魚類の消化酵素に関する研究 III

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Studies on Digestive Enzymes of Fishes—III Development of the Digestive Enzymes of Rainbow Trout after Hatching and the Effect of Dietary Change on the Activities of Digestive Enzymes in the Juvenile Stage

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The development of proteases, maltase and amylase activities in the digestive organs of rainbow trout, *Salmo gairdneri*, after hatching was investigated, and the responses of these enzymes to four kinds of experimental diets were also studied for 40 days in the juvenile stage of this fish. The results obtained are as follows.

Proteolytic activities of both pepsin-like and trypsin-like enzymes, and activities of maltase and amylase increased greatly during the 20 days after hatching, and 40–60 days after hatching these enzymes reached the activity level of young rainbow trout.

The activities of these four digestive enzymes in the juvenile rainbow trout fed various diets were all highest in the high-protein diet group (80% fish meal diet) and were generally proportional to the protein content. The growth rate paralleled the changes in enzyme activities. The results obtained with the juvenile rainbow trout were different from those found previously in the young carp.

From the point of view of fish culture, it is very important to study when digestive enzymes appear after hatching and how the enzyme activities increase along with growth. It is also interesting to investigate the enzyme adaptation to the dietary change in the larval or juvenile stage of fish.

There are very few studies about the development of digestive enzymes in fish[1,2], and there are no studies concerning the activities in a larval stage of fish.

In the present work, the development of protease and carbohydrase activities of rainbow trout were investigated after hatching, and effects of dietary change on the activities of these enzymes in juvenile stage of this fish were also studied.

Materials and Methods

Samples Eggs of rainbow trout, *Salmo gairdneri*, were supplied by a culturing pond at Samegai, Shiga prefecture, and were hatched in the experimental aquarium at 12°C. After hatching, the larvae were fed commercial pellets.

Preparation of enzyme solution All digestive organs were removed from 30–100 larvae and used for enzymic assay. The methods were described in the previous paper.[3]

Enzymic assay Activities of maltase and amylase were assayed by determining the glucose liberated from substrate with the TAUBER-KLEINER's method[4] or SOMOGYI-

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NELSON's method. The specific activity was expressed as μg of glucose liberated/mg of protein-N/hr., and total activity, mg of total glucose liberated/hr. Protease activity was assayed by determining the liberated tyrosine with the FOLIN's method. Milk casein was used as the substrate for the enzymic assay. Reaction was carried out at 37°C and pH 9.5 for trypsin-like enzyme, and pH 3.0 for pepsin-like enzyme. The specific activity was expressed as μg of tyrosine liberated/mg of protein-N/hr.

Results and Discussion

Development of Digestive Enzymes of Rainbow Trout after Hatching The development of proteolytic activities is shown in Fig. 1. A fairly high peptic activity was noticed in the eggs just before but decreased immediately after hatching. Therefore, this enzyme may have a role in hatching out. On the other hand, the tryptic activity was weak and showed no significant changes before and after hatching.

Twenty days after hatching, when the larvae had absorbed almost all of their yolk and started to feed, the peptic and tryptic activities increased greatly. Differing from other fish larvae, those of rainbow trout in this period had functional teeth on jaws and phalyngeal teeth, and developed gastric gland; Pyloric caeca also started to differentiate. The digestive systems in this period are reported to be almost similar to those of adults.[5]

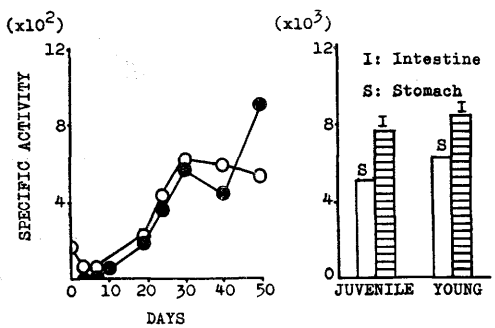

Fig. 1. Development of proteolytic activities of rainbow trout after hatching.
—●—, ▤ : Trypsin-like enzyme.
—○—, □ : Pepsin-like enzyme.
JUVENILE: 60 days after hatching. YOUNG: Body weight 70 g.

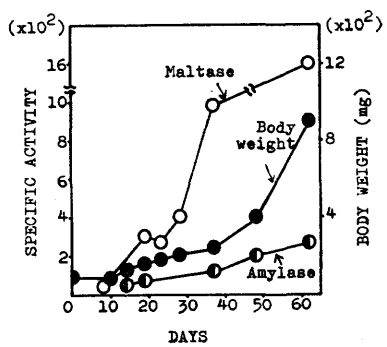

Fig. 2. Development of maltase and amylase activities of rainbow trout after hatching out.

Proteolytic activities, both peptic and tryptic, increased gradually in parallel to the growth, and 60 days after hatching they reached to the level of young rainbow trout weighing 70 g (Fig. 1).

On the other side, activities of maltase and amylase as shown in Fig. 2, were detected clearly from 20 days after hatching, and after another 20 days they reached to the level of young ones. These findings are interesting in reference to the fact that the food habits

of salmonidae have been considered to be carnivorous. Some carbohydrates such as glucose, sucrose and lactose have been reported to be actually digested and absorbed.[6]

From these results rainbow trout seem to complete development of their digestive systems morphologically and functionally within about two months after hatching.

Effect of Dietary Change on the Activities of Protease, Maltase and Amylase of Juvenile Rainbow Trout In the previous paper[7], the adaptation of digestive enzymes of young carp to various diets was described, and in the above experiment, development of some digestive enzymes of rainbow trout after hatching were described. This experiment was designed to elucidate how the adaptation to the dietary variation is achieved in juvenile rainbow trout. Juvenile rainbow trout, weighing 0.36 g, 45 days after hatching, were divided into four groups and fed in an experimental aquarium ($32 \times 62 \times 41$ cm^3) maintained at 10°C by tapping water. After being fed commercial standard pellets for 20 days, each group (200 fish) was fed different experimental pellets for a definite period that followed.

Table 1. Composition of experimental diets (g).

Component \ Group	A	B	C	D
Fish meal	80	60	40	20
Potato starch	15	15	15	15
α-Cellulose	0	20	40	60
Vitamin*	2			
Salts**	2			
α-Starch	5			

* "Kohkin Premix" for rainbow trout (Kohkin Chemical Co., Ltd.).
** McCollum's salt mixture No. 185.

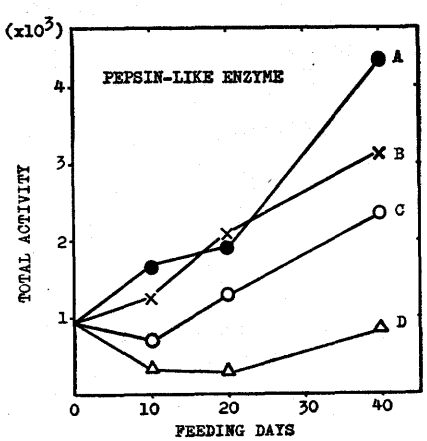

Fig. 3. Effect of dietary change on the pepsin-like enzyme activity in juvenile rainbow trout.

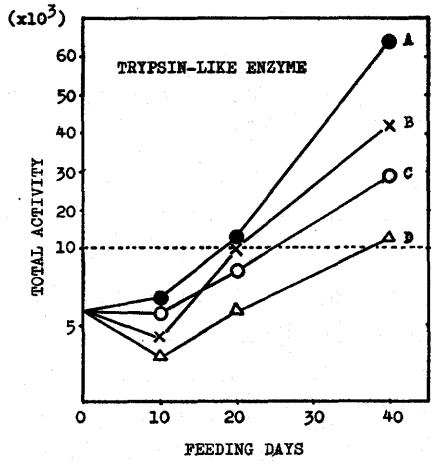

Fig. 4. Effect of dietary change on the activity of trypsin-like enzyme in juvenile rainbow trout.

Compositions of the experimental diets are shown in Table 1. The diets were fed as often as possible.

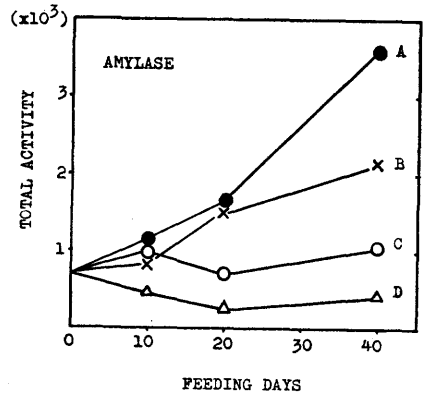

Fig. 5. Effect of dietary change on the amylase activity of juvenile rainbow trout.

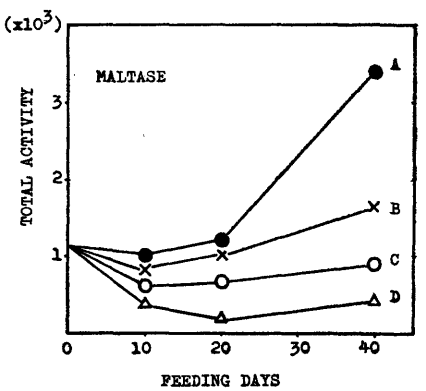

Fig. 6. Effect of dietary change on the maltase activity of juvenile rainbow trout.

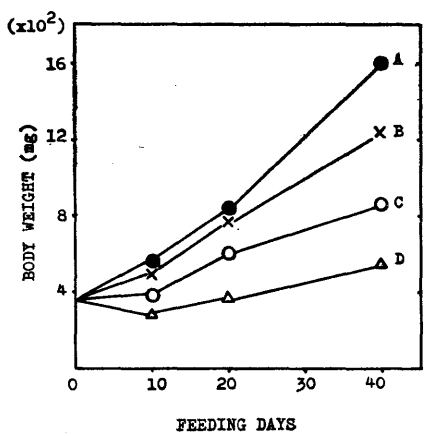

Fig. 7. Effect of dietary change on the body weight of juvenile rainbow trout.

The activities of pepsin-like and trypsin-like enzymes, amylase and maltase, as shown in Figs. 3–6, were all highest in Group A, in which the high-protein diet was given, generally throughout the whole experimental period, followed by Groups B and C. The velocity of the growth paralleled that of the increase of the enzyme activities (Fig. 7).

Among these four enzymes, only the activity of trypsin-like enzyme increased about 10-fold during the experimental period. Other enzymes developed only 3 or 4-fold during the same period.

The above results have led the authors to the conclusion that protein digestion in the juvenile stage of rainbow trout must be more dependent on the activity of trypsin-like enzyme than on that of pepsin-like one.

Similar results were reported that no differences were found between the peptic activity in juvenile rainbow trout and that in young or adult one, while the tryptic activity was extremely low in the juvenile stage and increased along the growth of fish.[1]

The adaptation of digestive enzymes to the diet composition must be different from that of young carp which was described previously.[7]

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