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Original article

Description of amino acid and fatty acid content during initial development of *Lophiosilurus alexandri* (Siluriformes: Pseudopimelodidae), a carnivorous freshwater catfish

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Samples of eggs, newly hatched larvae (NHL), and larvae at the end of the lecithotrophic period (eight days after hatching) (LPL) of *Lophiosilurus alexandri* were collected to determine the amino acid and fatty acid profiles. Crude protein did not change throughout initial development and the concentration of lipids was highest in NHL. The content of the indispensable amino acids (IAA) isoleucine, leucine, and valine decreased in LPL, while in eggs and NHL they remained high and similar in value. The dispensable amino acids (DAA), such as aspartic acid, tyrosine, and glycine, increased in LPL, while alanine decreased. The percentage of neutral lipids increased in LPL. The saturated fatty acid content decreased during ontogeny, while monounsaturated fatty acids decreased only in LPL. The polyunsaturated fatty acid content was highest in LP. Polar fatty acids were found in higher percentages in eggs and NHL, but lower in LPL. Saturated fatty acid content decreased during ontogenetic development, while that of monounsaturated fatty acids decreased only in LPL. Polyunsaturated fatty acid content was highest in LPL. Protein content was maintained during ontogenetic development, while amino acid classes experienced changes. *Lophiosilurus alexandri* preferentially uses saturated and monosaturated fatty acids as an energy source during its early development.

Keywords: Embryonic development, Nutritional requirement, Pacamã.

Amostras de ovos, larvas recém-eclodidas (NHL) e larvas no final do período lecitotrófico (oito dias após a incubação) (LPL) de *Lophiosilurus alexandri* foram coletadas para determinar os perfis de aminoácidos e ácidos graxos. A proteína bruta não alterou durante o desenvolvimento inicial e a concentração de lipídios foi maior na NHL. O conteúdo dos aminoácidos indispensáveis (IAA) isoleucina, leucina e valina diminuíram em LPL, enquanto nos ovos e NHL eles permaneceram com valores elevados e semelhantes entre si. Os aminoácidos dispensáveis (DAA), como ácido aspártico, tirosina e glicina, aumentaram em LPL, enquanto a alanina diminuiu. A porcentagem de lipídios neutros aumentou em LPL. O teor de ácidos graxos saturados diminuiu durante a ontogenia, enquanto os ácidos graxos monoinsaturados diminuíram apenas em LPL. O teor de ácidos graxos poliinsaturados foi maior em LPL. Os ácidos graxos polares foram encontrados em porcentagens mais altas em ovos e NHL, e menores em LPL. O teor de ácidos graxos saturados reduziu durante o desenvolvimento ontogenético, enquanto que os ácidos graxos monoinsaturados diminuíram apenas em LPL. O teor de ácidos graxos poliinsaturados foi maior em LPL. O conteúdo de proteínas foi mantido durante o desenvolvimento ontogenético, enquanto as classes de aminoácidos sofreram mudanças. *Lophiosilurus alexandri* usa preferencialmente ácidos graxos saturados e monossaturados como fonte de energia durante o desenvolvimento inicial.

Palavras-chave: Desenvolvimento embrionário, Pacamã, Requerimento nutricional.

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Introduction

From embryogenesis until first feeding all the nutrients required for larval growth, cell differentiation, and metabolism originate from yolk-sac reserves (Tocher, 2010). Proteins and lipids, as well as their respective amino acid and fatty acid constituents, represent a considerable portion of the composition of fish eggs (Finn, Fyhn, 2010).

During organogenesis, proteins are degraded and absorbed, and the amino acids used to either form tissues or be catabolized to generate metabolic energy (Li *et al*., 2009; Finn, Fyhn, 2010). Moreover, amino acids regulate osmotic pressure for oocyte hydration, regulate gene expression and serve as precursors for the synthesis of nitrogenous components of hormones (Wu, 2009). The composition of the profile of amino acids in larval tissue provides relevant information for understanding the nutritional requirements of larvae at the beginning of exogenous feeding (Gurure *et al*., 2007; Saavedra *et al*., 2015). Lipids and their constituent fatty acids (FA) are the second most abundant component of the egg, after proteins (Tocher, 2010). During larval development, FA are destined for energy production (mainly saturated and monounsaturated) (Dantagnan *et al*., 2007; Araújo *et al*., 2012), for the development of vision and nervous systems and to serve as membrane constituents and eicosanoid precursors (mainly Highly unsaturated fatty acids - HUFA and polyunstaurated fatty acids - PUFA) (Glencross, 2009; Tocher, 2010). The profiles of AA and FA in eggs and larvae have been used as indicators of the nutritional quality of fish larvae (Tong *et al*., 2017) and to estimate nutrient requirement indexes for young stages (Saavedra *et al*., 2015).

Pacamã, *Lophiosilurus alexandri* (Siluriformes: Pseudopimelodidae) (Steindachner, 1876), is a carnivorous freshwater fish that reproduces naturally in captivity (Costa *et al*., 2015). The use of *Artemia* during the larviculture has provided survival values around 96.9% (Takata *et al*., 2014). After the process of feeding training, juveniles respond positively to the exclusive consumption of pelleted feed (Cordeiro *et al*., 2016), a factor that is favorable for the potential captive production of the species.

Despite this knowledge, there remains no available information on the composition of amino acids (AA) and fatty acids (FA) of eggs and larvae of *L. alexandri*. Such information would serve as an important starting point for determining the dynamics of metabolic changes of AA and FA during the initial development of this species, and enable studies of nutritional requirements. The aim of this study was to analyze variation in the amino acid and fatty acid profiles of eggs, newly hatched larvae and larvae at the end of the lecithotrophic period of development of *L. alexandri*.

Material and Methods

The methodology used in the present study was approved by the Ethics Committee on Animal Use, Protocol 25/2010 - CEUA / UFMG. The number of a specimen placed in an ichthyological collection used in this experiment is MCNIP 3217 (Coleção de Ictiologia do Museu de Ciências Naturais da PUC Minas).

Broodstock used to collect eggs were wild-caught from the São Francisco River and then adapted to conditions of captivity in the Laboratory of Aquaculture (Laqua) of the Universidade Federal de Minas Gerais (UFMG). The broodstock were kept for about six months in 5-m^3 tanks, with a thin layer (approximately 5 to 10 cm deep) of sand (pool filter type) as a substrate, and supplemental aeration (dissolved oxygen > 5.5 mg/L), at an average temperature of 28 °C. The broodstock, weighing about 2.5 kg, were fed twice a week with tilapia fillets containing a vitamin premix capsule (600 mg of vitamin C + 30 mg of premix). The tilapia fillets contained on average 64.92 ± 3.34% of crude protein, 11.74 ± 4.13% of lipids and 9.97 ± 0.35% of moisture. The amino acid composition (% total AA) of the fillets included on average 5.63 ± 0.49 Lysine (Lys); 4.56 ± 0.45 Leucine (Leu); 4.08 ± 0.46 Arginine (Arg); 9.27 ± 0.95 Glutamine (Glu) and 6.52 ± 0.93 Aspartic acid (Asp), while the percentage of fatty acids (% of total FA) were 19.18 ± 2.04 Palmitic (C16: 0); 1.42 ± 0.17 Arachidonic (C20: 4 n-6); C20: 5n3 0.07 ± 0.01 Eicosapentaenoic (EPA) and 0.59 ± 0.11 C22: 6n3 Docosahexaenoic (DHA).

Amino acid and fatty acid profiles of *L. alexandri* were determined for eggs, newly hatched larvae and larvae at the end of the lecithotrophic period.

Eggs were collected from three random naturally-occurring spawns in captivity. Tanks containing the broodstock were surveyed on a daily basis, and when spawning was detected, the eggs were collected and cleaned by removing sand particles and fouled eggs.

The eggs were then placed in 40-L artificial incubators. Hatching occurred 24-h later and larvae were reared in the same place until the eighth day post-hatching (the end of the lecithotrophic period - LPL).

Incubators were kept at an average temperature of 28°C with a constant aeration system that maintained the oxygen concentration > 5.5 mg L^{-1} (measured using a Hanna HI9146 unit), a pH of 7.05 (measured using a Hanna HI98129 combo device), and zero total ammonia (measured by colorimetric test). Incubators were kept in a water recirculation system with 30% water renewal once a day to maintain water quality parameters. From each spawning, eggs (N = 175; 2.42 ± 0.11 mm and 21.63 ± 2.65 mg), NHL (N = 170; 6.48 ± 0.90 mm and 7.77 ± 0.33 mg) and LPL (N = 102; 12.07 ± 1.28 mm and 25.27 ± 0.48 mg) were collected for biochemical analyses. Samples were frozen in liquid nitrogen and stored in a freezer at -80°C for future lyophilization.

Biochemical analysis. Protein and dry matter analyses were performed at the Veterinary Nutrition Laboratory at UFMG. The samples were lyophilized for about 36 h. After drying, the samples were crushed for analysis of dry matter and nitrogen (protein). The moisture content was determined by drying the samples in an oven at 110°C for 24 h (A.O.A.C 1985). The nitrogen content was obtained using a LECO FP-528 nitrogen/protein analyzer. The nitrogen values found were subsequently multiplied by a correction factor of 6.25 in order to obtain the value of crude protein.

Amino acid profile. Amino acid (AA) analyses were performed at the Protein Chemistry Center of the Medical School of the Universidade de São Paulo in Ribeirão Preto, Brazil.

Protein-bound amino acid samples were hydrolyzed in 6 M hydrochloric acid at 108 °C for 24 h in nitrogen-flushed glass vials. Reversed-phase high-pressure liquid chromatography (HPLC), in a Waters Pico-Tag amino acid analysis system with norleucine as an internal standard, was used to determine amino acid content. The resulting chromatograms were analyzed with Breeze software (http://www.waters.com/waters/en). The results were expressed as mg/g of protein.

Fatty acid profile. Fatty acid analyses were carried out in the Laboratory of Natural Resources, Institute of Ecology and Environmental Science, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Samples were analyzed for total, polar, and neutral lipid content and for determining the fatty acid profile of total lipids. Lipids were extracted with chloroform:methanol (2:1), and quantified gravimetrically following the method described by Folch *et al*. (1957). Polar and neutral lipids were separated using adsorption chromatography on silica cartridges, as described by Juaneda, Rocquelin (1985). Fatty acid methyl esters were obtained using transesterification with methanol in sulphuric acid (Christie, 1982) and separated and quantified using gas chromatography. During lipid and fatty acid analysis, samples were protected from oxidation by maintaining them under nitrogen gas and using butylated hydroxy toluene (100 mg L^{-1} of solvent). Fatty acid methyl esters were analyzed using a gas chromatograph equipped with a flame ionization detector and a Supelcowax fused silica capillary column (30 m x 0.32 mm ID, Supelco) using nitrogen as a carrier. Samples were injected in split mode at 250 °C. Column temperature was maintained at 180°C for 12 min, increasing thereafter to 212°C at a rate of 2°C min^{-1} and maintained at 212°C for 13 min. Fatty acids were identified by comparison with the retention times of methyl ester standards (Supelco) and by reference to a well-characterized fish oil.

Statistical analysis. Statistical analysis was performed using the Statistical Analyses System software package, version 8.0 (SAS). The data were submitted to a Cramér-von Mises normality test and Levene homoscedasticity test. The data, shown as percentages, were previously transformed into arcsine for statistical analysis. Logarithmic transformation (natural logarithm) was used for the non-normal data. Data were tested by one-way ANOVA and compared by Tukey test at 5% probability.

Results

Values for moisture was differ between eggs and LPL ($P < 0.05$) (Tab. 1). NHL showed higher values of lipids than the other stages ($P > 0.05$), whereas protein content did not vary significantly during development.

Percentages of neutral lipids (NL) were higherst for LPL, while lower and similar percentages were observed for NHL and eggs ($P < 0.0001$). The percentages of PL were lowest in LPL, and higher for eggs and NHL, which were similar ($P < 0.0001$).

Tab. 1. Mean (± SD) values for moisture (%), protein (% dry weight), total lipids (% dry weight), neutral lipids (NL) (% dry weight) and polar lipids (PL) (% dry weight) of fertilized eggs, newly hatched larvae (NHL), and larvae at the end of the lecithotrophic period (LPL) of *Lophiosilurus alexandri*. Different letters indicate significant differences by Tukey test ($P < 0.05$).

Parameters	Eggs	NHL	LPL
Moisture	81.91 ± 2.97^{a}	78.22 ± 0.97^{ab}	76.26 ± 0.52^{b}
Protein	61.01 ± 3.86^{a}	64.85 ± 2.31^{a}	60.28 ± 0.20^{a}
Total lipids	17.50 ± 0.65^{b}	$23.65 \pm 0,25^{a}$	16.64 ± 1.45^{b}
Neutral lipids	38.01 ± 0.53^{b}	39.04 ± 0.69^{b}	45.59 ± 0.74^{a}
Polar lipids	61.98 ± 0.53^{a}	61.16 ± 0.44^{a}	54.29 ± 0.86^{b}

Amino acid content. During the ontogenetic development of *L. alexandri*, 18 amino acids (AA) were quantified, of which ten were indispensable amino acids (IAA) and eight dispensable amino acids (DAA) (Tab. 2). The amino acids with the highest concentrations in eggs were Glu (DAA) and Leu (IAA), respectively ($P < 0.0001$), followed by Ala and Asp (DAA), which had similar values ($P < 0.0001$). The AA with the lowest concentration in eggs was Cys (DAA). Additionally, many IAAs, such as His, Met, Phe, and Trp, were also found at low concentrations, with values similar to each other ($P < 0.0001$). In NHL, Glu and Leu also had the highest concentrations ($P < 0.0001$), followed by Asp, while Cys had the lowest concentration. Similar to what was found in eggs, NHL had IAAs in low concentrations, such as His, Met, Phe, and Trp, all with similar values. In LPL, Glu remained the AA with the highest concentration, followed by Asp and then Arg ($P < 0.0001$). The IAAs, such as His, Ile, Met, Phe, and Trp, were found in similarly low concentrations ($P < 0.0001$).

When compared among the stages of development the AAs His ($P = 0.2150$), Met ($P = 0.2846$), Phe ($P = 0.5073$), Thr ($P = 0.3585$) Cys ($P = 0.3806$) and Pro ($P = 0.8091$)

did not vary (Tab. 2). The AAs Arg (P = 0.0010), Lys (P = 0.0085), Asp (P = 0.0006), Glu (P < 0.0001), Gly (P < 0.0001) and Tyr (P < 0.0140) all had their highest concentrations in LPL, while eggs and NHL had lower and similar concentrations. In contrast, Ile (P = 0.0019), Leu (P < 0.0001), Val (P = 0.0014), and Ala (P < 0.0001) all had their lowest concentrations in LPL. The concentrations of these AAs were higher, and similar, in eggs and NHL than in LPL. Leu exhibited the greatest variation in concentration during development, being reduced in LPL to three quarters of its value in the previous stages (P < 0.0001). The concentration of Trp significantly decreased throughout the development (P < 0.0001). Ser had its highest concentration in eggs, intermediate values in NHL, and its lowest concentration in LPL (P = 0.0217).

Tab. 2. Mean (± SD) total amino acid content in eggs and newly hatched larvae (NHL), and larvae at the end of the lecithotrophic (LPL) period of *Lophiosilurus alexandri*. The statistical values of F and the probability are also presented. Lower case letters horizontally and vertically different capital letters indicate significant differences by Tukey test (P < 0.05).

Amino acids (mg/g of protein)	Eggs	NHL	LPL	P
Arginine (Arg)	71.0 ± 0.9^{Db}	75.2 ± 2.7^{Db}	81.9 ± 1.3^{Ca}	0.0010
Histidine (His)	29.0 ± 0.4^{Ga}	33.9 ± 3.9^{Ha}	30.3 ± 3.7^{Ha}	0.2156
Isoleucine (Ile)	55.9 ± 6.7^{EFa}	57.7 ± 0.4^{FGa}	35.2 ± 4.5^{Hb}	0.0019
Lysine (Lys)	54.4 ± 3.7^{EFb}	50.6 ± 4.1^{Gb}	63.8 ± 2.2^{DEa}	0.0085
Leucine (Leu)	104.2 ± 3.4^{Ba}	103.9 ± 2.6^{Ba}	74.6 ± 2.9^{CDb}	<0.0001
Metionine (Met)	34.9 ± 1.4^{Ga}	33.9 ± 0.2^{Ha}	31.4 ± 4.2^{Ha}	0.2846
Phenylalanine (Phe)	37.5 ± 1.4^{Ga}	36.5 ± 1.6^{Ha}	38.8 ± 3.3^{Ha}	0.5073
Treonine (Thr)	51.9 ± 1.9^{Fa}	53.6 ± 0.1^{Ga}	51.8 ± 2.0^{FGa}	0.3585
Tryptophan (Trp)	35.5 ± 0.2^{Ga}	32.6 ± 0.3^{Hb}	30.2 ± 0.1^{Hc}	<0.0001
Valine (Val)	65.5 ± 4.9^{Da}	65.7 ± 0.9^{EFa}	49.2 ± 3.0^{FGb}	0.0014
Alanine (Ala)	86.4 ± 2.5^{Ca}	80.3 ± 3.1^{Da}	59.6 ± 3.0^{EFb}	<0.0001
Aspartic acid (Asp)	93.8 ± 2.7^{Cb}	91.8 ± 0.5^{Cb}	112.8 ± 5.5^{Ba}	0.0006
Cysteine (Cys)	3.6 ± 0.7^{Ha}	2.5 ± 0.9^{Ia}	4.5 ± 2.7^{Ia}	0.3806
Glutamine (Glu)	128.9 ± 1.0^{Ab}	131.3 ± 1.4^{Ab}	160.6 ± 6.7^{Aa}	<0.0001
Glycine (Gly)	30.3 ± 2.1^{Gb}	31.8 ± 1.0^{Hb}	56.6 ± 1.8^{EFa}	<0.0001
Proline (Pro)	54.0 ± 1.8^{Fa}	54.8 ± 1.8^{FGa}	55.9 ± 5.6^{EFa}	0.8091
Serine (Ser)	62.5 ± 4.6^{DEa}	60.4 ± 0.2^{EFab}	54.3 ± 0.2^{EFb}	0.0217
Tyrosine (Tyr)	36.3 ± 0.7^{Gb}	36.0 ± 0.2^{Hb}	38.8 ± 1.3^{GHa}	0.0140
Prob.	<0.0001	<0.0001	<0.0001	

Fatty acid content. The fatty acid (FA) composition of neutral lipids (NL) is shown in Tab. 3. Palmitic acid (16:0) was the main FA found in NL, showing an important decrease in the last stage (LPL) (P < 0.001). Stearic acid (C18:0) content doubled in LPL (P < 0.0001), partially compensating for the decrease of 16:0. The ΣSFA content decreased throughout development from egg to LPL (P < 0.0001).

Oleic acid (C18:1n-9) was the most important MUFA in all three stages, representing circa 25% of all FA (P < 0.0001). NHL had the highest percentages of C18:1n-9, followed by eggs and LPL. The MUFAs C16:1n-7 (P < 0.0001) and C22:1n-7 (P < 0.0301) had their lowest percentages in LPL, while in eggs and NHL similar higher values were found. The other MUFAs had similar values throughout development. The values for ΣMUFA were highest and similar in eggs and NHL, and lowest in LPL (P < 0.0001).

The percentages of precursors of PUFA, C18: 2n-6 (P < 0.0001) and C18:3n-3 (P < 0.0001) increased significantly in LPL. Among HUFAs, a very important relative increase occurred in both the n-3 and n-6 series, with a very significant increase of DHA, DPA and AA in LPL.

Tab. 3. Mean (± SD) the neutral lipid FA profile expressed as % of total FA in eggs, newly hatched larvae (NHL), and the end of the lecitotrophic larval period (LPL) of *Lophiosilurus alexandri*. The statistical probabilities are also presented. Different letters indicate significant differences by Tukey test (P < 0.05). Pentadecanoic (C15:0), Palmitic (C16:0), Stearic (C18:0), Myristoleic (C14:1), Cis-7 hexadecenoic acid (C16:1n 9), Palmitoleic (C16:1n-7), Oleic (C18:1n-9), Vaccenic (C18:1n-7), 15-Docosenoic (C22:1 n-7), Linoleic (C18:2 n-6), Linolenic (C18:3n3), Dihomo-Gamma-Linolenic (C20:3n-6), Arachidonic (C20:4 n-6), Eicosapentaenoic (C20:5n-3) (EPA), Docosapentaenoic (22:5n-6), Docosapentaenoic (C22:5n-3), Docosahexaenoic (C22:6n-3) (DHA).

% of total FA	Eggs	NHL	LPL	P
16:0	35.90 ± 0.35^{a}	35.62 ± 0.06^{a}	26.15 ± 0.22^{b}	<0.0001
18:0	4.49 ± 0.06^{b}	4.69 ± 0.01^{b}	9.77 ± 0.21^{a}	<0.0001
∑ SFA	49.05 ± 0.07^{a}	48.38 ± 0.03^{b}	42.68 ± 0.28^{c}	<0.0001
16:1n-9	1.00 ± 0.13^{a}	1.12 ± 0.19^{a}	0.97 ± 0.07^{a}	0.3715
16:1 n-7	7.09 ± 0.14^{a}	6.92 ± 0.32^{a}	3.99 ± 0.17^{b}	<0.0001
18:1n-9	24.76 ± 0.65^{b}	26.34 ± 0.48^{a}	21.29 ± 0.02^{c}	<0.0001
18:1n-7	5.54 ± 0.31^{a}	5.15 ± 0.38^{a}	4.70 ± 0.51^{a}	0.0811
22:1n-7	0.28 ± 0.09^{a}	0.29 ± 0.05^{a}	0.13 ± 0.02^{b}	0.0301
∑ MUFA	42.07 ± 0.44^{a}	42.91 ± 0.03^{a}	33.89 ± 0.83^{b}	<0.0001
18:2n-6	4.01 ± 0.10^{c}	4.29 ± 0.04^{b}	6.38 ± 0.05^{a}	<0.0001
18:3n-3	0.27 ± 0.01^{b}	0.16 ± 0.01^{c}	0.36 ± 0.01^{a}	<0.0001
20:3n-6	0.57 ± 0.08^{b}	0.61 ± 0.03^{b}	2.95 ± 0.14^{a}	<0.0001
20:4n-6	0.31 ± 0.02^{b}	0.31 ± 0.05^{b}	3.60 ± 0.32^{a}	<0.0001
20:5n-3	0.32 ± 0.06^{b}	0.26 ± 0.01^{b}	0.50 ± 0.01^{a}	0.0003
22:5n-6	0.28 ± 0.09^{b}	0.32 ± 0.04^{b}	1.67 ± 0.29^{a}	<0.0001
22:5n-3	0.24 ± 0.06^{b}	0.23 ± 0.07^{b}	0.75 ± 0.06^{a}	<0.0001
22:6n-3	0.37 ± 0.08^{b}	0.31 ± 0.02^{b}	3.39 ± 0.25^{a}	<0.0001
∑ PUFA	7.67 ± 0.36^{b}	7.77 ± 0.06^{b}	22.51 ± 1.20^{a}	<0.0001
n-9	26.53 ± 0.20^{a}	27.16 ± 0.15^{a}	17.49 ± 0.09^{b}	<0.0001
n-3	1.54 ± 0.14^{b}	1.21 ± 0.04^{b}	5.59 ± 0.27^{a}	<0.0001
n-6	5.74 ± 0.15^{b}	6.12 ± 0.06^{b}	16.41 ± 0.96^{a}	<0.0001
n-3HUFA	1.19 ± 0.12^{b}	0.95 ± 0.04^{b}	5.11 ± 0.28^{a}	<0.0001
n-6HUFA	1.30 ± 0.02^{b}	1.38 ± 0.04^{b}	9.04 ± 0.96^{a}	<0.0001
n-3/n-6	0.27 ± 0.02^{b}	0.20 ± 0.01^{c}	0.34 ± 0.00^{a}	<0.0001
DHA/EPA	1.22 ± 0.50^{b}	1.20 ± 0.10^{b}	6.82 ± 0.59^{a}	<0.0001
DHA/ARA	1.22 ± 0.32^{a}	1.02 ± 0.22^{a}	0.94 ± 0.01^{a}	0.3722

The PUFAs C20:3n-6 ($P < 0.0001$), C20:4n-6 ($P < 0.0001$), C20:5n-3 ($P = 0.0003$), C22:5n-6 ($P < 0.0001$), C22:5n-3 ($P < 0.0001$) and C22:6n-3 ($P < 0.0001$) had lower and similar amounts in eggs and NHL, being highest in LPL. ΣPUFA was highest in LPL ($P < 0.0001$), and lower and similar in eggs and NHL.

The FAs of the n-9 series were lowest in LPL ($P < 0.0001$). The n-3 ($P < 0.0001$) and n-6 series ($P < 0.0001$) were highest in LPL. The highly unsaturated FAs (HUFAs) of the n-3 ($P < 0.0001$) and n-6 series ($P < 0.0001$) were highest in LPL. The DHA/EPA ratio was highest in LPL. The DHA/ARA ratio remained the same throughout ontogenetic development ($P < 0.0001$).

The FA composition of polar lipids (PL) is shown in Tab. 4. The SFAs C16:0 ($P = 0.0003$) and C18:0 ($P = 0.0002$) were lower in LPL than in eggs and NHL.

Tab. 4. Mean (± SD) the FA profile of PL expressed in % of total FA in eggs, newly hatched larvae (NHL), and the end of the lecitotrophic larval period (LPL) of *Lophiosilurus alexandri*. The statistical probabilities are also presented. Different letters indicate significant differences by Tukey test (P<0.05). Pentadecanoic (C15:0), Palmitic (C16:0), Stearic (C18:0), Myristoleic (C14:1), Cis-7 hexadecenoic acid (C16:1n 9), Palmitoleic (C16:1n-7), Oleic (C18:1n-9), Vaccenic (C18:1n-7), 15-Docosenoic (C22:1 n-7), Linoleic (C18:2 n-6), Linolenic (C18:3n3), Dihomo-Gamma-Linolenic (C20:3n-6), Arachidonic (C20:4 n-6), Eicosapentaenoic (C20:5n-3) (EPA), Docosapentaenoic (22:5n-6), Docosapentaenoic (C22:5n-3), Docosahexaenoic (C22:6n-3) (DHA).

% of total FA	Eggs	NHL	LPL	P
16:0	24.26±0.99^a	23.44±0.11^a	20.26±0.16^b	0.0003
18:0	17.50±0.43^a	17.94±0.51^a	15.07±0.03^b	0.0002
∑SFA	46.77±0.60^a	45.36±0.69^b	39.25±0.13^c	<0.0001
16:1n-9	0.81±0.03^a	0.64±0.18^a	0.63±0.05^a	0.1588
16:1n-7	3.10±0.25^a	2.88±0.17^a	1.38±0.03^b	<0.0001
18:1n-9	23.78±0.49^a	24.58±0.39^a	15.48±0.08^b	<0.0001
18:1n-7	5.68±0.62^a	4.74±0.01^b	3.48±0.01^c	0.0009
22:1n-7	0.44±0.02^a	0.34±0.05^a	0.18±0.07^b	0.0038
∑MUFA	36.83±1.35^a	35.94±0.47^a	22.99±0.10^b	<0.0001
18:2n-6	4.88±0.43^b	6.27±0.13^a	4.92±0.12^b	0.0013
18:3n-3	0.24±0.01^a	0.18±0.02^b	0.14±0.00^c	0.0004
20:3n-6	1.72±0.36^c	2.50±0.12^b	4.41±0.26^a	<0.0001
20:4n-6	2.65±0.40^b	3.10±0.16^a	9.05±0.03^a	<0.0001
20:5n-3	0.73±0.07^a	0.56±0.05^a	0.60±0.00^a	0.2092
22:5n-6	0.78±0.26^b	1.02±0.17^b	4.03±0.08^a	<0.0001
22:5n-3	0.52±0.03^b	0.42±0.05^b	1.39±0.04^a	<0.0001
22:6n-3	2.31±0.37^b	2.24±0.34^b	9.57±0.02^a	<0.0001
∑PUFA	15.70±1.84^b	17.71±0.87^b	37.35±0.23^a	<0.0001
n-9	26.73±0.30^a	27.53±0.56^a	17.86±0.05^b	<0.0001
n-3	4.08±0.37^b	3.60±0.45^b	11.97±0.02^a	<0.0001
n-6	11.96±1.55^c	15.18±0.46^b	25.94±0.29^a	<0.0001
n-3HUFA	3.78±0.37^b	3.37±0.43^b	11.80±0.02^a	<0.0001
n-6HUFA	5.54±1.08^b	7.04±0.34^b	18.96±0.17^a	<0.0001
n-3/n-6	0.36±0.03^b	0.25±0.02^c	0.48±0.00^a	<0.0001
DHA/EPA	3.15±0.42^b	3.97±0.54^b	15.86±0.06^a	<0.0001
DHA/ARA	0.87±0.05^b	0.72±0.08^b	1.06±0.00^a	0.0011

The MUFA fraction of PL exhibited the same pattern observed for NL, with an important decrease in LPL, and an increase in PUFAs content. The MUFA fraction in eggs and NHL showed the highest percentages for C16:1n-7 ($P < 0.0001$) and C18:1n-9 ($P < 0.0001$). Also, C18:1n-7 decreased from eggs to LPL ($P = 0.0009$), while C22:1 n-7 had its lowest percentages in LPL ($P = 0.0038$).

The C20:5n-3 PUFA had constant percentages throughout ontogenetic development, while C18:2n-6 exhibited higher values in NHL than in eggs and LPL. The percentage of C18:3n-3 decreased slightly during development ($P = 0.0004$), but in general was always low. The percentages of C20:4n-6 ($P < 0.0001$), C22:5n-3 ($P < 0.0001$) and C22:6n-3 ($P < 0.0001$) were highest in LPL. In particular, DHA and AA exhibited important increases in their content in LPL. The ΣPUFA was also higher in LPL than in the previous stages ($P < 0.0001$). The percentages of 20:3 n-6 ($P < 0.0001$) and 22:5 n-6 ($P < 0.0001$) also increased in LPL.

As n-3 and n-6 increased, n-9 decreased to its lowest percentage in LPL ($P < 0.0001$) (Tab. 4). The highest values for n-3 and n-6 were observed in LPL ($P < 0.0001$). The percentages of the n-3 and n-6 HUFAs were low and similar in eggs and NHL, and higher in LPL ($P < 0.0001$). The n-3/n-6 ratio was highest in LPL, intermediate in eggs, and lowest in NHL ($P < 0.0001$). The DHA/EPA and DHA/ARA ratios were slightly higher in LPL than in the other stages.

Discussion

During embryogenesis, and until the first feeding, all the required nutrients for cell differentiation, organ development and growth comes from yolk reserves (Wiegand, 1996; Tocher, 2010). Thus, fish eggs should contain all the essential nutrients required for embryo development and larval growth (Rønnestad *et al*., 1999).

Among the developmental stages studied, the eggs of *L. alexandri* had higher of moisture when compared the LPL percentage. This higher moisture content can be directly related to the absorption of water by the egg during the formation of the embryo (Fyhn, Finn, 2010).

Crude protein remained constant throughout the three stages of development, indicating that this component is preserved through ontogeny. Variation in protein consumption, content and sequence during development seems to differ among species (Gunasekera *et al*., 1999a; Finn, Fyhn, 2010; Tong *et al*., 2017). In species with carnivorous habits, such as *Maccullochella macquariensis*, *Maccullochella peelii peelii*, *Scophthalmus maximus* and *Dicentrarchus labrax*, protein concentration decreases immediately after hatching, showing that this nutrient serves as an energy source during development (Finn *et al*., 1996; Rønnestad *et al*., 1998; Gunasekera *et al*., 1999a). The constant protein content in *L. alexandri* may be a result of the mobilization of protein for the formation of structural tissues. On the other hand, amino acid composition varied during ontogenetic development.

The diet provided by broodstock can change the fatty acid and amino acid profiles of fish eggs and larvae (Sink *et al*., 2010; Fuiman, Ojanguren, 2011). Hence the synthesis of nutrients contained in eggs is totally dependent on the supply of maternal nutrients (Finn, Fyhn, 2010; Lubzens *et al*., 2017). Soybean meal represents a significant percentage of the composition of tilapia diets as a protein component, and provides high concentrations of amino acids such as Leu (Guimarães *et al*., 2008; Furuya *et al*., 2010). In present experiment, broodstock were fed tilapia fillets, and so the amino acid profile of the composition of this feed may have significantly influenced the amino acid content found in the eggs, NHL and LPL, with the predominance of amino acids such Leu in all stages of development, although more detailed studies need to be carried out to investigate this premise.

The biochemical composition and sequence of consumption of amino acids in eggs and larvae are species-specific (Saavedra *et al*., 2015; Tong *et al*., 2017). In *L. alexandri*, the IAAs of His Met, Phe, and Thr maintained constant concentrations throughout development. Conservation of IAAs is a nutritional strategy geared toward maintenance of larvae before they start exogenous feeding (Conceição *et al*., 2002). According to Li *et al*. (2009), His is preserved because it is responsible for maintaining homeostasis of the body, and Met because it is considered AA limiting (Zhou *et al*., 2011). Phe is the precursor of Tyr synthesis and so is conserved for the synthesis of specific hormones (Li *et al*., 2009), however, Tyr increased in larvae after hatching. Tyr is the immediate precursor to the synthesis of several hormones including thyroxin (T4) and melatonin (Li *et al*., 2007). Conceição *et al*. (1997) suggested that in *S. maximus* the retention or increase of this AA may be associated with early thyroid gland activity. Furthermore, fish thyroid hormones have a significant role in skin pigmentation and process and structure formation (Li *et al*., 2007).

Concentrations of the IAAs Ile, Leu, and Val decreased in LPL, while Trp decreased throughout development. This suggests that some of these AAs may have been mobilized, preferentially, as an energy source when the yolk reserves became depleted and they were needed for the implementation of physiological functions (Li *et al*., 2009). During the development of turbot larvae, the predominant amino acid in eggs and larvae was Leu (Conceição *et al*., 1997; Tong *et al*., 2017). Leu is essential for growth and works by stimulating protein synthesis in muscle tissue (Abidi, Khan, 2007). Hence, according to Conceição *et al*. (1997), the elevated concentrations of Leu found in the composition of larval *S. maximus* were correlated with a high demand for this particular amino acid in feeding of this species. Trp is the main precursor of serotonin and is known to affect food intake and aggression in fish (Li *et al*., 2007). Portella *et al*. (2013) stated that in *Piaractus mesopotamicus*, Trp was probably maintained for the synthesis of serotonin and melatonin. A similar behavior was reported throughout development of *Latris lineata* (Brown *et al*., 2005). In *L. alexandri*, a sedentary species that occupies the bottom of rivers, this reduction in Trp levels during ontogeny may indicate that the concentration of this AA is reduced dramatically before the first feeding. One has to also consider that this species exhibits cannibalistic behavior in the early stage of life, which can be stopped when a source of live food is offered (Santos, Luz, 2009; Takata *et al*., 2014).

Val is involved in many metabolic pathways and is considered an indispensable AA. Its reduction may be associated with protein synthesis and fish growth (Abidi, Khan, 2004; Ahmed, Khan, 2006), which could explain the results of the present study in which reduced quantities were found in *L. alexandri* during LPL.

Arg and Lys were the only IAAs whose proportions increased in LPL. Arg is an essential amino acid involved in several metabolic pathways, such as protein synthesis, production of urea and nitric oxide, and the synthesis of creatine and polyamines (Li *et al*., 2009; Wu *et al*., 2009; Cheng *et al*., 2011). In *M. macquariensis* and *M. peelii peelii* (Gunasekera *et al*., 1999a), Arg was found to decrease in larvae after yolk absorption, which can be an indication that this AA may be required as an energy source when the endogenous reserves are depleted. In *L. alexandri,* an increase in the concentration of this AA was observed in LPL, showing that this AA may be being conserved and mobilized for protein synthesis. A similar behavior was observed for the percentage of Lys, an amino acid essential to the growth of animals and with no known pathways for endogenous synthesis. Lys is involved with increased immune response in fish and functions of the central nervous system (Zhang *et al*., 2008).

The high concentrations of AAs, such as Leu and Ala, found in the eggs and NHL during development are similar to those observed for other freshwater species such as trout cod, Murray cod (Gunasekera *et al*., 1999a), and *Ictalurus punctatus* (Sink *et al*., 2010). As recorded for *L. alexandri* in the present study, the concentration of Glu was elevated in LPL, as has been observed for other species, such as *Salvelinus alpinus*, *Solea senegalensis*, *D. labrax* and *S. maximus* (Cara *et al*., 2007; Gurure *et al*., 2007; Tong *et al*., 2017). Meanwhile, Cys is present in low concentrations in all the life stages analyzed for *L. alexandri*, as well as in the development of *S. senegalensis* and *Argyrosomus regius* (Aragão *et al*., 2004; Saavedra *et al*., 2015). The higher concentrations of glutamic acid is closely linked to its roles in protein synthesis and energy production by the pathway of gluconeogenesis (Li *et al*., 2007; Cheng *et al*., 2011), and so the high content of glutamic acid in *L. alexandri* may be due to conversion from other low-level amino acids such as Gly and Cys.

The proportions of Ala and Ser decreased strikingly in LPL of *L. alexandri*. These AAs are the main glucogenic precursors and are important energy sources for fish that can be synthesized through certain biochemical pathways in the body itself (Li *et al*., 2009).

Amino acid profiles of eggs and larvae after hatching can be used as indicators of the nutritional quality of larvae (Jaya-Ram *et al*., 2008; Oberg *et al*., 2015) and to estimate the requirements of early stages of development (Saavedra *et al*., 2006; 2015). From AA profiles obtained from sampled eggs and larvae it is possible to identify potential AA deficiency in diets and correct the profiles towards a diet more balanced in AA (Saavedra *et al*., 2006, 2007, 2015). This approach has been successfully adopted to study amino acid requirements in fish larvae (Oberg *et al*., 2015; Saavedra *et al*., 2015). Thus, based on data from this study, future research can be carried out to analyze the compositions of different sources of live food offered to *L. alexandri* larvae in order to meet the requirements of this stage of life.

The proportion of most of the fatty acids and fatty acid classes, including n-3HUFA, in eggs were well correlated with that of the respective formulated diets (Luo *et al*., 2015). The high amounts of C16:0 and C18:1n9 present in the fillets of tilapia (offered as food to the broodstock) were also found in eggs and larvae of *L. alexandri*. Several authors have reported that the FA composition of total lipids in eggs is a reflection of maternal diet (Sink *et al*., 2010; Zakeri *et al*., 2011).

In *L. alexandri,* the content of total lipids decreased from NHL to LPL, clearly indicating the catabolism of this nutrient for energy production during ontogenetic development, as has been reported for *Carassius auratus* (Wiegand, 1996), trout cod and Murray cod (Gunasekera *et al*., 1999b). In *Brycon orthotaenia* (Martins *et al*., 2017), *Pseudoplatystoma reticulatum* and its hybrid *Pseudoplatystoma corruscans* X *Pseudoplatystoma reticulatum*, all carnivorous freshwater species (Mello *et al*., 2012), total lipid percentages remained constant in eggs and larvae. The initial development of these species, however, is faster, lasting approximately three days, compared to the seven days of *L. alexandri* larvae. For Wiegand (1996), the maintenance of lipid content can be attributed to its storage and later use during a period of food restriction, when it can act as an energy reserve in larvae after the absorption of the yolk sac.

Neutral lipids are generally regarded as primarily energy reserves (Wiegand, 1996). In the present study, the increased percentage of neutral lipid observed in LPL shows that during embryonic development (eggs and NHL), both neutral and polar lipids were utilized for energy production. Polar lipids are found in greater proportions during early life stages due to their function as a structural component of organs and tissue (Sargent *et al*., 2002; Tocher, 2010). As reported during the development of *C. auratus,* the depletion of the proportion of polar lipids in FA may indicate that this class of FA serves a role as the primary fuel provider for energy production, rather than as a structural lipid (Wiegand, 1996). Tocher *et al*. (1985) stated that the consumption of this class of FA has the advantage of providing choline and phosphate to developing fish embryos, and for the synthesis of neurotransmitters and macromolecules.

In general, in both neutral and polar lipid fractions, SFA decreased during development mainly due to the exhaustion of C16:0, indicating that FA are being preferentially used as an energy source, as has been observed for other species, such as *Thunnus thunnus* (Ortega, Mourente, 2010) and *Salminus hilarii* (Araújo *et al*., 2012). Rainuzzo *et al*. (1992) reported that larvae containing a low lipid content mainly use C16:0 as the energy source, thus retaining n-3 HUFAs. According to Abi-Ayad *et al*. (2004), the reduction in the percentage of this FA in *Sander lucioperca* larvae is an indication that these are the energy substrates preferentially used by the larvae, as also observed during the development of *B. orthotaenia* (Martins *et al*., 2017) and *Sarda sarda* (Ortega, Mourente, 2010).

MUFA may be required preferentially as a source of energy throughout ontogenetic development (Abi-Ayad *et al*., 2004; Dantagnan *et al*., 2007). In eggs and NHL these FAs were conserved, while in LPL they were reduced, both the neutral and polar lipids fractions. The decrease in the percentages of C16:1n-7, C18:1n-9 and C22:1n-7 only in the late lecithotrophic period of *L. alexandri* could be evidence that these nutrients are being catabolized as a source of energy during this phase, or that they are being mobilized to form other FAs with larger chains, since larvae in this period require more energy for swimming in order to find food because of the depletion of their endogenous reserves. A similar reduction of MUFAs in LPL has been reported for other freshwater fish species, such as goldfish (Wiegand, 1996), and *M. macquariensis,* and *M. peelii peelii* (Gunasekera *et al*., 1999b).

In *L. alexandri,* the decrease of SFAs and MUFAs were offset by an increase in PUFAs in LPL, both the neutral and polar lipid portions. The increase in PUFAs in LPL could be related to the increase of n-6 and n-3 fatty acids, such as C20:4n-6 and C22:6n-3, indicating the importance of these FAs at this stage of life.

The decrease of n-9 in LPL may indicate that the consumption of this specific class of PUFA meets some metabolic demand in this life stage, thus the maintenance of n3 and n-6 FA. In *Thunnus thynnus* and *Sarda sarda* (Ortega, Mourente, 2010), a decrease of this FA series was observed, together with a decrease in n-3 and n-6, indicating a high consumption of PUFAs in these species. On the other hand, in *L. alexandri*, there was a tendency to increase n-3 and n-6 in both the neutral and polar lipid fractions, as well as the n-3 and n-6 HUFAs, suggesting their importance for larvae after depletion of their endogenous reserves, similar to what has been reported for *C. auratus* (Wiegand, 1996) and *M. macquariensis* (Gunasekera *et al*., 1999b). This is due to fact that they act as eicosanoid precursors and structural constituents of the phospholipids of cell membranes, particularly of neural and retinal tissue (Sargent *et al*., 1999a; Tocher, 2010). The increase of PUFAs in the n-3 and n-6 series can be an indication that these FAs are stored for later use in the formation of new tissues, such as the nervous and visual systems, as well as participation in the immune system and in cell signaling (Li *et al*., 2007; Wu *et al*., 2015).

Channa striata, a carnivorous freshwater fish species, is able to synthesize DHA from EPA, with both genes responsible for this synthesis being regulated by the dietary level of C18 (Kuah *et al*., 2015). Freshwater species are capable of producing biologically active HUFA from PUFA C18, a process called bioconversion through desaturase and elongase enzyme activity (Jaya-Ram *et al*., 2008; Tocher, 2010). Studies have indicated that the expression of the genes for desaturase and elongase occurs shortly after hatching, indicating the possibility of longer chain PUFA synthesis at this time of development (Morais *et al*., 2012; Cunha *et al*., 2013). In the present study, EPA and DHA values increased considerably in LPL, that is, during mouth opening and the start of exogenous feeding.

The higher concentration of the HUFA C20:5n-3 (EPA) and C22:6n-3 (DHA) in *L. alexandri* LPL was followed by reduced levels of their precursor, the C18:3n-3 PUFA, found in the polar fraction, and increased levels of ARA in LPL was followed by a reduction of its precursor, PUFA C18: 2n-6, which shows the bioconversion potential of this type of fatty acid as reported for other species, such as the freshwater *Salminus hilarii* (Araújo *et al*., 2012), *P. reticulatum* and its hybrid *P. corruscans* X *P. reticulatum* (Mello *et al*., 2012). The increase in DHA in LPL, in both neutral and polar lipid fractions, is consistent with that found for *C. auratus* (Wiegand, 1991; 1996). DHA is quantitatively the most important FA of the group and has important structural roles in cell membranes, particularly in the neural tissue (Sargent, 1999b). Likewise, EPA has an important physiological role in the modulation of the eicosanoid action and tends to be largely maintained during ontogenetic development (Tocher, 2010).

20:4n6 (ARA), increased soon after hatching in both the neutral and polar lipid fractions, with its content increasing three-fold from NHL to LPL. ARA has an essential role in growth, survival, and resistance to stress through the production of eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes (Bessonart *et al*., 1999; Glencross, 2009; Tocher, 2010). According to Gunasekera (1999b), ARA may be maintained during development due to its importance in the further development of *M. macquariensis* and *M. peelii peelii*, and also for its possible structural use. Different from the results of the present study, in freshwater species, such as the *Perca fluviatilis* (Abi-Ayad *et al*., 2000), and *Sander lucioperca* (Abi-Ayad *et al*., 2004), ARA levels in larvae showed a considerable reduction during development. Figueiredo *et al*. (2012) reported that the consumption of ARA during embryogenesis makes it necessary to add a food source that contains this nutrient.

It is very interesting to observe that the DHA/ARA ratio remained almost constant in the polar lipid fraction during the entire period of development examined, evidencing the importance of the conservation of this ratio, rather than the DHA/EPA ratio, when DHA level increases. It has been suggested that this occurs to maintain balance in eicosanoid production (Bessonart *et al*., 1999).

The fatty acid profile of eggs and larvae has been widely used to evaluate the quality of spawn and larvae (Tocher, 2010). It is also an indicator of nutritional needs, particularly in marine species (Garrido *et al*., 2012). The relative decrease in linoleic and linolenic acid content during LPL, and the magnitude of the increase in the relative content of DHA and ARA, suggest that the larvae are producing HUFA from C18:3 n3 and C18:2 n6 precursors. Therefore, the decrease in the percentages of C18:3n-3 and C18:2n-6 in LPL of *L. alexandri* suggests that foods that provide sufficient amounts of these fatty acids may be needed at the beginning of exogenous feeding of *L. alexandri* to ensure proper intake of EPA, DHA, and ARA.

In summary, these results show that during the different stages of development of *L. alexandri*, protein is conserved during ontogeny, although there were significant changes in the composition of some AAs during development. Furthermore, the percentages of lipids decreased in LPL, showing that they are used as the main source of energy during this development phase. The consumption of SFA and MUFAs in developing embryos or larvae of *L. alexandri* allows temporary storage of essential fatty acids, mainly PUFA, which can be mobilized for structural needs.

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