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Fatty Acids Profiles and Growth Performances of *Artemia franciscana* Fed with Different Types of Microalgae

(Pemprofilan Asid Lemak dan Prestasi Pertumbuhan *Artemia franciscana* yang Diberikan Pelbagai Jenis Mikroalga)

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ABSTRACT

Artemia has been considered as one of the most important live diets for crustacean and finfish larviculture as well as broodstocks. However, the basal nutrient of *Artemia* has been reported to be poor in polyunsaturated fatty acids (PUFA's) especially eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA), essential fatty acids for larval normal growth and gonad maturity in shrimp broodstocks. Thus, the present study aimed at investigating the effect of different microalgal diets on fatty acid content, growth performances and survival rate of *Artemia francisciana.* The study was performed by culturing instar I nauplii of *A. franciscana* for 12 days at a stocking density of 100 nauplii/L and fed with one of these microalgae: *Chaetoceros calcitrans* (T1), *Dunaliella salina* (T2)*, Tetraselmis chuii* (T3), and *Nanochloropsis oculata* (T4). The results showed that the different microalgal diets affected fatty acid content, growth and survival rate of *A. fransicana*. The highest DHA content was obtained from those *Artemia* fed on *D. salina*, $p<0.05$. While DHA content of *A. fransciscana* fed with the other three microalgae was not significantly different, $p>0.05$. Another result indicated that EPA contents in the *Artemia* biomass were not significantly affected by the microalgal diets, $p>0.05$. In terms of growth and survival rate, *A. franciscana* fed on *C. calcitrans* and *T. chuii* had better growth and survival rate compared to that of *Artemia* fed on either *D. salina* or *N. oculata,* $p<0.05$. Due to the faster growth, it was also observed that *Artemia* fed on *T. chuii* started producing eggs on day 12. Further studies by feeding the *Artemia* with a mix of microalgal species either a mix of *T. chuii* and *D. salina* or a mix of *C. calcitrans* and *D. salina* are highly recommended for better PUFA contents, specific growth rate (SGR) as well as survival rates of *Artemia*.

Keywords: Brine shrimp; diet; docosahexaenoic acids (DHA); eicosapentaenoic acids (EPA); microalgae

ABSTRAK

Artemia dianggap sebagai salah satu diet hidup yang paling penting untuk krustasea, larvikultur ikan bersirip dan stok induk. Walau bagaimanapun, kandungan nutrien asas *Artemia* telah dilaporkan rendah dalam asid lemak tak tepu (PUFA) terutamanya asid eikosapentanoik (EPA) dan asid dokosaheksanoik (DHA), yang merupakan asid lemak perlu untuk pertumbuhan normal larva dan kematangan gonad dalam stok induk udang. Oleh itu, kajian ini bertujuan untuk mengkaji kesan diet mikroalga yang berbeza ke atas kandungan asid lemak, prestasi pertumbuhan dan kadar kebolehidupan *Artemia francisciana*. Kajian dilakukan dengan mengkultur instar I nauplii *A. franciscana* selama 12 hari pada ketumpatan stok 100 nauplii/L dan diberi makan dengan salah satu mikroalga ini: *Chaetoceros calcitrans* (T1), *Dunaliella salina* (T2), *Tetraselmis chuii* (T3) dan *Nanochloropsis oculata* (T4). Hasil menunjukkan bahawa diet mikroalga yang berbeza mempengaruhi kandungan asid lemak, kadar pertumbuhan dan kemandirian *A. fransicana*. Kandungan DHA tertinggi diperoleh daripada *Artemia* yang diberi makan *D. salina*, $p<0.05$. Manakala kandungan DHA *A. fransciscana* yang diberi makan dengan tiga mikroalga yang lain tidak berbeza secara signifikan, $p>0.05$. Walau bagaimanapun, kandungan EPA dalam biojisim *Artemia* tidak terjejas dengan ketara oleh diet mikroalga, $p>0.05$. Dari

segi pertumbuhan dan kadar kemandirian, *A. franciscana* yang diberi makan *C. calcitrans* dan *T. chuii* mempunyai kadar pertumbuhan dan kemandirian yang lebih baik berbanding *Artemia* yang diberi makan sama ada *D. salina* atau *N. oculata*, $p<0.05$. Disebabkan pertumbuhan yang lebih cepat, juga diperhatikan bahawa *Artemia* yang diberi makan *T. chuii* mula mengeluarkan telur pada hari ke-12. Kajian lanjut dengan memberi makan *Artemia* dengan campuran spesies mikroalga sama ada campuran *T. chuii* dan *D. salina* atau campuran daripada *C. calcitrans* dan *D. salina* sangat disyorkan untuk kandungan PUFA, kadar pertumbuhan khusus (SGR) serta kadar kebolehidupan *Artemia* yang lebih baik.

Kata kunci: Asid dokosaheksanoik (DHA); asid eikosapentanoik (EPA); diet; mikroalga; udang air garam

Introduction

Artemia has been considered as the most common live diet for larviculture of finfish and crustaceans due to their physical characteristics e.g., soft-bodied prey and high nutrient contents (Basford et al. 2020; Sorgeloos et al. 2001). However, some studies have also documented that *Artemia* is deficient in polyunsaturated fatty acids (PUFAs), particularly in eicosapentaenoic acids (EPA, 20:5*n*-3), docosahexaenoic acid (DHA, 22:6*n*-3) and arachidonic (ARA, 20:4*n*-6) (Nieves-Soto et al. 2021). These PUFAs play important roles in improving the quality and growth of marine crustaceans and fish larvae (Francis et al. 2019; Lund et al. 2018). The importance of EPA and DHA has been also described by Lavens et al. (1995) as compounds for promoting growth, stress resistance and pigmentation. Therefore, most attention has now been given to enhance the presence of the PUFAs especially EPA and DHA in *Artemia*.

As a consequence, research on enriching nutrient content of Artemia before their use as live prey for larvae has received considerable attention in the last few decades. Regarding the feeding characteristics of *Artemia*, the manipulation of its nutritional value can be performed through diets. Artemia has been described to be a continuous, non-selective, obligate phagotrophic filter feeder and starts to ingest food at the Instar II stage using their larval antennae (Le et al. 2019). Furthermore, Fernández (2001) showed that *Artemia* filtered particles range from 1 - 50 µ and the optimal size is about 16 µ. Due to these feeding characteristics, several enrichment methods have been developed up until now such as the supplementation of fish oils (Nieves-Soto et al. 2021), n-3 HUFA commercial methyl ester oil (Roo et al. 2019), gamat emulsion (Putra et al. 2018) and microalgal feeding (Paulo et al. 2020). Several commercial PUFA enrichment formulations have been also developed (Roo et al. 2019). Among the available methods, the use of microalgae with high PUFAs has been recommended by many researchers. A study by Basford et al. (2020) stated that microalgae were better than commercial lipid emulsion at enhancing the fatty acids content of the live diet. Several microalgal species are reported to have high contents of PUFAs such as *Chaetoceros calcitrans* (Méndez-Martínez et al. 2018), *Tetraselmis* spp. and *Dunaliela* spp. (Dineshbabu et al. 2019). In addition, high protein and fat content make some microalgal species are potential to support the growth and survival rate of *Artemia* including *C. calcitrans* (protein 43% dw, lipid 23.80% dw), *N. oculata* (protein 34.11% dw, fat 12.51% dw) (Banerjee et al. 2011), *T. chuii* (protein 56% dw, lipid 9.4% dw, carbohydrate 16.6% dw) (Arkronrat et al. 2016; El-Sayed et al. 2020) and *D. salina* (protein 40.46% dw, lipid 15.51% dw, carbohydrate 20.44% dw) (Dineshbabu et al. 2019). Several studies have previously reported that these microalgal species improved either fatty acid or growth of *Artemia*. However, those studies were performed separately. Therefore, a study that compares those different species of microalgae (which were previously reported to have the best impact but in a separate study) is required to find the best microalgal diet.

Thus, the present study was performed to quantify fatty acid profiles and growth performances of *A. fransciscana* fed on different microalgal species which were *Chaetoceros calcitrans*, *Tetraselmis chuii*, *Dunaliella salina,* and *Nanochloropsis oculata*. The experimental results are expected to give an overview on how to enhance fatty acids profiles and the growth of *Artemia* which will be very useful for marine hatcheries.

Materials and Methods

Experimental design

Four diet treatments (T1: *Chaetoceros calcitrans*, T2: *Tetraselmis chuii*, T3: *Dunaliella salina*, and T4: *Nanochloropsis*) with triplicates of each were assigned to a completely randomized design (CRD). Thus, a total of 12-6 L plastic gallons were used in the experiment. The plastic gallons were labelled according to the treatments. In addition, an aeration tube was installed in each gallon connected to an aerator (RESUN LP 100) for oxygen supply (Figure 1).

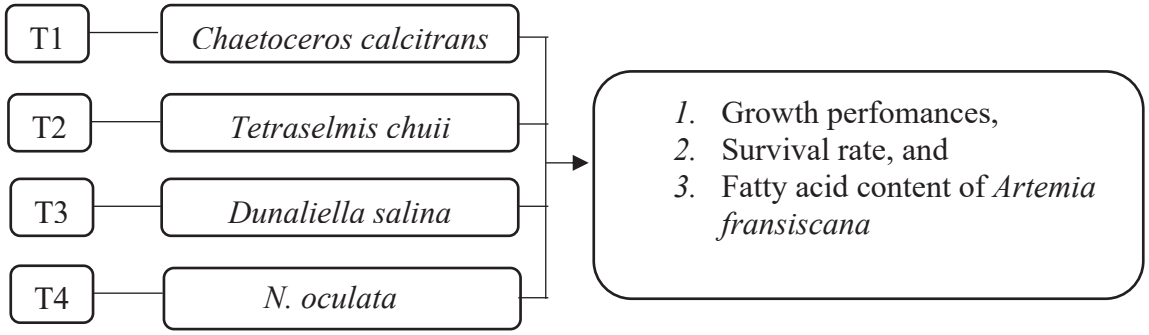

FIGURE 1. Experimental design

DECAPSULATING *Artemia*

The decapsulation of *Artemia* cysts was performed according to a protocol previously described by Sorgeloos et al. (1977) with slight modifications. In brief, the commercial *Artemia* cysts (Supreme PLUS) were dehydrated by immersing in freshwater for 2 h. Then, the dehydrated cysts were filtered using a 120 µ plankton net and washed repeatedly with sterile freshwater. Afterwards, the cysts were mixed with a chlorine solution at a dose of 1.5 mL per 1 g of cyst and stirred with a spatula until the colour becomes red. Thereafter, the cysts were immediately filtered again using the 120 µ plankton net and rinsed with fresh water until the chlorine smell disappears. Then, the chlorinated cysts were placed in ~34 ppt seawater for hatching. After 18-24 h, the cyst started to hatch and free-swimming nauplii were harvested and stocked to the experimental unit at a density of 100 nauplii/L.

MICROALGAL CULTURE

Four microalgae (*C. calcitrans*, *T. chuii*, *D. salina*, and *N. oculata*) were cultured in a 500 mL Erlenmeyer individually. The microalgal culture was started by sterilizing seawater with an autoclave at a temperature of 121 °C for 15 min at a pressure of 1 atm. Then, commercial microalgal media (WALNE) and vitamins with a composition of 1 mL/l L were added to the sterilized seawater. Additional µ nutrient (silicate) was added to *C. calcitrans* medium as diatom required the chemical compounds for building their cells (Egge & Aksnes 1992). Furthermore, the media was inoculated with plankton seeds with an initial concentration of ~1.0 × 10^4 cells/mL. Aeration was provided by inserting an aeration tube connected to the aerator machine (RESUN LP100) with 24 h light supply. The culture process was carried out for 5 days to reach the late exponential stage (~10^6 cells/mL), at which the microalgae were harvested to feed *Artemia*.

FEEDING EXPERIMENT

The feeding rate was calculated according to a protocol previously described by Lavens and Sorgeloos (2018) with a slight modification. Firstly, microalgal concentration was measured by counting the cell under a binocular microscope. Thereafter, a certain volume of microalgal culture was collected using a sterile pipette and added directly to the *Artemia* culture at a dose of 10^2 cell/nauplii (day 1- day 6), and ~10^4 cells/mL from day 7 until day 12.

MEASURED VARIABLES

Several variables were measured during the experimental periods including eicosapentaenoic acids (EPA), docosahexaenoic acids (DHA), total length (mm), specific growth rate (SGR) (µg/day), microalgal density in the rearing water (Log cells/mL), survival rate (%), and water quality (temperature, dissolved oxygen, salinity, pH, ammonia and nitrite).

ANALYSIS OF FATTY ACIDS

Fatty acid profiles in *Artemia* biomass harvested in each treatment were analyzed by the Laboratory of Biochemical Analysis, Faculty of Public Health, Universitas Airlangga with the following protocol. Fifty mg of *Artemia* biomass was homogenized in 1 mL of hexane and 2 mL of 0.5 N NaOH in methanol. The homogenate was then heated to 50 °C for 10 min and

cooled down at room temperature. Thereafter, 100 µL acetic acid glacial and 5 mL aquadesh was added and allowed to stand until 2 layers had been formed. One mL of the upper layer was collected and put into a vial, while the bottom layer was reextracted twice with 5 mL hexane. The three filtrates were afterwards mixed in a ratio of 1:1:1 and analyzed using a gas chromatograph (Shimadzu GC 2014) with Flame Ionization Detector (FID). A capillary column (Thermo Scientific-FAME) of 30 m length × 250 µm and a film thickness of 0.25 µm, and helium as the carrier gas is set at 75 kPA. One µL of the sample was injected with a Split 1/10 T. The column temperature was from 120 °C for 7 min and then raised to 240 °C at a rate of 10 °C/min and maintained for 5 min at a constant temperature of 250 °C.

TOTAL LENGTH

Fifteen *Artemia* were collected using a scope net and their total lengths were measured by taking a picture using a DSL camera (Canon EOS D30) with a scale (ruler) under a dissecting microscope. The pictures were then measured with the help of ImageJ software (Figure 2). The measurement was performed on day 0, day 3, day 6, day 9 and day 12.

FIGURE 2. A scaled photograph of *Artemia* taken using a DSLR camera under a dissecting microscope as a raw material for measurement of total length using ImageJ software

SPECIFIC GROWTH RATE (SGR)

The *Artemia* weight was measured using analytic scales with a precision level of 0.0001 g or 0.1 mg. The total wet biomass from each treatment was collected on a 250 µm mesh size sieve and the excess of water was removed by means of absorbent paper (Gómez et al. 1999). Then, the growth was calculated as previously described in Evjemo and Olsen (1999) based on a dry weight basis. Specific growth rates (SGR) were calculated according to Lavens and Sorgeloos (1991):

$$SGR = (\text{Ln } W2 - \text{Ln } W1)/(\text{Culture Period}) \times 100 \quad (1)$$

where W1 is the *Artemia* nauplii weight at day 1; and W2 is the *Artemia* weight at the end of the culture period.

SURVIVAL RATE

The survival value of *Artemia* was determined after 12 rearing days, by counting the number of surviving and dead *Artemia* in each treatment. The survival rate was calculated as previously described by Amin et al. (2020) with some modifications:

$$\text{Survival rate (\%)} = \frac{\text{total number of live } \textit{Artemia at the end of trial}}{\text{the initial number of } \textit{Artemia} \text{ stocked}} \times 100\% \quad (2)$$

MICROALGAL DENSITY IN THE REARING WATER

Microalgal concentrations in the rearing water of *Artemia* was measured with a haemocytometer under a binocular

microscope. The measurements were performed at three-day intervals to study how the microalgae or bacteria fluctuate over time in the rearing systems.

WATER QUALITY

Water quality parameters were also measured during the experiment to assure that the *Artemia* lived under optimal conditions. The measured water quality parameters are dissolved oxygen (DO), temperature, pH, salinity, ammonia, and nitrite concentrations. These measurements were performed on day 0, 3, 6, 9, and 12. DO, pH and temperature were measured with a DO meter probe (HI98193 - Waterproof Portable Dissolved Oxygen Meter), and salinity with a refractometer (ATAGO 20M). While ammonia and nitrite concentrations were measured with commercial kits (SERA test kit for ammonia and nitrite).

DATA ANALYSIS

Data of the specific growth rate and survival data were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test in order to determine significant differences among treatments at $p<0.05$. Prior to the statistical analysis, data were tested for linearity, homogenous and independent.

RESULTS

FATTY ACID COMPOSITIONS

Microalgal diets appeared to influence fatty acid content of *Artemia* biomass especially docosahexaenoic acids (DHA), but not eicosapentanoic acids (EPA) content. As presented in Figure 3, *A. franciscana* receiving *D. salina* had the highest DHA content (10.37% dw), compared to *Artemia* biomass fed on *T. chuii, C. calcitrans* or *N. oculata* which were 1.84% dw, 3.86% dw, and 3.79% dw, respectively. Meanwhile, EPA content of *Artemia* biomass fed with the four different microalgal species appeared to be quite similar which were ~1.47% dw, 2.41% dw, 2.05% dw and 4.25% dw for *T. chuii*, *C. calcitrans, D. salina,* and *N. oculata,* respectively, $p>0.05$.

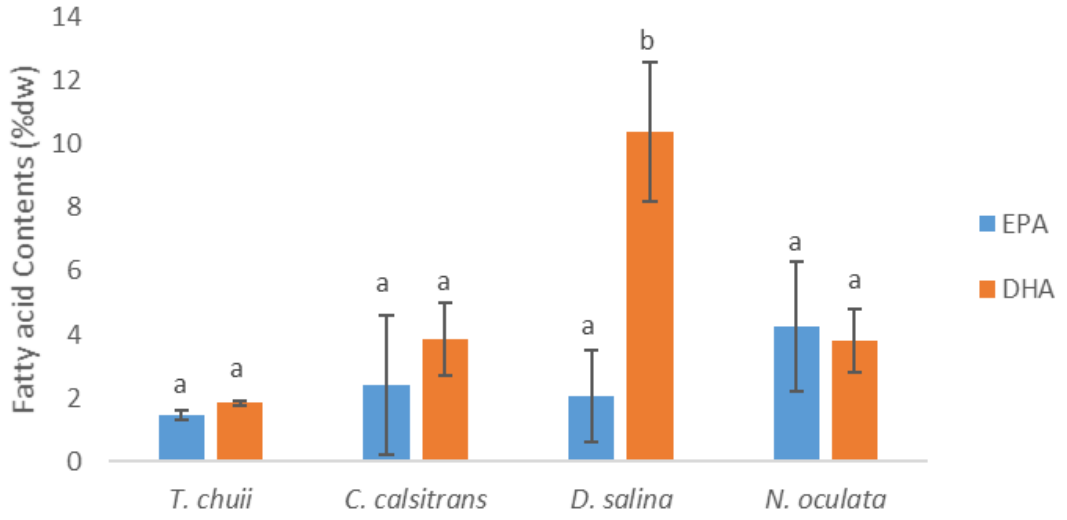

FIGURE 3. Composition of eicosapentaenoic acids and docosahexaenoic acids in the body mass of *Artemia fransiscana* fed with different microalgal species for 12 days. The fatty acids content was calculated based on the dry weight. Values are the average and bars are standard deviations of triplicate units. Different letters indicate that there was a significant difference in fatty acid content at $p<0.05$

GROWTH RATE

The total length of *Artemia* is presented in Figure 4(a). In general, there was a significant difference ($p<0.05$) in the total length of *Artemia* receiving different diets. The highest total length was obtained from the *Artemia* group fed on *Tetraselmis chuii* (6.23 mm), whereas the lowest length was observed in *Artemia* receiving *Dunaliella salina* (2.79 mm).

Similarly, the microalgal diets significantly affected the specific growth rate (SGR) of *A. fransciscana*

calculated based on dry weight (F=11.42, df 3,8, p=0.003). As presented in Figure 4(b), the highest SGR was obtained from *Artemia* fed on *T. chuii* (45.33 µg/day), although there was a significant difference with that of *Artemia* receiving *C. calcitrans* (0.41 µg/day). Meanwhile, the lowest SGR was obtained from those *Artemia* receiving *N. oculata* which was 0.25 µg/day.

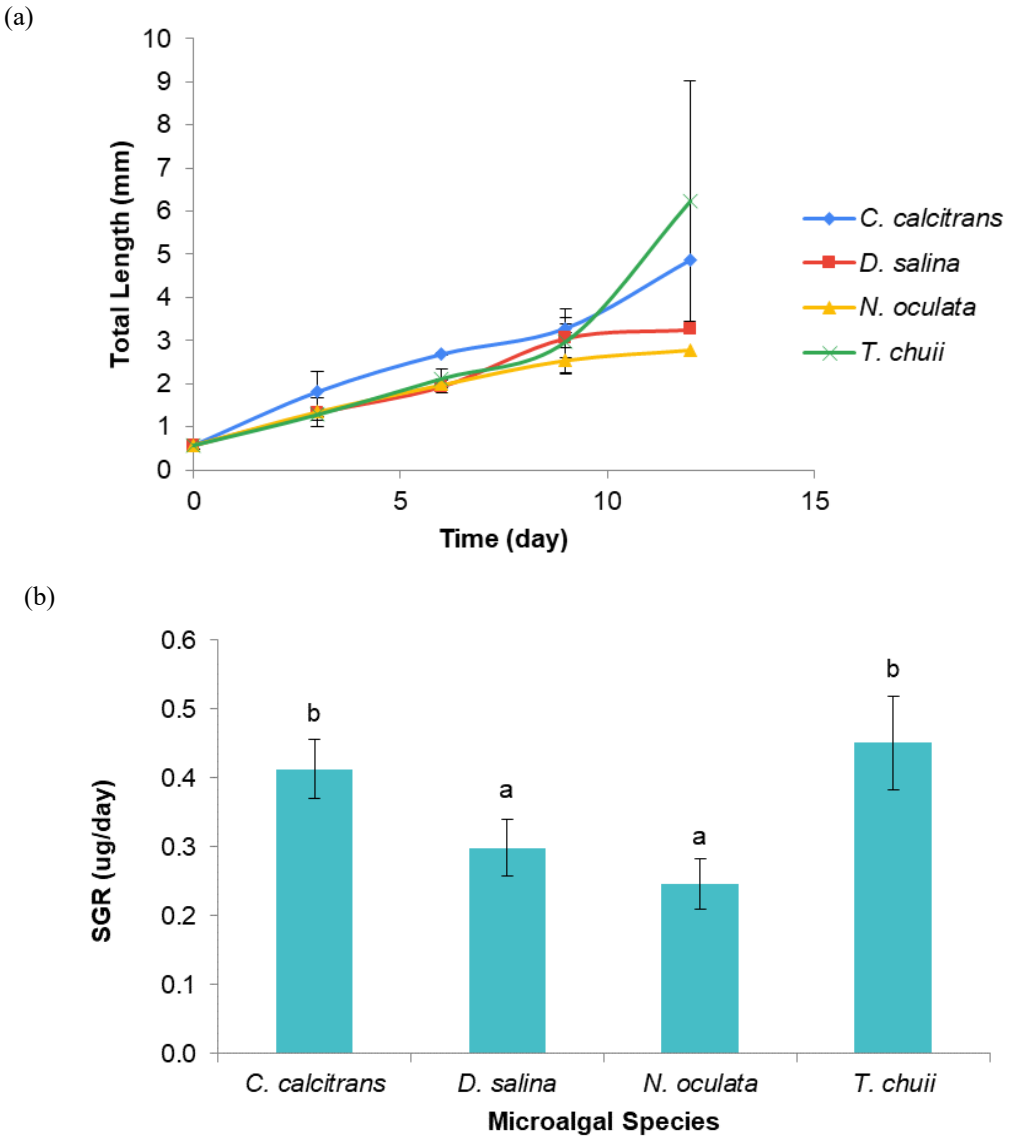

FIGURE 4. Total length (a) and specific growth rate (b) of *Artemia franciscana* fed with four different microalgae for 12 days. Values are the average and bars are standard deviations of triplicate units. Different letters indicate that there was a significant difference in survival rate at $p<0.05$

Other results also showed that *Artemia* fed on *Tetraselmis chuii* started to breed on day 10, and eggs production was observed on day 12. As presented in Figure 5, about 40 eggs have been produced in the egg sacs. No egg was observed on those *Artemia* fed on the other three microalgal species (*C. calcitrans*, *D. salina,* & *N. oculata*).

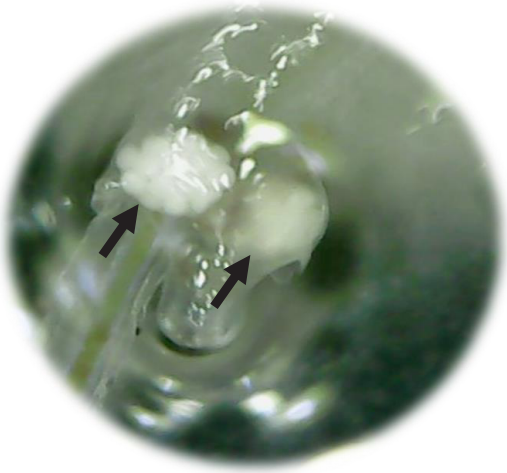

FIGURE 5. Eggs observed in egg sacs of *Artemia fransiscana* after 12 day of feeding with *Tetraselmis chuii*

SURVIVAL RATE

There was a significant difference in the survival rate (SR) of *A. fransiscana* fed with different microalgal (F=10.26, df 3,7, p=0.006). As presented in Figure 6, high survival rates were obtained from those *Artemia* feed on *C. calcitrans* and *T. chuii* (~36% and 45%, respectively), followed by *D. salina* at ~16%. The lowest SR was observed in *Artemia* received *Nanochloropsis oculata*, ~10%.

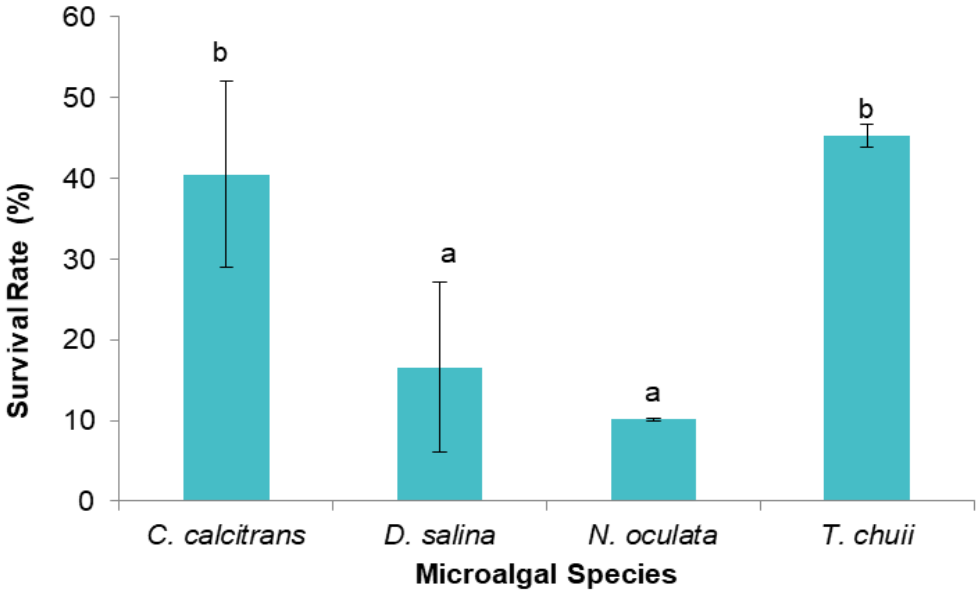

FIGURE 6. The survival rate of *A. franciscana* fed with different microalgal species for 12 days. Values are the average and bars are standard deviations of triplicate units. Different superscripts indicate that there was a significant difference in survival rate at $p<0.05$

DIET CONCENTRATION AND WATER QUALITY

The diet concentrations measured in the rearing water of *Artemia franciscana* among the treatments were not significantly different ($p>0.05$). As presented in Table 1, microalgal concentration was calculated at ~4.61, 4.59, 4.54, and 3.99 log unit cells/mL for the rearing water of *C. calcitrans, D. salina*, *N. oculata,* and *T. chuii*, respectively.

All water quality parameters measured in the present study including dissolved oxygen, temperature, pH, salinity, ammonia, and nitrite were not significantly different among treatments ($p>0.05$). The results also showed that all water quality, in general, was in the optimal range for the growth of *Artemia*, except for the concentration of ammonia and nitrite. Dissolved oxygen in the rearing medium was around 4.76 - 4.90 mg/L and tended to be stable during the maintenance period. Likewise, the temperature was in the range of 28 - 29 °C, and the pH was 7.8 - 7.9. Ammonia and nitrite appeared to be slightly high in all treatments (Table 1).

TABLE 1. Average values of water quality parameters including temperature, dissolved oxygen, pH, salinity, ammonia and nitrite measured in the rearing water of *A. fransciscana*

Parameters	Average ± St.dev			
	C. calcitrans	*D. salina*	*N. oculata*	*T. chuii*
DO (mg/L)	4.90 ± 0.39	4.76 ± 0.32	4.85 ± 0.53	4.82 ± 0.46
Temperature (°C)	29.26 ±0.31	29.20 ± 0.37	29.18 ± 0.34	29.22 ± 0.37
pH	7.91 ± 0.02	7.87 ± 0.01	7.91 ± 0.02	7.89 ± 0.02
NH_3 (ppm)	0.18 ± 0.11	0.18 ± 0.11	0.15 ± 0.10	0.18 ± 0.11
NO_2 (ppm)	1.07 ± 0.92	1.28 ± 0.31	0.93 ± 0.88	0.29 ± 0.25
Salinity (ppt)	38.38 ±1.77	38.38 ± 1.68	38.48 ± 1.77	38.81 ± 2.04
Microalgae (Log cells/mL)	4.61 ± 0.40	4.59 ± 0.49	4.54 ± 0.29	3.99 ± 0.28

DISCUSSIONS

Artemia is relatively poor in essential polyunsaturated fatty acids (PUFAs) particularly eicosapentaenoic acid (EPA) and especially docosahexaenoic acid (DHA) (Sorgeloos et al. 2001). On the other hand, EPA and DHA are regarded as the most important n3 PUFAs for many marine larval species. Thus, it is common practice to enrich *Artemia* spp. before it being used as a live prey for larvae in the last few decades (Francis et al. 2019; Méndez-Martínez et al. 2018; Roo et al. 2019; Sorgeloos et al. 2001). Up to now, there have been several formulations of PUFA enrichment methods that are commercially produced. However, due to several issues such as the shorter-shelf life, there has been a growing interest in marine microalgae which have high PUFAs content. The high content of the essential fatty acids in some microalgae has made them excellent diets for boosting the fatty acid content of live food *Artemia*. The present study reported fatty acid profiles, growth and survival rate of *A. franciscana* fed with four different microalgal species: *Chaetoceros calcitrans*, and *Nannochloropsis oculata*, *Dunaliella salina,* and *Tetraslemis chuii*. The result indicated that the dietary sources significantly affected DHA, specific growth rate and survival rate of *A. franciscana* after being cultured for 12 days. *Artemia* fed with *D. salina* had the highest levels of DHA (10.37% dw) followed those *Artemia* fed on *C. calcitrans, N. oculata,* and *T. chuii*. The result might be due to the high content of DHA content in *D. salina* (Lora-Vilchis et al. 2004). Similarly, Millamena et al. (1988) confirmed that *D. salina* was rich in n-3 series of fatty acids, especially 18:3 (n-3), which might explain the high DHA accumulation in the *Artemia* biomass. Lower EPA and DHA contents obtained in *Artemia* fed on *C. calcitrans* and *T. chuii* might be due to the lower content of EPA and DHA in these microalgal compared to that of *D. salina* (Chakraborty et al. 2007). DHA contents of *A. franscisana* obtained in the present study were higher than previous studies which were 9.10-10.20 ppm (Balachandar & Rajaram 2019). These results confirm previous studies suggesting that microalgal diets could be used to enhance fatty acid profiles in *Artemia.*

Furthermore, the microalgal diets had also significant effects on the growth and survival rate of

A. fransiscana. The highest specific growth rate and survival rate were obtained from those *Artemia* receiving *T. chuii* and *C. calcitrans*. While the lowest growth was observed in that of *Artemia* receiving *N. oculata* or *D. salina*. This result might be due to the higher protein content of *C. calcitrans* and *T. chuii,* which are 43 and 54% (El-Sayed et al. 2020), respectively, compared to 35% in *Nanochloropsis* (Banerjee et al. 2011), and 40% in *D. salina* (Dineshbabu et al. 2019). The average length of *A. fransciscana* obtained in the present study was the same as previously reported by Gómez et al. (1999) with the same microalgal diets and the same culture period. However, our result was higher than in previous studies. Average lengths of *A. franciscana* measured in the present study were 6 mm and 4.7 mm for *Artemia* fed on *T. chuii* and *C. calcitrans,* respectively. While the average length of *Artemia* with the same culture periods (12 days) was 3-4 mm (Dineshbabu et al. 2019). However, the present study showed contradictory with several previous studies with the same type of experiments. Sick (1976), for instance, reported that *A. franciscana* had the highest growth rate when they were fed *Dunaliella viridis*. Similarly, Mohebbi et al. (2016) described that *Dunaliella* sp. gave the best growth of *Artemia urmiana* while showed the lowest growth with *Tetraselmis suecica*. It was explained that *Dunaliella* sp. gave better growth for *Artemia* because the microalgae had a high protein, energy content (Dineshbabu et al. 2019), and total DHA and EPA (Chakraborty et al. 2007) as well as lacking a cell wall (Sick 1976). The different results might be due to the difference in *Artemia* species used for studies. Mohebbi et al. (2016), for instance, used *Artemia umima*, while the present study used *A. fransciscana*. The different species can have different abilities in different microalgal digestibility.

The present study also showed that *Artemia* receiving *T. chuii* produced eggs faster than *Artemia* fed on the other three microalgae. It was observed that the *Artemia* fed on *T. chuii* started mating on day 10 and day 12, where eggs had been observed in some female yolk sacs. The same result was previously reported by Balachandar and Rajaram (2019) where *A. franciscana* was started to produce nauplii on day 12. However, the growth of *Artemia* in this study is also faster compared to a study by Rode et al. (2011) which took 23 days in a wild condition. This happens might be due to faster growth and better environmental conditions including diet quantity and quality.

Results obtained in the present study can be as an additional source on how to enhance nutrient content especially EPA and DHA content as well as to increase the growth and survival rate of *Artemia fransciscana*. *Artemia* biomass which is high in EPA and DHA is not only good for fish and crustacean larviculture, but also fasten gonadal maturity in shrimp broodstock (Dineshbabu et al. 2019; Kaparapu 2018). In addition, these results can be used to culture *Artemia* for broodstock and produce *Artemia* nauplii for larviculture. This approach can reduce the high dependency of fish and crustaceans' hatcheries on *Artemia* cyst import. The future direction of studies would be highly recommended to blend the range of microalgal species to achieve the highest PUFAs and growth. A selected mixture of microalgae such as *D. tertiolecta* with a high PUFAs content and *T. chuii* with gave highest SGR and survival rate can offer an excellent nutritional package for various larvae. The use of the right size of on-grown *Artemia* for feeding ensures a better energy balance in food intake and assimilation, thereby improving the performance of the fish.

Conclusion

Microalgal diets had a significant effect on fatty acid profiles, the growth and survival rate of *Artemia fransciscana*. *Dunaliela salina* yielded the highest docosahexaenoic acids (DHA) in the *Artemia* biomass. However, *Chaetocero calcitrans* and *Tetraselmis chuii* gave the best growth and survival rate of *Artemia franciscana*. Based on the different results in terms of fatty acid content and growth performances, it is highly recommended to investigate a combination of more than one microalgal species, for instance, *T. chuii* and *D. salina*, to obtain higher growth and survival rate as well as increase PUFAs content.

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