Aquaculture Studies, 25(1), 40-46, AQUAST1995
http://doi.org/10.4194/AQUAST1995

RESEARCH PAPER

The Effects of Formulated Diet Inclusion on Growth and Reproductive Performances of *Artemia franciscana*

Muhamad Amin[1,*], Mahfudz Dzotul Hasanah[1], Akhmad Taufiq Mukti[1], Mochammad Amin Alamsjah[1], Dita Wisudyawati[1], Laila Musdalifah[2]

[1]Universitas Airlangga, Faculty of Fisheries and Marine, Department of Aquaculture, Surabaya-East Java, Indonesia, 60115.
[2]National Research and Innovation Agency of the Republic of Indonesia, Research Center for Fishery, Jakarta, Indonesia, 10340.

How to Cite

Amin, M., Hasanah, M.D., Mukti, A.T., Alamsjah, M.A., Wisudyawati, D., Musdalifah, L. (2025). The Effects of Formulated Diet Inclusion on Growth and Reproductive Performances of *Artemia franciscana*. *Aquaculture Studies*, *25(1), AQUAST1995*. http://doi.org/10.4194/AQUAST1995

Article History
Received 29 June 2024
Accepted 26 December 2024
First Online 27 December 2024

Corresponding Author
E-mail: muhamad.amin@fpk.unair.ac.id

Keywords
Brine shrimp
Microalgae
Formulated diet
Growth
Fecundity

Abstract

Artemia is an important live diet for shrimp and fish larvae in Indonesian hatcheries, yet its supply is highly dependent on imported products. Few hatcheries have been trying to culture and produce Artemia nauplii indoors. However, the artemia diet such as *Tetraselmis chuii* is very costly and laborious in preparation. Thus, this study aimed to investigate the effect of microalgal substitution with a formulated diet on the growth, fecundity, nauplii production, and survival rate of *A. franciscana*. A formulated diet (48% crude protein) was prepared and tested in a completely randomized design with five treatments (T1: 100% *T. chuii* as control, T2: 75% *T. chuii* and 25% formulated diets, T3: 50% *T. chuii* dan 50% formulated diets, T4: 25% *T. chuii* dan 75% formulated diets, T5: 100% formulated diets), and four replications. The results showed that the formulated diet can substitute microalgal diet up to 50% without significantly affecting the growth, fecundity, nauplii production, and survival rate of *A. franciscana*, P<0.05. These data are useful to improve *A. franciscana* culture to produce artemia biomass for feeding larvae of marine species.

Introduction

Artemia franciscana, commonly known as brine shrimp, is one of the most important live diets for fish and shrimp aquaculture. *A. franciscana* nauplii, for instance, is widely used for fish and shrimp larvae (Dhont et al., 1993). While adult *Artemia* is often used to accelerate gonad maturation (Adloo et al., 2020). However, Artemia supply in Indonesia has been highly dependent on cysts imported from countries such as Belgia or the United States of America. As a consequence, the price of Artemia becomes very expensive and sometimes difficult to find.

One way to reduce the dependency and availability issues of the exported artemia cysts is by culturing the artemia cysts to brood stock and producing artemia nauplii. Few studies have reported successfully culture Artemia and producing artemia nauplii (Amin et al., 2022; Amin et al., 2023). Nonetheless, the main challenge faced is the use of microalgae as a live diet. The use of microalgae is not only costly due to requiring expensive fertilizers to grow, but also laborious work (Amin et al., 2022). Thus, a solution for this issue such as the development of a formulated diet is an important research topic to perform.

Previous studies reported that the best live diet for artemia culture is *Tetraselmis chuii* (Amin et al., 2022). The nutrient content in *Tetraselmis chuii,* is 48.42% protein, 12.10% carbohydrates, and 9.70% fat (Brown et al., 1997). The nutrient content of *T. chuii* has been used as a basic idea to develop a formulated diet of Artemia culture. With comparable nutritional content, more affordable prices, and easy accessibility, a formulated diet may become a good alternative for artemia culture.

Acknowledging the issue, the present study aimed to investigate the effect of microalgal substitution with a formulated diet on the growth, fecundity, nauplii production, and survival rate of *A. franciscana*.

Materials and Methods

Experimental Design

The present study used a completely randomized design (CRD) consisting of five treatments (T1: 100% *T. chuii* as control, T2: 75% *T. chuii* and 25% formulated diet, T3: 50% *T. chuii* dan 50% formulated diet, T4: 25% *T. chuii* dan 75% formulated diet, T5: 100% formulated diet) with four replications of each.

Preparation of Formulated Diet

Formulated diet was prepared using a solver, an additional tool in Excel software as previously described by Amin et al. (2023). In brief, the raw materials used consisted of fish meal, rice bran, fish oil, tofu processing waste, and a vitamin and mineral premix. The raw materials, including the fish meal, rice bran, and tofu processing waste, were previously ground into a fine powder and sifted through a mesh size of 80 to achieve a homogeneous particle size of approximately 170 micrometers. The final nutritional profile was adjusted to 48% crude protein, 6.16% crude fat, and 18.95% crude carbohydrate. The proportions of each raw material are presented in Table 1.

Preparation of *Tetraselmis chuii*

T. chuii was prepared according to a protocol previously described by Amin et al. (2022). In brief, 500 ml seawater in a 1,000 ml Erlenmeyer flask was sterilized by putting it in an autoclave (121˚C for 15 min). After cooling, fertilizers containing 0.25 ml of Walne (0.5 ml/L) and 0.5 ml of vitamins (1 ml/L) were added to the sterilized seawater. Thereafter, *T. chuii* inoculant was added to the seawater according to the calculation results (final concentration was 1.0 x 10^5 cells/ml).

$$V1 * N1 = V2 * N2$$

Where; V1 = Volume of seeds for initial stocking (ml), N1 = Cell density of *T. chuii* inoculation/ (cells/ml), V2 = Desired volume of culture medium (ml/l), N2 = Desired stocking density (cells/ml)

Then, *T. chuii* culture was carried out for 4-5 days to obtain a density of ~10^6 cells/ml. The growth of *T. chuii* was monitored daily by taking a 1 ml sample in the culture container using a pipette and counting using a hemocytometer under a binocular microscope (Olympus-CX21FSI Japan), and a hand counter. The population density was calculated using the following formula.

$$\text{Number of cells/ml} = \frac{\text{Total number of cells in 4 blocks}}{\text{Total blocks (4)}} \text{ X10.000}$$

Calculation of Diet Biomass

The total diet was calculated based on dry weight in order to have the same feeding rate among treatments (0.269 mg.nauplii^{-1}.day^{-1} or 80.7 mg.day^{-1} for 300 Artemia nauplii (Amin et al., 2023)

Feeding Trial

Artemia was first decapsulated as previously described by Amin et al. (2023). In brief, Artemia cysts (2 gr) were weighed using an analytical balance (CNME060708-5) and placed into a 5-litre jar previously filled with freshwater for dehydration for 2 hours. Thereafter, the dehydrated cyst was immersed in 5 ml chlorine and stirred using a spatula for 10-15 min until the color of the cyst changed to light brown. Then, the cysts were filtered out with a 120-micron plankton net and washed using running water. The cysts were placed into a decapsulation container containing 4 liters of seawater with a salinity of 33 ppt. During the decapsulation process, aeration was provided to maintain adequate dissolved oxygen levels. After 24 hours, the Artemia cysts hatched, and the resulting Artemia nauplii were harvested for use in subsequent experiments.

Artemia nauplii were manually counted in a petri dish and stocked into a 4L -plastic container with a density of 100 individuals/L (Mohebbi *et al.*, 2016). Then, the Artemia nauplii was fed daily according to the following treatments:

T1 : 100% *T. chuii* as the control

T2 : 75% *T. chuii* + 25% formulated diet

T3 : 50% *T. chuii* + 50% formulated diet

Table 1. Nutrient content of raw materials, and final nutrient content of a formulated diet used in Artemia culture

Raw Materials	Inclusion (g)	Nutrient content		
		Crude Protein (%)	Crude Fat (%)	Crude Carbohydrate (%)
Fish meal	69.13	40.77	4.52	4.49
Rice bran	1.00	0.11	0.15	0.34
Topu processing waste	27.37	7.54	1.35	14.12
Fish oil	1.50	0.00	0.14	0.00
Vitamin premix	0.50	0.00	0.00	0.00
Mineral Mix	0.50	0.00	0.00	0.00
Total	100 g	48.42	6.16	18.95

T4 : 25% *T. chuii* + 75% formulated diet

T5: 100% formulated diet

Nauplii Production

Broodstock culture was started from day 15 (D15) when *A. franciscana* reached the instar XV phase (this stage at which *A. franciscana* was ready for mating). Reproduction begins with mating, during which a male and female form a riding pair and swim together. The female *A. franciscana* leads, and the male *A. franciscana* "embraces" her using claspers from behind. Mating/copulation only takes a short time, but riding pairs lasts quite long. Thereafter, nauplii production was counted daily for one week.

Observer Parameters

Growth Performances

The growth measurement was conducted using two data points: the length and weight of *A. franciscana*. The body length measurement was performed by randomly sampling ten individuals from each treatment. To facilitate measurement, the samples were placed on a glass slide with minimal water content. Observations were conducted under a stereo microscope with a magnification of 80x. The required application for this measurement was Optilab Advance Plus, which was used to connect the microscope camera to the laptop screen. This application was also utilized to capture images of *A. franciscana.* The second application used was ImageJ, a tool for accurately measuring the length of *A. franciscana* through scale settings with calibration as the initial step. The individual growth of *A. franciscana*, according to Amin et al. (2023), was calculated using the formula:

$$L=(Lt-L0)$$

Where; L = Growth in length, Lt = Length growth at the end of the maintenance period, L0 = Initial length at the beginning of the maintenance period.

The specific growth rate was calculated using the formula from Lora-Vilchis *et al.* (2004) as follows:

$$SGR=\frac{(LnWt-LnWo)}{ti} \times 100\%$$

Where; SGR = Specific Growth Rate (%), Wo = Average weight at the beginning of the study (g), Wt = Average weight at the end of the study (g), ti = Culture period (day)

The weight of *A. franciscana* was measured on the first day of maintenance (instar I) and on day 15 (instar XV). The weight measurement was conducted by randomly sampling ten individuals and placing them on pre-weighed filter paper. The water on the filter paper was absorbed using blotting paper to reduce the sample's water content. The samples were weighed using an analytical balance with a precision of 0.1 mg (ADWAG AS 220-R2). The individual weight gain was calculated using the formula from Akhsin *et al*. (2014):

$$W=(Wt-Wo)$$

Where ; W= Weight gain; Wt = Weight gain at the end of the study; Wo = Weight gain at the beginning of the study.

Fecundity

Fecundity was observed by collecting Artemia brood stock with mature gonads and had spawned. A mature gonad was marked by the brown color in the uterus. Observations were made on the 5th day of brood rearing or 20 days after hatching (Baxevanis et al., 2004). The procedure for observing fecundity is to take all *A. franciscana* brood stock that has mature gonads in each treatment. Then the number of cysts or eggs in the uterus or egg sac of individual females is counted using a microscope camera.

Nauplii Production

The number of *A. franciscana* nauplii was observed by exploiting the characteristic of nauplii moving towards light (positive phototaxis). The basic principle is to place light in one corner or gap. Nauplii were gathered in areas with higher light intensity, making them easier to harvest (Panggabean, 1984). The first step was to turn off aeration for 10-15 minutes; this caused the unhatched cysts to settle at the bottom of the rearing container. The hatched cysts would float to the surface. A flashlight was turned on to make *A. franciscana* move towards the light. The counting of offspring was done manually using a hand counter.

Survival Rate

The survival rate of *A. franciscana* compares the number of *Artemia* organisms alive at the end of the study with the number of organisms alive at the beginning. The survival rate was calculated by counting all *A. franciscana* in each treatment. The survival rate formula, according to Abdel-Tawwab et al. (2010), is as follows:

$$SR=(Nt/No)\text{x}100$$

Where; SR = Survival rate (%); Nt=Total biomass alive at the end of the study; No=Total biomass alive at the beginning of the study.

Water Quality

Water quality consisted of temperature, pH, salinity, dissolved oxygen (DO), nitrate, nitrite, and ammonia. Temperature and dissolved oxygen

measurements using a DO meter (YSI-Pro20i, USA), pH using pH meter (Hanna-HI98107, Romania) salinity measurements using a refractometer (Atago, Japan), nitrate, nitrite, and ammonia measurements using an appropriate test kit (HANNA instruments). Water quality measurements were carried out on maintenance days 3, 6, 9, 12, 15, 18, 21, 24, 27 and day 30.

Data Analysis

The obtained data were analyzed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) to determine the best treatment at P<0.05 using a IBM SPSS statistic software version 23. Then, the results of the data analysis were described descriptively.

Results

Absolute Growth Performances

The growth of *A. franciscana* was presented in terms of length-specific growth rate (SGR_L) and weight-specific growth rate (SGR_W). The present study showed that the inclusion of formulated diets significantly affected the growth performances (in terms of length and weight) of *A. franciscana* (P<0.05), but not reproductive performance and survival rate, (P>0.05).

Specific Growth Rate

The best growth was obtained from *A. franciscana* receiving 100% *T. chuii*, in which average length reached 9.13±0.23 mm, and total weight reached 3.24±0.18 mg, Table 2. Additionally, the specific growth rate in terms of length (SGR_L) reached 17.98±0.36% BL/day, and in terms of weight (SGR_W) reached 35.36±0.36% BW/day. Substitution of 25% microalgae with formulated diet (T2) resulted in growth one level lower than the control. SGR_L in P2 and P4 did not significantly differ from the control treatment (P<0.05), suggesting that formulated diets could replace microalgae by 75% based on SGRL data. The treatment of formulated diets resulted in slightly lower growth but did not significantly differ from the control treatment. This is because the similar nutritional content in the formulated diets still meets the dietary requirement of *A. franciscana*. The growth of *A. franciscana* is influenced by the nutritional content and the environmental conditions in the rearing media. This can be minimized by siphoning and periodic water changes to maintain water quality in the rearing media.

Fecundity and Nauplii Production

Artemia receiving 100% *T. chuii* feed (T1) provided the highest fecundity, ranging from 34 - 37 eggs per brood, but not significantly different from Artemia fed on 25% microalgal diet substitution with formulated diet, ranging from 31 to 33 eggs per brood. A lower fecundity was observed from those Artemia in T3 (28 eggs), T4 (25 eggs), and T5 (22 eggs), Table 3.

The best treatment resulting in a high average number of nauplii was the provision of 100% *T. chuii* feed (P1), where the number of hatched offspring per brood ranges from 27-29 nauplii per brood. P1 and P2 did not significantly differ, suggesting that formulated diets could replace 50% of microalgae as *A. franciscana* feed.

Survival Rate

The results showed in general that substitution of microalgal diet with the formulated diet had no significant effect on the survival rate of *A. Franciscana*, P>0.05. As presented in Figure 1, the survival rate was considered high enough since all treatments had >90%.

Table 2. Growth performances initial weight, final weight, absolute growth, and specific growth rate of *A. franciscana* fed with different types of diets for a 15-day culture period

Parameters	Treatments				
	T1	T2	T3	T4	T5
IL (mm)	0.62±0.05	0.62±0.05	0.60 ±0.05	0.63±0.03	0.61±0.04
FL (mm)	9.13±0.23	7.94±0.12	7.43±0.18	7.42±0.24	6.73±0.19
ΔL (mm)	$8.51±0.28^{a}$	$7.32±0.16^{b}$	$6.83±0.20^{c}$	$6.79±0.23^{c}$	$6.12±0.21^{d}$
SGR_L(bl/d)	$17.99±0.71^{a}$	$16.97±0.59^{a,b}$	$16.75±0.67^{a,b}$	$16.43±0.35^{b}$	$16.01±0.58^{b}$
Iw (mg)	0.02	0.02	0.02	0.02	0.02
Fw (mg)	3.24±0.18	2.79±0.06	2.64±0.13	2.55±0.09	2.46±0.11
ΔW (mg)	$3.22±0.18^{a}$	$2.77±0.06^{b}$	$2.62±0.13^{b,c}$	$2.53±0.07^{b,c}$	$2.44±0.10^{c}$
SGRw (%bw/d)	$33.91±0.36^{a}$	$32.91±0.15^{a,b}$	$32.54±0.31^{a,b}$	$32.32±0.24^{b}$	$32.08±0.30^{b}$

Table 3. Fecundity of *A. franciscana* receiving different types of diets

Treatments	Fecundity (eggs/broodstock) Average ± SD	Nauplii production (nauplii/Brood) ± SD
T1	$35.00±1,41^{d}$	$27.75± 0.96^{d}$
T2	$31.50±1.00^{cd}$	$24.50±1.29^{cd}$
T3	$28.50±1.29^{bc}$	$21.25±1.71^{bc}$
T4	$25.00±0.82^{ab}$	$18.75±082^{ab}$
T5	$21.75±3.30^{a}$	$15.25±3.59^{a}$

Water Quality

Water quality parameters appeared to be the same among treatments, Table 4. Dissolved oxygen (DO), for instance, was recorded at ~8 mg/l which is within the tolerance range for *Artemia* to grow and reproduce. Furthermore, nitrite concentration was recorded to be less than 0.025 mg/L. However, ammonia concentration appeared to be quite different, ranging from <0.025 mg/L in the control (T1), increased to 0.14 mg/L in T2, 0.17 mg/L in T3, 0.5 mg/L in T4, and up to 0.62 mg/L in T5.

Discussion

One way to reduce the dependency on exported artemia cysts is by culturing Artemia and producing artemia nauplii. However, preparing live diets such as *Tetraselmis chuii* or *Chaetoceros calcitrans* in Artemia culture is very costly and laborious work (Amin et al., 2022; Amin et al., 2023). Thus, the present study developed a formulated diet to replace the microalgal diet and investigated its effect on the growth, fecundity, nauplii production, and survival rate of *A. franciscana*. The results, in general, showed that the formulated diet was able to substitute 50% of the microalgal diet without significantly affecting growth performance (SGR_L), reproductive performances (egg and nauplii production), and the survival rate of *A. franciscana*, $P<0.05$.

The final length of *A. franciscana* after a 15-day culture period with 50% inclusion of formulated diet was 7.43 mm. The total length was slightly higher compared to a study by Tampubolon et al. (2020), in which the total length of Artemia was only 5.22 mm with the same culture period (15 days). A study by Turcihan et al. (2021) also showed that the final length of *A. franciscana* was only ~2.5 mm after being cultured for 15 days and fed either single or a combination of *Amphora viridis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, or *Dunaliella salina*. The present study shows that the final length of the present study was comparable total length to a study of Shanmugam Balachandar and Rajendran Rajaram (2019) which was about 7.29 mm when the *A. franciscana* was fed with *Nanochloropsis* sp. According to Turcihan et al. (2021), the growth of Artemia is highly determined by the size and nutritional contents of the diet. As a filter feeder (Fernández, 2001), Artemia commonly eats at small particles (7- 28 µm), but the optimum size is described as 16 µm. Besides particle size, the growth of *A. franciscana* was highly affected by the protein content of the diet. The formulated diet developed in the present study was in fine powder and protein content was adjusted to be similar to the microalgal diet in the control (~48%) (Brown et al., 1997). The developed

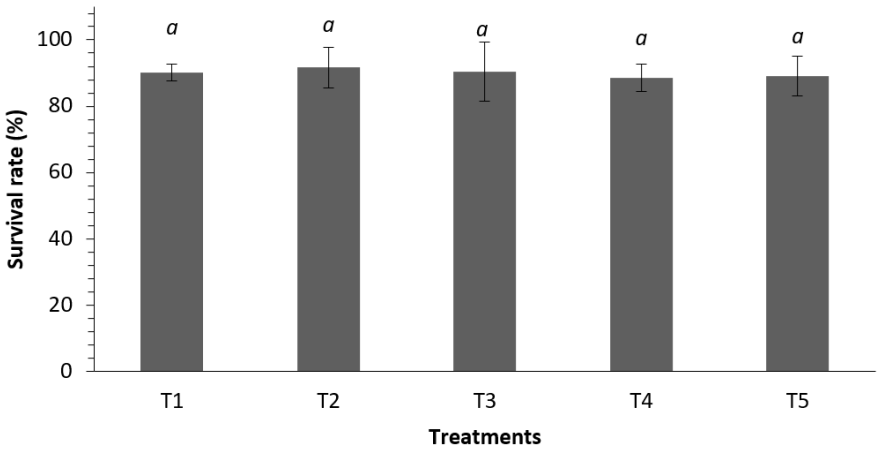

Figure 1. Survival rate of *A. franciscana* fed with different feeding types for 15 days. The same superscript indicates no significant differences (P>0.05). T1 is Artemia fed with 100% *T. chuii*; T2 is Artemia fed with 75% *T. chuii*+25% formulated diet; T3 is Artemia fed with 50% *T. chuii*+50% formulated diet; T4 is Artemia fed with 25% *T. chuii*+75% formulated diets; T5 is Artemia fed with 100% formulated diet.

Table 4. Water quality during the 15day-culture period of Artemia

	Average ± Stdev				
Parameters	T1	T2	T3	T4	T5
Temperature (°C)	28.26±0.81	28.76±0.87	28.65±0.74	28.77±0.91	28.94±0.92
Salinity (ppt)	38.89±0.57	39.00±0.00	39.00±0.00	39.00±0.00	39.00±0.00
DO (mg/l)	8.24±0.21	8.19±0.29	8.26±0.28	8.14±0.52	8.18±0.46
pH	7.09±0.09	7.09±0.09	7.04±0.07	7.03±0.07	7.03±0.07
Nitrate (mg/l)	<0.025	<0.025	<0.025	<0.025	<0.025
Nitrite (mg/l)	<0.025	<0.025	<0.025	<0.025	<0.025
Ammonia (mg/l)	<0.025	0.14±0.27	0.17±0.39	0.50±0.50	0.62±0.44

formulated diet appears to be quite suitable as a diet for Artemia by showing good growth although still at 50% inclusion. Subamia et al. (2017) stated that diet nutritional content significantly influences *Artemia's* growth. In terms of specific growth in terms of length (SGR_L) at the 50% microalgal diet replacement was recorded at 16.75%BL/day, and in terms of weight was 32.54%BW/day.

The present study also indicated that 50% replacement of *T. chuii* with a formulated diet did not significantly affect fecundity and nauplii production of *Artemia*, with a value of 27-30 eggs/brood and 19-22 nauplii/brood. The present result was higher compared to the fecundity of *A. francicana* previously reported by Shanmugam Balachandar and Rajendran Rajaram (2019), which ranged from 10 – 25 eggs/brood when they were fed with either *Tetraselmis* sp. *Isochrysis* sp., *Thallassiosira* sp., rice bran, or soybean meal. The higher fecundity of *A. franciscana* in some treatments is presumed to be due to faster gonad maturation, resulting in higher fecundity of the broodstock (Widhiyanto, 2006). The yolk strongly influences egg hatching, which contains essential substances the embryo needs to hatch. The yolk content is affected by the energy from the nutritional intake of the *A. franciscana* broodstock (de Courcelles & Kondo, 1980). Similarly, the number of nauplii produced by Artemia brood fed with a 50% formulated diet was not significantly different compared to the control.

The present result also indicated that the 50% inclusion of a formulated diet had a good survival rate (>90%). This result was higher compared to a study by Sivaji (2016) which was only 35%-80%, and a study result by Putra et al. (2018) which was about 86%. The survival rate obtained in the present study was also higher compared to the survival rate by a study by Shanmugam Balachandar and Rajendran Rajaram (2019) which was 52-73% when *A. franciscana* was fed with either *Tetraselmis* sp., *Isochrysis* sp. *Chaetoceros* sp. or *Nanochroropsis* sp. In this study, one of the highest survivals was found in the Artemia group fed *C. vulgaris*. This could be related to the antioxidant effect of these microalgae (Turcihan et al., 2021).

However, the present study also indicated that >50% replacement of the microalgal diet with the formulated diet reduced growth performance and reproductive performance. The present result may be subjected to the water quality during the culture period. According to Vos and de la Rosa (1980), environmental factors including temperature, dissolved oxygen (DO), salinity, and pH may affect the growth of cultured organisms. Similarly, Alcaraz et al. (1999) reviewed that ammonia and nitrite are toxic compounds to aquatic animals including artemia. Ammonia (NH_3) in rearing water of T3 (75% microalgal replacement with formulated diet), and T5 (100% formulated diet) was higher compared to T0 or control, T1, and T2. The average ammonia concentrations in T4 and T5 were recorded at 0.5 mg/L and 0.62 mg/L respectively, compared to control (0.025mg/L), T1 (0.14 mg/L), and T2 (0.17mg/L). The values suggest that the higher the replacement of the microalgal diet with the formulated diet, the higher the ammonia concentration in the rearing water. This might be due to the formulated diet being degraded easily and decomposed to produce dissolved toxic ammonia in the rearing water. According to Sorgeloos (1980), the Ammonia concentration in rearing water of Artemia culture should be less than 0.1 mg/L. As stated by Schram et al. (2010), ammonia at higher concentrations may have an acute effect or kill the culture organism, or at lower concentrations may have a chronic effect in which it may reduce the growth of culture animals.

Meanwhile, other water quality parameters including temperature, pH, salinity, and dissolved oxygen level were all at optimal values for Artemia culture. The optimal temperature for the growth of *A. franciscana* is 25-30 $^{\circ}$C (Trisnabatin et al., 2021), and the temperature recorded during this experiment was ~28 $^{\circ}$C in all treatments. Salinity during maintenance was 37 ppt, which is the optimum range for *A. franciscana*. Biomass culture of Artemia sp. which is good at a salinity of 30-50 ppt (Isnansetyo & Kurniastuty, 1995). The dissolved oxygen content in all maintenance media is at high DO conditions, namely >7 mg/L. DO concentration is good for the growth of Artemia sp. is more than 5 ppm (Van Hoa et al., 2011). According to Sorgeloos (1980), the pH of the maintenance medium is good for the growth of *Artemia* sp. ranging from 7 to 8.5. In this study, the pH for maintenance was between 7 – 7.3 so it was still considered normal.

Conclusion

The substitution of microalgae with formulated diets can be up to 50% without significantly affecting growth performance, reproductive performances (egg and nauplii production), and the survival rate of *A. franciscana*. With 50% substitution, specific growth in terms of length (SGR_L) was recorded at 16.75%BL/day, and in terms of weight (SGRW) was 32.54%BW/day. Average fecundity and nauplii production of *Artemia*, were recorded at 27-30 eggs/brood and 19-22 nauplii/brood, with survival rate >90%. These study results may be useful knowledge to improve *A. franciscana* culture and produce artemia biomass for live aquafeeds.

Ethical Statement

The study requires no ethic statement.

Funding Information

The present research was financially supported by LPDP Republic of Indonesia under grand number: 1804/B/UN3.LPPM/PT.01.03/2024.

Author Contribution

M. Amin, M.A. Alamsjah, A.T. Mukti., Y. Cahyoko: Conceptualization, Data curation, Formal analysis, Writing original draft. M. Hasanah, L. Musdalifah, D. Wisudyawati, A.T. Mukti., Y. Cahyoko: Data curation, Methodology, Investigation, ms preparation.: Data curation and Experiment setup. M. Amin, L. Musdalifah, D. Wisudyawati, Y. Cahyoko A.T. Mukti: Supervision and Data analysis. M.A. Alamsjah, M. Amin: Funding acquisition, Investigation, Supervision, Writing - review & editing.

Conflict of Interest

Hereby declare that there were no conflicts of interest among all authors when writing and publishing the manuscript.

Acknowledgements

The authors would like to thank colleagues at Laboratory of Fish Nutrition, Faculty of Fisheries and Marine, Universitas Airlangga, who have given support and advice during the experiments.

References

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. (2010). *Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.)*. Aquaculture, 298(3-4), 267-274.

Adloo, M. N., Agh, N., Salarzadeh, A., & Bahri, A. (2020). *The effect of lipid-enriched Artemia franciscana on reproductive performance of broodstock and larval quality of Pacific white shrimp Litopenaeus vannamei*. Iranian Journal of Fisheries Sciences, 19(4), 1928-1943.

Alcaraz, G., Chiappa-Carrara, X., And, V. E., & Vanegas, C. (1999). *Acute toxicity of ammonia and nitrite to white shrimp Penaeus setiferus postlarvae*. Journal of the world aquaculture society, 30(1), 90-97.

Amin, M., Erwinda, M., Nissa, M., Nindarwi, D. D., HSetyantini, W., & Alamsjah, M. A. (2022). *Fatty Acids Profiles and Growth Performances of Artemia franciscana Fed with Different Types of Microalgae*. Sains Malaysiana, 51(8), 2449-2459.

Amin, M., Intan, B., Putri, M. A. R., Mukti, A. T., & Alamsjah, M. A. (2023). *Effect of protein sources in formulated diets on growth performance, feed utilization, survival rate, and reproductive performance of Artemia franciscana*. Aquaculture International, 31(4), 1893-1910.

Baxevanis, A. D., El-Bermawi, N., Abatzopoulos, T. J., & Sorgeloos, P. (2004). *Salinity effects on maturation, reproductive and life span characteristics of four Egyptian Artemia populations (International Study on Artemia. LXVIII)*. Hydrobiologia, 513, 87-100.

Brown, M., Jeffrey, S., Volkman, J., & Dunstan, G. (1997). *Nutritional properties of microalgae for mariculture*. Aquaculture, 151(1-4), 315-331.

de Courcelles, D. d. C., & Kondo, M. (1980). *Lipovitellin from the crustacean, artemia salina. Biochemical analysis of lipovitellin complex from the yolk granules*. Journal of biological Chemistry, 255(14), 6727-6733.

Dhont, J., Lavens, P., & Sorgeloos, P. (1993). *Preparation and use of Artemia as food for shrimp and prawn larvae*. CRC handbook of mariculture, 1, 61-93.

Fernández, R. G. (2001). *Artemia bioencapsulation I. Effect of particle sizes on the filtering behavior of Artemia franciscana*. Journal of Crustacean Biology, 21(2), 435-442.

Isnansetyo, A., & Kurniastuty, E. (1995). *Teknik Kultur Phytoplankton dan Zooplankton*. Pakan Alami untuk Pembenihan Organisme Laut. Penerbit Kanisius, Yogyakarta.

Panggabean, M. G. L. (1984). *Teknik penetasan dan pemanenan Artemia salina*. Jurnal Oseana, 9(2), 57-65.

Putra, D., Trisyahdar, T., Dewiyanti, I., & Muhammadar, A. (2018). Effect of enhanced Artemia with gamat emulsion on growth performance and survival rate of white shrimp Litopenaeus vannamei larvae. IOP Conference Series: Earth and Environmental Science,

Schram, E., Roques, J. A. C., Abbink, W., Spanings, T., de Vries, P., Bierman, S., Flik, G. (2010). *The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus)*. Aquaculture, 306(1), 108-115. https://doi.org/https://doi.org/10.1016/j.aquaculture.2010.06.005

Shanmugam Balachandar, S. B., & Rajendran Rajaram, R. R. (2019). *Influence of different diets on the growth, survival, fecundity and proximate composition of brine shrimp Artemia franciscana (Kellog, 1906)*.

Sivaji, S. (2016). *Evaluation of different feeds for the culture of Artemia parthenogenetica*. Advance Research Journal of Medical and Clinical Sciences, 2(3), 8-14.

Sorgeloos, P. (1980). *The use of the brine shrimp Artemia in aquaculture*. The brine shrimp Artemia, 3, 25-46.

Subamia, I. W., Suhenda, N., & Tahapari, E. (2017). *Pengaruh Pemberian Pakan Buatan Dengan Kadar Lemak Yang Berbeda Terhadap Pertumbuhan Dan Sintasan Benih Ikan Jambal Siam (pangasius hypophthatmus)*. Jurnal Penelitian Perikanan Indonesia, 9(1), 37-42.

Tampubolon, K., Effendi, I., & Tanjung, A. (2020). *The effect of different feed on the growth rate of Artemia salina*. Asian Journal of Aquatic Sciences, 3(1), 77-83.

Trisnabatin, G. A., Julyantoroa, P. G. S., & Wijayantia, N. P. P. (2021). *Biomassa dan kandungan nutrisI Artemia sp. yang diberi pakan alami Thalassiosira sp. dan Chlorella sp.* Current Trends in Aquatic Science IV, 1, 57-62.

Turcihan, G., Turgay, E., Yardımcı, R. E., & Eryalçın, K. M. (2021). *The effect of feeding with different microalgae on survival, growth, and fatty acid composition of Artemia franciscana metanauplii and on predominant bacterial species of the rearing water*. Aquaculture International, 29(5), 2223-2241.

Van Hoa, N., Anh-Thu, T., Ngoc-Anh, N., & Thanh-Toi, H. (2011). *Artemia franciscana Kellogg, 1906 (Crustacea: Anostraca) production in earthen pond: improved culture techniques*. Int. J. Artemia Biol, 1, 13-28.

Vos, J., & de la Rosa, N. L. (1980). *Manual on Artemia production in salt ponds in the Philippines*.

Widhiyanto, D. (2006). *Pengaruh Pakan Silase Ikan Petek (Leiognathus sp) terhadap Produksi dan Daya Tetas Kista Artemia sp*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan UNDIP. Semarang.