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Comparison of Ontogenic Development and Digestive Enzymes in Ornamental Goldfish (*Carassius auratus auratus* L.) Larvae Fed with Decapsulated Cysts and Nauplii of *Artemia*

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Abstract.- The purpose of this study is to compare the growth of goldfish, *Carassius auratus auratus* L. larvae fed on *Artemia* nauplii and decapsulated cyst from 4 days after hatching (DAH) to 40 DAH. In detail, specific growth and survival rates, specific activities of total protease, lipase, amylase, and chitinase were examined in both experimental groups. At the end of the study, the highest survival rate was found in the group fed on cyst with 75.60±10.72% while the rate of the group fed on *Artemia* nauplii was found as 72.20±13.88%. Specific growth rate was calculated for larvae were fed by *Artemia* nauplii and cyst as 11.59±8.92%.d^{-1} and 12.02±4.94%.d^{-1}, respectively. Total protease activity presented increased profile during the early ontogeny in both experimental group. The highest total protease specific activity was determined on day 34 as 3.81±0.26 U/mg protein^{-1} in nauplii group. The maximum value for total protease (3.81±0.26 U.mg^{-1} protein) and lipase (557.38±24.77 mU/mg) specific activities were determined on day 34 in nauplii group ($p>0.05$). The peak of amylase (2.77±0.25 U.mg^{-1} protein) and chitinase (769.27±74.74 mU.mg^{-1} protein) specific activities were determined on day 34 in cyst group ($p<0.05$). Results of the study indicated that feeding on decapsulated cyst contributed positively to the larval development goldfish as much as *Artemia* nauplii.

Keywords: *Carassius auratus auratus*, ornamental fish, *Artemia* cysts, larval feeding, digestive enzymes.

INTRODUCTION

Aquaria which are known to have positive impact on human health in psychological and sociological aspects (Brodie and Biley, 1999; Herzog, 2011) have led to the development of a huge sector throughout the world (Livengood and Chapman, 2007). Many aquarium fish species need nourishing feeds which are suitable for spread and digestive system in the early larval period. Within this scope, manufacturers may not find a sufficient amount of live preys in the nature due to seasonal changes or other factors. Feed problems to be encountered while ontogenic development of larvae is one of the most crucial factors in terms of larval quality, health and feeding. In this regard, *Artemia* sp. is the most appropriate live prey for the larvae of the aquarium fish out of all live prey sources and has been used commonly since 1950s. *Artemia* species which are collected as egg in the nature could be given to the larvae either in the form of *Artemia* nauplii or as decapsulated cyst. As it has 30-40% more energy content, it can be used directly as food for larval feeding (Bengston *et al*., 1991; Vanhaecke *et al*., 1983). However, digestive enzyme activities mainly correlated with variety of the offered food to the larvae, the use of *Artemia* cyst in the larval feeding depends on the specific species since the larval nutritional requirements could be generated by the feeding habits during the early feeding of larvae.

Goldfish, a member of the Cyprinidae family, is the leading ornamental fish species in the aquarium sector in terms of its popularity and economy (Mischke, 2012). Although the development of this species (Kwasek *et al*., 2008), feed intake behaviours (Hidalgo *et al*., 1999; Silva *et al*., 2010) and enzyme activities according to the feeding times (Vera *et al*., 2007) were examined, there is no study examining the impact of feeding made with decapsulated *Artemia* eggs in the larval period on the digestive enzymes. Within this scope, in the present study, development, survival and digestive enzyme activities of the goldfish larvae fed by both *Artemia* nauplii and also cyst.

MATERIALS AND METHODS

The study was carried out in an aquarium fish farm, named Orta Doğu Akvaryum, found in Bergama, İzmir. Ten brood fish were placed into 6

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glass aquaria of 200 liter volume with the same fresh water temperature (which was 26°C). The aquaria were filled with filtered fresh water and located so as to receive natural sunlight through the southern window of the room and to remain in daylight for 15 h and in darkness for 9 days. Two simple sponge filters were put in the middle of the experiment aquaria. Kakabans (fish spawning place that made of nylon ropes) were put in the aquariums. The brood fish mated randomly. They were taken out from aquariums at the end of spawn. After hatching, kakabans were removed from the aquaria carefully and about 400 larvae were placed randomly each aquarium. This experiment was conducted in triplicate, with 2 trial groups: group 1 fed on *A. nauplii*; group 2 fed on decapsulated cyst.

External feeding started on the morning of the 4th day after hatching. Uneaten food were removed from experimental aquariums every day. Randomly, 30 larvae were sampled from each aquarium and were used to determine length, weight and analyses before feeding in the early morning of days 4, 7, 10, 13, 16, 19, 22, 25, 28, 31 and 34 after hatching.

Water quality parameters were measured on days when larvae samples were taken. Water temperatures were between 25.2±0.04 and 26.07±0.1°C. pH value were found to be minimum 8.61±0.02 and maximum 8.92±0.03 ppm.

Preparation of experiment feeds

For both experimental groups commercial Great Salt Lake- Utah origin *Artemia* sp. cyst were used (Salt Lake Cooperative GSLA, USA) and also nutritional value were reported 56% protein, 13% fat and 4% ash by the manufacturer. While decapsulated cysts were used directly, the unprocessed cysts were prepared in the previous evening for being reaped on the ensuing morning in order to achieve the *A.* nauplii Instar I phase (Bengston *et al*., 1991). On the other hand, in order to obtain of *A.* nauplii, cysts were decapsulated by 5% Hypochloride for 10 min and also incubated by during the 18 h at 28°C and also newly hatched nauplii were collected immediately. For each larvae, feed amount was determined by calculating 10% of its body weight (Mohanta and Subramanian, 2002).

Sampling and analytical procedure

In order to measure of the growth, larvae were sampled individually from each tank at 3 days interval (30 larvae sample group^{-1}). Specific growth rate was calculated by formulae SGR = 100 (Ln FBW−Ln IBW)/Δt, with IBW, FBW: initial, final body weight of fish (mg), Δt: time interval (day). At the end of the experiment, larval survival was determined by this formulae SR: (NFE/NFI)*100, SR: survival rate, NFE: number of fish at the end, NFI: number of fish initially. Pooled samples of larvae (30 individuals) were collected for enzyme analysis at 4, 7, 10, 13, 16, 19, 22, 25, 28, 31 and 34 DAH. Whole body homogenates were used for enzymatic assays and samples were taken at the same hour, before food distribution. Samples were collected and homogenized in 5 volumes v/w of ice-cold distilled water. Extracts utilized for enzyme assays were obtained after homogenization of larvae (35 mg ml^{-1}) in cold 50 mM Tris-HCl buffer, pH 8.0, followed by centrifugation (13.500xg; 30 min at 4°C). Total protease activity was assayed at 25°C and pH 9.0 using casein as the substrate (Walter, 1984). Amylase activity was measured at 37°C and pH 7.4 and pH 4.4 using starch as the substrate (Métais and Bieth, 1968). Chitinase activity was analysed at 37°C using chitin azure as the substrate (Hackman and Goldberg, 1964). Lipase activity was analysed at 37°C and pH 9.0 using the method of Mckellar and Cholette (1986) as modified by Versaw *et al*. (1989), using β-naphtyl caprylate as substrate. One unit of lipase activity was defined as 1 mg of β-naphtol released per minute. Enzyme activities were expressed as specific activities, *i.e.* U mg-1 soluble protein. Protein was determined by the Bradford procedure (Bradford, 1976). All spectrophotometric analyses were performed by Jenway 6300 UV-visible Spectrophotometer.

Statistical analysis

In this experiment, variance analysis was used between differences of days for enzyme analyses. One Way Anova was used to determine between days. While Levene's test was applied for the homogeneity of variances and also Kolmogorov-Smirnov test was performed for normality before this application. After ANOVA test, SNK (Student Newman Keuls) were used to compare the

differences for enzymes.

Significance test (t test) of the difference between two average values was conducted in order to reveal the differences of feeding on *A.* nauplii and decapsulated *Artemia* cyst while Levene's test was applied for the homogeneity of variances before this application.

SPSS 15 package software (SPSS INC. Chicago, IL, USA) was used in the statistical calculations.

RESULTS

Growth and survival

Survival rates were found satisfactory at the end of the experiment for both experimental groups. The highest survival rate was found in the group fed on cyst by 75.60±10.72%. This rate was found as 72.20±13.88% in the group fed on *Artemia* nauplii.

When standard length values were examined in the growth values of the nauplii and cysts groups (Fig. 1), the difference between the differences was found statistically significant on 13, 16, 22, 25, 28 and 31 DAH ($p<0.05$) while a difference could not be found in the differences of other days ($p>0.05$). When weights were compared, differences between the groups were found to be different and significant on 7, 10, 28, 31 and 34 DAH ($p<0.05$).

At the end of the experiment, the overall SGR was calculated as 11.59±8.92 and 12.02±4.94 for both *Artemia* nauplii and cyst group, respectively. In terms of specific experimental period, the highest SGR was found at the 1st period (days 4-10 after hatching) for both groups during the experiment. In this period, the highest SGR was detected in the nauplii feeding group (24.19±40.61%). This value was followed by the 2nd period (days 4-16 after hatching), the 3rd period (days 4-22 after hatching), the 4th period (days 4-28 after hatching) and the 5th period (days 4-34 after hatching) of the larvae fed on nauplii with 18.44±27.90%, 15.83±20.53%, 13.00±14.98% and 11.59±12.11%, respectively. The same trend was also observed in the group fed on decapsulated cyst and the highest SGR was found as 21.35±31.60% at the 1st period while SGR values were detected as 17.90±22.98%, 16.67±20.34%, 14.33±14.09% and 12.02±12.73% at the 2nd, 3rd, 4th and 5th periods, respectively.

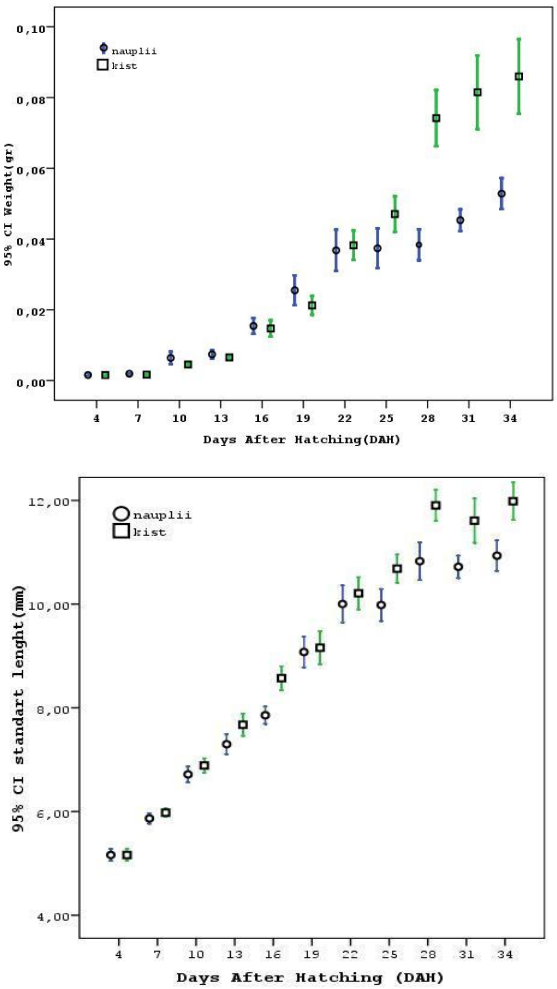

Fig. 1. Growth of *Carassius auratus auratus*, L. larvae weight (A) and standard length (B) fed with *Artemia nauplii* and decapculated cysts during the experiment. Each mean ±SD represents a pool of 30 larvae.

When the group fed on *Artemia* nauplii and the group fed on decapsulated cyst were compared, the SGR values of the 1st and 2nd periods were found higher in the nauplii group while SGR values of the 4th and 5th periods were found higher in the groups fed on cyst.

Digestive enzyme activities

Total protease activity presented increased profile during the early ontogeny in both group (Fig.2A). In nauplii group, total protease activity slowly increased until 16 DAH, after this date it

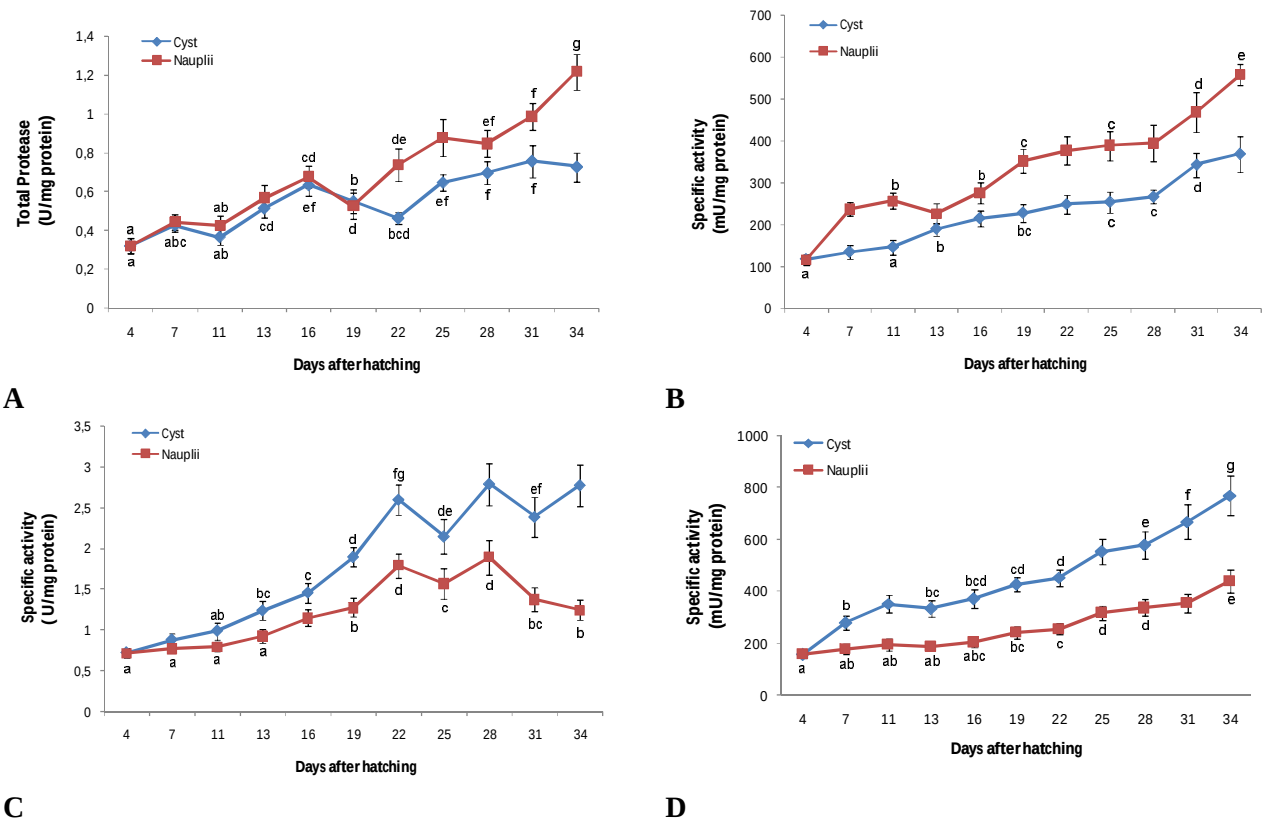

Fig. 2. Specific activities of total protease (A), lipase (B), amylase (C) and (D) chitinase during in *Carassius auratus auratus*, L. the experiment. Results are expressed as means ±SD (n=3). Different superscripts indicate significant differences between means.

suddenly decreased to 19 DAH and then it sharply rose up until 34 DAH. In addition to these, similar pattern was observed in cyst group while slowly increase followed by sudden decline and also slight increase was measured until 34 DAH. The highest total protease specific activity was determined on day 34 as 1.21±0.09 U/mg protein^{-1} in nauplii group (Fig.2A). There is significant differences between experimental groups ($p<0.05$).

In similar with total protease, specific activities of lipase were showed parallel pattern for both experimental groups. Although slight fluctuations in lipase activity of nauplii group were measured between 11 and 16 DAH, specific activity of lipase slowly rose up in both groups until 34 DAH. The highest lipase specific activity was determined on day 34 as 557.38±24.77 mU/mg protein^{-1} in nauplii group (Fig.2B). Significant differences were observed between experimental groups ($p<0.05$).

During the first 22 DAH, specific activities amylase constantly increased in nauplii and cyst groups and followed by important fluctuations up to 28 DAH. Specific activity of amylase showed slight variations in cyst group, however, this activity sharply decreased until 34 DAH. Amylase activities presented a complex profile in experimental groups. The highest amylase specific activity was determined on day 34 as 2.77±0.25 U/mg protein^{-1} in cyst group (Fig.2C). There is significant differences between experimental groups ($p<0.05$).

In contrast to amylase, specific activity of chitinase presented constant increased profile in both groups. At 31 DAH, chitinase activities slightly rose up in both groups, after this date specific activities suddenly increased until 34 DAH. The highest chitinase specific activity was determined on day 34 as 769.27±74.74 mU/mg protein^{-1} in cyst

group (Fig. 2D). There is significant differences between experimental groups ($p<0.05$).

DISCUSSION

Feed sources and feeding strategies used in larval feeding constitute a key point of success. This importance is also valid for goldfish which is a commonly farmed species throughout the world. Larval feeding still intensely relies on the forms of nauplii and metanauplii obtained from *Artemia* species. However, due to difficult process of *Artemia* decapsulation, certain practices are developed to eliminate the procedure and infrastructure dependency of obtaining *Artemia* nauplii by the manufacturers. Within this scope, the present study gave similar findings to those obtained in a previous studies (Kaiser et al., 2003) which was carried out on the goldfish (*Carrascius auratus auratus*) by using *Artemia* nauplii, cyst and both of them. Likewise, findings of Kaiser´s (2003) study related to feeding in the larvae of goldfish showed parallelism to the results of our study in terms of survival rates. Furthermore, it was reported that carp (*C. carpio*), red mullet (*Barbus barbus*) and herbivorous grass carp (*Ctenopharyngodan idella*) fed on decapsulated *Artemia* displayed a better growth in comparison to other commercial feeds and *Poecilia reticulate* fed on decapsulated *Artemia* eggs displayed better growth and survival rates than the group fed on moina (Dhert *et al*., 1997). Additionally a direct comparison between *Artemia* and decapsulated cysts revealed no differences in the growth of carp larvae, while decapsulated *Artemia* cysts produced significantly better growth than *Artemia* nauplii in *Leuciscus cephalus* (L.) and *C. idella* (Jahnichen and Kohlmann, 1999). In addition, it is known that decapsulated *Artemia* eggs are successfully applied in feeding of *Panaeus indicus* and *Astacus astacus* in the larval period (Ribeiro, 1998; Kouba *et al*., 2011). However, it was reported that survival rates of *Poecilia sphenops* and tetra fish fed on decapsulated *Artemia* eggs were found to be lower (Lim *et al*., 2002). This difference of survival rates can be attributed to nutritional quality of *Artemia* eggs used for feeding, biological properties of the fish species and the difference between the application procedures.

Specially, digestion mechanisms in fish larvae have been particularly studied during the last two decades as a means of understanding the nutritional needs of animals and the effect of dietary constituents on enzyme activity (Zambonino Infante and Cahu, 2001; Zambonino Infante *et al*., 2009). Moreover, knowledge in detail about digestive enzyme activity could be used as comparative indicator tool for better understanding of nutritional conditions and capabilities of fish larvae. Therefore, numerous detailed studies have been carried out to clarify and establish feeding strategies and also to adequate larval feeding protocols under culture conditions (Zambonino-Infante and Cahu, 2001; Zambonino-Infante *et al*., 2009; Suzer *et al*., 2006, 2007a,b). In the present investigation all the enzymes studied were detected at the first feeding of the gold fish. The specific activity of total protease is the main indicator for food acceptance in terms of protein content. In this study, findings supported this phenomenon were observed during the early ontogeny concurrently with exogenous feeding. Especially, relatively higher activities of total protease were measured and it is thought that it is strongly correlated with protein content of nauplii (Leger *et al*., 1987), since this activity was relatively lower in cyst group. At the end of the experiment, there was significant differences in the both experimental groups and it is also thought that this difference could be related with malnutrition by cysts in terms of protein content. Similar profiles about protease were reported in carp hybrids (Chakrabarti *et al*., 2006) and koi (*Cyprinus carpio* var. *koi*) larvae (unpublished data). The first detection of proteases in hybrids were measured concurrently with starting of exogenous feeding of gold fish larvae. In addition to these, this phenomenon was supported not only for ornamental fresh water but also for marine cultured species. In Mediterranean cultured species such as common dentex *Dentex dentex* (Gisbert *et al*., 2009), common pandora *Pagellus erythrinus* (Suzer *et al*., 2006), sharpsnout seabream *Diplodus puntazzo* (Suzer *et al*., 2007a), red porgy, *Pagrus pagrus* (Suzer *et al*., 2007b) and meagre *Argyrosomus regius* (Suzer *et al*., 2013), the first detection time of protease was occurred at the mouth opening and exogenous feeding at 3 DAH.

It is well known that lipase is produced mainly in the pancreas and it is thought to play a relatively minor role in lipid digestion in fishes, and catalyses the breakdown of triacylglycerol first to diacylglycerol and then to monoacylglycerol (Zambonino-Infante and Cahu, 2001; Kolkovski, 2001). It seems that lipid content of live prey and compound diet deeply effected response of lipase to its substrate. In this study, lipase expression in both groups presented constant increased profile in spite of fed with both *Artemia* nauplii and cyst. Similar findings about lipase profile were reported in carp hybrids whereas slightly increased profile followed between 4 and 26 DAH by sudden rise up until 34 DAH (Chakrabarti *et al*., 2006).

As reported by some researchers, amylase has been found to be an integral component of the enzymatic equipment in developing larvae of carnivorous fish and also enzymatic expression of amylase is usually stimulated by glycolytic chains, glycogen, and starch additionally food characteristics and feeding regime affected amylase activities in fish larvae (Zambonino-Infante and Cahu, 2001; Zambonino-Infante *et al*., 2009). In this study, complex profiles and also important variations of amylase activities were recorded in nauplii and cyst group. similar in carps hybrids whereas sudden increase followed by sharp decrease during the early ontogeny (Chakrabarti *et al.*, 2006). This fluctuated profile could be related with larval age, pancreatic organogenesis and also starch content of *Artemia* nauplii and cysts. Besides, in the study conducted by Leger *et al*. (1987), it was reported that *Artemia* had impact on amylase and trypsin enzymes and it might have played a key role in the digestive system of the larvae through its voyage to the intestines of the larvae.

It is well known that exoskeletons of crustacean and other marine organisms are the most usual chitinous structure, but chitin is also synthesized by many other invertebrates, bacteria and algae (Gutowska *et al*., 2004). Considering the results concerning the chitinase activity, it was detected that the specific activity displayed an increasing tendency in both groups during early ontogeny. Although this result shows parallelism with the amylase enzyme activity, it is thought that high amount of chitin found in the structures of cyst and shell, in particular, directly affects the increase of this activity. Increases in the amylase and chitinase activities in the groups fed on nauplii are closely related to larval age and the growth of pancreas. In addition, the amount of carbohydrate found in the feeds taken by the fish is thought to affect this change closely.

As a conclusion, the importance paid to the feeding applications made with *Artemia* eggs for larval feeding of many species in terms of ease of application and energy content has increased. On the other hand, contents of nutritional substances based on the origins of eggs should be evaluated in detail by also taking the survival, growth and cost factors into account. Intensifying the future studies on this framework is of great importance to evaluate this issue objectively.

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