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Effects of the Alga *Aurantiochytrium mangrovei* FIKU008-Enriched Artemia on Early Stages of the Green Tiger Shrimp, *Penaeus semisulcatus*

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Abstract

The first aim of the present study was to determine appropriate artemia enrichment levels by using *Aurantiochytrium mangrovei* FIKU008 served as DHA enrichment source and secondly to test the effects of enriched-Artemia on growth and survival during the early larval stages (M1-PL7) of *Penaeus semisulcatus*. In the 1st experiment, four different enrichment levels of AUR (0.0,0.6,0.8 and 1.0g/L) against a control commercial solution (S-presso) were tested for 12/24-hours enrichment while in the 2nd experiment, three levels (0.0, 0.6 and 0.8g/L) of AUR enriched-Artemia were fed to larval stages. At the end of both enrichments, DHA and EPA levels were found to be significantly enhanced by using AUR at 0.6 and 0.8g/L. In the 2nd experiment, fatty acid composition of the PL was significantly affected by AUR and the PL fed with enriched diets showed higher n-3 LC-PUFA accumulation in their tissues. The larvae grown from M1 to PL7 displayed higher survivals and growth in the enriched AUR-groups compared to the control (0.0AUR). In conclusion, this study has demonstrated that the use of AUR as enrichment significantly elevated EPA and DHA levels to 0.6-0.8g/L enrichment periods and the enriched-Artemia at such levels were proven to effectively improve growth and survival of the early larval stages of *P. semisulcatus*.

Introduction

Successful production of marine fish and shrimp larvae depended on high-quality live feed. Microalgae play a vital role in the growing global aquaculture industry. Various heterotrophic and photosynthetic microalgae are already widely used in hatcheries to provide both EPA and DHA to live feeds such as rotifers (*Brachionus* sp.) and brine shrimp (*Artemia*) for the cultivation of larval crustacean and fish species, (Muller-Feuga, 2004). More recently, among the other microalgae being used in the aquaculture industry, the fungus-like clades of Thraustochytrids are of interest to the industry (Gupta et al., 2012; Singh et al., 2014; Nagappan et al., 2021). Thraustochytrids are a heterotrophic group of protists commonly found in marine and estuarine waters and comprising several genera such as *Aurantiochytrium* sp., *Parietichytrium* sp., *Schizochytrium* sp. and *Thraustochytrium* sp. (Liu et al., 2017; Marchan et al., 2018). Among these,

Aurantiochytrium mangrovei is produced by heterotrophic production and is considered a microorganism with significant potential for industrial DHA production (Osmond et al., 2021; Sarker et al., 2016). Whole cell *Aurantiochytrium* is a rich source of long-chain (C_{20-24}) polyunsaturated fatty acids (LC-PUFAs), particularly DHA. Also, *Aurantiochytrium* sp. has been recognized as promising ingredients in aquatic feeds due to its high content of LC-PUFAs and results in similar or better growth performance compared to fish oils for various marine fish and crustaceans (Patnaik et al., 2006; Wang et al., 2017; Allen et al., 2019). It is well known and widely documented that EPA and DHA are essential for enhancing growth performance, increasing survival rate, egg and larval quality, and helping in the development of tissues in the nervous system. Thus, similar to DHA-enriched oils, algae cells such as *Aurantiochytrium* sp. may also be suitable for the enrichment of live prey items for larval fish and crustaceans (Sorgeloos et al., 2001; Dhont and Sorgeloos, 2002; Glencross, 2009; Felix et al., 2021; Monroig et al., 2022).

It is known that the LC-PUFA requirements of penaeid shrimps vary in their early larval stages as they live in different ecosystems and at different trophic levels (Bell and Koppe, 2010). Consequently, the addition of high-quality supplements to the diet is crucial for the growth performance and survival of penaeid shrimp at mysis and post-larval stages. In this context, the use of heterotrophic or photosynthetic microalgae as single-celled oil sources increases the larval quality and production efficiency of marine fish and crustaceans. In addition, adding some microalgae as enrichment media to both live feed organisms and formulated feeds also improves feed utilization, stress tolerance, growth, disease resistance and carcass quality (Kumar et al., 2018).

Successful larval culture of marine fish and crustaceans is depended on cost-effective production of live feeds. In many commercial marine hatcheries, shrimp post-larva production relies on the microalgae and *Artemia* (Bengtson et al., 2018). Even though the use of *Artemia* in hatchery production offers many advantages, most importantly ease of storage and transport (Lavens and Sorgeloos, 1992), n-3 PUFAs levels in newly hatched *Artemia* nauplii are either low or highly variable depending on origin (Figueiredo et al., 2009). It is well known that *Artemia* is deficient in some essential lipids, particularly phospholipids and LC-PUFAs (*i.e.* arachidonic acid (AA), EPA and DHA) for the larval stages of fish (Monroig et al., 2003; Dhont et al., 2013; Cavrois-Rogacki et al., 2020). Therefore, their nutritional content must be manipulated, especially enriching it with n-3 LC-PUFAs being a pivotal for larval performance. This enrichment has been tested in many commercially produced emulsions and/or algae sources (Watanabe, 1993; Leger et al., 1985; Glencross and Smith, 2001; Immanuel et al., 2004). Artemia, as a suitable "carrier" vehicle, ensures the transfer of certain enhancers, such as essential fatty acids, to the targeted organism (Watanabe et al., 1982; Barclay and Zeller 1996; Furuita et al., 1999; Sorgeloos et al., 2001). Previous researchers have demonstrated that using enrichment with n-3 HUFA showed better survival and growth in various crustacean species (Rees et al., 1994; Cavalli et al., 2000; Martins et al., 2006; Immanuel et al., 2007; Sui et al., 2007; Jinbo et al., 2013; Mutti et al., 2017).

The green tiger shrimp, *Penaeus semisulcatus*, is an Indo-Pacific species that is distributed from southeast Africa to Japan and North Australia and it is an important component of commercial shrimp fisheries (Somers, 1994). The slow growth, low survival rates and cannibalism creates production barriers for this species. Studies on its nutritional requirement are limited (Al-Mohanna and Nott, 1989; Genc et al., 2007; Sivakamavalli and Vaseeharan, 2013; Al-Musalam et al., 2014; Yılmaz, 2020) and further research on larval survival and development is needed to enable successful hatchery management practices. Especially information on the pre- and post-supplementation of emulsified concentrates of enriched *Artemia* and its resultant use on larval growth and survival of this shrimp species are not available. As a result, this study was, therefore, explores the effects of *A. mangrovei* as n-3 LC-PUFAs enrichment media, by using *Artemia* as a carrier, on growth, survival and fatty acids accumulation of the green tiger shrimp *P. semisulcatus* during mysis and early post-larval stages.

Materials and Methods

Materials

The freeze-dried powder of heterotrophic micro-algae, *A. mangrovei* FIKU008 was obtained from the Algal Bioresources Research Center, Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Thailand.

Larvae used in the experiment; green tiger shrimp brood stocks were caught off the Mediterranean Sea and were spawned at Mariculture Research Station of Fisheries Faculty, Cukurova University, Adana, Türkiye. After hatching, shrimp larvae were cultured in a static water hatchery system, as described by Kumlu et al., (2000). Larval culture was carried out as described by Yılmaz (2020). A gentle aeration was maintained through a silicone rubber tube with a glass rod at the tip. Water quality parameters were maintained at salinity of 35±1.3 ppt, temperature of 28.5±1.8 ºC, pH 7.8±0.5, dissolved oxygen of 6.5–7.7 mg/L, and total ammonia levels of <0.5 mg/L. A photoperiod of 12:12 dark-light cycle was provided during the study.

Experimental Design

The main purpose of this study was to firstly enrich Artemia, as a carrier, with optimal ratio of *A. mangrovei* and then investigate the effects of enriched Artemia on

the growth, survival rate and fatty acid profiles of the shrimp *P. semisulcatus* during mysis and early post-larval stages. For this purpose, the study was designed as two successive stages.

Stage1: Enrichment of Artemia with Different Doses of AUR

Artemia used in this study were hatched from the Great Salt Lake cysts (INVE Aquaculture, Belgium). During the incubation, 3.0 g of cysts per liter of seawater were placed in four 20 L conical glass containers at 28 ºC and 35 ppt for 24 h. The nauplii were harvested through a 120-µ sieve, rinsed with UV-irradiated seawater, and then stocked into 2-L round glass flasks at a density of 5000 nauplii/L at 28 ºC/35 ppt under vigorous aeration for enrichment trial. Five different Artemia-enrichment treatments were set up with four different doses of microalga (*A. mangrovei*: AUR) at 0.6 g/L (0.6AUR), 0.8 g/L (0.8AUR) and 1.0 g/L (1.0AUR) against S-Presso-enriched group (Positive Control; pCONT) or non-enriched group (Negative control; nCONT). S-presso® (INVE Aquaculture, Belgium) was used at a dose of 0.5 g/L according to manufacturer enrichment protocol. Each enrichment dose for both AUR and S-presso was split into two and applied twice a day with 12 h intervals. After 12-h and 24-h of enrichment, the nauplii were harvested through a 120-µm sieve, rinsed with UV-irradiated seawater and distilled water before sampling for fatty acids analyses. All the samples were kept at -20 ºC until further analysis.

Stage 2: Effects of Enriched-Artemia on Growth and Survival of *Penaeus Semisulcatus* Larvae and Post-Larvae

At mysis 1 (M1) stage, larvae were cultured in 2-L round bottom glass flasks at a density of 100 pieces/L on one of the experimental feeding regimes as described above for ten days to determine the effect of different AUR-enrichment levels of Artemia on larval growth and survival. The experiment was a completely randomized design with four replicates per dietary treatment. The experimental groups were; 1) 0.0AUR (CONT), 2) 0.6AUR, 3) 0.8AUR. The whole enrichment period lasted 24 h and the shrimp larvae/post-larvae fed newly harvested/thoroughly rinsed Artemia. During the trial, the larvae were fed twice daily (08.00 and 20.00 h) with Artemia offered all feeding time at an initial density of 3 nauplii/mL, but gradually increased to 7 nauplii/mL towards the end of the experiment. The experimental system had a 100% water change every two days and fifteen post-larvae (PL) in each flask were counted (N=60 per treatment) to determinate survival rate, while on alternate days, only 50% of the flask water was changed. The sampled PLs were measured under a microscope from the tip of the rostrum to the end of telson. Larval growth index was calculated by using the method of Kanazawa et al., (1985).

Analytical Methods

Lipids were extracted according to the procedure of Folch et al., (1957). Following the lipid extraction, fatty acid methyl esters (FAME) were prepared according to Metcalfe and Schmitz (1961) and analyses as described previously (Czesny and Dabrowski, 1998) with some modifications in Czesny and Dabrowski (1998). Briefly, the FAME obtained were separated by gas chromatography (Agilent 6820 A), equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 µm). The injector temperature program was 190 ºC for 35 min then increasing at 30 ºC pre min up to 220 ºC where it was maintained for 5 min. The carrier gas was hydrogen (2 mL/min and split ratio was 30:1). The individual fatty acids were identified by comparing their retention times to that of a standard mix of fatty acids (Supelco 37 component FAME mix). Resulting peaks were corrected by the theoretical relative FID response factors and for methyl transformation, and then they were quantified as moles, relative to the internal standard, and ultimately reported as mole% of total fatty acids.

Statistical Analysis

Growth performance data (n=4) and proximate composition and fatty acids data (n=3) were reported as means ± standard deviation (SD) throughout the text. Percentage values were arcsine square root transformed and after normality and homogeneity of variance were confirmed and data were analyzed by one-way analysis of variance (ANOVA) at a significance level of 0.05% following confirmation of normality and homogeneity of variance. Where significant differences were detected, data were subjected to Student-Newman-Keuls post hoc tests for identifying homogeneous subsets. All computations were performed using SPSS16.0 (SPSS Inc., Chicago, IL, USA).

Results

Fatty Acids Composition of Enricher Sources

When the fatty acid profiles were compared, although both microalgae AUR and S-Presso contained similar amounts of DHA, S-Presso was richer in EPA and docosapentaenoic acid (C22:5n-3, DPA) contents and therefore richer in overall n-3 HUFA (Table 1). The amount of EPA was 13.2 times higher while of DPA 14.9 times higher in S-Presso enhancer compared to AUR. These two enrichment sources had very similar DHA contents. The level of total SFA (saturated fatty acids), especially C16:0, was higher in AUR while MUFAs (mono-unsaturated fatty acids), n-6 PUFAs, and total PUFA were higher in S-Presso enricher than AUR. As shown in Table 1, the enhancers differed in the amounts of PUFAs and HUFAs but most monounsaturated and polyunsaturated were similar. The n-3 PUFA content of

S-presso was 1.2 times higher than that of AUR. DHA/EPA ratio in AUR was 147.22, while 10.52 in S-presso.

Stage1: Fatty Acids Composition of Enriched-Artemia

Fatty acids profiles of the newly-hatched Artemia nauplii and enriched-Artemia presented significant differences (Table 2). Fatty acids composition of enriched-Artemia was affected both by algae dosages and enrichment time. The algal enrichment for 12 h and 24 h (Table 2, 3) significantly affected fatty acids composition of Artemia. Enrichment with AUR increased fatty acids content, total SFA primarily C16:0 and DHA in artemia at the end of both enrichment times.

At 12 h enrichment, 1.0AUR invoked the highest level of total SFA and C16:0, though lower levels of AUR enrichment also sustained higher levels of these fatty acids compared to nCONT in Artemia. Similar results were also clearly evident at 24 h enrichment duration (Table 2). However, at both enrichment times, C18:0 as well as total MUFA were found to be highest in nCONT. Oleic acid (C18:1n-9) was the most abundant fatty acid of monoenes in artemia and there were no significant differences among all enrichment groups at 12 h enrichment time (P>0.05), while 0.0AUR was found contain higher oleic acid than all the enriched-groups at the end of 24-h enrichment period (P<0.05).

The content of 18:2n-6 in enriched-Artemia decreased with increasing AUR level and was significantly lower in pCONT and nCONT groups (P<0.05). Also, 18:2n-6 was lowest in all AUR including groups in both enrichment times. No significant differences were observed in 18:3n-3 levels among the experimental groups (P>0.05). The highest DHA (20:6n-3) content (6.81%) was found to be in 0.8AUR group, while DHA content lowest was found in the 0.0AUR group (0.05%). The fatty acid profile of the enriched-Artemia reveals that S-Presso-enrichment induced higher levels of EPA while AUR- enrichment led to higher levels of DHA in Artemia. EPA and DHA as well as total n-3 HUFA levels were found to be significantly higher in 0.6AUR and 0.8AUR groups than all other treatment groups after 24 hours enrichment (Table 3). In general, while non-enriched-Artemia (nCONT) contained very low levels of DHA (0.04-0.05%), enrichment by either S-Presso or especially AUR led to significant increases (between 3.35-6.87%) in 24 h. The fatty acid composition of artemia has been found to be closely related to the fatty acids in the enrichment sources and the type of enricher.

Table 1. Fatty acids composition (% of total fatty acids) of enrichment sources.

	Enrichment Sources	
Fatty Acids	AUR	S-Presso
14:0	1.88±0.01	1.58±0.01
15:0	0.04±0.00	0.71±0.07
16:0	26.94±0.17	11.71±0.21
17:0	1.64±0.02	0.23±0.00
18:0	0.86±0.01	1.83±0.02
24:0	10.17±0.03	nd
∑SFAs	*41.60±0.15*	*18.86±0.26*
14:1	0.11±0.00	0.03±0.00
15:1	7.46±0.02	n.d.
16:1n-7	0.30±0.04	1.16±0.03
18:1n-7	0.28±0.05	1.06±0.01
18:1n-9	0.23±0.00	9.52±0.09
22:1n-9	0.04±0.00	0.90±0.01
24:1n-9	0.04±0.00	0.47±0.08
∑MUFAs	*8.60±0.10*	*14.90±0.11*
18:2n-6	0.19±0.00	7.92±0.27
18:3n-6	0.41±0.00	0.21±0.00
20:3n-6	0.19±0.01	0.25±0.00
20:4n-6	1.24±0.04	0.58±0.03
∑n-6	*2.01±0.09*	*9.05±0.24*
18:3n-3	0.15±0.00	0.42±0.00
20:3n-3	0.10±0.01	1.07±0.04
20:5n-3 (EPA)	0.31±0.01	4.11±0.10
22:5n-3 (DPA)	0.43±0.01	6.40±0.01
22:6n-3 (DHA)	45.64±0.49	43.24±0.48
∑n-3	*46.63±0.89*	*55.23±0.35*
∑PUFAs	48.79±0.47	64.28±0.11
n3/n6	23.10±0.75	6.10±0.35
DHA/EPA	147.22±0.47	10.52±0.89

The results were express as mean ± SD (n=3), *nd: not detected

Stage 2: Postlarvae Fatty Acids Composition and Growth Parameters

As expected, the fatty acid composition of Artemia was affected by the enrichment, which in turn affected the composition of the larvae feeding on them. The fatty acid composition of post-larvae was influenced by the algal levels that was used as enrichment media for Artemia (Table 4). Statistical differences were detected between treatments in the amounts of PUFAs and HUFAs (P<0.05). The whole body C16:0, C17:0, and total SFA levels of the shrimp fed with 0.6AUR or 0.8AUR diets were significantly higher than those fed nCONT diet (P<0.05). The accumulation of C18 fatty acid levels were similar among the treatments and did not significantly different (P>0.05).

The fatty acid profile of the post-larvae revealed that SFA accumulated more in 0.6AUR and 0.8AUR compared to CONT over the experiment, while the situation was just the opposite for MUFAs (P<0.05) (Table 4). Post-larvae fed enriched-Artemia with 0.6AUR and 0.8AUR presented significantly higher levels of total n-3 PUFA levels than post-larvae fed 0.0AUR in the same period. While DHA did not differ among the groups, DHA levels at PL7 were 17.27 to 18.68-fold higher in 0.6AUR and 0.8AUR groups than for CONT. In general, post-

Table 2. Fatty acids composition (% of total fatty acids) of enriched Artemia for 12 h.

Fatty Acids	Experimental treatments - 12 h				
	pCONT	nCONT	0.6AUR	0.8AUR	1.0AUR
16:0	10.84±0.01^{C}	11.09±0.00^{B}	11.89±0.02^{BC}	11.77±0.37^{B}	12.24±0.25^{A}
18:0	6.21±0.32^{B}	7.58±0.24^{A}	6.17±0.01^{BC}	6.63±0.69^{AB}	6.35±0.02^{B}
ΣSFAs	*19.23±0.48^{D}*	*19.97±0.55^{C}*	*20.32±0.05^{BC}*	*20.71±0.41^{AB}*	*21.14±0.25^{A}*
14:1	0.86±0.03^{B}	0.97±0.03^{A}	0.84±0.01^{B}	0.84±0.03^{B}	0.82±0.01^{B}
16:1n-7	1.64±0.11^{B}	1.52±0.04^{C}	1.77±0.03^{A}	1.75±0.06^{A}	1.74±0.02^{A}
18:1n-7	7.05±0.06^{C}	8.22±0.16^{A}	7.27±0.17^{B}	7.34±0.00^{B}	7.40±0.07^{B}
18:1n-9	18.83±0.88	19.42±0.63	19.34±0.29	18.96±0.52	18.98±0.18
ΣMUFAs	*29.88±0.52^{B}*	*32.09±0.94^{A}*	*30.72±0.40^{B}*	*30.39±0.66^{B}*	*30.68±0.29^{B}*
18:2n-6	6.61±0.10^{A}	6.83±0.42^{A}	5.80±0.09^{B}	5.78±0.12^{B}	5.71±0.05^{B}
18:3n-6	0.56±0.10^{B}	0.71±0.00^{A}	0.36±0.03^{B}	0.56±0.01^{B}	0.54±0.04^{B}
20:4n-6	0.83±0.12^{B}	1.04±0.02^{A}	0.85±0.02^{B}	0.87±0.02^{B}	0.91±0.01^{B}
Σn-6	*8.21±0.06^{A}*	*8.84±0.42^{B}*	*7.38±0.13^{C}*	*7.42±0.12^{C}*	*7.38±0.02^{C}*
18:3n-3	28.03±1.33	29.53±0.00	28.96±1.12	29.15±0.41	29.21±0.14
20:3n-3	1.08±0.18^{B}	1.38±0.06^{A}	1.04±0.04^{B}	1.01±0.03^{B}	1.02±0.03^{B}
20:5n-3	3.64±0.29^{A}	1.68±0.06^{D}	2.43±0.11^{C}	2.56±0.19^{C}	2.93±0.01^{B}
22:6n-3	3.63±0.32^{C}	0.05±0.98^{D}	5.82±0.08^{B}	6.81±0.18^{A}	5.79±0.44^{B}
Σn-3 [4]	*39.03±1.76^{A}*	*35.52±0.44^{C}*	*38.25±1.12^{AB}*	*39.53±0.68^{A}*	*37.35±0.46^{B}*
∑PUFAs	47.82±0.06^{A}	44.97±0.78^{B}	46.14±1.24^{AB}	47.70±0.84^{A}	45.33±0.47^{B}
n3/n6	4.75±0.16^{C}	4.03±0.16^{D}	5.18±0.06^{AB}	5.32±0.02^{A}	5.06±0.06^{B}
DHA/EPA	1.00±0.11	0.03±0.00	2.40±0.01	2.66±0.02	1.98±0.10

The results were express as mean ± SD (n=3), the values in the same row with different superscript letters are significantly different from each other (P<0.05).

Table 3. Fatty acids composition (% of total fatty acids) of enriched Artemia for 24 h.

Fatty Acids	Experimental treatments - 24 h				
	pCONT	nCONT	0.6AUR	0.8 AUR	1.0 AUR
16:0	11.67±0.07^{B}	10.86±0.04^{C}	12.52±0.30^{A}	12.39±0.17^{A}	12.58±0.09^{A}
18:0	7.32±0.04^{B}	8.53±0.86^{A}	7.44±0.40^{B}	7.90±0.03^{B}	7.88±0.04^{B}
ΣSFAs	*21.45±0.50^{B}*	*20.92±0.81^{B}*	*22.61±0.61^{A}*	*23.03±0.18^{A}*	*21.45±0.50^{A}*
14:1	0.80±0.01^{B}	0.88±0.06^{A}	0.72±0.01^{C}	0.70±0.01^{C}	0.68±0.01^{C}
16:1n-7	1.44±0.10^{B}	1.49±0.08^{B}	1.62±0.08^{A}	1.52±0.01^{AB}	1.55±0.10^{AB}
18:1n-7	7.73±0.25^{C}	9.35±0.70^{A}	8.55±0.20^{B}	8.49±0.05^{B}	8.49±0.01^{B}
18:1n-9	19.13±0.52^{B}	20.55±0.88^{A}	19.10±0.82^{B}	18.91±0.17^{B}	19.13±0.52^{B}
ΣMUFAs	*30.84±1.57^{B}*	*34.17±0.80^{A}*	*31.72±0.81^{B}*	*31.27±0.01^{B}*	*31.30±0.20^{B}*
18:2n-6	6.17±0.29^{A}	6.00±0.06^{A}	5.33±0.08^{B}	5.23±0.01^{B}	5.21±0.01^{B}
18:3n-6	0.49±0.03^{B}	0.66±0.01^{A}	0.50±0.01^{B}	0.49±0.01^{B}	0.49±0.01^{B}
20:4n-6	0.75±0.05^{C}	0.92±0.02^{A}	0.84±0.05^{B}	0.87±0.07^{AB}	0.86±0.02^{AB}
Σn-6	*7.70±0.39^{B}*	*8.78±0.01^{A}*	*6.95±0.02^{C}*	*6.83±0.09^{C}*	*6.84±0.07^{C}*
18:3n-3	25.24±0.96^{B}	26.66±0.84^{A}	26.54±0.23^{A}	26.10±0.00^{AB}	25.84±0.27^{AB}
20:3n-3	1.09±0.00^{D}	1.32±0.01^{B}	1.27±0.07^{C}	1.52±0.02^{A}	1.56±0.01^{A}
20:5n-3	5.18±0.36^{A}	1.11±0.36^{D}	4.62±0.23^{AB}	4.49±0.02^{B}	4.50±0.05^{B}
22:6n-3	3.35±0.09^{C}	0.04±0.12^{D}	6.81±0.72^{A}	6.87±0.09^{A}	5.88±0.05^{B}
Σn-3	*35.80±0.31^{A}*	*34.21±0.87^{B}*	*35.16±1.25^{A}*	*34.97±0.05^{AB}*	*34.77±0.36^{AB}*
∑PUFAs	44.04±0.08^{A}	43.77±0.82^{A}	41.65±1.27^{B}	42.16±0.19^{B}	42.13±0.31^{B}
n3/n6	4.66±0.28^{B}	3.84±0.09^{C}	5.05±0.20^{A}	5.12±0.06^{A}	5.09±0.10^{A}
DHA/EPA	0.64±0.02^{C}	0.04±0.00^{D}	1.47±0.23^{A}	1.53±0.12^{A}	1.31±0.01^{B}

The results were expressed as mean ± SD (n = 3), the values in the same row with different superscript letters are significantly different from each other (P<0.05).

larvae fed with enriched-Artemia showed higher n-3 fatty acid accumulation in the tissues and n-3 HUFA in 0.6AUR and 0.8AUR were 27.06-27.07% compared to 25.78% in 0.0AUR group. The n3/n6 ratios in enriched-Artemia-fed groups ranged from 4.10% to 5.01%, while this value was 4.21 in CONT groups (Table 4).

Higher growth performance (as total length) in shrimp larvae during the 10-day study period was detected in the groups fed with algae-enriched Artemia (Table 5). Larvae grown from the M1 stage to the M3 stage had the highest survival rate in the 0.8AUR (99.09%) group, while the lower survival rate was found in the CONT (93.48%) and 0.6AUR (94.24%) groups ($P<0.05$). Larvae grown from the PL1 stage to the PL7 stage had the best survival rate in the AUR-enriched artemia groups, but yet also the lowest in the CONT group ($P<0.05$). During the experiment, both levels of AUR-enriched Artemia (0.6AUR and 0.8AUR) constantly sustained better post-larval (PL7) survival (85.00%) than that of (75.00%) with non-enriched Artemia ($P<0.05$). Growth performance and survival of shrimps increased proportionally as the inclusion level of alga increased in Artemia as diet (Table 5). Significant differences in survival rate, growth rate, and total length were found

Table 4. Whole body fatty acids composition (% of total fatty acids) of *P. semisulcatus* larvae fed with AUR-enriched Artemia from M1 to PL7 stages.

Fatty Acids	Experimental treatments			
	M1		PL7	
	Initial	CONT	0.6AUR	0.8AUR
16:0	25.72±0.04	16.36±0.54^{B}	17.65±0.28^{A}	17.23±0.04^{A}
18:0	10.22±0.10	12.33±0.57	12.21±0.24	12.47±0.05
Σ SFAs	*37.32±0.14*	*30.73±0.42^{B}*	*32.71±0.55^{A}*	*31.87±0.07^{A}*
14:1	0.27±0.02	0.36±0.02^{A}	0.31±0.02^{B}	0.34±0.01^{AB}
16:1n-7	4.39±0.19	1.95±0.03^{A}	1.81±0.02^{B}	1.74±0.00^{C}
18:1n-7	6.80±0.25	9.14±0.18^{A}	7.96±0.14^{C}	8.71±0.06^{B}
18:1n-9	10.28±0.43	18.18±0.27^{A}	16.44±0.22^{B}	16.69±0.52^{B}
Σ MUFAs	*23.81±0.57*	*32.97±0.26^{A}*	*29.78±0.33^{C}*	*30.79±0.50^{B}*
18:2n-6	3.60±0.16	4.45±0.44^{B}	4.90±0.10^{A}	3.75±0.02^{C}
18:3n-6	0.46±0.00	0.36±0.04	0.35±0.01	0.36±0.00
20:4n-6	0.47±0.02	0.54±0.02^{A}	0.34±0.01^{C}	0.40±0.01^{B}
Σ n-6	*5.70±0.23*	*6.34±0.51^{A}*	*6.61±0.12^{A}*	*5.40±0.04^{B}*
18:3n-3	7.86±0.31	14.60±0.20^{A}	10.95±0.17^{C}	11.43±0.04^{B}
20:3n-3	1.74±0.16	2.23±0.10^{B}	2.87±0.03^{B}	2.91±0.00^{A}
20:5n-3	13.71±0.95	10.27±0.16	10.47±0.13	10.50±0.01
22:6n-3	3.81±0.16	0.22±0.01^{C}	4.11±0.51^{A}	3.80±0.25^{AB}
Σ n-3	*31.43±0.93*	*25.78±0.33^{B}*	*27.07±0.26^{A}*	*27.06±0.21^{A}*
∑PUFAs	37.13±0.88	32.12±0.71^{B}	33.68±0.17^{A}	32.47±0.15^{B}
n3/n6	5.51±0.23	4.21±0.33^{B}	4.10±0.11^{B}	5.01±0.08^{A}
DHA/EPA	0.28±0.01	0.02±0.00^{B}	0.40±0.01^{A}	0.36±0.03^{A}

The results were expressed as mean ± SD (n=3), the values in the same row with different superscript letters are significantly different from each other ($P<0.05$).

Table 5. Growth performance of the green tiger shrimp *Penaeus semisulcatus* larvae fed *A. mangrovei*-enriched Artemia from M1 to PL7.

Growth Parameter	*Experimental Groups*		
Survival rate (%)	CONT	0.6AUR	0.8AUR
M1	100	100	100
M3	93.48±4.86^{B}	94.24±5.46^{B}	99.09±1.20^{A}
PL1	84.85±3.81^{C}	92.88±6.00^{B}	97.58±0.26^{A}
PL7	75.00±5.52^{B}	85.00±2.16^{A}	85.00±2.29^{A}
Growth rate (mm/day)			
M1 to M3	0.45±0.02^{B}	0.50±0.09^{A}	0.49±0.10^{A}
PL1 to PL7	0.50±0.00	0.51±0.03	0.51±0.03
Total length (mm)			
M1	3.96	3.96	3.96
M3	4.88±0.03	4.96±0.20	4.94±0.22
PL1	5.89±0.55	5.92±0.23	6.04±0.11
PL7	8.77±0.03^{B}	9.04±0.08^{A}	9.05±0.05^{A}

The results were expressed as mean ± SD (n=3), the values in the same row with different superscript letters are significantly different from each other ($P<0.05$).

between the experimental groups (P>0.05). According to our results, the growth rate (mm day^{-1}) of the larvae fed with the 0.8 and 0.6 AUR artemia were significantly higher than that of CONT between M1 and PL1 stages (Table 5). Nevertheless, there were no significantly differences in the growth rate PL1 to PL7 stages (P>0.05). Postlarvae-fed enriched Artemia also presented a higher total length at the end of the larviculture period. The least growth performance was observed in the larvae fed non-enriched artemia group.

Discussion

As expected, in the present study, the fatty acid composition of Artemia was affected by enrichment protocols including duration and nutrient sources. Our results showed significantly higher levels of n-3 HUFA, particularly EPA and DHA, in alga-enriched (AUR) Artemia compared with non-enriched Artemia (nCONT). Similar to our findings, Watanabe (1993) previously found that rotifers and *Artemia* nauplii were successfully enriched with another alga species of the same family (Thraustochytrids) from different genera (*Schizochytrium* sp.) and suggested that, as an n-3 HUFA source, the alga can provide the best strategy for the enrichment of live forage zooplankton used in aquaculture. Like other sources of algae, Thraustochytrids have been potentially cultured and applied in food and feed industries with application in larval culture primarily due to their high EPA and DHA contents (Raghukumar, 2008; Jaseera et al., 2021). Recent studies have shown that dietary *A. mangrovei* meal and oils improve growth performance, immunological responses, disease resistance in different crustacean species (Wang et al., 2017; Kumar et al., 2018; Allen et al., 2019; Jaseera et al., 2021; Rocha et al., 2021). Since the production of shrimp post-larvae in commercial hatcheries is largely dependent on Artemia as the main living food organism (Sorgeloos et al., 1998; Cobo et al., 2015), expanding the use of artemia enhancers in hatcheries should provide the farmers some advantages to operate with higher seed performance and productivity.

In this study, AUR-enriched Artemia had higher levels of DHA compared to CONT, which ultimately affected the fatty acids composition of shrimp larvae fed on this food. This fits well with previous findings showing the positive effect of dietary n-3 HUFA in enhancing larval growth and development (Rees et al., 1994; Cavalli et al., 2000; Palacios et al., 2004; Immanuel et al., 2007). It is also known that microalgae can improve survival in shrimp larval culture even when added directly to water (Lober and Zeng, 2009). However, as noted in previous studies, post-hatching period of larval development, shrimp larvae obtain their nutritional needs by hunting, so shrimp larvae consuming Artemia enriched with algae are able to reassemble better larval nutritional requirements compared to algae alone or brood only (Lavens et al., 2000; Bhavan et al., 2010). Studies of dietary HUFA requirements have generally focused on the post-larval or juvenile shrimp and therefore additives have been generally added to their feed (Patnaik et al., 2006; Kangpanich and Senanan, 2015; Wang et al., 2017; Kangpanich et al., 2017; Kumar et al., 2018; Visudtiphole et al., 2018; Guimarães et al., 2019; Allen et al., 2019; Jaseera et al., 2021; Rocha et al., 2021). In agreement with the studies above, our results also found that the fatty acids in the algae largely reflected the total fatty acids content of Artemia. Enriched Artemia (pCONT, 0.6 and 0.8 AUR) contained high proportions of high essential fatty acid groups such as PUFA, EPA and DHA. With the supplementation protocol in which n-3 HUFA-rich compound or algae (AUR) were fed to Artemia, n-3 HUFA levels increased in both 12 and 24 hours of enrichment periods, thus increasing the amount of nutrients.

The post-larval shrimp fed the AUR-enriched artemia exhibited greater growth and survival than the CONT group confirming the literature in that growth and/or survival will be higher with diets enhanced by n-3 HUFA in penaeid shrimps (*P. japonicus* by Kanazawa et al., 1979, Kontara et al., 1997; *P. chinensis* by Xu et al., 1993, 1994; *P. indicus* by Read 1981; *P. stylirostris* by Léger et al., 1985; *P. monodon* by Rees et al., 1994, Millamena et al., 1988; *Farfantepenaeus paulensis* by Mutti et al., 2017). As in all aquatic animals, DHA is essential for maintaining the structural and functional cell membranes and nervous system development in shrimp, whereas arachidonic acid and EPA are required for the production and modulation of eicosanoids (Méndez-Martínez et al., 2018). Although fatty acid enrichment diets have been reported to generally improve growth performance in a number of studies (Lavens and Sorgeloos, 1992; Rees et al., 1994; Cavalli et al., 2000; Palacios et al., 2004; Immanuel et al., 2007; Zelaya et al., 2007; Mutti et al., 2017; Felix et al., 2021), some other studies report no growth enhancement or even adverse results in post-larval stages of penaeid shrimps (Rees et al., 1994; Pontes et al., 2003). The positive effect is suggested to be due to the increased post-larval fatty acid metabolism of shrimps resulting in a faster growth rate by Hurtado et al., (2007) and Martins et al., (2006).

The fatty acid composition of Artemia was affected by enrichment, which in turn affected the fatty acid composition of shrimp larvae fed on them in this study. Accordingly, post-larvae fed AUR-enriched Artemia (0.6AUR and 0.8AUR) had higher levels of n-3 HUFA, particularly DHA, compared to those fed non-enriched Artemia (CONT). In this study, there were significant differences in fatty acids of the whole body of *P. semisulcatus* post-larvae due to artemia enrichment with AUR. In particular, C16:0, C20:3n-3, C22:6n-3 and Σn-6, ΣSFA and n-3 PUFA were significantly higher in AUR-enriched artemia-fed post-larvae compared to CONT-group. Previous reports have determined that the fatty acid profile of shrimp tissue varies positively with

the fatty acid profile of the diet (Gonzalez-Felix et al., 2010; Kumar et al., 2018; Soller et al., 2019; Jaseera et al., 2021; Rocha et al., 2021). Our finding with *P. semisulcatus* is generally in line with other studies showing that the fatty acid profile largely reflects the diets consumed by shrimp larvae (Gonzalez-Feliz et al., 2010; Allen et al., 2019; Felix et al., 2021). In our study, the delay in post-larval growth in the CONT-group was due in part to a lack of adequate essential fatty acids in the diet, particularly a deficiency of DHA. It has been reported that most crustacean marine animals have generally limited ability to biosynthesize LC-PUFAs from one of the precursors of C18 PUFAs, due to their markedly limited fatty acid elongation and desaturase enzymatic activities (Watanabe, 1993). In this study, the disappearance of DHA, that had previously existed at M1 stage, by the PL7 stage in the non-enriched groups confirms this view. Similar results were found in studies reporting that *P. monodon* and *L. vannamei* use shorter chain fatty acids for their metabolism and can retain LC-PUFA in the abdomen (Deering et al., 1997; Palacios et al., 2004; Felix et al., 2021). Shrimp larvae use fatty acids for energy and to maintain the functionality of biological membranes (Méndez-Martínez et al., 2018). Therefore, the use of live foods enriched with high levels of HUFAs is recommended (Romdhane et al., 1995; Méndez-Martínez et al., 2018), since enriched Artemia nauplii can successfully transfer HUFAs to shrimp post-larvae as also previously suggested (Lavens and Sorgeloos, 2000; Wang et al., 2017; Kangpanich et al., 2017; Kumar et al., 2018).

In conclusion, the present study confirms that Artemia fed with *A. mangrovei* meal showed high levels of EPA and DHA depositions (0.6-0.8 g/L) in both 12 and 24h enrichment periods. Dietary supplementation of AUR at 0.6 g/L and 0.8 g/L levels via Artemia had positive effects on growth parameters, survival rate and fatty acid profile in the early post-larval stages of *P. semisulcatus.* Consequently, more detailed studies are recommended to optimize and fully finalize the details about dietary inclusion of *A. mangrovei* for more successful post-larvae production of *P. semisulcatus*. Future developments in algae growing technology could maintain a nutritional quality final product in terms of HUFA content as an alternative to other enrichment sources.

Ethical Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors

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Author Contribution

Hatice Asuman Yılmaz: Conceived and designed the study, lab technical assistant, performed the analysis, collected the data, contributed data or analysis tools, performed the analysis, wrote the paper; Metin Kumlu: design of the study, contributed data or analysis tools, read and wrote the paper; Ece Evliyaoğlu: collected the data, lab technical assistant, read and wrote the paper; Jantana Praiboon: contributed data or analysis tools, performed the analysis, wrote the paper; Mehmet Bedrettin Duman: collected the data, lab technical assistant; Burcu Ak Çimen: Supply of microalgae and lab technical assistant; Oya Işık: supply of microalgae, read and wrote the paper; Orhan Tufan Eroldoğan; supervised the project, conceived and designed the study; collected the data, contributed data or analysis tools, performed the analysis, wrote the paper. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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