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Fatty Acid Utilization and Digestive Enzyme Activity during Early Larval Development of *Anabas testudineus*

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ABSTRACT: *Anabas testudineus* is one of the potential candidates for species diversification in freshwater aquaculture. The present study aimed to evaluate the consumption pattern of endogenous nutritional components available in the form of yolk and digestive enzymes status during early larval development of *A. testudineus*. The fatty acid profiles *viz*., saturated fatty acid (SFAs), monounsaturated fatty acid (MUFAs), polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA), n-3/n-6 ratio of eggs and different stages of unfed larvae of Anabas were determined. The digestive enzyme activities were also studied from the day of fertilization to the 25-day post-hatching (DPH). The results indicated that the MUFAs were the predominant fatty acid available in eggs and all the larval stages. The total MUFAs, n-6 contents and DHA: EPA were decreased significantly ($p<0.01$) from the eggs to 6 days old non-feeding larvae. Total n-3, EPA, DHA, ARA and ARA: EPA were significantly increased ($p<0.01$) in developing larvae. Surprisingly, EPA and DHA contents were significantly less ($p<0.05$) during 0 to 2 DPH and increased later on from 3 to 6 DPH. Proteases like trypsin and chymotrypsin activities were significantly ($p<0.01$) increased from the day of fertilization and they reached a peak on 15 DPH and 12 DPH, respectively and then a decreasing trend was observed up to 21 DPH. However, the pepsin activities were negligible up to 12 DPH and subsequently increased from 15 to 25 DPH. In contrast to pepsin, the lipase activities increased from day of fertilization to 12 DPH, then decreases up to 18 DPH and increased thereafter. Amylase activities were negligible ($p<0.05$) up to 2 DPH, then increased from 3 DPH to 18 DPH followed by significantly reduced ($p<0.05$) from 18 to 25 DPH. The culture potential of this species has not realized so far due to the lack of sufficient quantity of quality seed and the non-availability of appropriate larval feed. To produce a good quality larval feed, the basic knowledge on nutrient requirement of larvae is the pre-requisite. Therefore, the present investigation revealed the activities of digestive enzymes and utilization of total fatty acids from the day of fertilization to 25 DPH in the early larval development of *Anabas testudineus* which are very much essential for developing the quality larval diets for this species.

Keywords: Anabas, larvae, n-3, n-6, n-3/n-6, EPA, DHA, PUFA, fatty acid, digestive enzyme.

INTRODUCTION

Anabas testudineus, commonly known as "climbing perch" or "Koi carp" is a commercially important high valued food fish in Southeast Asian countries including India. It is widely distributed in fresh and brackish water as well as estuaries (Chondar, 1999; Jayaram, 2010; Pal and Chaudhry 2010). It possesses accessory respiratory organs such as labyrinth (Olson *et al.,* 1986; Munshi *et al.,* 1986) which help to culture this species in swampy areas, derelict and sewage water as well as paddy fields unsuitable for carp culture (Dehadrai and Kamal 1993). Anabas can also be a potential culture species for Recirculatory Aquaculture Systems (Singh *et al.,* 2022). These characteristics along with easy acceptance of artificial feeds make this species one of the potential candidates for species diversification in aquaculture. The culture of this species has not yet received attention due to poor seed availability owing to non-availability of quality brooders (Dehadrai and Kamal 1993; Kumar *et al.,* 2012). Although it is easily bred under controlled conditions, the high larval mortality has been reported by many researchers (Amornsakun *et al*., 2005; Trieu and Long 2001).

The nutrition required for the embryonic and early larval development of fish comes from endogenous food stored in the form of yolk. The pattern of utilization and conservation of endogenous nutrients available in the yolk sac during embryonic and early larval developmental stages could be an important approach to know the nutritional requirement of the fish larva. The nutrient available in the yolk sac are the basis for the development of fish embryos. The early larval stages also determine the early health and

survival rate of larvae and juveniles (Tong *et al*., 2017). It also indicates the consumption pattern of endogenous nutritional components available in the form of yolk which helps determine the nutritional requirement of larvae. It directly affect the metabolic capacity of larvae development (Lubzens *et al.,* 2010; Tong *et al.,* 2017). During the embryonic and larval stages, maternally derived nutrition (yolk) is the only source of energy and materials for metabolism, development, and growth. The n-3 fatty acids effectively modulate the growth performance, immune response, and disease resistance in fish against various stresses (Kumar *et al.,* 2022). Lipids and fatty acids are the main component of yolk and serve as a major energy source, structural components of cell membranes, and precursors of various important biologically active molecules, such as eicosanoids (Bell *et al.,* 1986; Heming and Buddington 1988; Sargent *et al.,* 1999).

Fish eggs yolk are rich in polyunsaturated fatty acids (PUFA) especially essential fatty acids such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6). The PUFAs are essentially required for the proper development of embryos and larvae (Izquierdo, 1996; Izquierdo *et al.*, 2000; Wang *et al*., 2006).

The composition and content of fatty acids varied with fish species (Shi *et al.,* 2010; Huang *et al*., 2013). The fatty acid composition of eggs within a species may vary as a result of variances in maternal dietary food intake (Izquierdo, 1996; Hou *et al.,* 2020). For fish, egg fatty acid composition can vary quickly in response to changes in the maternal diet (Fuiman and Faulk 2013; Hou *et al.,* 2020). The exact fatty acid composition in the larval diet should be similar to that of fertilized eggs or yolk-sac larvae (Hou *et al.,* 2020). Therefore, the study on the utilization and conservation pattern of fatty acids during the embryonic stage of fish and the development of yolk-sac larvae is valuable in understanding the requirements of dietary fatty acids in the early larval stages of fish.

The digestive enzymes during larval stages are reliable indicators of digestive capacity and are noted for age, species, sensitivity and short latency. It varies from proteolytic pancreatic enzymes to intestinal brush border and cytosolic enzymes (Cara *et al*., 2007).

Considering these aspects, a study was conducted to determine the utilization and conservation pattern of fatty acids (available maternal nutrition in yolk) during the endogenous feeding period and digestive enzyme activities in early stages of Anabas larvae. This information could lead to the formulation of appropriate broodstock and larval diet that may improve gonadal maturation and larval quality.

MATERIAL AND METHODS

Experimental materials and sampling. Anabas broodstock of the same age, size and physiological condition was collected from air-breathing fish culture unit of ICAR-Central Institute of Freshwater Aquaculture Bhubaneswar, Odisha, India. Brood fishes were fed at 2% of body weight with a commercial diet containing 35% crude protein and 6% ether extract for a month. Three sets of fully matured female and male Anabas were induced-bred after hormonal injection of ovatide. Each breeding set constitutes two numbers of females (average weight 35±4g) and four numbers of males (average weight 28±3g). After spawning, the spent fish were removed from the spawning tank. Eggs shells, unfertilized eggs and dead larvae were removed from the spawning tank. For the fatty acid utilization study, the fertilized eggs samples were collected and kept in separate FRP tanks of capacity 200 L in triplicates. The fertilized eggs samples were collected just after the spawning. Larvae were hatched after 18 h of spawning (at water temperature of 24-26 ºC) in the circular FRP tank. Larvae were not fed during this study. Sampling was done for newly hatched larvae (0 DPH), 1 DPH, 2 DPH, 3 DPH, 4 DPH and 5 DPH at 10 AM. At every sampling time point, a fresh weight of 8 g sample was collected from each parallel (in triplicates) rearing tank. The collected samples were spread over a plankton net, and the excess moisture was removed with tissue paper and then stored in a 15 ml capped centrifuge tube. The processed samples were frozen in the refrigerator at -20 °C for 24 h, then transferred and stored at -80 °C.

For the digestive enzyme study, the larvae were reared separately in 400 L FRP tank and they were fed with mixed zooplanktons collected from fertilized pond after filtering with a fine muslin cloth. In every morning, the unutilized food and excreta were siphoned out from the larval-rearing tank. Larvae were collected every day from hatching to 3 DAH, and after that on 5, 8, 11, 15, 18, 21, and 25 DAH at 09:00 AM before the feeding in order to minimize the effects of exogenous enzymes from undigested live food in the gut. To determine the digestive enzyme activity, at each sampling, the pooled sample of 500 larvae from hatching to 3 DAH, 300 larvae at 3 and 5 DAH, 200 larvae at 5 and 8 DAH, 100 larvae at 11 and 15 DAH, 50 larvae at 15 and 18 DAH and 25 larvae at 21 and 25 DAH were collected in triplicates from Air-breathing Fish Culture Unit of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar. Collected larvae were kept in glass beakers filled with clean water for 2 h to allow any remaining food in the gut to be assimilated or excreted. The larvae were frozen at -20 °C until further processing. The frozen larvae were homogenized in four volumes (v/w) of ice-cold phosphate buffer at pH 7 (Dawson *et al.,* 1986) with a tissue grinder, followed by centrifugation at 10,000 g for 20 min at 4 °C. Supernatants or crude enzyme extract was stored at -20°C until analysis. Soluble protein in the crude enzyme was measured by the Lowry method (Lowry *et al.,* 1951) using bovine serum albumin as a standard.

Fatty acid analysis. The fatty acid profile of seven samples of fertilized eggs and unfed larvae up to 6 DPH were determined. The lipid was extracted from fertilized eggs and unfed larval samples using Folch *et al.* (1957). The fatty acid methyl esters (FAME) were prepared using Morrison and Smith (1964). In this process, the FAMEs were separated by a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-2010, Kyoto, Japan) using

capillary column DB225. The hydrogen gas was used as the carrier for separation and set up the initial injection temperature for 30 sec at 35 °C. The conditioning for the column temperature as increased at 25 °C min^{-1} to 195 °C, then at 3 °C· min^{-1} to 205 °C, at 8 °C· min^{-1} to 240 °C. The standard of FAMEs was injected as 1 µl (Sigma-Aldrich, Inc., St. Louis, MO, USA) (Supelco 37 Component FAME Mix; CRM47885).

Digestive enzyme. Trypsin (E.C.3.4.21.4) activity was determined in the whole body of the larva. The N-α-benzoyl-L-arginine-p-nitroanilide (BAPNA) was used as substrate. The reaction mixture of BAPNA (1 mM in 50 mM Tris–HCl, pH 7.5, 20 mM $CaCl_2$) and enzymes was incubated at 37 °C. The reading of absorbance was recorded at 410 nm (Erlanger *et al.,* 1961). Similarly, whole body chymotrypsin (EC. 3.4.21.1) activity was determined. The reaction mixture of 0.1 mMSuc-Ala-Ala-Pro-Phe-p-nitroanilide (SAPNA) in 50 mM Tris–HCl, pH 7.5, 20 mM $CaCl_2$ and incubated with the substrate at 37 °C and observed the reading at 410 nm for 3 min. (Erlanger *et al.,* 1961). Chymotrypsin /Trypsin activities were calculated by the following equation:

Unit/mg protein = [(Abs410/min) × 1000 × ml of reaction mixture]/ 8800 × mg protein in reaction mixture

Pepsin (E.C.3.4.23.1) activity was determined in the whole body of larva using Worthington (1991) and Anson (1938) method. The substrate was mixed with enzyme (2% haemoglobin solution in 0.3 N HCl at pH = 2.0) and incubated at 37 °C for 10 min. Trichloroacetic acid (TCA) (5%) was added to stop the reaction and centrifuge at 4000 g at 4 °C for 6 min. The absorbance was recorded at 280 nm. One unit of pepsin activity was defined as the µg of tyrosine released at 37 °C min^{-1} mL^{-1}, considering the extinction coefficient ($\epsilon 280 = 1250\ M^{-1}\ cm^{-1}$).

Amylase activity was determined using Rick and Stegbauer (1974) by which reducing sugar was produced due to the action of glucoamylase and α-amylase on carbohydrates using dinitro salicylic acid (DNS). The reaction mixture consisted of phosphate buffer, tissue homogenate and 1% (w/v) starch solution. The reaction mixtures were incubated for 30 min at 37 ^{o}C followed by the addition of DNS and boiled for 5 min. The reaction mixture was diluted with distilled water after cooling and observed the reading at 540 nm. The maltose was used for standard. Amylase activity was expressed as millimoles of maltose released from starch per min at that temperature.

Lipase (EC. 3.1.1.3) activity was determined in whole body of the larva using Cherry and Crandell (1932) method. The samples were marked as control and test and the 3 ml of distilled water and 1 ml of homogenate added to it. The control sample was kept in boiling water at 100 ºC for 5 min and cooled. Then 2 ml of olive oil emulsion and 0.5 ml of phosphate buffer solution (pH 7) were added to both the tubes; shaken well and incubated for 24 h at 37 ºC. Then 2 drops of phenolphthalein solution were mixed with 3 ml of 95% alcohol and then titrated with 0.05 N NaOH up to the appearance of permanent pink colour.

Statistical Analysis. Data were analyzed using Statistical Package for Social Sciences Program Version 16.0 (SPSS Inc., Chicago, IL, USA). The data were tested for homogeneity of variance and normality using Levene's test and Shapiro-Wilk's. The data were expressed as mean ± standard error. Then the data were analyzed using one-way ANOVA with Duncan's multiple range tests (DMRT) for the statistically significant difference at $p<0.01$and $p<0.05$.

RESULTS

Fatty acid profiling of early developmental stages of *A. testudineus* larvae. In the present investigation, the total lipid profiling was determined in early developmental stages of *A. testudineus* and data is presented in Table 1. The results of total SFA (saturated fatty acids) were increased significantly ($p<0.01$) from 0 DPH to 4 DPH as compared to the day of fertilization. Moreover, the MUFAs as (monounsaturated fatty acids) were noticeably higher during days of fertilization and 0 DPH as compared to 1 to 6 DHP. The results of n-3 were significantly ($p<0.01$) lowered up to 0 to 2 DPH and subsequently increased ($p<0.01$) from 3 to 6 DPH, whereas, the n-6 fatty acids were significantly higher ($p<0.01$) from the day of fertilization up to 3 DPH and then it was decreased significantly ($p<0.01$) at 4 and 6 DPH. Results of EPA and DHA were similar ($p>0.01$) to n-3 and interestingly, the ratio of DHA: EPA was significantly higher ($p<0.05$) at 0 and 1 DPH and it was subsequently decreased ($p<0.05$) from 2 to 6 DPH. The ratio of ARA: EPA was significantly lower ($p<0.05$) up to 1 DPH and later on it was increased ($p<0.05$) from 2 to 3 DPH and then again it reduced ($p<0.05$) from 4 and 6 DPH.

Digestive enzyme activities during the early developmental stages of *A. testudineus*

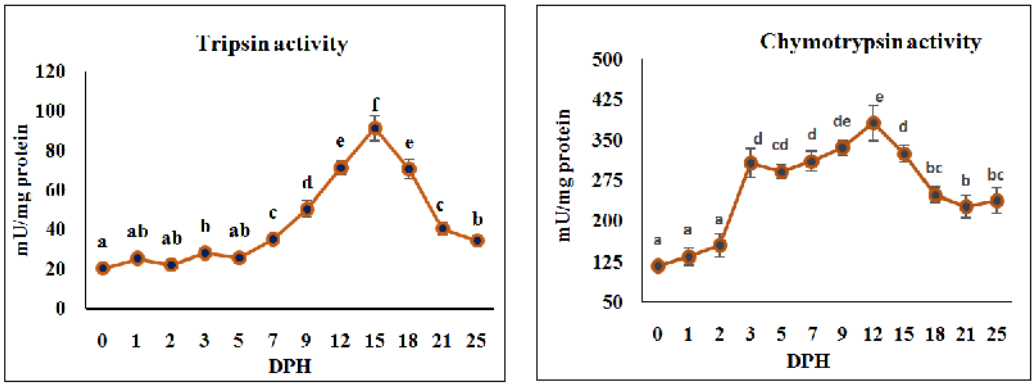
Trypsin and chymotrypsin activity. In the present investigation, the trypsin and chymotrypsin enzyme activities of the early development stage of *A. testudineus* were determined and presented in Fig. 1. The trypsin activities were significantly higher ($p<0.01$) at 15-day post-hatching (DPH). Later on the trypsin activity was decreased ($p<0.01$) and the least activity was found on 25 DHP. The results also showed that the trypsin activity was increased ($p<0.01$) from 5 DPH to 15 DPH. Similarly, the chymotrypsin activity was significantly ($p<0.01$) lower up to 2 DPH and later on it was significantly increased ($p<0.01$) from 3 DPH to 15 DPH. The chymotrypsin enzymatic activities were significantly decreased ($p<0.01$) after 15 DPH and it was lowest at 25 DPH.

Table 1: Fatty acid profile of fertilized eggs and unfed anabas larvae up to six days post hatch (DPH). Fatty acids having concentration more than 0.25 % at least one point are reported. Data expressed as % weight of total identified fatty acids and values are represented as means±SE. n=3. Letters indicate significant differences within the same row (p<0.05).

% Total FA	Fertilized Egg	0 DPH	1 DPH	2 DPH	3 DPH	4 DPH	6 DPH
C:14	0.37^{b}±0.01	0.38^{b}±0.01	0.16^{a}±0.01	0.13^{a}±0.00	0.51^{c}±0.01	0.85^{d}±0.01	1.42^{e}±0.04
C:15	0.22^{bc}±0.06	0.10^{a}±0.01	0.07^{a}±0.01	0.12^{a}±0.01	0.15^{ab}±0.02	0.23^{c}±0.01	0.32^{d}±0.02
C:16	5.56^{a}±0.03	5.87^{b}±0.06	6.14^{c}±0.02	7.80^{d}±0.04	8.91^{e}±0.03	7.20^{f}±0.04	6.50^{g}±0.05
C:17	0.14^{a}±0.01	0.14^{a}±0.01	0.43^{b}±0.01	0.96^{c}±0.03	1.15^{d}±0.01	1.87^{e}±0.01	1.98^{e}±0.15
C:18	1.61^{a}±0.02	1.71^{b}±0.02	1.85^{c}±0.01	1.93^{d}±0.02	3.80^{e}±0.02	3.98^{f}±0.02	3.93^{f}±0.02
C:21	0.12^{a}±0.01	0.41^{c}±0.01	0.30^{b}±0.02	0.41^{c}±0.01	0.37^{c}±0.01	0.85^{e}±0.02	0.64^{d}±0.02
C:22	0.14^{a}±0.03	0.31^{b}±0.02	0.88^{c}±0.02	1.03^{d}±0.01	2.10^{e}±0.02	3.97^{f}±0.01	4.39^{g}±0.02
C:23	0.34^{a}±0.01	0.44^{c}±0.01	0.38^{b}±0.00	0.57^{d}±0.01	0.60^{e}±0.01	1.81^{f}±0.00	2.30^{g}±0.01
C:24	0.06^{a}±0.01	0.08_{a}±0.02	0.14^{b}±0.00	1.96^{c}±0.01	3.43^{d}±0.03	4.18^{e}±0.01	4.33^{f}±0.01
C14:1	1.88^{g}±0.01	1.64^{f}±0.01	1.55^{e}±0.01	1.39^{d}±0.01	1.33^{c}±0.00	0.82^{b}±0.01	0.31^{a}±0.00
C15:1	0.24^{c}±0.01	0.25^{d}±0.00	0.26^{d}±0.01	0.27^{d}±0.01	0.25^{d}±0.00	0.19^{b}±0.00	0.12^{a}±0.00
C16:1	3.36^{c}±0.04	3.19^{bc}±0.10	3.00^{b}±0.12	3.29^{c}±0.12	3.38^{c}±0.08	3.18^{bc}±0.01	2.43^{a}±0.01
C17:1	0.33^{f}±0.01	0.32^{f}±0.01	0.28^{e}±0.00	0.20^{d}±0.01	0.16^{c}±0.00	0.09^{b}±0.00	0.05^{a}±0.00
C18:1n-9	41.17^{f}±1.59	41.35^{f}±0.87	39.22^{e}±1.25	34.94^{d}±0.67	30.77^{c}±0.36	27.65^{b}±0.93	26.23^{a}±0.35
C20:1	0.55^{c}±0.01	0.62^{c}±0.03	1.00^{d}±0.15	0.47^{bc}±0.01	0.36^{b}±0.01	0.29^{ab}±0.01	0.13^{a}±0.01
C24:1	0.43^{d}±0.01	0.13^{c}±0.00	0.05^{b}±0.01	0.02^{a}±0.01	0.02^{a}±0.01	0.03^{ab}±0.01	0.02^{a}±0.00
C18:3n-3	2.02^{a}±0.03	2.14^{ab}±0.04	2.25^{b}±0.09	2.14^{ab}±0.03	2.79^{c}±0.10	3.21^{d}±0.06	3.80^{e}±0.07
C203n-3	0.78^{a}±0.01	0.87^{ab}±0.04	0.92^{b}±0.02	1.10^{c}±0.07	1.26^{d}±0.05	1.44^{e}±0.03	2.37^{f}±0.06
C205n-3	0.97^{a}±0.01	0.83^{a}±0.01	0.74^{a}±0.01	0.94^{a}±0.05	1.32^{a}±0.54	2.54^{b}±0.04	3.23^{c}±0.18
C226n-3	5.72^{bc}±0.08	5.91^{c}±0.09	5.06a±0.09	5.31^{ab}±0.23	6.48^{d}±0.13	6.45^{d}±0.24	7.39^{e}±0.06
C182n-6	18.85^{b}±1.15	18.86^{b}±1.48	18.98^{b}±0.86	17.96^{b}±0.50	18.45^{b}±0.78	16.01^{a}±0.61	15.45^{a}±0.54
C183n-6	10.32^{d}±0.57	10.13^{d}±0.28	10.25^{d}±0.21	9.60^{cd}±0.14	8.55^{bc}±0.39	7.90^{b}±0.43	6.49^{a}±0.16
C203n-6	1.10^{b}±0.02	1.05^{b}±0.08	0.97^{b}±0.05	1.69^{c}±0.13	0.95^{b}±0.05	0.64^{a}±0.04	0.54^{a}±0.04
C204n-6	0.32^{a}±0.06	0.31^{a}±0.01	0.42^{a}±0.06	0.90^{b}±0.17	1.28^{c}±0.15	1.45^{c}±0.02	1.46^{c}±0.04
C20:2	0.49^{b}±0.02	0.50^{b}±0.01	0.58^{c}±0.02	0.49^{b}±0.02	0.48^{b}±0.04	0.42^{ab}±0.02	0.22^{a}±0.02
C22:2	0.17^{ab}±0.01	0.13^{ab}±0.01	0.25^{b}±0.12	0.11^{ab}±0.02	0.09^{ab}±0.03	0.05^{a}±0.01	0.02^{a}±0.01

Table 2: Evolution of selected fatty acids in fertilized eggs and unfed anabas larvae of 0-6 day post hatching. Values are means±SE, n=3. Letters indicate significant differences within the same row (p<0.05).

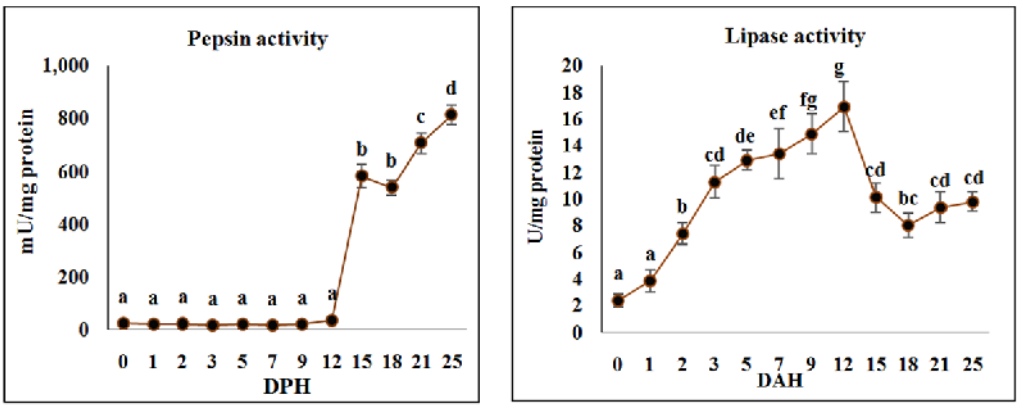
% Total FA	Fertilized Egg	0 DPH	1 DPH	2 DPH	3 DPH	4 DPH	6 DPH
SFA	8.56^{a}±1.08	9.44^{b}±0.90	10.35^{c}±0.71	14.91^{d}±0.84	21.03^{e}±1.12	24.94^{f}±2.09	25.21^{f}±2.01
MUFA	47.96^{f}±1.57	47.49^{f}±1.31	45.36^{e}±1.13	40.57^{d}±2.16	36.27^{c}±1.62	32.26^{b}±0.87	29.29^{a}±1.34
PUFA	40.73±1.05	40.71±0.72	40.40±1.15	40.22±1.31	41.67±1.11	42.10±0.66	40.98±0.90
n-3	9.48^{a}±1.08	9.75^{a}±1.07	8.97^{a}±0.82	9.48a±0.47	11.86^{b}±1.69	13.63^{c}±0.66	16.80^{d}±1.26
n-6	30.59^{c}±1.59	30.35^{c}±1.27	30.62^{c}±1.87	30.15^{c}±1.35	29.24^{c}±0.77	26.00^{b}±0.84	23.94^{a}±1.05
n3:n6	0.31^{a}±0.01	0.32^{a}±0.00	0.29^{a}±0.00	0.32a±0.01	0.41^{b}±0.02	0.52^{c}±0.01	0.70^{d}±0.01
DHA: EPA	5.92^{c}±0.22	7.10^{d}±0.21	6.84^{cd}±0.10	5.72^{c}±0.44	4.41^{b}±0.82	2.54a±0.09	2.30^{a}±0.11
ARA: EPA	0.33^{a}±0.07	0.38^{a}±0.01	0.57^{ab}±0.09	0.98^{b}±0.21	1.70^{c}±0.97	0.57^{ab}±0.01	0.45^{a}±0.02



(a) (b)

Fig.1. Trypsin and Chymotrypsin activity during early larval stages.

Pepsin and Lipase activity. In early larval stages, the pepsin enzymatic activity was significantly lower (p<0.01) up to 11 DPH. However, it was significantly higher (p<0.01) from 21 to 25 DPH (Fig. 2a). Interestingly, the lipase enzymatic activity was noticeably (p<0.01) increased from 1 DPH to 11 DPH. The lipase activity was significantly highest (p<0.01) at 12 DPH and thereafter it was decreased till 18 DPH and again it was increased (p<0.01) from 21 to 25 DPH (Fig. 2b).



(a) (b)

Fig. 2. Pepsin and lipase activity during early larval stages.

Amylase activity. In the present investigation, the amylase enzymatic activities were determined in the early developmental stages of *A. testudineus* and data are presented in Fig. 3. The amylase activities were significantly decreased ($p<0.01$) up to 1 DPH and after that, it increased ($p<0.01$) from 2 DPH to 3 DPH. The amylase activities were significantly decreased ($p<0.05$) from 5 DHP to 15 DHP and again, increased ($p<0.05$) and observed highest at 18 DHP. Moreover, the activity was lowest at 25 DAH.

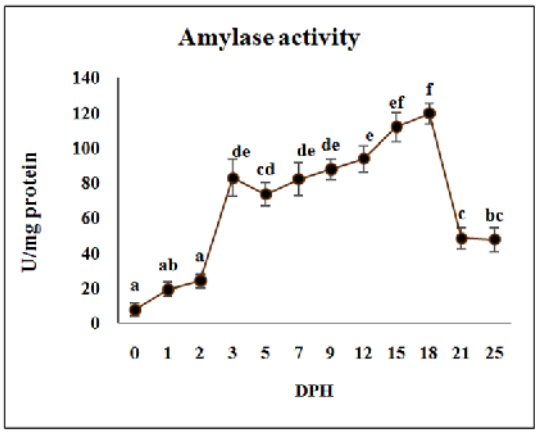


Fig. 3. Amylase activity during early larval stages.

DISCUSSION

The present investigation deals with nutrient utilization especially fatty acids and the status of different digestive enzymes during the early larval development of *A. testudineus*. Fatty acid is an important component during embryogenesis as it provides metabolic energy during early larval development. Generally, the fish larvae depend exclusively upon the nutrient availability in the yolk sac for up to 3-4 days. Moreover, the major component of the yolk sac is derived from fatty acids and amino acids along with other nutrients. Moreover, the yolk sac is an essential component in providing nutrients during organ development, cell differentiation and growth during embryogenesis (Tocher, 2010; Costa *et al.,* 2018). The fish larva is sensitive and vulnerable during the first stages of development. It is reported that high lipid content and an oil globule enhance fast eggs development (Morais *et al.,* 2011). Similar results were reported in this study as higher lipid levels were observed during the initial stage of egg development. Moreover, the neutral lipids are rich in MUFAs and preferred for energy (Kamler, 2007), hence, in the present study, the MUFAs were higher in the initial period of embryonic development and larvae. Polyunsaturated fatty acids have an important role in the structural maintenance of phospholipids with phosphatidylcholine. It is a higher level of phosphatidylinositol with a high level of 18:0 associated with C20 PUFAs and phosphatidylserine incorporating with high level of 18:0 associated with C22 PUFAs, particularly with arachidonic acid (Sargent *et al.,* 2002; Tocher, 1995). Interestingly, in the present investigation, the n-3 and n-6 fatty acid was higher after 4 to 6 DPH which indicate that the utilization of this nutrient was obtained in the later stage of larval development. Further, the quality of larvae and spawn, and the fatty acid profile are commonly used to measure the nutritional requirement of fish in its early stages (Tocher, 2010; Garrido *et al.,* 2012). Results of ARA indicated that it requires in the early development of *Anabas testudineus* larva (3 DPH). Moreover, it is reported that the DHA and ARA retained phospholipid fraction during the early development of the larva (Huang *et al.,* 2022). These nutrients are also very much essential for survival, brain development and other neurological organs in fish (Hauville *et al.,* 2016). In the early developmental stage of larva, the deficiency of EPA and DHA induces abnormalities in vision, neurons and behavior (Forsyth *et al.,* 2017; Garg *et al.,* 2017).

The digestive enzymes activities such as trypsin, chymotrypsin, pepsin, lipase and amylase are very important during early larval development in fish. The enzyme activities are dependent upon genetics, and food administration in terms of quality and quantity (Martinez-Lagos *et al.,* 2014). In the present investigation, the chymotrypsin and trypsin activities were detected on the 4th and 8th day, respectively during

the early larval development. Activities of these enzymes indicate that the proteolytic expression is observed during hatching and embryogenesis (Civera-Cerecedo *et al.,* 2004; Sveinsdóttir *et al*., 2006). Moreover, the synthesis of digestive enzymes are considered to be genetically pre-programmed in early larval development before mouth opening and exogenous feeding (Cara *et al.,* 2007; Zouiten *et al*., 2008). The trypsin and chymotrypsin activities at 4 and 8 days, respectively were due to the mouth opening of the larvae and ingestion of exogenous food for the first time (Alvarez-Gonzalez *et al.,* 2005). It might be also possible that the development of exocrine pancreas during larval development (Gisbert *et al.,* 2004). However, the lowest activities of trypsin and chymotrypsin could be due to the quality of the spawns, age of larvae and the food availability and quality. Further, the amylase activities initiated after 2 DPH indicate the use of carbohydrates at mouth opening (Cara *et al.,* 2003). Moreover, in the present investigation, the lipase activity was initiated at 1 DPH. It is associated with lipid catabolism to provide the energy for developing larvae and is highly essential for larval development (Rainuzzo *et al*., 1997).

CONCLUSIONS

The results of present studies provide the basic information on conservation and utilization pattern of different fatty acids during early embryonic and larval development of Anabas larvae. Our findings also provide the information on digestive enzymes that are available during the early larval stages of Anabas. This information also gives an idea of fatty acid requirements during the early embryonic development and the digestive capacity of Anabas larvae at different stages.

FUTURE SCOPE

The present findings will be helpful to understand the nutritional requirement of larvae and also lipid and fatty acid requirement of Anabas broodstock. This study also gives information on digestive capacity in the sense of digestive enzymes available at different stages of early larval development. The information generated here may be helpful for the larval and broodstock feed development of Anabas in near future.

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Conflict of Interest. None.

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