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Enzymatic Characteristics and Growth Parameters of Ornamental Koi Carp (*Cyprinus carpio* var. *Koi*) Larvae Fed by *Artemia nauplii* and Cysts

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Abstract

The purpose of this study was to compare the larval growth and digestive enzyme activities of koi carp (*Cyprinus carpio* var. Koi) larvae fed on *Artemia nauplii* (AN) and decapsulated cyst (ADC). The experiment was carried out in triplicate and lasted 27 days. Specific growth and survival rates, specific activities of total protease, lipase, amylase and chitinase were examined in experimental groups. At the end of the experiment for group fed with AN total length, weight and SGR were 20.56±3.23 mm, 0.9±0.05 g and 2.03 % d^{-1}, respectively and for group fed with ADC it was 28.16±4.81 mm, 0.28±0.16 g and 6.02 d^{-1} ($P<0.05$). However, survival rates in the group fed on ADC was 98.88% while in the group fed on AN was 97.77% ($P>0.05$). Besides, there were significant differences on specific enzyme activities (total protease, amylase, chitinase, lipase) ($P<0.05$). Obtained results suggested that koi larvae fed by ADC presented relatively higher growth parameters.

Keywords: *Cyprinus carpio* var. Koi, ornamental fish, *Artemia* cysts, larval feeding, digestive enzymes.

Artemia nauplii ve Kisti ile Beslenen Koi (_Cyprinus carpio_ var. _Koi_) Larvalarında Enzimatik Özellikler ve Büyüme Parametrelerinin İncelenmesi

Özet

Bu çalışmanın amacı, *Artemia nauplii* (AN) ve dekapsüle *Artemia* kist (ADC) ile beslenen Koi (*Cyprinus carpio* var. Koi) larvalarının sindirim enzimlerindeki değişimleri ve larval gelişimleri ortaya koymaktır. Bu kapsamda, 27 gün süren ve 3 tekrarlı yapılan çalışmada gruplar arasındaki ağırlık ve boy gelişimi, spesifik büyüme ile hayatta kalma oranları ve sindirim enzimlerinden total proteaz, lipaz, amilaz ve kitinaz aktiviteleri incelenmiştir. Çalışmada AN ile beslenen grupta total boy, canlı ağırlık ve SGR değerleri sırası ile 20,56±3,23 mm, 0,9±0,05 g ve 2,03% d^{-1} tespit edilmiş olup, ADC ile beslenen grupta ise bu değerler 28,16±4,81 mm, 0,28±0,16 g ve 6,02 d^{-1} olarak saptanmıştır ($P<0,05$). Bununla birlikte, gruplar arasında yaşama oranı bakımından farklılık tespit edilmemiş olup, ADC ile beslenen grupta %98,88, AN ile beslenen grupta ise %97,77 yaşama oranı tespit edilmiştir $P>0,05$). Ayrıca her iki grubun enzim aktiviteleri incelendiğinde total proteaz, lipaz, amilaz ve kitinaz spesifik aktiviteleri farklı bulunmuştur ($P<0,05$). Sonuç olarak elde edilen bulgular dekapsüle edilmiş *Artemia* kisti ile beslenen koi larvalarının, *Artemia* nauplii ile beslenen Koi larvalarından daha iyi gelişim gösterdiğini ve yetiştiricilikte bu kistlerin etkin biçimde kullanılabileceğini ortaya koymuştur.

Anahtar Kelimeler: *Cyprinus carpio* var. Koi, akvaryum balığı, *Artemia* kisti, besleme, larva üretimi, sindirim enzimleri.

Introduction

Asia-origin koi carp are currently listed among the most important ornamental species as they can be reared in all countries throughout the world (Hickling *et al.*, 2007). Economic values of koi carp can be increased with appropriate mechanisation and feeding techniques depending on an effective broodstock management. However, certain problems can be encountered in koi carp breeding in the larval period depending on the nutritional requirements and larval feeding conditions as in all other species (Haniffa *et al.*, 2007; Carvalho *et al.*, 1997). Thus, studies on ideal protocols for feeding koi carp and appropriate water conditions for rearing them as of the larval period continuously improve. Within this scope, *Artemia* sp. is commonly used in feeding the larvae of the cultured marine and freshwater fish constitute the most important live food source for Koi larvae. It is known that, today, applications with decapsulated

cysts also exist in addition to the period of larval feeding made with the recently hatched *Artemia* nauplii (Bengston *et al.*, 1991). The most important reason of this application is that decapsulated *Artemia* cyst contain 30-40% more energy than the newly hatched *Artemia* nauplii (Vanhaecke *et al.*, 1983). It is thought that cyst is a favourable food for feeding the larvae of the koi carp as it is 50% smaller than the newly hatched *Artemia* nauplii (instar I nauplii) (Leger *et al.*, 1987), it is disinfected while it splits off the outer shell during decapsulation and it does not consume energy while it comes out of the shell. However, examining the impacts of such applications on the digestive system of the larval period is of great importance to investigate the survival and development parameters.

Over the last decade, there are an increasing number of studies focused on the onset and development of digestive enzyme activities during larval development of both marine and fresh water fishes used in aquaculture. During the early ontogeny, functional changes of certain enzymes (e.g. trypsin, pepsin) may be used as an indicator of larval development, as well as a predictor of their future survival (Zambonino Infante and Cahu, 2001; Kolkovski, 2001; Cahu and Zambonino Infante, 2001; Zambonino Infante *et al.*, 2008). Therefore, digestive enzymes could be more effective for digestion of all nutritional components. Some studies have been focused on digestive enzyme activites in ornamental fishes especially pointed out that some diets could be more appropriate for the effective larval feeding these species. Although reproductive behaviours, fertilisation, egg development, growth (Gosch *et al.*, 2012) and skin pigmentation (Liang *et al.*, 2012) of the larvae and detection of parasites (Eun Han *et al.*, 2010) have been examined in koi carp, there is no study on the impact of feeding with decapsulated *Artemia* cysts in the larval period on digestive enzymes. In this respect, the present study aimed at examining the development, survival rates and digestive enzyme activities of the koi carp larvae fed on *Artemia* cyst.

Materials and Methods

Experimental Animals and Design

The study was carried out in an aquarium fish farm, Orta Doğu Aquarium Inc., founded in Bergama, İzmir, Turkey. Egg-full nylon ropes (kakaban is the general name of this ropes) were taken from the tank where 10 spawners fish mated randomly. Kakabans were separated and placed into 6 glass aquaria; each aquarium was 200 liter volume. The aquaria were located so as to receive natural sunlight through the southern window of the room and to remain in daylight for 15 hours and in darkness for 9 hours.

After spawning, breeders were removed from aquaria and also obtained eggs were incubated at 26°C and larvae hatched out at the end of the 86th hours. Mouth opening was observed at 4 DAH (day after hatching) and larvae were fed by *Artemia* nauplii until 7 DAH. Then larvae were randomly distributed into 6 aquarium for 2 experimental groups in triplicate and total 150 specimen were stocked into each experimental aquarium (100 L). Water temperature, dissolved oxygen, and pH were monitored daily. Water temperature and average pH value were kept 26.01±0.1°C, 6.25-7mg/L and 8.61±0.02, respectively. The aquarium water was partially replaced 5% daily. Light was supplied by fluorescent tubes, with a power of 800 lux at water surface. Photoperiod was set on a 16 h light 8 h dark cycle (16L:8D) daily until the end of experiment.

For initial measurement at 7 DAH, larvae were sampled from each aquarium and also randomly selected 30 larvae were measured wet weight with electronic balance (0.001 g) and total length in terms of detection of growth parameters and enzymatic analysis before food distribution. After this date, these sampling were carried out regularly 3 and 6 days interval for measurements of enzymatic and meristic parameters, respectively.

Preparation of Experiment Feeds

Artemia sp. decapsulated cysts and non-decapsulated cysts of Great Salt Lake- Utah origin supplied from a commercial company (USA Salt Lake Cooperative GSLA) were used in the study. Both cysts were reported by the manufacturer that had the same nutritional value and contained 56% protein, 13% fat and 4% ash. While decapsulated cysts were used directly, the unprocessed cysts were prepared in the previous evening for being used on the ensuing morning in order to achieve the *Artemia* nauplii instar I phase (Bengston *et al.*, 1991).

After taking out for measurement and enzymatic procedure, the total amount of food for each regime were decreased in proportion to the estimated number of fish remaining. All *Artemia* cysts were weighted same amount of both regime group. Then, for one group and its replicated groups were prepared *Artemia* nauplii (AN) instar I before feeding time (Bengston *et al.*, 1991). For the second experiment group and its replicated groups, it was used directly the decapsulated and dried commercial *Artemia* cysts (ADC). Daily feed amount was determined by calculating 10% of total larva biomass for each aquarium of decapsulated cysts feeding larvae (Mohanta *et al.*, 2002). Moreover, for larvae fed with *Artemia* nauplii, *Artemia* cysts were weighed at the same rate which was calculated by hatching rate of *Artemia* cysts. They were incubated at 28°C by strong aeration and obtained nauplii were administered to the experimental aquarium.

Sampling and Analytical Procedure

In order to measure of growth rate larvae from each aquarium were sampled (30 larvae sample

group^{-1})[Repetition]. Specific growth rate was calculated by formula

SGR= 100 (Ln FBW −Ln IBW)/Δt,

where IBW, FBW= initial and, final body weight of fish (mg),

Δt= time interval (day).

At the end of the experiment, larval survival rate SR was determined by this formula

SR: (NFE/NFI)*100,

Where NFE = were number of fish at the end,

NFI = Initial number of fish initially.

For enzymatic assays whole body homogenates were used 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 (35mg 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 using casein as the substrate (Walter, 1984). Amylase activity was measured using starch as the substrate (Métais *et al.*, 1968). Chitinase activity was analyzed using chitin azure as the substrate (Hackman *et al.*, 1964). Lipase activity was analyzed using the method of Mckellar *et al.* (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

Significance test (T test) of the difference between two average values was conducted in order to reveal the differences of feeding on *Artemia* nauplii and decapsulated cyst while Levene's test was applied for the homogeneity of variances before this application. SGR values were tested Chi- square test. SPSS 15.0 software was used in the calculations.

Results

Growth and survival

In general, the survival rates were found to be considerably successful in both groups. The survival rate observed in the ADC groups was 98.88% at the end of the experiments. On the other hand, this rate was 97.40% in the groups fed on AN. As presented in figures, survival rate is achieved in the groups fed on ADC ($P>0.05$).

At the end of the experiment, total length developments were estimated as 28.16±4.81 mm and 20.56±3.23 mm for ADC and AN group, respectively. In addition, larval weights were 0.28±0.16 g and 0.9±0.05 g ($P<0.05$) for ADC and AN group, respectively. Moreover, SGR values were calculated as 6.02 and 2.03% d^{-1} for larvae were fed by ADC and AN, respectively. At the end of the experiments, final SGR data were found significantly different between two experimental groups ($P<0.05$) (Figure 1).

Digestive Enzyme Activities

Total protease activity presented increased profile during the experiment in both group (Figure 2). The highest total protease specific activity was determined on day 34 as 3.64±0.46 U/mg protein^{-1} in AN group. However, total protease activity for ADC group was 1.37±0.13 U/mg protein^{-1}. There were 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. In AN group, lipase activity continuously increased until end of the experiment. Although similar increase was observed in ADC group to 25 DAH, from 28 DAH this activity slowly increased until end of the experiment. The highest lipase specific activity was determined on day 34 as 555.17±30.20 mU/mg protein^{-1} in AN group (Figure 3). There were significant differences between experimental groups ($P<0.05$).

Amylase activities were presented complex profile in experimental group. During the first 28 DAH, specific activities constantly increased, and then they showed opposite pattern between 28 and 31 DAH, after that these activities sharply increased until end of the experiment. The highest amylase specific activity was determined on day 34 as 3.54±0.25 U/mg protein^{-1} in ADC group (Figure 4). There were significant differences between experimental groups ($P<0.05$).

Compared to amylase, specific activity of chitinase presented variable profile in both group. For the first 16 DAH chitinase activities were lower, until 28 DAH they decreased in both group. After this date, these activities showed fluctuated profile to 34 DAH. The highest chitinase specific activity was determined on day 34 as 775.69±15.17 mU/mg protein^{-1} in ADC group (Figure 5). There were significant differences between experimental groups ($P<0.05$).

Discussion

Larval feeding of ornamental fish still mainly depends on nauplii and metanauplii of *Artemia* species. Direct application of decapsulated *Artemia* cysts to the larvae is the leading attempt to eliminate the dependency on procedure of obtaining *Artemia* nauplii. Koi carp, which were discussed in the present study, is one of the aquarium fish species that can be found in almost all regions of the world and it is economically valuable. In the study, development

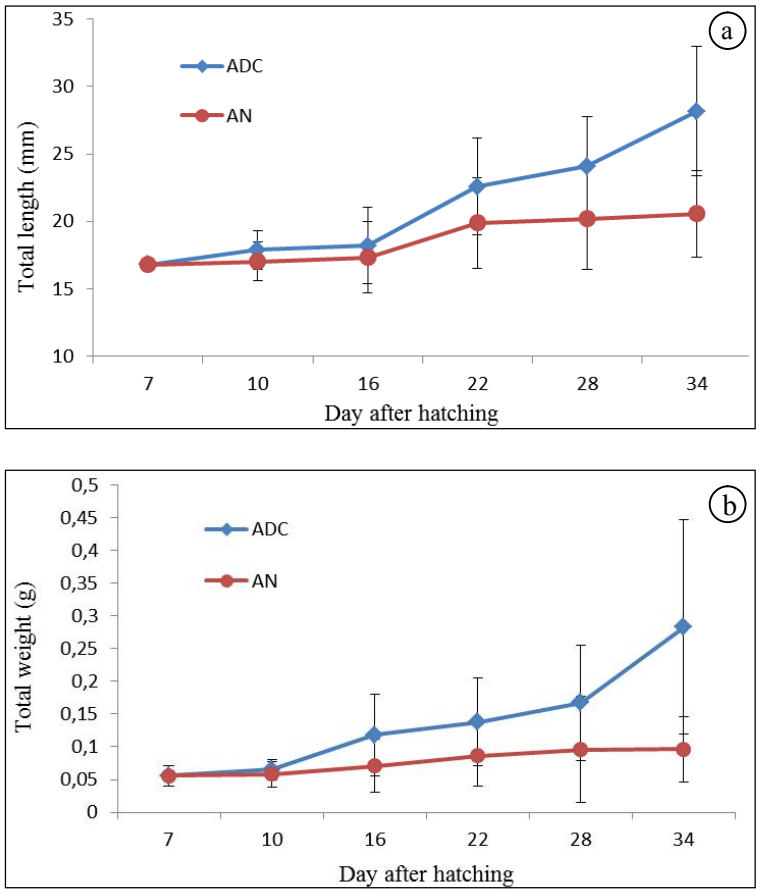

Figure 1. Growth of total length (a) and weight (b) *Cyprinus carpio* var. *Koi* larvae feeding with *Artemia* nauplii (a) (AN) and decapsulated cysts (b) (ADC) during the experiment. Each mean ±SD represents a pool of 30 larvae ($P<0.05$).

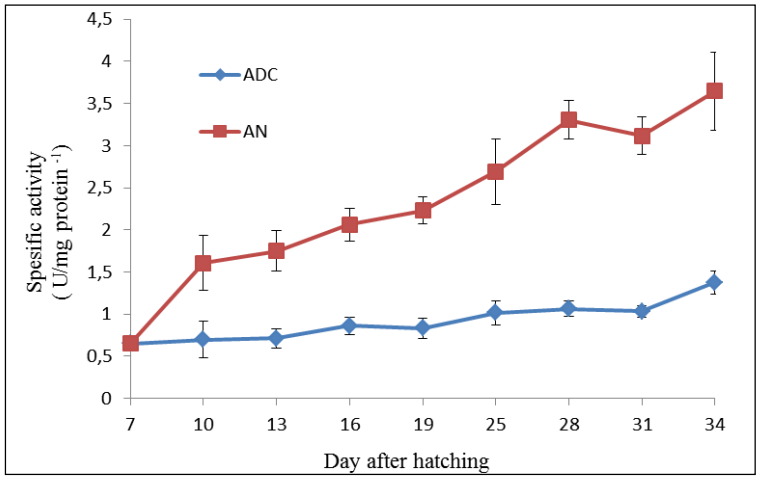

Figure 2. Specific activities of total protease in *Cyprinus carpio* var. Koi during the experiment. Results are expressed as means ±SD (n=3)($P<0.05$).

values of the Koi larvae fed on nauplii and cyst on day 34 were found as 20.56±3.23 mm and 28.16±4.81 mm, respectively. Likewise, in the study conducted by Haniffa *et al.*, (2007) on the feeding (with rotifer and moina *ad libitum* twice a day) of Koi larvae at 26-28°C, total length increases were determined as 12-14 mm on day 23 and 24-32 mm at 28 DAH. Although the findings of the present study are relatively parallel

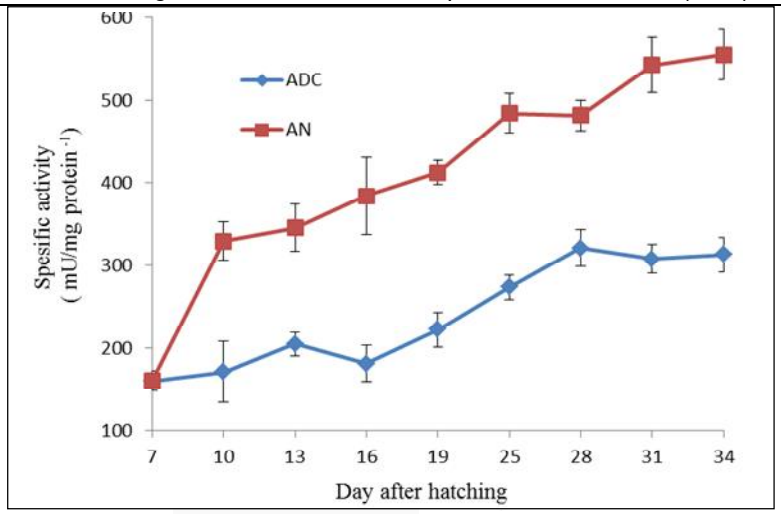

Figure 3. Specific activities of lipase in *Cyprinus carpio* var. *Koi* during the experiment. Results are expressed as means±SD (n=3) (P<0.05).

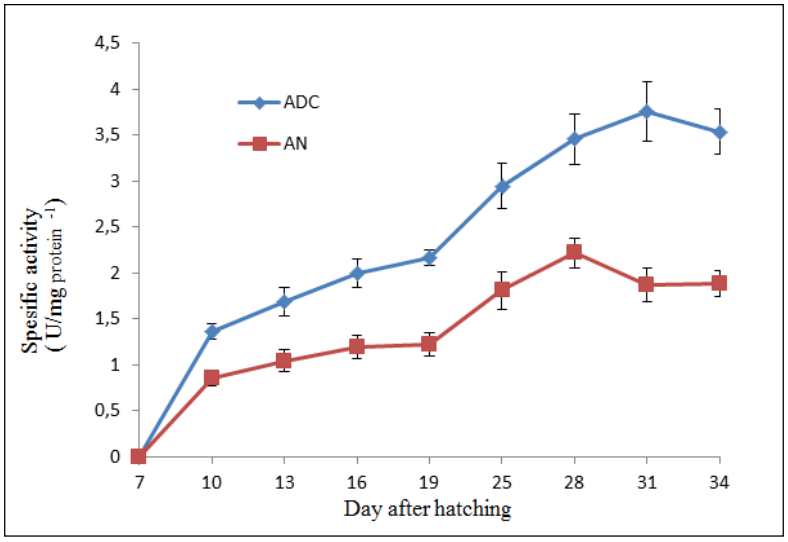

Figure 4. Specific activities of amylase in *Cyprinus carpio* var. Koi during the experiment. Results are expressed as means ±SD (n=3) (P<0.05).

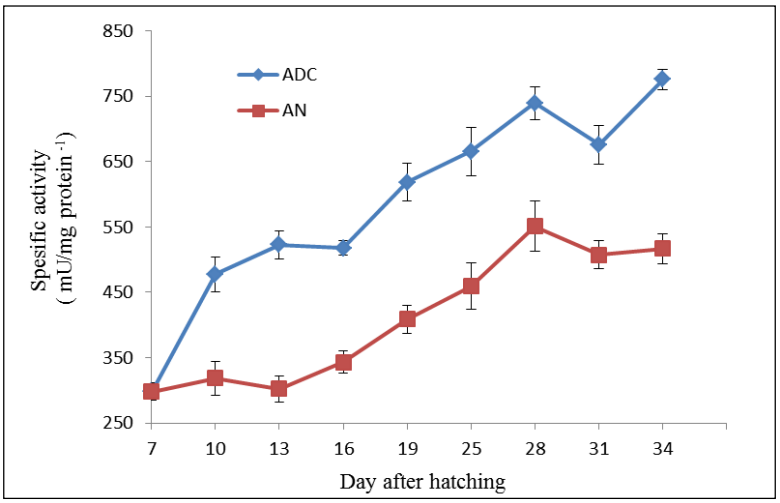

Figure 5. Specific activities of chitinase during in *Cyprinus carpio* var. Koi the experiment. Results are expressed as means±SD (n=3) (P<0.05).

to this study (even the experiments were not done in the same conditions), it is thought that the difference results could be sourced from the experimental feeding regime. It is envisaged that this difference might be resulted from the differences in the larval feeding as well as the rearing conditions. Survival rates obtained in present study were found relatively higher than the gold fish fed with *Artemia* nauplii, daphnia and commercial feed in Kaiser *et al.* (2003).

There are different studies concerning the feeding techniques applied in the larval and juvenile periods in koi carp culture. It was reported that the Koi larvae fed on the animal-origin feeds gained more weight than the larvae fed on feeds containing plant-origin ingredients (Jha *et al.*, 2005).

In recent years, one of the most common techniques of larval feeding were the direct application of decapsulated *Artemia* cysts to the larvae after undergoing a series of processes (Lim *et al.* 2011). In this technique, as nauplii will not consume energy to hatch, the available high energy will be transferred to the larvae directly (Bengston *et al.*, 1991). Significant differences found between the growth parameters of both groups in the present study support this finding. Such a similar finding was also achieved for the *Tinca tinca* larvae fed on *Artemia* nauplii and decapsulated cyst and the author, Garcia *et al.* (2011) stated that "Both fresh and brine cysts are a suitable food from the onset of exogenous feeding and dried cysts can be successfully used after 7 days feeding on nauplii. As reported in previous studies, it is well estimated that obtained relatively higher specific growth rate from larvae were fed by *Artemia* cysts could be related higher nutritional composition of cysts (Lim *et al.*, 2011). Similar findings were recorded in both common (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) and also ornamental guppies (*P. reticulata*) and barbel (*Barbus barbus*) larvae were fed with *Artemia* cysts compared to commercial starter microdiet (Dhert *et al.*, 1997). For all that, Mamcarz *et al.* (2011) reported that *Tinca tinca* larvae feed *Artemia* nauplii showed better growth value (total length, weight, SGR) and survival rate than decapculated cycts.

The digestive physiology of many fish larvae undergoes numerous morphological and functional changes during the early ontogeny that can substantially influence larval growth and survival under culture conditions. Therefore, expression of digestive enzyme activity can be used as an indicator of larval food acceptance and to some extent serve as an indicator for the digestive capacity in relation to the type of feed offered (Ueberschär, 1995; Zambonino Infante and Cahu, 2001; Kolkovski, 2001; Zambonino Infante *et al.*, 2008). Leger *et al.*, (1987) reported that *Artemia* was effective on amylase and trypsin enzymes and *Artemia* nauplii may play an essential role in the digestive system of the larva through its transition towards the intestines of the larva. Examinations on the enzyme activities of the koi carp larvae in our study revealed that the larvae could digest the decapsulated cysts as much as the *Artemia* nauplii and accordingly, length and weight values increased. Thus, we determined that both forms of *Artemia* could meet the nutritional requirements of this experiment and control groups. On the other hand, enzymatic changes in the early larval stage were examined in a study carried out with carp hybrids and it was reported that trypsin, chymotrypsin and total protease enzymes had a tendency to increase constantly until the 34th day during the experiment (Chakrabarti *et al.*, 2006). Besides, researchers stated in their experiment on protein inhibition that protein inhibition levels increased considerably between 28 and 34 DAH and digestion of the protein taken from the feed also increased. Results of our study show major similarities to the results of the abovementioned research. In this study, total protease activity showed a regular increase during the experiment and sharp increases were recorded as of the 31st day, in particular. The increase in this enzymatic activity presented that feeds were digested and the larvae made use of the protein source of the feeds as much as possible. Protein inhibition values were not examined in this experiment. However, when it was considered the sharp increases, it was observed in the enzymatic results of koi carp, a congener of the common carp, as of the first month. Besides, it is possible to report that protein reactions increased significantly following the first month and feeds were digested and became beneficial to the metabolism of the fish as of this period. Results supporting this argument of current study were also reported for the discus (*Symphysodon aequifasciata*) which holds an important place among aquarium species. Chong *et al.* (2002a, b) examined the protein inhibition reactions of discus juveniles in their study and reported that fishmeal was highly digestible in terms of dry matter (67.22–7.52%) and protein ratio of 76.8–91.18% was an adequate amount for digestion in the fishmeal. Additionally, same authors (2002b) reported that specific protease inhibitors also showed the presence of eight distinct proteases based on molecular weights ranging from 19.2 to 76.5 kDa.

It is well defined that most of the fish larvae presented higher developmental profile of amylase during the first week of life and after then decrease in specific activity of this enzyme was uncorrelated with carbohydrate concentration of larval feeds and also this is hypothesized that this opposite pattern could be genetically programmed during larval development (Péres *et al.*, 1996; Zambonino Infante and Cahu, 2001; Zambonino Infante *et al.*, 2008). However, nutritional component and biochemical composition of offered diet could be effected secretion of this enzyme through the former stages. For instance, in some marine fishes, although similar developmental profile was observed, it is detected that microdiet supplementation increased amylase activity in

cultured Sparid larvae such as common pandora *Pagellus erythrinus*, sharpsnout sea bream *Diplodus puntazzo* and red porgy *Pagrus pagrus* during early ontogeny (Suzer *et al.*, 2006, 2007a,b). In similar with this finding, it is clearly estimated that high level in glycogen in *Artemia* might be stimulated synthesis and secretion of this enzyme in larvae of yellow croacker *Pseudociaena crocea* (Ma *et al.*, 2005). As seen in the results, amylase activities of the larvae in the group fed on cyst were found relatively higher than the group fed on *Artemia* nauplii. It is thought that the difference between the groups mainly resulted from the intensity of starchy agents within the cyst. However, in the study carried out with the carp hybrids, amylase activity displayed a sharp increase in the first 10 days; fluctuations were observed until 32 DAH and afterwards, it started to increase once more. This difference between our study and the abovementioned one in terms of amylase activity results is attributed to the fact that the study carried out with the carp hybrids did not focus on feeding. In the latter study, mixed feeding with artificial diet was used from 14 DAH and different dietary combinations were used during routine feeding. On the other hand, *Artemia* nauplii and cyst were used as food substance in our study (Chakrabarti *et al.*, 2006). Considering the intense shell formation both in the *Artemia* nauplii and cyst, the difference between two studies becomes more clear and understandable.

As it is a generally known fact that, although chitin can be synthesized by other invertebrates, bacteria and algae, the most natural chitinase structure pertains to the exoskeletons of crustaceans and the other marine organisms (Gutowska *et al.*, 2004). Results concerning the chitinase enzyme activity were also similar to the amylase activity profile. This is because of the fact that chitinase also takes part in the digestion of carbonhydrates just like amylase. It is thought that the intensity of starchy agents both in *A.* nauplii and cyst and intense shelled structure of both food substances directly triggered this enzyme activity. Gutowska *et al.* (2004) monitored chitinase activities in saltwater fish in their study and concluded that feeding profile and food substance compositions were directly associated with this activity. Besides, the same researchers reported that feeding habits and its frequency may affect the evolution of the digestive enzyme systems in saltwater fish.

It is pointed out that lipase is produced mainly in the pancreas and thought to play a relatively minor role in lipid digestion in fishes, and catalysis the breakdown of triacylglycerol first to diacylglycerol and then to monoacylglycerol (Kolkovski, 2001; Zambonino Infante and Cahu, 2001; Zambonino Infante *et al.*, 2008). Considering the results of this study, it is possible to express that lipase activity has increased steadily day by day especially in nauplii group. The main reason why there were differences between two groups is probably that lipid contents of *Artemia* nauplii due to nutritional properties and advanced lipid metabolism of nauplii (synthesis and secretion) than the cysts in particular. Likewise, the study carried out with carp hybrids also revealed that lipase activity increased steadily depending on the age of the larva (Chakrabarti *et al.*, 2006). Additionally, weaning and/or shifting of offered food by microdiet could be triggered activity of lipase due to higher lipid content of compound artificial feed. This pattern clearly observed that starting of weaning by compound extruded diet was stimulated secretion and synthesis of this enzyme in some cultured marine fish larvae such as *Pagellus erythrinus*, sharpsnout sea bream *Diplodus puntazzo* and red porgy *Pagrus pagrus* during early ontogeny (Suzer *et al.*, 2006, 2007a, b).

Decapsulated *Artemia* cyst has the same nutritional value as the *Artemia* nauplii and its use as feed is more advantageous in terms of the required labour force and preparation time. In this study, *Artemia* nauplii and decapsulated cysts were used as feed and morphological growth rates of the larvae were found slightly higher in the group fed on cysts. Digestive enzyme activities were found out to be similar in both groups. According to these results, it could be suggested that use of decapsulated *Artemia* cysts to feed the koi carp larvae is effective and reasonable.

In contrast, a disadvantageous aspect is that it may not appeal to the larvae as it is not capable of swimming like nauplii and submerges after a while. Although koi carp was a benthic species, it was observed that it leapt towards the cysts within the first several minutes following the feeding in its larval stage but did not take an interest in the cysts which submerged in the course of time. Thus, it can conclude that the necessity of cleaning the bottom of the aquarium arouses after the fish are fed with cysts. Furthermore, we recommend using additional fans with moderate speeds in order to increase the visibility of cysts by the larvae by circulating the air in the rearing tank.

In conclusion, it is clearly suggested that decapsulated *Artemia* cysts could be administered as larval feeds instead of nauplii form of *Artemia* during early feeding of Koi larvae. As the ornamental fish sector develops, the necessity of making feeding, which is considered as an important step in farming and larval rearing, in accordance with the species, age and size of the fish arouses. Besides, it is recommended to conducted similar studies on the larvae of the other aquarium fish species in the future.

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