|---|---|
| 0 | 100* | | | |
| 5 | 133 | 52 | 42 | 6 |
| 22 | 99 | 59 | 33 | 24 |
| 43 | 74 | 23 | 16 | 3 |
| 69 | 72 | 42 | 32 | 18 |

* The activity ratio at 0 day is expressed as 100.

On the other hand, aspects of tryptic activity are shown in Table 2. Group D generally showed lower activity throughout the experimental period. As shown in Table 3, the ratio of tryptic activity to amylase activity decreased generally in accordance with the increase of starch content in diet. This fact also indicates the enzyme adaptation to the diet.

Relation between the growth rate and the compositions of diets are presented in Table 4. The growth was estimated during 21 days from 22 days to 43 days after start of feeding. Among these four groups, only group D showed low growth rate and distinct differences were not noticed among the other groups.

**Table 4.** Effect of diet variation on the growth rate of carp.

| Group | A | B | C | D |
|---|---|---|---|---|
| Initial total weight(g) | 635 | 669 | 564 | 630 |
| Increase in total weight (g) | 86 | 82 | 67 | 27 |
| Growth rate (%) | 114 | 112 | 112 | 104 |

Each group consisting of 10 fishes was fed for 21 days.

## Experiment II. Effect of carbohydrate source in diet on the activities of maltase and amylase.

In the above experiments, the source of carbohydrate used was starch only. In the next place, adaptation of maltase and amylase to four kinds of carbohydrate source were examined in young carp, averaging 7.5 g in body weight and 7.5 cm in fork length. Fishes were fed in the experimental aquaria, 20 carps in each aquarium. Compositions of the experimental diets are shown in Table 5. Diets, which were equivalent to 5% of body weight of carp, were given twice a day. Five carps from each group were killed after 10 days feeding.

**Table 5.** Compositions of experimental diets used (Expt. II).

| | |
|---|---|
| Fish meal | 50 g |
| Carbohydrate* | 40 |
| $\alpha$-Cellulose | 10 |
| Vitamins | 1 |
| Salts | 2 |
| CMC | 2 |

* Maltose, sucrose, lactose or starch was used as carbohydrate source, respectively.

Fig. 3 shows the intestinal maltase and amylase activities of carp fed various carbohydrate diets. The maltase activity was highest in the lactose group and lowest in the maltose group. The amylase activity was higher in the starch and lactose groups than in the sucrose and maltose groups. According to these results starch and lactose might have the power to induce digestive enzymes such as intestinal maltase and amylase.

## Experiment III. Effect of protein content in diet on the protease activity.

In this experiment, protease and carbohydrase activities in young carp, averaging

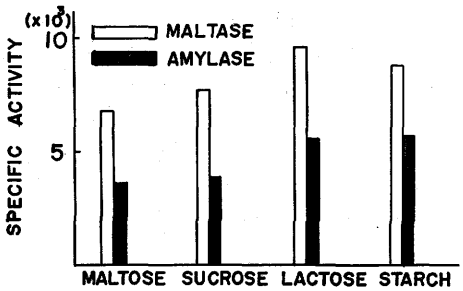


Fig. 3. Aspects of intestinal maltase and amylase activities in young carp fed various carbohydrate diets for 10 days.

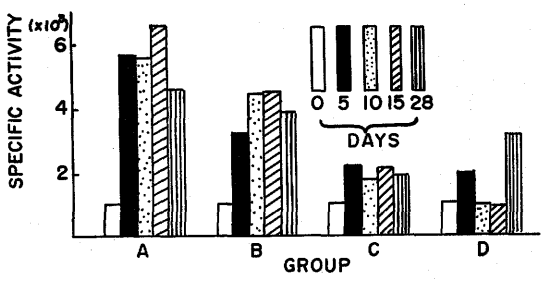


Fig. 4. Effect of diet variation on the protease activity in young carp intestine.

18 g in body weight and 10 cm in fork length, were examined when the starch content in diet was kept constant. Fishes were fed in the experimental aquaria, 35 carps in each one. Compositions of the experimental diets are shown in Table 6. Diets, which were equivalent to 4% of body weight of carp, were given twice a day.

Protease activity of each group, as shown in Fig. 4, was generally increased in accordance with the content of fish meal in the diets during experimental period and the adaptation of protease to diet composition was more evident than in Experiment I.

The relation between growth rate and diet compositions is shown in Table 7. Growth rate was determined after feeding each diet for 28 days and it increased in accordance with the content of fish meal in the diet.

In this experiment, effect of $\alpha$-cellulose content in the diet was not discussed. The diet for group D contained about 60% of $\alpha$-cellulose, and the content may affect con-

**Table 6.** Compositions of experimental diets (Expt. III).

| Group / Composition | A | B | C | D |
|---|---|---|---|---|
| Fish meal | 80 g | 60 g | 40 g | 20 g |
| Potato starch | 20 | 20 | 20 | 20 |
| $\alpha$-Cellulose | 0 | 20 | 40 | 60 |
| Salts | 2 | 2 | 2 | 2 |
| Vitamins | 1.5 | 1.5 | 1.5 | 1.5 |

**Table 7.** Effect of diet variation on the growth rate of young carp.

| Group | A | B | C | D |
|---|---|---|---|---|
| Initial total weight (g) | 133.5 | 109.5 | 104.4 | 103.9 |
| Increase in total weight (g) | 42.9 | 29.5 | 19.0 | 11.7 |
| Growth rate (%) | 132 | 127 | 118 | 111 |

Each group consisting of 10 fishes was fed for 28 days.

siderably the activities of digestive enzymes. FURUKAWA *et al.*[6] reported that the digestibility of protein in diet with various quantities of cellulose did not show clear differences, but the growth rate decreased in accordance with the increase of the cellulose content.

## References


1) J. P. REBOUD, A. BEN ABDELJLIL and P. DESNUELLE: *Biochim. Biophys. Acta*, **58**, 326–337 (1962).
2) A. BEN ABDELJLIL, A. M. VISANI, and P. DESNUELLE: *Biochim. Biophys. Res. Com.*, **10**, 112–117 (1963).
3) J. P. REBOUD, G. MARCHIS-MOUREN, L. PASERO, A. COZZONE, and P. DESNUELLE: *Biochim. Biophys. Acta*, **117**, 351–367 (1966).
4) G. NAGASE: *Zeit. vergl. Physiol.*, **49**, 270–284 (1964).
5) S. KAWAI and S. IKEDA: This Bull., **37**, 333–337 (1971).
6) A. FURUKAWA and Y. OGASAWARA: *ibid.*, **17**, 255–258 (1952).