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Amino acid and fatty acid compositions of various stages of *Chanos chanos* larvae: Implications for larval feed formulation

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ABSTRACT

Amino acid (AA) and fatty acid (FA) composition of the fertilised eggs and different larval stages (at 0, 3, 6, 9, 12, 15 and 21 days post-hatch, dph) of *Chanos chanos* was investigated. The total indispensable amino acids (IAA) contributed to 55.62% of the total AA in the egg which reduced to 52.54% on 6 dph. The AA profile of *C. chanos* was found to be rich in valine (7.99%), leucine (7.51%) and lysine (6.98%) and poor in histidine (2.36%) and methionine (2.47%), indicating a high valine, leucine and lysine requirement. The docosahexaenoic acid (DHA) content recorded for egg, newly hatched larvae (NHL) and 21 dph larvae were 2.77, 1.36 and 1.94 mg g^{-1}, respectively. The reduction of fatty acids (FAs) was found to be very high in newly hatched larvae (NHL), especially that of DHA (51%), ARA (26%) and EPA (24%), which indicates the significance of these FAs during the embryogenesis of milkfish egg. The trend observed during different stages of AAs and FAs content indicates their requirement during the larval period and those values are to be considered while formulating feeds for larval stages of milkfish.

Keywords: Embryogenesis, Larval feed, Milkfish larvae, Nutrient requirement

Introduction

During early life stages, from embryogenesis until first feeding, finfish larvae require various essential nutrients for growth, cell differentiation and metabolism. The nutritional requirements of larvae are sourced from yolk-sac reserves initially and later through feeding on live feeds (Tocher, 2010). Fish eggs contain a significant amount of proteins, lipids and their corresponding profiles of fatty and amino acids (Finn and Fyhn, 2010). In fish larvae, amino acids derived from the diet are either utilised to create vital tissues or catabolised to produce metabolic energy (Li *et al.,* 2009; Finn and Fyhn, 2010). Additionally, amino acids (AA) affect gene expression, osmotic pressure for oocyte hydration and act as precursors for the synthesis of nitrogenous hormone components, which in turn regulates egg quality and then larval health (Wu, 2009). To assess the dietary needs of larvae at the start of external feeding, the profile of amino acids in larval tissue provides pertinent information (Gurure *et al.,* 2007; Saavedra *et al.,* 2015).

Following proteins, lipids and the fatty acids (FA) form the second-highest share in fish egg (Tocher, 2010). The majority of highly unsaturated fatty acids (HUFA) and polyunsaturated fatty acids (PUFA) are used as membrane constituents and eicosanoid precursors during the larval development and are primarily used for energy production (mostly saturated and monounsaturated fatty acids) for the development of eye sight and neurological systems (Dantagnan *et al.,* 2007; Glencross, 2009; Tocher, 2010; Araujo *et al.,* 2012). Tong *et al.* (2017) used the profiles of AA and FA in eggs and larvae to determine the nutritional quality of fish larvae and to calculate nutrient demand indices during the early life stages (Saavedra *et al.,* 2015).

Milkfish (*Chanos chanos*) is an important euryhaline food fish cultured in the Indo-Pacific region with bulk of the production coming from Philippines, Indonesia and Taiwan (Lim *et al.,* 2002). Milkfish is among the top twenty aquaculture species and cultured widely in South-East Asian countries, contributing to 2.8% of the total fish production in 2018 (Bera *et al.,* 2019; FAO, 2020). ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) had made a significant breakthrough in captive maturation and hatchery seed production of milkfish in 2015 and fine-tuned protocols towards extended seed production for eight months (March-October) (Bera *et al.,* 2021). For developing efficient larval feeds, it is essential to have adequate knowledge on the nutritional requirements, especially of vital nutrients and its composition, which are critical during the early life stages. In this scenario, it is imperative to understand

larval nutritional requirement of milkfish during hatchery phase for mass scale production of healthy seed (Sivaramakrishnan *et al.,* 2021).

Information on AA and FA composition of hatchery produced *C. chanos* eggs and larvae are scanty and no organised studies have been carried out on changes in the nutrient composition during various stages of the larvae. Such details would enable investigations of dietary needs and serve as the scientific basis for understanding the dynamics of metabolic alterations of AAs and FAs in early life stages. In this back drop, the objective of the current study was to compare the amino acid and fatty acid profiles of fertilised eggs, newly emerged larvae and larvae at various developmental stages up to 21 days post-hatch (dph).

Materials and methods

Larval rearing and feeding

Fertilised eggs of *C. chanos* were collected from the fish hatchery at Muttukadu Experimental Station, ICAR-CIBA, Chennai and transferred to an incubation tank of 250 l capacity. The eggs were hatched out 24-26 h after spawning (Bera *et al.,* 2021). Milkfish larvae were reared in a green water system as described in Bera *et al.* (2019). The details of the larval rearing protocol and feeding regime are depicted in Fig. 1.

Sampling

Fertilised eggs, freshly hatched larvae (NHL) and fed larvae of 3, 6, 9, 12, 15 and 21 dph were collected. Fish larvae of above said stages were kept in a 100 l tank containing filtered seawater (devoid of live feed) to evacuate the digestive tract content, eliminating the influence of gut contents on the composition of larvae (Dayal *et al.,* 2003). The samples collected from triplicate tanks were washed briefly with distilled water and stored at -80°C until further analysis.

Analysis of amino acid composition

The larval samples were hydrolysed using 6 N HCL in a sealed tube at 110°C in an oven for 22 h (Finlayson, 1965). The entire content was transferred to a round bottom flask and the acid was evaporated in a vacuum rotary evaporator (IKA, RE 10 C S84). The residual matter was re-solubilised with a known volume of 0.1 N HCl. Pre-column HPLC gradient system (Shimadzu Corp, LC- 30AD) was used to analyse the essential amino acids (Sivaramakrishnan *et al.,* 2022). The column used for AA separation was YMC-Triart C18, RRH (1.8 µm, 2.1 x 100 mm). Gradient elution was performed using phosphate buffer (20 m mol) and a combination of acetonitrile: methanol: water (45:40:15) was used as mobile phases A and B, respectively. Mercaptopropionic acid, O-phthalaldehyde and fluorenylmethoxycarbonyl chloride were used as derivatising agents. All the reagents, including samples, were filtered through a 0.2 µm membrane syringe filter. The eluted amino acids were detected by a fluorescent detector (RF- 20AXS) and quantified using the AA mixer as an external standard (Sigma Aldrich,) and norleucine as an internal standard

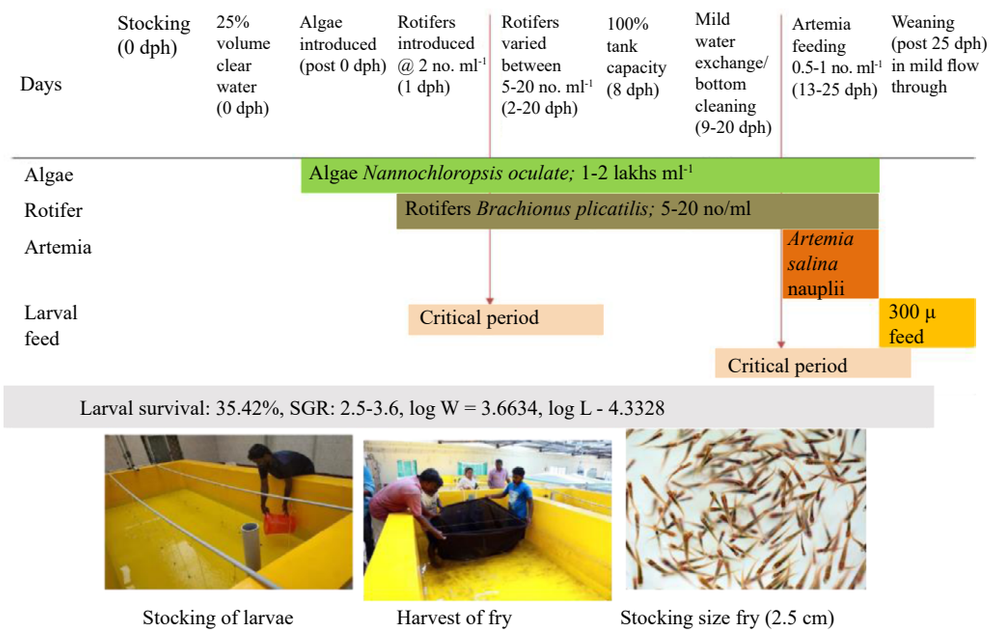

Fig. 1. Schematic representation of larval rearing protocol and feeding regime

(Jannathulla *et al.,* 2019). Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis using a spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). To prevent partial oxidisation of sulphur-containing AAs, particularly methionine, due to acid digestion, 0.1% phenol was added along with digestive agents (Jajic *et al.,* 2013).

Analysis of fatty acid composition

The extraction of total lipids from fertilised eggs and whole larvae samples was done by Folch method (Folch *et al.,* 1957) with slight modification in weight of sample (200-300 mg) and based on the sample weight, chloroform and methanol extractions were carried out. Following this, fatty acid methyl esters (FAME) were prepared by acid catalysed transmethylation of total lipids using the standard AOAC method (AOAC, 1995). For fatty acid analysis, a gas chromatograph (GC-2014, Shimadzu, Japan) separated FAME on an RT wax capillary column (100 m length x 0.25 mm internal diameter x 0.2 µm film thickness). Nitrogen was used as a carrier gas at a linear velocity of 20.9 cm s^{-1} with 3 ml min^{-1} of purge flow. The oven temperature was initially set at 100°C for 4 min and increased to 225 at 3°C min^{-1} and held for 5 min, followed by a temperature increase of 1°C min^{-1} to 240°C and held for 10 min. Operating temperatures for injection ports and flame ionisation detector were 225 and 250°C, respectively. Individual FAs were identified by comparing the retention times with a 37-component standard FAME mixture (Supelco-Sigma, USA) (Sivaramakrishnan *et al.,* 2021).

Statistical analysis

In the tables, figures and text, data are presented as mean±standard error (SE). Using the Kolmogorov-Smirnoff and Leven's tests, respectively, the normality and homogeneity of variance of all the response variables were examined. Prior to further statistical analysis, non-normally distributed data were transformed using the arcsine function. One-way analysis of variance (ANOVA) was used to compare the dietary regimens statistically and Duncans' *post-hoc* multiple range test was used to confirm the results. Using SPSS 21.0 for all statistical calculations, differences were deemed significant at $p<0.05$ (n=3).

Results

Amino acid profile of larvae

The amino acid profile of *C. chanos* larvae during early developmental stages are given in Table 1 and Fig. 2. Seventeen amino acids (AA) were quantified during the ontogenetic development of milkfish, of which ten were indispensable amino acids (IAA) and the remaining seven were dispensable amino acids (DAA). Histidine (His) and methionine (Met) constituted only 1.84 and 2.67% of the total AA content, respectively, the lowest among IAA. The total IAA accounted for 55.61% of the total AA in the egg and reduced to 52.54% at 6 dph. The sum of valine (Val), leucine (Leu), lysine (Lys), aspartic acid (Asp), isoleucine (Ile) and arginine (Arg) accounted for more than 50% of the total AA in the egg; glutamic acid (Glu) was predominant among the dispensable AA (DAA). The larvae of milkfish was rich in Val (7.99%), Leu (7.51%) and Lys (6.98%) and poor in His (2.36%) and Met (2.47%), suggesting a high Val, Leu and Lys requirement during the early larval development of milkfish.

The most AA in eggs were Glu (DAA) and Val (IAA), followed by Lys (IAA) and Asp (DAA), which had levels that were comparable. His was the AA with the least amount of alcohol in the eggs (IAA). Numerous IAAs, including Met, Phe and Cys, were also discovered at low quantities with comparable values. Glu and Val likewise had the highest amounts in newly hatched larvae (NHL), followed by Asp and Leu. His was the amino acid with the lowest concentration among those analysed, following a pattern seen in eggs. NHL also contained low levels of His, Met, Phe and Cys IAAs (2.273-4.199). AA analysis revealed changes in the feeding regime contributed by the nature of diets consumed during the various stages of milkfish larval development.

Fatty acid profile of larvae

Hatchery produced milkfish egg, and larvae (0, 3, 6, 9, 12, 15 and 21 day) were analysed for fatty acid (FA) profiles and presented in Table 2 and Fig. 3. The DHA content of egg, NHL and 21 day old larvae were 2.77, 1.36 and 1.94 mg g^{-1}, respectively. The reduction of FAs like DHA (51%), ARA (26%) and EPA (24%) is very high in NHL, indicating their involvement during the embryogenesis of milkfish egg. The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n3 and n6 fatty acids content of 21 day old larvae were 21.49, 11.03, 8.93, 3.80 and 5.13 mg g^{-1} respectively. The increasing trends of total PUFA, n3 and n6 were found to be very high in 21 dph, indicating their important role during formulation of weaning feed.

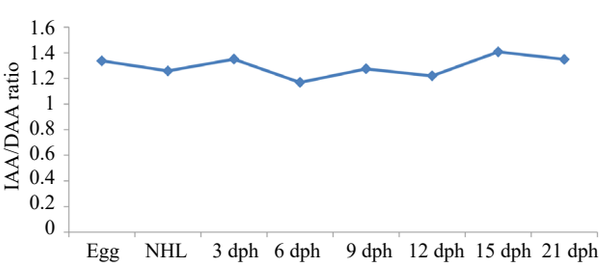

Fig. 2. Changes in IAA/DAA ratio during larval development of milkfish

Table 1. Amino acid profiles (%) of the eggs and larvae of milkfish (n=3)

Amino acid	Eggs	NHL	3 dph	6 dph	9 dph	12 dph	15 dph	21 dph	SEM
Indispensable amino acids (IAA)									
ARG	5.505^{b}	5.200^{b}	5.385^{b}	5.404^{b}	5.187^{b}	5.346^{b}	4.828^{a}	4.788^{a}	0.104
LYS	7.064^{a}	7.682^{b}	8.120^{bc}	8.128^{bc}	7.661^{b}	8.323^{c}	6.573^{a}	6.984^{a}	0.243
HIS	1.848^{ab}	2.273^{b}	2.192^{b}	2.208^{b}	2.109^{b}	2.222^{b}	1.385^{a}	2.369^{b}	0.126
ILE	5.679^{c}	5.246^{b}	4.297^{a}	4.396^{a}	4.590^{ab}	4.555^{ab}	4.436^{a}	4.440^{a}	0.128
LEU	9.209^{g}	8.533^{f}	7.768^{d}	7.788^{d}	7.596^{d}	7.569^{c}	7.360^{a}	7.510^{b}	0.256
THR	4.676^{b}	4.529^{a}	5.097^{f}	5.034^{f}	4.841^{d}	4.732^{c}	5.078^{g}	4.974^{e}	0.076
MET	2.673^{e}	2.529^{d}	2.248^{b}	2.251^{b}	2.786^{f}	2.166^{a}	2.807^{g}	2.473^{c}	0.094
VAL	10.895^{g}	9.397^{f}	8.018^{b}	8.021^{b}	8.242^{d}	8.271^{e}	8.145^{c}	7.993^{a}	0.407
PHE	3.702^{f}	4.105^{g}	3.503^{a}	3.506^{a}	3.644^{d}	3.545^{b}	3.615^{c}	3.696^{e}	0.074
CYS	4.359^{b}	4.199^{a}	5.701^{c}	5.808^{c}	7.074^{e}	6.410^{d}	12.513^{g}	11.125^{f}	1.225
Total IAA	55.61^{d}	53.693^{c}	55.329^{e}	52.544^{a}	53.73^{c}	53.139^{b}	56.74^{g}	56.352^{f}	1.889
Dispensable amino acids (DAA)									
ALA	4.373^{f}	3.192^{d}	3.186^{d}	3.209^{e}	3.164^{c}	3.192^{d}	3.028^{a}	3.125^{b}	0.176
GLY	3.858^{a}	4.439^{b}	6.400g	6.433^{g}	5.280^{d}	5.729^{e}	4.688^{c}	5.974^{f}	0.344
PRO	3.899^{b}	4.735^{g}	4.017c	4.026^{c}	4.155^{d}	4.615^{f}	4.435^{e}	3.751^{a}	0.140
ASP	7.030^{a}	9.000^{ab}	9.510c	9.538^{c}	9.210^{ab}	9.201^{ab}	8.528^{b}	9.209^{ab}	0.381
GLU	12.958^{a}	13.033^{b}	13.902f	13.967^{f}	13.242^{e}	13.561^{d}	12.940^{a}	13.140^{c}	0.142
SER	4.103^{c}	3.452^{b}	3.911^{d}	3.877^{d}	3.526^{c}	3.562^{c}	3.359^{a}	3.441^{b}	0.105
TYR	5.389^{g}	4.824^{f}	3.917e	3.929^{e}	3.588^{c}	3.727^{d}	3.351^{b}	3.149^{a}	0.108
Total DAA	41.61^{b}	42.675^{c}	40.865^{a}	44.979^{e}	42.165^{c}	43.587^{d}	40.329^{a}	41.789^{b}	1.834

Values bearing different superscripts in a row differ significantly (One-way ANOVA; $p<0.05$). ARG-Arginine; LYS-Lysine; HIS-Histidine; ILU-Isoleucine; THR-Threonine; MET-Methionine; VAL-Valine; PHE-Phenlyalanine; CYS-Cystine; ALA-Alanine; GLY-Glycine; PRO-Proline; ASP-Asparagine; GLU-Glutamine; SER-Serine; TYR-Tyrosine

Table 2. Fatty acid profiles (mg g^{-1}) in different stages of milkfish larvae

Fatty acid	Egg	NHL	3 dph	6 dph	9 dph	12 dph	15 dph	21 dph	SEM
C14:0	1.082^{c}	0.982^{b}	1.122^{e}	0.869^{a}	1.102^{d}	1.182^{f}	0.982^{b}	1.102^{d}	0.035
C16:0	12.384^{f}	10.278^{b}	9.872^{a}	11.23^{d}	10.869^{c}	9.872^{a}	12.342^{e}	16.52^{g}	0.776
C18:0	3.476^{d}	3.132^{c}	2.899^{a}	3.102^{b}	3.685^{e}	3.712^{f}	3.751^{g}	3.872^{h}	0.128
ΣSFA	16.942	14.392	13.893	15.201	15.656	14.766	17.075	21.497	0.858
C16:1	2.542^{e}	2.372^{c}	2.352^{b}	2.444^{d}	2.301^{a}	2.956^{f}	3.125^{g}	3.256^{h}	0.135
C18:1c	3.441^{c}	3.094^{a}	3.395^{c}	3.312^{b}	3.642^{d}	3.752^{e}	3.872^{f}	3.922^{g}	0.102
C18:1t	3.685^{d}	3.251^{b}	3.132^{a}	3.752^{e}	3.438^{c}	3.625^{d}	3.812^{ef}	3.852^{f}	0.094
ΣMUFA	9.668	8.717	8.879	9.508	9.381	10.333	10.809	11.030	0.105
C18:2	3.512^{b}	3.473^{a}	3.624^{c}	3.782^{d}	3.812^{e}	3.952^{f}	4.102^{g}	4.442^{h}	0.115
αC18:3	0.432^{bc}	0.382^{a}	0.392^{a}	0.411^{b}	0.424^{c}	0.466^{d}	0.513^{e}	0.555^{f}	0.021
C20:4	0.652^{g}	0.483^{a}	0.524^{b}	0.552^{d}	0.525^{c}	0.594^{e}	0.612^{f}	0.689^{h}	0.024
C20:5	1.244^{ab}	1.101^{a}	1.084^{a}	1.213	1.254	1.244	1.264	1.312	0.031
C22:6	2.771^{h}	1.361^{b}	1.286^{a}	1.393^{c}	1.416^{d}	1.593^{e}	1.623^{f}	1.941^{g}	0.176
ΣPUFA	8.611	6.799	6.910	7.350	7.430	7.848	8.114	8.939	0.277
Σn3	4.447	2.844	2.762	3.016	3.093	3.302	3.400	3.808	0.198
Σn6	4.165	3.955	4.148	4.334	4.337	4.546	4.714	5.131	0.132
n3/n6	1.068	0.719	0.666	0.696	0.713	0.726	0.721	0.742	0.078
ARA/EPA	0.525	0.439	0.483	0.456	0.419	0.477	0.485	0.525	0.133
DHA/EPA	2.229	1.236	1.187	1.148	1.129	1.281	1.285	1.479	0.128

Values bearing different superscripts in a row differ significantly (One-way ANOVA; $p<0.05$).
SFA-Saturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids; ARA-Arachidonic acid; EPA-Eicosapentaenoic acid; DHA-Docosahexaenoic acid

Discussion

In most marine finfish hatcheries, larval nutrition plays a critical role in the growth and survival of early larval stages. Proper larval nutrition gives the optimum amount of dietary micronutrients, HUFA and phospholipids, which form necessary physiological requirements for metamorphosis (Koven *et al.,* 1993). All the nutrients needed for cell differentiation, organ

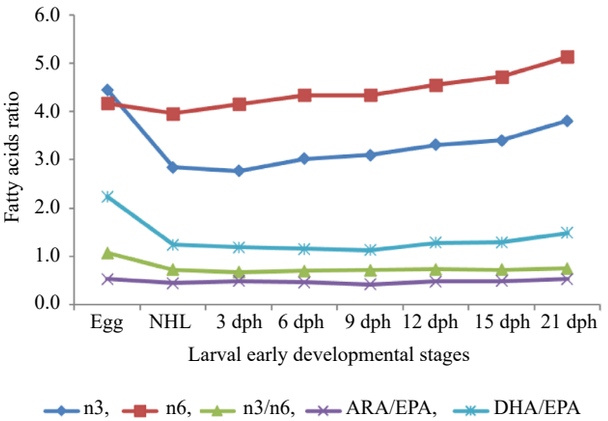

Fig. 3. Changes in fatty acids ratio in milkfish larvae, from 0 to 21 dph (One-way ANOVA; n=3; $p<0.05$)

development and growth during embryogenesis until the first feeding, originates from the yolk reserves (Wiegand, 1996; Tocher, 2010). Consequently, fish eggs should be fully nutrient-dense to support embryonic development and larval growth (Ronnestad *et al.,* 1999).

Species-specific differences exist in the composition and order of amino acid (AA) intake in eggs and larvae (Saavedra *et al.,* 2015; Tong *et al.,* 2017). IAAs like His, Met, Phe and Thr were kept at stable levels in *C. chanos* throughout ontogeny. A dietary technique for maintaining larvae before they begin external feeding is conservation of IAAs (Conceiao *et al.,* 2002). His is preserved since it is in charge of preserving biological homeostasis and Met is preserved because it is the first limiting AA (Li *et al.,* 2009; Zhou *et al.,* 2011). Phe is retained for the biosynthesis of particular hormones since it is the precursor to Tyr synthesis (Li *et al.,* 2009). A number of hormones, including thyroxin (T4) and melatonin, can be produced directly from Tyr (Li *et al.,* 2007). According to Conceicao *et al.* (1997), early thyroid gland activity may be linked to retention of this AA in the turbot *Scophthalmus maximus*.

Ile, Leu and Val concentrations dropped throughout the developmental stages, whereas Cys concentrations increased. This implies that some of these AAs may have been used as an energy source, preferentially, when the yolk reserves were depleted and they were required for the physiological processes (Li *et al.,* 2009; Costa *et al.,* 2018). The most prevalent AA in eggs and larvae during the development of turbot was Leu (Conceicao *et al.,* 1997; Tong *et al.,* 2017). Leu increases protein synthesis in muscle tissue, which is necessary for growth (Abidi and Khan, 2007). Conceicao *et al.* (1997) concluded that the higher need for this specific amino acid in feeding of this species was detected based on the higher concentrations of Leu detected in the composition of *S. maximus* larvae.

Val is a crucial AA that participates in numerous metabolic processes. According to Abidi and Khan (2004) and Ahmed and Khan (2006), its decrease may be related to protein synthesis and fish growth, which could account for the lower levels discovered in *C. chanos* at all developmental stages.

The only IAAs whose proportions increased on 6 dph were Arg and Lys. The formation of protein, urea, nitric oxide, creatine and polyamines are a few metabolic processes where the important amino acid Arg plays a role (Li *et al.,* 2009; Wu *et al.,* 2009; Cheng *et al.,* 2011). Arg was shown to decrease in larvae of *Maccullochella macquariensis* and *Maccullochella peelii peelii* after yolk absorption (Gunasekera *et al.,* 1999), which may be a sign that this AA may be needed as an energy source when the endogenous supplies are depleted. This AA was shown to be more abundant in *C. chanos* at 6 dph, indicating that it might be preserved and mobilised for protein synthesis. The two IAAs, Arg and Lys, were retained in larval body in greater proportion, as these were less used for oxidation in seabass (*Lates calcarifer)* larvae, *Hippoglossus hippoglossus* (Applebaum and Ronnestad, 2003) and in post-larvae of *S. senegalensis* (Ronnestad *et al.,* 2003). Similar behaviour was seen for the amount of Lys, an amino acid necessary for animal growth but without known endogenous production routes (Dayal *et al.,* 2003). Fish immune responses are boosted by Lys and it also affects how the central nervous system works (Zhang *et al.,* 2008).

The higher levels of Glu are closely related to its functions in protein synthesis and energy production through the gluconeogenesis pathway (Li *et al.,* 2007; Cheng *et al.,* 2011; Costa *et al.,* 2018), so the high concentration of Glu in milkfish may be the result of conversion from other low-level amino acids like Gly and Cys. The ratios of Ala and Ser dramatically reduced in *C. chanos* on 15th dph. These AAs can be produced by specific metabolic pathways in the body and are the primary glucogenic precursors and significant energy sources for fish (Li *et al.,* 2009).

The AA profile of eggs and larvae after hatching can be used as indicator for the nutritional condition of larvae and to calculate the requirements of the early stages of developing larvae (Jaya-Ram *et al.,* 2008; Oberg *et al.,* 2015; Saavedra *et al.,* 2015; Costa *et al.,* 2018). It is possible to identify probable AA deficiency in diets from AA profiles of eggs and larvae and modify the profiles towards a diet more balanced in AAs (Saavedra *et al.,* 2006, 2007, 2015). This strategy has been effectively used to investigate the needs for amino acids in fish larvae (Oberg *et al.,* 2015; Saavedra *et al.,* 2015). Future research can thus be conducted using the information from this study

to examine the compositions of various sources of live food provided to *C. chanos* larvae in order to meet their nutritional needs at this stage of development. Further, these values form the basis for larval diet development for this fish species.

The fatty acid (FA) profile has been widely used to evaluate the quality of spawn and larvae (Tocher, 2010). It is also an indicator of fatty acid requirements, particularly for marine species (Garrido *et al.,* 2012). The DHA content of egg, NHL and 21 days old larvae are 2.77, 1.36 and 1.94 mg g^{-1}, respectively. The reduction of FAs like DHA (51%), ARA (26%) and EPA (24%) is very high in NHL, indicating their significance during the embryogenesis of milkfish egg.

Saturated fatty acids (SFA) reduced during the first stage of larval development, mostly as a result of the use of C16:0, showing that FA is preferred as an energy source. Similar observations have been made in other species, including *Salminus hilarii* and *Thunnus thunnus* (Ortega and Mourente, 2010; Araujo *et al.,* 2012). According to Rainuzzo *et al.* (1992), larvae with minimal lipid content primarily utilise C16:0 as an energy source, retaining n-3 HUFAs. The decrease in the percentage of FA in *Sander lucioperca* larvae (Abi-Ayad *et al.*, 2004) suggests that these are the energy substrates that the larvae preferentially consume, as was also seen throughout the development of *B. orthotaenia* (Martins *et al.,* 2017) and *Sarda sarda* (Ortega and Mourente, 2010). As a source of energy during ontogenetic development, MUFA may be preferred (Abi-Ayad *et al.,* 2004; Dantagnan *et al.,* 2007).

The IAA and EFA recorded during different life stages of milkfish larvae may reflect their early nutrient requirements for better larval growth and survival. Based on the nutrient profiling database, functional larval feeds can be formulated for milkfish aquaculture.

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