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Amino Acid Profile of Caspian Sea Carp (*Cyprinus carpio*) during Ontogenetic Development: Applications to Feed Formulation

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ABSTRACT Fish larvae have a high requirement of amino acid (AA) for energy production and growth. This study was aimed to increase knowledge of AA profile during feral carp larval ontogeny and estimate larval AA requirements. Larvae were collected randomly at 1, 3, 7, 11, 15, 19, 26 and 33 days post hatch for growth and AA analysis. The composition of total AA changed significantly during ontogeny. The essential AA profile of marine carp showed low correlation with rotifers (R^2=0.22). High correlation was found between dry food and early larval ages ($R^2 \geq 0.7$) but was lower in late stage ($R^2 \geq 0.37$). At day 7, when larvae were fed on rotifers, methionine seemed to be the limiting AA and when larvae were fed dry food at day 11, 15, 19, 26 and 33, arginine seemed to be the limiting AA. Larval indispensible AA profile can be used as index of the IAA requirements of carp larvae. Supplementation of larval diet used with limiting AA is one way for compensating the deficient amino acid.

Key words: *Amino Acids, Cyprinus Carpio, Feral Carp, Larval Growth, Ontogeny*

1 INTRODUCTION

Cyprinus carpio, commonly called 'marine carp' in Iran, is a warm water fish which can be found in southern of Caspian Sea, Iran. Feral carp is one of the most important economically species for aquaculture and restocking purposes of the Caspian Sea. The most suitable habitat for feral carp is an estuary witrh salinity of 12 to 15 ppt in the southeast coasts of the Caspian Sea. This species is anadromous and after maturation in an estuarine environment, broodfish from these populations migrate to spawn in a freshwater environment.

Knowledge on larval nutritional requirements is limited and often qualitative rather than quantitative (Conceição *et al.*, 2007). During the last decade, the production of marine carp has decreased due to low larval survival and unsuitable nutritional protocols. Therefore, it is crucial to improve the rearing technology of this species. Amino acid (AA) is critical biochemical compound for living organisms (Ventura and Catalan, 2010). During early developmental stages, AA is important fuel molecules and major substrates for the synthesis a large number of bioactive

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molecules and proteins (Finn and Fyhn, 2010). Fish need high AA levels due to high growth and energy production during the larval stage (Aragão *et al.,* 2004 b). Growth is mainly due to muscle protein deposition and therefore, a high flow of AA is required from food to growing biomass (Rønnestad *et al.,* 2003). The AA profile of the diet affects larval growth (Conceição *et al.,* 2003). In order to maximize larval growth rate, the AA profile of the diet should be as close as possible to the larval AA requirements (Saavedra *et al.,* 2009). In this sense, the indispensable amino acid (IAA) profile seems to be a competent indicator for estimation of AA requirements in fish larvae (Mambrini and Kaushik, 1995). The knowledge of changes in the AA profile during larval development may provide a better understanding of nutritional requirements of exogenous feeding larvae (Wilson and Poe, 1985). There is very little information on the changes of AA profile of marine carp at early larval stages of development. The main objective of the present study was to provide a preliminary knowledge on ontogenetic changes of AA profile in marine carp larvae and compare them with AA profiles in the diet, in order to identify possible deficiencies during ontogeny.

2 MATERIALS AND METHODS

2.1 Broodstock spawning and larval transfer

This study was carried out at the governmental warm water fish aquaculture center of Shahid Rajaee in Sari, Mazandaran, Iran. Feral carp fertilized eggs were obtained from feral broodstocks captured from a freshwater environment. Broodstock spawned between April and May 2009. Caught broodstock were transferred to the center and were kept in spawning tanks (2*3*1m dimension with 5 m^3 water and natural photoperiod). Water temperature ranged 24.4 to 25.7 °C. Males (weight 0.7±0.1 kg) and females (weight 1.1±0.01 kg) were stripped and eggs were transferred to a vase incubator (with 15 liter water). Water temperature and pH in the vase incubator ranged from 19.6 to 22.80°C and 7 to 8.5 respectively during the study period. Dead eggs were removed daily to prevent fungal contamination. Eggs hatched after 6-7 days and 3 days after hatching (DAH) larvae were transferred to rearing pools (three pools, square shape, 100 m^2 with 1m depth, natural photoperiod and periodic water change). Larvae were reared in freshwater. Water temperature was 24.2 to 25.5°C. In this study, the rotifers were produced naturally in the pond. After increasing rotifer density in the pond the larvae were transferred to the pond for feeding and growth. Rotifers were fed with different microalgae such as *Chlorella* sp. and *Scenedesmus* sp.

Larvae started feeding on rotifers at day 3 to day 7 at a density of 10 rotifers/ ml. Co-feeding of rotifers and a commercial diet was between 7 and 11 DAH. From that point until the end of the experiment larvae were fed a commercial diet (SFC-1, 3-4 times per day, 40% protein of dry weight, Chineh Company, Iran). The duration of the experiment was 33 days.

2.2 Sample preparation

Larvae were collected randomly at 0, 3, 7, 11, 15, 19, 26 and 33 days after hatching (DAH). The first 24 h after hatching was considered as day 0. Two grams of sample was collected at each larval stage. Samples were washed with distilled water to remove excess salts, and frozen in liquid nitrogen and then freeze-dried (Operon-Model: OPRFDU 7012, Korea).

2.3 Amino acids analysis

For determination of AA content in larvae, the samples were hydrolysed in 6 N HCl for 24 h at 110 °C in glass vials replaced with nitrogen.

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Derivatization of AA in the samples, was used by *o*-phthaldialdehyde (OPA) as a pre-column derivatization reagent, followed by high pressure liquid chromatography with fluorescence detection and spherical type column, all parts were from Knauer Corporation, Berlin, Germany, using the method of Lindroth and Mopper (1979) as modified by Flynn (1988). The fluorescence excitation and emission wavelengths were set 330 nm and 450 nm, respectively. In this study tryptophan was not estimated, because this amino acid is destroyed by acid hydrolysis. All determinations were carried out in triplicate.

2.4 Data analysis

Indispensable amino acid (IAA) data are expressed in weight percentage of the IAA pool ((weight of one IAA) × (weight of all IAA)$^{-1}$ ×100) (Saavedra *et al.*, 2007).

The first limiting AA was determined by the formula: (IAA diet - IAA larvae) × 100 × (IAA larvae)$^{-1}$ according to Conceição *et al.* (2003). The IAA index was determined by the formula: IAA= $((a_1 /A_1) \times (a_2 /A_2) \times \dots (a_n /A_n))^{1/n}$, where a_n is the indispensable AA in the diet and A_n is the indispensable AA in the larval body (Peñaflorida, 1989).

2.5 Statistical analysis

Data are expressed as mean ± S.D. Differences between mean values in AA contents larvae at day 0 to day 33 were determined by a One-way analysis of variance (ANOVA) followed by Duncan test. Statistical analysis was performed using the SPSS for Windows software, version 11.5 (SPSS Inc., Chicago, IL, USA). Mean values were considered significantly different at $P<0.05$.

3 RESULTS

Marine carp larval growth as wet weight (mg) and standard length (mm) is showed in Figureg 1. Larval growth in weight and standard length followed a linear curve (y = 32.063x - 46.55, R^2= 0.93 and y = 4.0987x - 0.3954, R^2= 0.93, respectively). The weight of larvae in the beginning and at the end of experiment was 3.8±0.11 mg and 199.5±0.02 mg, respectively. The length of larvae at the beginning and at the end of experiment was 3.36±0.34 mm and 29.09±0.17 mm, respectively.

The AA profiles of the feed sources (rotifers and compound diet) used for feeding marine carp larvae are presented in table 1, Ontogenetic changes in AA profiles in marine carp larvae are shown in Table 2. Whole body AA composition of marine carp changed significantly from hatching until 33 DAH ($P<0.05$) (Table 2).

The changes observed in AA contents of yolk sac larvae (from hatching to day 3) were significantly different ($P<0.05$). Most EAA and NEAA decreased during the endogenous feeding stage, except phenylalanine, threonine, glutamic acid, aspartic acid and glycine.

In marine carp all but four EAA (leucine, threonine, valine, methionine) and two NEAA (alanine and tyrosine) decreased ($P<0.05$) from day 7 to day 33. The predominant AAs observed in marine carp larvae were glutamic acid, aspartic acid, isoleucine and threonine, whereas tyrosine and histidine presented the lowest relative AA levels.

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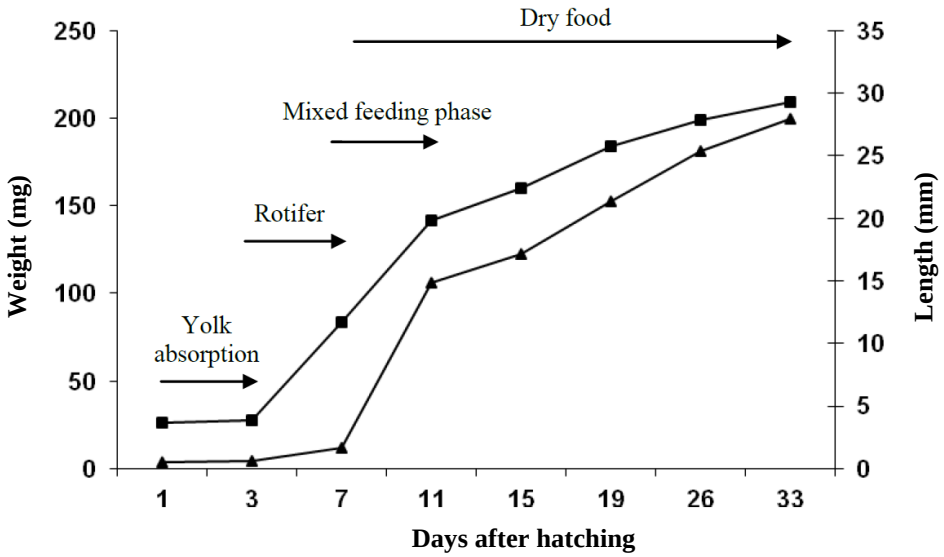

Figure 1 Growth in length (■) and weight (▲) of *Cyprinus carpio* during larval develop

Table 1 The amino acid profiles of live and dry feeds distributed to marine carp larvae (g/100g protein)

Amino acids	Rotifer	Dry food
Arg	6.14±0.03[a]	0.61±0.02[b]
His	0.97±0.02[a]	0.64±0.04[b]
Ile	6.83±0.03[a]	5.07±0.04[b]
Leu	8.34±0.03[a]	8.29±0.04[a]
Lys	2.93±0.04[a]	2.47±0.03[a]
Phe	3.39±0.03[a]	3.01±0.04[a]
Thr	5.83±0.05[a]	5.32±0.04[a]
Val	8.98±0.06[a]	8.03±0.05[b]
Met	2.62±0.04[b]	7.98±0.04[a]
Glu	12.59±0.03[b]	15.16±0.03[a]
Ser	0.29±0.01[b]	7.01±0.06[a]
Asp	9.36±0.04[a]	7.60±0.05[b]
Gly	5.43±0.04[b]	7.06±0.03[a]
Ala	9.23±0.03[a]	6.38±0.02[b]
Tyr	1.81±0.01	2.01±0.02

All data are expressed as a percentage of all amino acids. Each value is the mean of three replicates ± SD. Mean values with different superscripts are significantly different from each other. (Duncan significance level is defined as $P>0.05$).

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Table 2 The amino acid profile of *Cyprinus carpio* at different periods of larval development (g/100g protein)

	Larvae age (DHA)							
Amino acid	**1**	**3**	**7**	**11**	**15**	**19**	**26**	**33**
Arg	7.99±0.10^{a}	6.87±0.03^{b}	1.33±0.03^{f}	7.92±0.02^{a}	5.41±0.03^{d}	5.77±0.02^{c}	5.71±0.03^{c}	2.53±0.04^{e}
His	1.12±0.01^{b}	1.05±0.02^{cd}	1.14±0.03^{b}	1.15±0.04^{b}	2.12±0.04^{a}	1.11±0.02^{bc}	0.94±0.02^{e}	1.02±0.04^{d}
Ile	9.78±0.11^{a}	8.40±0.01^{c}	8.70±0.05^{b}	8.39±0.02^{c}	5.36±0.04^{f}	5.65±0.04^{e}	8.07±0.02^{d}	8.46±0.06^{c}
Leu	8.51±0.01^{a}	7.24±0.02^{c}	6.10±0.00^{e}	7.31±0.02^{b}	7.34±0.03^{b}	7.30±0.03^{b}	6.28±0.04^{d}	7.28±0.06^{bc}
Lys	4.68±0.04^{a}	3.62±0.04^{b}	2.96±0.02^{f}	3.47±0.01^{c}	2.72±0.03^{g}	3.38±0.03^{d}	3.25±0.04^{e}	2.28±0.04^{h}
Phe	1.44±0.06^{g}	1.52±0.01^{f}	5.97±0.02^{a}	3.49±0.02^{c}	3.25±0.04^{d}	3.20±0.01^{de}	3.16±0.04^{e}	3.58±0.02^{b}
Thr	5.12±0.02^{fg}	5.15±0.02^{f}	6.03±0.03^{e}	6.16±0.03^{d}	7.39±0.04^{a}	6.75±0.02^{b}	5.08±0.02^{g}	6.48±0.02^{c}
Val	2.76±0.09^{d}	2.41±0.02^{e}	2.84±0.04^{d}	7.72±0.03^{c}	7.81±0.04^{b}	7.92±0.03^{a}	7.83±0.03^{b}	7.93±0.05^{a}
Met	8.27±0.29^{a}	7.90±0.03^{b}	8.07±0.02^{b}	5.15±0.04^{d}	6.87±0.07^{c}	5.27±0.05^{d}	4.72±0.04^{e}	2.82±0.03^{f}
TEAA	**49.62±0.26^{b}**	**44.20±0.08^{f}**	**43.18±0.14^{g}**	**50.78±0.04^{a}**	**48.26±0.17^{c}**	**46.37±0.16^{d}**	**45.07±0.08^{e}**	**42.41±0.08^{h}**
Glu	12.45±0.09^{f}	12.54±0.04^{e}	14.60±0.05^{a}	13.17±0.04^{d}	12.50±0.02^{ef}	13.43±0.03^{c}	13.13±0.04^{d}	14.11±0.02^{b}
Ser	6.17±0.21^{b}	3.47±0.10^{c}	7.04±0.01^{a}	6.09±0.00^{b}	1.86±0.05^{e}	6.14±0.03^{b}	3.23±0.04^{d}	6.24±0.04^{b}
Asp	8.45±0.05^{g}	8.96±0.17^{f}	10.69±0.04^{a}	9.63±0.03^{c}	9.10±0.02^{e}	9.53±0.03^{c}	9.33±0.05^{d}	10.20±0.03^{b}
Gly	5.65±0.15^{g}	6.91±0.04^{e}	8.44±0.04^{a}	7.68±0.02^{c}	1.23±0.04^{h}	7.35±0.03^{d}	8.10±0.02^{b}	6.53±0.04^{f}
Ala	2.31±0.09^{f}	2.14±0.04^{g}	2.58±0.03^{e}	7.49±0.02^{c}	7.33±0.05^{d}	7.45±0.05^{c}	7.63±0.04^{b}	8.35±0.04^{a}
Tyr	0.03±0.00^{e}	0.03±0.00^{e}	0.06±0.02^{e}	2.45±0.04^{d}	2.55±0.04^{c}	2.64±0.04^{b}	2.40±0.03^{d}	2.94±0.05^{a}
TNEAA	**35.08±0.31^{e}**	**34.06±0.23^{e}**	**41.88±0.85^{d}**	**46.53±0.04^{b}**	**34.60±0.02^{e}**	**46.53±0.06^{b}**	**43.84±0.13^{c}**	**48.07±0.51^{a}**
TAA	**84.70±0.57^{e}**	**78.26±0.31^{g}**	**85.06±1.03^{e}**	**97.31±0.12^{a}**	**82.86±0.19^{f}**	**92.90±0.21^{b}**	**88.91±0.21^{d}**	**90.48±0.59^{c}**

All data are expressed as a percentage of all amino acids. Each value is the mean of three replicates±SD. Mean values with different superscripts are significantly different from each other. (Duncan significance level is defined as $P>0.05$). **TEAA**: Tottal Essential Amino Acids, **TNEAA**: Tottal Non Essential Amino Acids, **TAA**: Tottal Amino Acids.

At day 7, the IAA in deficiency were methionine, phenylalanine, arginine and isoleucine. At this stage, methionine seemed to be the AA in most deficiency. Leucine seemed to be present in excess at day 7 (Table 3).

At day 11, arginine, histidine, isoleucine and lysine had shown lower relative contents in the diet. At day 15, arginine, histidine and threonine were in deficiency. At day 19, essential AA in deficiency were arginine, histidine and lysine. At day 26 arginine, histidine, isoleucine and lysine were in deficiency. At day 33 arginine, histidine, isoleucine, phenylalanine and threonine were apparently in deficiency. At day 11, 15, 19, 26 and 33 arginine was the AA in most deficiency and presented the highest difference between the AA profile from the larvae and the dry food. Methionine seemed to be present in excess from day 11 until day 33 (Table 3).

The essential AA profile of marine carp showed low correlation with rotifers (R^2=0.22) and dry food in late larval stage ($R^2\geq0.37$) but higher correlation was found between dry food and early larval ages. ($R^2\geq0.7$). (Table 4).

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Table 3 Relative differences between Indispensable amino acids from the diet and *Cyprinus carpio* larvae (apparent first limiting amino acid for each larval age is marked in bold)

Amino acids	**Larvae age (DHA)**					
	L-7	L-11	L-15	L-19	L-26	L-33
Arg	-53.93[a]	**-90.57**[d]	**-86.87**[c]	**-88.18**[d]	**-88.38**[d]	**-75.33**[b]
His	-8.29[a]	-40.76[e]	-64.92[f]	-35.56[c]	-25.96[b]	-35.83[d]
Ile	-15.16[c]	-35.20[e]	10.27[b]	49.26[a]	-31.62[d]	-38.61[f]
Leu	47.83[a]	20.97[e]	31.64[c]	27.12[d]	43.64[b]	16.60[f]
Lys	7.07[b]	-24.07[f]	5.67[c]	-18.13[e]	-17.33[d]	10.98[a]
Phe	-38.61[f]	-7.98[d]	7.87[a]	5.21[b]	3.56[c]	-13.98[e]
Thr	4.54[b]	-7.82[c]	-16.33[f]	-11.75[d]	13.93[a]	-15.91[e]
Val	24.21[a]	10.93[e]	19.77[b]	13.46[c]	11.50[d]	3.69[f]
Met	**-64.89**[f]	65.32[c]	35.34[d]	69.24[b]	83.95[a]	19.06[e]

Diet given to the larvae: rotifers (7DAH), dry food (11DAH to 33 DAH).

Table 4 Indispensable amino acid index (IAA) for larvae and diet, according to larvae age

Larvae age (DHA)	**Diet**	**IAA Index**	**R^2**	***P***
7	Rotifers	0.89	0.22	0. 690
11	Dry food	0.80	0.71	0.004
15	Dry food	0.66	0.62	0.008
19	Dry food	0.71	0.52	0.026
26	Dry food	0.75	0.37	0.08
33	Dry food	0.84	0.43	0.042

Diet given to the larvae: rotifers (7DAH), dry food (11DAH to 33 DAH).

4 DISCUSSION

The present study is the first focusing on the changes in the AA profiles during feral carp larval development (*C. carpio*). AA profile of *C. carpio* changed significantly. These changes may be related to changes in the rates of synthesis of different proteins at the different larval stages (Conceição *et al.*, 1998). Changes in the AA profile during larval development have been reported for several fish species. Aragão *et al.* (2004a) reported AA profiles of *Solea senegalensis* changed significantly, especially during metamorphosis. The AA profile of African catfish (*Clarias gariepinus*) larvae changed during ontogeny, especially before the start of exogenous feeding (Conceição *et al.*, 1998). On the contrary, AA profile of common dentex (Tulli and Tibaldi, 1997) did not change significantly during larval exogenous feeding (from 12 DAH until 30 DHA) and Saavedra *et al.* (2006) reported that AA profiles of *Diplodus sargus* did not change significantly from hatching to 45 DHA.

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During the embryonic and yolk sac stages, yolk proteins are broken down into AA for organogenesis or energy production. Therefore, until first feeding developing larvae depend entirely on the nutritional material in the yolk (Gunasekera *et al.,* 1999). In marine carp larvae, all EAA except Alanine and Threonine, and three NEAA (Glutamic acid, Aspartic acid and Glysine) decreased (P <0.05) their levels with development, from newly hatched larvae to yolk-sac reabsorption. This suggests that AA are used for energy production during endogenous feeding stage in marine carp larvae. The decrease in total amino acid (TAA) after hatching (from day 1 until day 3) is consistent with the above observation. Conceição *et al.* (2003) and Saavedra *et al.* (2008) reported that AA are an important energy source during fish larval stages. The same has been shown for alevins of cultured and wild Atlantic salmon *Salmo salar* (Srivastava *et al.,* 1995), trout cod and Murray cod larvae (Gunasekera *et al.,* 1999). The IAA profile of rotifers seems to be deficient in methionine for 7 DAH marine carp larvae. Methionine is an essential AA for optimal growth and metabolic pathways (Luo *et al.,* 2005). This IAA is precursor of choline, a vitamin required for fish homeostasis and growth (Kasper *et al.,* 2000).

The indispensable AA profiles obtained in this study showed low correlation with rotifers (R^2=0.22). This low correlation indicates rotifers have strong AA imbalances for this species. This means larvae must spend more time preying on this type of live food to compensate the dietary AA losses (Saavedra *et al.,* 2006; Aragão *et al.,* 2004 a).

In this study, the phenylalanine and tyrosine content increased or maintained at constant levels around the first feeding. The increase in these AA might be associated with the onset of thyroid gland activity (Conceição *et al.,* 1997). These aromatic AA have important physiological functions, since they are the precursors of tyroid hormones, melanin, dopamine and cathecolamines (Aragão *et al.,* 2004b). The same has been reported for Asian seabass (Sivaloganathan *et al.,* 1998), lemon sole (Rønnestad *et al.,* 1992) and Senegalese sole (Parra *et al.,* 1999).

Arginine seems to be the limiting AA at 11, 15, 19, 26 and 33 DHA. Therefore, the dry food did not present balanced arginine content. Arginine is involved in many metabolic pathways such as: protein synthesis, urea production, metabolism of glutamic acid and proline, and synthesis of creatine and polyamines (Alam *et al.,* 2002), and is considered for optimal growth of fish (Wilson, 1989).

Also, comparing larval and dry food AA profiles, high correlation were found in early larval stage but from day 11 to day 33, the correlations decreased, probably due to AA requirements change during ontogeny. Beside this, these low correlations in common carp larvae may indicated that dry diet used failed to support fish AA requirements and the profile of the dry food seems to be deficient in some IAA. The use of diets with unsuitable AA profiles will lead to AA losses and increase nitrogen excretion (Aragão *et al.,*). Therefore, fish will need to increase their food consumption (Saavedra *et al.,* 2006). The choice of the best zooplankton enrichment and/or mixture of enrichments for the different fish larval stages and supplementation of dry food with essential AA, should take into account the AA composition, in order to fulfill the energetic and nutritional requirements of the larvae (Aragão *et al.,* 2004 b; Saavedra *et al.,* 2006).

5 CONCLUSION

The composition of total AA changed significantly during ontogeny, even if significant differences were not very marked. Larval indispensible AA profile can be used as index of the IAA requirements whenever it is not determined. Comparisons between larval

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and diet AA profiles at different stages indicated that rotifers and dry food are deficient in methionine and arginine respectively. Since feral carp (*Cyprinus carpio*) is one of the most important economically species for aquaculture and restocking purposes of Caspian Sea, indispensable AA supplementation in *Cyprinus carpio* diets seems necessary to fulfill the nutritional requirements of the larvae.

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تغییرات اسیدهای آمینه ماهی کپور دریای خزر (*Cyprinus carpio*) در دوره تکامل لاروی: کاربرد آن در جیره نویسی

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چکیده لارو ماهیان نیاز به مقادیر بالای اسیدهای آمینه برای تولید انرژی و رشد دارند، بنابراین تحقیق حاضر به‌منظور آگاهی از تغییرات ترکیب اسیدهای آمینه ماهی کپور دریای خزر طی مراحل تکامل لاروی جهت تعیین احتیاجات غذایی و بهبود کیفیت تولید، صورت گرفت. نمونه‌برداری از لاروها در مرکز تکثیر و پرورش ماهیان گرمابی شهید رجایی ساری به‌صورت کاملاً تصادفی به‌مدت ۳۳ روز در روزهای ۱، ۳، ۷، ۱۱، ۱۵، ۱۹، ۲۶ و۳۳ پس از تفریخ انجام شد. ترکیب اسیدهای آمینه تغییرات معناداری طی مراحل تکامل لاروی را نشان داد. پروفیل اسیدهای آمینه لارو کپور دریایی و روتیفر ضریب همبستگی بسیار پایینی (R^2 =۰/۲۲) را نشان دادند. ضریب همبستگی بین پروفیل اسیدهای آمینه لارو کپور دریایی و غذای خشک در روزهای اولیه بالا (R^2≥۰/۷) ولی در مراحل بالاتر پایین بود (R^2≥۰/۳۷). در روز هفتم، در مرحله تغذیه از روتیفر، به‌نظر می‌رسد متیونین اسید آمینه محدود کننده باشد و از روز ۱۱ تا پایان دوره که تغذیه با غذای خشک انجام شد، به‌نظر می‌رسد آرژنین اسید آمینه محدود کننده باشد. تا زمانی‌که احتیاجات اسیدهای آمینه این ماهی تعیین نگردید پروفیل اسیدهای آمینه ضروری (IAA) بدن لارو ماهی به عنوان یک شاخص مناسب برای تخمین و تعیین احتیاجات آمینو اسیدی غذای لارو ماهی می‌تواند به‌کار رود. اضافه کردن اسیدهای آمینه مورد نظر به غذای مصرفی لارو می‌تواند یکی از راه‌های جبران کمبود اسیدهای آمینه باشد.

کلمات کلیدی: اسید آمینه، رشد لارو، کپور دریایی، مراحل تکامل لاروی، *Cyprinus carpio*

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