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Physiology

PRELIMINARY STUDIES ON PROTEOLYTIC ACTIVITY IN CARP (*CYPRINUS CARPIO* L.) LARVAE INTESTINES

WSTĘPNE BADANIA AKTYWNOŚCI PROTEOLITYCZNEJ W PRZEWODACH POKARMOWYCH LARW KARPIA (*CYPRINUS CARPIO* L.)

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Proteolytic activity and pH in intestines of carp (*Cyprinus carpio* L.) larvae aged 1 to 12 days were determined. The larvae were fed with natural food, artificial feeds, and starved. Diet-dependent growth rates were determined.

INTRODUCTION

To-date attempts to feed carp larvae with artificial feeds proved unsuccessful: insofar as the fishes did grow, their growth rate was much slower than that of fishes kept on an appropriate natural food offered in sufficient quantity (Opuszyński and Onoszkiewicz, 1973; Anwand et al., 1976; Albrecht et al., 1977; Appelbaum and Dor, 1978; Szlamińska, 1980). The principal cause of this phenomenon was looked for in the larval inability to digest an artificial feed. The inability is suggested to result from the lack, or deficiency, of endogenous digestive enzymes in juvenile developmental stages of carp (Dąbrowski and Glogowski, 1977 a).

The aim of studies undertaken at the Department of Pond Management, Inland Fisheries Institute, Żabieniec was to found out if intestinal pH of larval carp of up to 12 days would be sufficient for alcaline proteases, and to compare activities of trypsin-like proteases in larvae fed with natural food, artificial feeds, and starved.

MATERIAL AND METHODS

The materials consisted of carp larvae aged 1 do 12 days, fed with pond plankton and feeds (celamina, salmon and trout starters manufactured by Ewos, and trout starter manufactured by ZPP Słupsk) the insuitability of which to rear juveniles of the species had been proved in several tests.

The intestinal content pH was determined by grinding intestinal contents on a pH paper (0.2 g-ion/l accuracy), the resulting colour being then compared to colour spots produced by squirting buffers of a known pH on the paper. The pH of the buffer that left a spot of a colour most resembling that caused by the intestine content tested was considered to be the latter's pH.

The materials to be used in proteolytic activity assays were collected every evening. Alimentary tracts were dissected from the fishes caught. The contents of some intestines were squeezed out (empty intestines), the remaining alimentary tracts being left intact (full intestines). Microscopis examination failed to show any content in the starved fish intestines; thus they were treated as empty ones.

A sample consisted of 50, 25, or 10 dissected intestines, depending on size of the larvae. Each sample was homogenized, small amounts of toluene being added to the homogenizate to prevent microfloral growth.

Proteolytic activity of homogenizates was determined with a modified Remmert technique (Aleksandrowicz et al., 1971, 1973). As the substrate, sterile, agar-solidified 1.5% caseine solution of pH 0.8 was used. A filter paper circle was covered with a homogenizate placed on a substrate, and incubated at 44°C for 24 or 12 h. Under the influence of proteases, the matt substrate surface around the circle turned brighter, the area of the brighter zone being proportional to the amount of caseine lysed. As a proteolytic activity unit, 1 mg of caseine lysed during 1 h incubation was used and related to 1 mg wet weight of the larvae tested.

RESULTS

The mean intestinal content pH values of the carp larvae aged up to 12 days are reported below.

Type of food	Intestinal content pH (mean ± standard error)
Słupsk granulate	7.81 ± 0.11

Ewos granulate	7.70 ± 0.30
Natural food	7.49 ± 0.34
Starved fish	7.38 ± 0.11

No acidic pH zone was revealed in the intestines examined; the intestinal content pH did not drop below 7.0. The growth rate analysis of carp larvae fed with natural food, feeds and the starved ones shows that after 9 days of feeding, i.e., at the age of 12 days the natural food-fed larvae attained a mean individual weight of 18.9 mg, the weight of those larvae kept on artificial feeds amounting to 2.9 mg. Starved fishes of 12 days yielded their mean individual weight to be 1.2 mg on the day of the larval die-off. The 7th day of larval life was taken as termination of the yolk sac resorption.

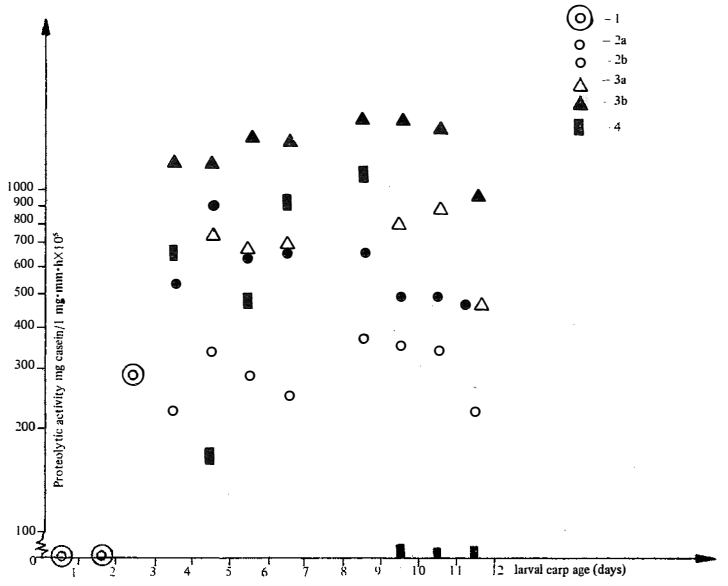

Fig. 1. Intestinal proteolytic activity vs. larval carp age. 1. Larvae before feeding, 2a. Larvae fed with natural food: activity in empty intestines, 2b. Larvae fed with natural food: activity in full intestines, 3a. Larvae fed with feeds: activity in empty intestines, 3b. Larvae fed with feeds: activity in full intestines, 4. Starved larvae. x – total absorption of yolk sac.

The results of growth-related proteolytic activity assays in the larvae are presented in Fig. 1. The proteolytic activity was first recorded in a homogenizate yielded by the larvae of 3 d, i.e., before feeding. The first feeding brought about a differentiation in the larval proteolytic activity. Three activity levels can be discerned:

- minimum: protease activity in empty intestines of the natural food-fed larvae;
- intermediate: protease activity in empty intestines of the feeds-fed larvae as well as protease activity in full intestines of the natural food-fed ones;
- maximum: protease activity in full intestines of the feed-fed larvae.

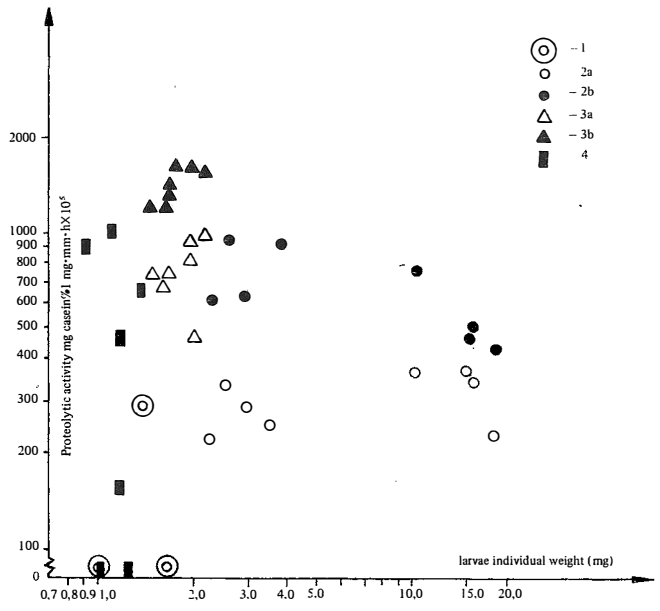

Fig. 2. Intestinal proteolytic activity vs. carp larvae body weight. 1. Larvae before feeding, 2a. Larvae fed with natural food: activity in empty intestines, 2b. Larvae fed with natural food: activity in full intestines. 3a. Larvae fed with feeds: activity in empty intestines. 3b. Larvae fed with feeds: activity in full intestines. 4. Starved larvae

The proteolytic activity of empty intestines of the larvae fed with natural food was on the level comparable to that of the larvae too young to be fed. In the initial stage of starving, the larval proteolytic activity remained on the level defined above as "intermediate" to vanish in the 10-d-old larvae, 3 days before the total stock die-off.

Fig. 2 illustrates changes in the larval proteolytic activity as related to the mean individual weight of the larvae in each age group. A slight increase in the individual weight of the larvae fed with feeds was accompanied by a strong increase in the proteolytic activity of these larvae, in both empty and full intestines. In those larvae fed with natural food the empty intestines proteolytic activity is slightly higher in heavier fishes than in those with a lower individual weight. The full intestines proteolytic activity clearly decreases with increasing individual weight.

The intestine content proteolytic activity is shown in Fig. 3. A drop in the activity with increasing individual weight is clearly marked in the natural food-fed larvae, while the feeds-fed larvae intestinal content shows a higher proteolytic activity than that in the larvae offered natural food.

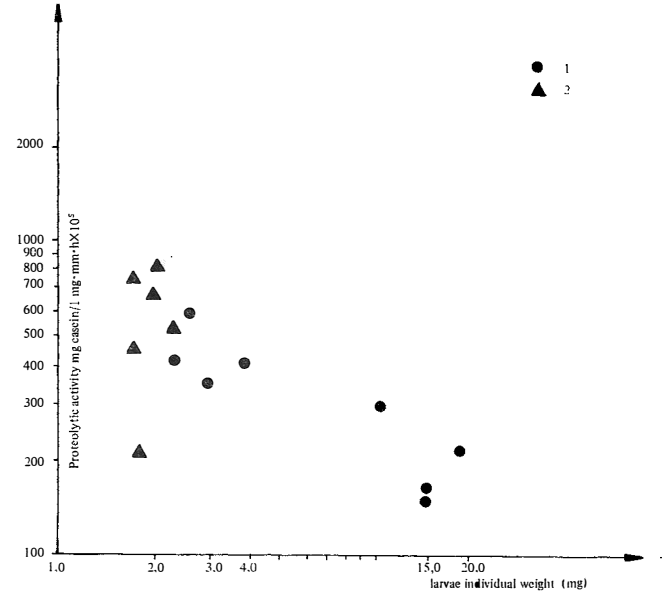

Fig. 3. Intestinal content proteolytic activity vs. carp larvae body weight. 1. Larvae fed with natural food. 2. Larvae fed with feeds.

DISCUSSION

No organ that would play the role of stomach has been found in post-larval stages of carp (Al Hussaini, 1949). Intestinal content pH of these fishes is usually 6.8–7.4 (Scerbina and Kazlauskene, 1971), which is close to the range found in the present study. According to Brehmer (1978), larvae of *Aristichthys nobilis,* a cyprinid, have a proto-stomach at their very early developmental stage. However, the author gave no details on functioning of the organ, its ability to excrete HCl and pepsin in particular. The optimal pH for the pepsin activity is 2 to 3 (Kitamikado and Tachino, 1960). Dąbrowski and Głogowski (1977 b) found the optimal pH of pepsin-like enzymes of natural food to cover a similar range. The present studies failed to demonstrate if the larval carp intestine had a pH enabling pepsin-like enzymes to work.

The *Carassius auratus* trypsin-like proteases have their maximum activity at pH slightly exceeding 8.0 (Jany, 1976). Dąbrowski and Głogowski (1977 b) determined the activity of natural food trypsin-like enzymes at pH 9.5, having earlier found them to exhibit a similar activity within the pH range of 8–10. The pH observed in the carp larvae studied here allows the trypsin-like enzymes of cyprinids and their food organisms to act.

Proteolytic activity assays of whole carp larvae intestines give no information on the digestive fluids enzymatic content, i.e. on a true digestive potential of an organism. In carp, the hepatopancreas surrounds and interlaces the intestine (Al Hussaini, 1949). It is

thus impossible to dissect an intestine only, to separate the intestinal tissue from the hepatopancreatic one. The pancreatic zymogenes become quickly activated in the presence of intestinal digestive fluids (Jany, 1976). In the present studies, the activity of empty larval intestines was presumably resulting from the activation of pancreatic zymogenes. When carp larvae become capable of active feeding, their pancreatic duct is well-developed, the pancreatic cells containing zymogenes (Tanaka, 1969), which suggests a possibility of enzymes being excreted into the intestine. The natural food, however, shows a specific activity of proteolytic enzymes (Dąbrowski and Glogowski, 1977 b). Although the dry feeds used showed themselves no proteolytic activity, they might have constituted a substrate for microorganisms that were probably a source of proteolytic enzymes in the intestinal contents of those larve offered the feeds. Thus, in the light of the present studies, the question whether or not the larval intestinal content proteolytic activity was brought about exclusively by the enzymes contained in food, or a part of this activity resulted from the fish proteases activity remains an open problem.

The absence of proteolytic activity observed in the larvae of up to 2 days (Fig. 1) was found also by Kawai and Ikeda (1973). At this stage the morphogenetic processes in the pancreas and intestine are very intensive (Kłębukowska, 1962). Pancreatic zymogenes synthesis occurs preseumably on the 3rd day of life.

In their initial stage of life, the starved larvae showed a relatively high proteolytic activity (Fig. 1), which would indicate a continued, in spite of the lack of food, enzyme synthesis. It is interesting to note that the disappearance of proteolytic activity observed in the starved larvae aged 10 days (Fig. 1) converges with the so-called "point of no return" (May, 1974) which occurs on the 10th day of life of starved carp larvae at 24°C (Wolnicki, pers. comm.). The phenomenon of proteolytic activity disappearance may be taken as an evidence of a lack of zymogenes in the fishes' pancreas or else may be a sign of a destruction of enzymatic activators in the intestinal epithelium (Noaillac-Depeyre, 1974).

The studies presented allow one to suppose that a slight increase in growth of larval carp fed with feeds enriched with extracts of larval carp intestines showing a proteolytic activity, as observed by Dąbrowska et al. (1979) was brought about by small amounts of vitamins or other feed deficiency-compensatory substances added to the feeds used rather than by the activity of digestive enzymes contained in the extracts.

CONCLUSIONS

1. The larval carp intestinal pH is favourable for the activity of alcaline proteases of fishes and of natural food.
2. The technique of proteolytic activity determination in the larval carp intestines, used in the present study, allows to conclude on the potential proteolytic activity, i.e., the simultaneous identification of the activity of already active enzymes and that of zymogenes activated during incubation of samples is possilbe.

3. In spite of a much higher proteolytic activity found in the intestinal content enzymes and those in the intestines of the artificial feeds-fed carp larvae, growth increments of those larvae were much lower then those in the larvae kept on natural food.
4. The clearly higher activity of proteases in the intestines of those carp larvae fed with feeds compared to that in the natural food-fed larvae suggest an attempt of an organism to adapt to feeding on an unusual food.
5. Starving, in its initial phase, poses no restriction on the intestinal proteolytic enzymes activity in carp larvae, the activity disappearing after several days of starving.

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WSTĘPNE BADANIA AKTYWNOŚCI PROTEOLITYCZNEJ W PRZEWODACH POKARMOWYCH LARW KARPIA (*CYPRINUS CARPIO* L.)

STRESZCZENIE

Przebadano pH i aktywność proteolityczną przewodów pokarmowych larw karpia w wieku od 1 do 12 dni, żywionych pokarmem naturalnym, paszą i głodzonych. Stwierdzono, że odczyn panujący w jelicie larw umożliwia działalność trypsonopodobnych enzymów trawiennych ryb. Najniższą aktywność proteolityczną zaobserwowano w pozbawionych treści przewodach pokarmowych ryb żywionych pokarmem naturalnym, wyższą – w pozbawionych treści przewodach pokarmowych ryb żywionych paszą oraz w wypełnionych przewodach pokarmowych ryb żywionych pokarmem naturalnym, najwyższą – w wypełnionych przewodach pokarmowych larw żywionych paszą.

U ryb głodzonych w początkowym okresie głodzenia aktywność proteolityczna utrzymywała się na poziomie nazwanym uprzednio wyższym. Zanikała zaś u larw w wieku 10 dni.

Mimo, że aktywność proteolityczna przewodów pokarmowych ryb żywionych paszą była wyższa niż u ryb żywionych pokarmem naturalnym, tempo wzrostu ryb żywionych paszą było znacznie powolniejsze, niż ryb żywionych pokarmem naturalnym.

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ПРЕДВАРИТЕЛЬНЫЕ ИССЛЕДОВАНИЯ ПРОТЕОЛИТИЧЕСКОЙ АКТИВНОСТИ В ПИЩЕВОМ ТРАКТЕ ЛИЧИНОК КАРПА (CYPRINUS CARPIO L.)

Р е з ю м е

Исследовали pH и протеолитическую активность пищевых проводов личинок карпа в возрасте 1-го до 12 дней, которых кормили натуральной пищей, комбикормами и лишали пищи. Обнаружили, что реакция действующая в кишках личинок карпа способствует действию трипсиноподобных пищеварительных энзимов. Самую низкую протеолитическую активность наблюдали в лишенных содержимого пищевом проводе рыб комплетных натуральной пищей, высокую- в лишенных содержимого пищевых трактах рыб кормленых комбикормами а также в заполненых пищевых проводах рыб кормленых натуральным кормом, самую высокую в заполненых пищевах проводах личинок кормленых комбикормами.

У голодающих рыб в начальном периоде голодания протеолитическая активность удерживалась на уровне называемом ранее высшим. Исчезала она у личинок в возрасте 10 дней.

Несмотря на то, что протеолитическая активность пищевых проводов рыб кормленых комбикормами является высшей, чем у рыб кормленых натуральной пищей скорость роста рыб кормленых кормом была меньшей, чем у рыб кормленых натуральной пищей.

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