Aquaculture International

Springer

Aquaculture International

Journal of the European Aquaculture Society

european aquaculture society

Editors

For authors

Submission guidelines

Manuscript editing services

Ethics & disclosures

Open Access fees and funding

Contact the journal

Aquaculture International
https://doi.org/10.1007/s10499-023-01059-x

Effect of protein sources in formulated diets on growth performance, feed utilization, survival rate, and reproductive performance of Artemia franciscana

Muhamad Amin[1] · Bunga Intan[2] · Mashielda Arbias Ridwan Putri[2] · Akhmad Taufiq Mukti[1] · Mochammad Amin Alamsjah[3]

Received: 15 September 2022 / Accepted: 15 January 2023

Abstract

The best diet for *Artemia* culture is phytoplankton; yet it is expensive and laborious work. Thus, cheaper and easily prepared feed such as formulated diet is required. The present study aimed to investigate the effect of different protein sources in formulated diets on growth performance, feed utilization, survival rate, fecundity, and nauplius production of *Artemia franciscana*. A total of 6,000 Artemia nauplii were randomly distributed equally into 20 rearing tanks (5 treatment groups with 4 replicates): *Artemia* fed with *Tetraselmis chuii* as control (T1), *Artemia* fed with a formulated diet with fish meal inclusion (T2), *Artemia* fed with the formulated diet with sergestid shrimp meal inclusion (T3), *Artemia* was fed with the formulated diet with soybean meal inclusion (T4), and formulated diet with Black Soldier Fly meal inclusion (T5). The results showed that protein sources in formulated diets had a significant effect on growth performances, feed utilization, survival rate, fecundity, and nauplius production of *A. fransciscana* ($p < 0.05$). The best result, in general, was obtained from the soybean meal-fed group (T4): specific growth rate in length (17.78% BL.d^{-1}), the specific growth rate in body weight (35.95% BW.d^{-1}), feed conversion ratio (1.37), feed efficiency (74.00 %), protein retention (64.07%), fat retention (55.93%), fecundity (44 eggs.broodstock^{-1}), and nauplius production (35 nauplii.broodstock^{-1}). While the highest energy retention was obtained from the control group (60.90%). These results suggest that soybean meal can be used as the protein source in formulated diets to replace phytoplankton usage in *Artemia* culture.

Keywords Brine_shrimp · Formulated_diet · Growth · Life_below_water · Protein_sources · Reproduction

Handling Editor: Gavin Burnell

✉ Muhamad Amin
muhamad.amin@fpk.unair.ac.id

Extended author information available on the last page of the article

Introduction

Nauplii of brine shrimp, *Artemia franciscana*, is one of the most important live diets for fish and shrimp larvae due to its high and complete nutrient contents (Méndez-Martínez et al. 2018; Nascimento et al., 2020; Sorgeloos et al., 1998). Up until now, the fulfillment of *Artemia* nauplii in Indonesia still relies on imported commercial cysts from several countries such as Belgium and USA. According to the Indonesian Ministry of Marine Affair, the total import of *Artemia* cyst increased every year, from 102.51 tons in 2017 to 150.92 tons in 2018, and became 149.01 tons in 2019, which means that Indonesia has spent more than US$ 15 million year^{-1} (https://kkp.go.id). As the number of fish and shrimp hatcheries in Indonesia is continuously increasing in the last few years, the demand for the *Artemia* cyst shall increase significantly in the coming years. To reduce the high dependency on imported *Artemia* cysts, several strategies have been developed including nauplius production through aquacultures. Several studies have reported that *Artemia* nauplii have been successfully produced through the culture process (Bwala 2019), and nowadays *Artemia* nauplii have been commercially available in frozen form. However, one of the main obstacles in *Artemia* aquaculture is diet preparation. Many studies reported that the best diet for *Artemia* culture is microalgae such as *Tetraselmis chuii*, *Dunaliella* sp., and *Chaetocheros calcitrans* (Balachandar and and Rajaram 2019), *Chlorella* sp., or *Nannochloropsis* sp. (Dan et al., 2022) or a combination of those microalgal species (Turcihan et al., 2021). However, the live diet preparation is laborious work and expensive, especially due to culture media and fertilizer requirements. Thus, an alternative diet which is easily prepared as well as cheap such as formulated diet is required.

Research on formulated diets for *Artemia* culture has been reported previously in several studies including the use of rice bran (Le et al. 2019; Méndez-Martínez et al. 2018), fish silage, (Djunaedi 2016), shrimp-head meal, corn meal wheat meal and the use of tapioca meal (Tampubolon et al. 2020). However, these study results have still some weaknesses, including the low survival rate of *Artemia*, which was 31% or even less. Acknowledging these study results, more research to find a better-formulated diet is still required. Some studies suggest that a good formulated diet can be developed by mimicking the nutrient content of a natural diet (Amin et al. 2022c; Perera and Simon 2015). Similarly, a formulated diet for *Artemia* culture should also be developed based on the *Artemia* natural diet. Balachandar and Rajaram (2019) and Amin et al. (2022b) reported that the best natural diet for Artemia culture is *T. chuii.* This might be due to the high and suitable composition of nutrient content of *T. chuii;* 48.42% crude protein, 9.70% crude fat, and 12.10% carbohydrate (El-Sayed et al. 2020). Thus, the nutrient composition should be initially used to make a formulated diet for *Artemia* culture. Besides nutrient content, sources of nutrients used in formulated diet have also a significant effect on the responses of aquaculture animals including *Artemia* because they may significantly affect its digestibility and the availability. Thus, a study of the formulated diet with different protein sources is very important to determine the best source for *Artemia*. There are 4 protein sources commonly and easily found in Indonesia including fish meal, sergestid shrimp meal, black soldier butterfly (BSF) meal, and soybean meal.

Therefore, the present study aimed to investigate the effect of different protein sources (fish meal, sergestid shrimp meal, BSF meal, and soybean meal) in formulated diets on the growth performances, survival rate, nutrient utilization, fecundity and nauplius production of brine shrimp, *A. franciscana*.

Materials and methods

Experimental design

Five different diets (five treatments with 4 replicates) were assigned to a completely randomized design (CRD). Those five treatments were: *Artemia* fed with microalgae (*T. chuii*) as the control (T1), *Artemia* fed with a fish meal-based formulated diet (T2), *Artemia* fed with a sergestid shrimp meal *(Acetes japonicas*)–based formulated diet (T3), *Artemia* fed with a soybean meal-based formulated diet (T4), and *Artemia* fed BSF (*Hermetia illucens*) meal-based formulated diet (T5).

Feed formulation

Nutrient composition of each formulated diets was adjusted according to the nutrient composition of *T. chuii*: 48.42% crude protein, 9.70% crude fat, and 12.10% carbohydrate (El-Sayed et al. 2020). Firstly, nutrient content of the main raw materials (fish meal, sergestid shrimp meal, soybean meal, BSF meal, as well as *T. chuii*) was analyzed by proximate analysis (crude protein, crude fat, carbohydrate, and energy content). Based on the proximate results (Table 1), diets were formulated using "linear Programming" and protein content was set at ~48% as the protein content of *T. chuii*. While nutrient contents of other materials such as fish oil, rice bran, and Tofu waste were based on published papers (Budaarsa et al. 2015; Lestari et al. 2013). The final formula for every diet was presented in Table 1. Thereafter, all materials were weighed according to the formula and mixed for homogenization. Each diet was then packed in a sealable plastic bag and stored at 4°C until further use.

Cultivation of *T. chuii*

The cultivation of *T. chuii* was performed according to a protocol previously described by Amin et al. (2022b) with slight modification. In brief, a pure *T. chuii* inoculum obtained from Brackish Water Aquaculture Centre, (Jepara, Central Java, Indonesia) was cultured in a 1L Erlenmeyer with an initial concentration of ~1.0×10^3 cells.mL^{-1}. The culture media consisted of 500 mL sterile seawater, 0.5 mL.L^{-1} WALNE, and 1 mL.L^{-1} VITAMIN. The culture process was carried out for 4–5 days to obtain a microalgal density of 10^6 cells.mL^{-1}.

Feeding rate

Artemia nauplii at instar I were cultured for 7 days and fed with *T. chuii* at four different cell concentrations (10^2, $10^{4,}$ and 10^6 cells.nauplii^{-1}). The best result (growth and survival rate) was obtained from a diet concentration of 10^6 cells.nauplii^{-1}. The cell concentration of *T. chuii* was afterward converted into biomass according to Lu et al. (2017).

269 pg	: dry weight of a single *T. chuii* cell
10^6 cells	: the optimal dose of *T. chuii* for *Artemia* nauplii
300	: number of *Artemia* nauplii in each rearing tank
	= 269 pg or 2.69×10^{-7} mg,
	= 2.69×10^{-7} mg $\times 10^6$= 0.269 mg.
	= 0.269 mg × 300 nauplii = 80.7 mg.d^{-1}

Table 1 Feed formulation and nutrient content of raw materials and formulated diets

Raw materials	Nutrient composition of main protein sources				
	Crude protein (% DM)		Crude fat (%DM)	Carbohydrate (%DM)	Energy (Kcal.DM)
Fish meal	59.24		6.54	6.49	320.95
Sergestid shrimp meal	59.40		3.6	3.2	413.68
Soybean meal	47.68		15.81	25.11	390.45
BSF meal	50.20		17.84	16.97	407.44
T. chuii	48.09		9.72	12.89	357.16
Formulation	**Proportions (%)**				
	T1	**T2**	**T3**	**T4**	**T5**
T. chuii	100	-	-	-	-
Fish meal	-	69.13	-	-	-
Sergestid shrimp meal	-	-	74.26	-	-
Soybean meal	-	-	-	96.00	-
BSF meal	-	-	-	-	91.92
Rice bran	-	1.00	12.89	1.00	2.00
Tofu waste	-	27.37	10.35	0.50	3.58
Fish oil	-	1.50	1.50	1.50	1.50
Vitamin Premix	-	0.50	0.50	0.50	0.50
Mineral Premix	-	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Proximate analysis					
Crude protein (%)	48.09	48. 61	48.42	46.02	47.35
Crude fat (%)	9.72	6.16	5.25	15.49	17.01
Carbohydrate (%)	12.89	18.95	12.10	9.70	18.13
Energy (Kcal)	357.16	404.90	370.99	490.98	460.24

Based on this calculation, it was assumed that a total of 80.7 mg DM of formulated diet should be added to each rearing tank containing @300 Artemia nauplii. As the moisture content of the formulated diet was ~15%, 80.7 mg DM was equal to ~92 mg formulated diet. The amount was afterward used to determine the feeding rate of *Artemia* in the main feeding experiment.

Feeding experiment

The feeding experiment was performed according to a protocol previously described by Amin et al. (2022b) with slight modification. A total of 6000 *Artemia* nauplii at instar I were distributed into 20 plastic gallons previously filled with 3L sterile seawater each (@300 nauplii.gallon^{-1} or at stoking density of 100 nauplii.L^{-1} (Nieves-Soto et al. 2021). The *Artemia* nauplii were fed daily at 10 am, and the first feeding was given when the *Artemia* nauplii entered the second instar phase, which was 8 h after hatching. The feeding rate was *T. chuii* (control) and artificial feed with protein sources was based on a preliminary study, 10^{6} cells.individual^{-1} and 92 mg.day^{-1} formulated diet. Before feeding, uneaten feed, feces and other waste were siphoned daily.

Observed parameters

Absolute growth and specific growth rate

Growth performace was monitored by measuring total length and weight of *A. franciscana* on days 0, 3, 6, 9, 12, and 15 according to a protocol as previously described by Amin et al. (2022b). In brief, total length was measured by collecting 15 *Artemia* from each rearing tank and photographed under a dissecting microscope (ST-30-2LF, binocular dissecting microscope, 20×–40×) using a DSL camera (Canon EOS D30). Each *Artemia* photograph was then measured with ImageJ software. While weight was measured using an analytical balance (KERN: ABS 220-4 Analytical Balance) with a precision level of 0.0001 g or 0.1 mg. Then, absolute growth and specific growth rates were calculated according to a formula previously described by Amin et al. (2020).

$$L = L_t - L_0$$
$$W = W_t - W_0$$

$$SGR = \frac{Ln(W_t) - Ln(W_0)}{t} \text{ x } 100\%$$

Where: L is absolute growth in length (mm), L_t is the total length at the end of the experiment (mm), and L_0 is Total length at the beginning of the experiment (mm). W is absolute growth in weight (mg), W_t is total weight at the end of the study (mg), and W_0 is total weight at the beginning of the study (mg). SGR is the specific growth rate (% BW.day^{-1}); W_t is the weight of *Artemia* at the end of the study (mg); W_0 is the weight of *Artemia* at the beginning of the study (mg); t is the culture period (days).

Survival rate

The survival rate of *Artemia* was determined by counting the number of dead *Artemia* in each treatment during the experimental period. The survival rate was calculated using a formula previously described by Amin et al. (2020). Any dead *Artemia* was taken out directly from the rearing system to avoid water quality deterioration.

$$SR = \frac{Nt}{No} x\ 100\%$$

Where: SR is survival rate (%), Nt is the number of Artemia at the end of the experiment, and N0 is the number of nauplii at the beginning of the experiment.

Fecundity and nauplius production

Fecundity was measured by calculating the total number of eggs produced by each broodstock according to a protocol of Balachandar and Rajaram (2019) with slight modification. Mature *Artemia* indicated by dark brown of the uterus was taken out individually and placed under a binocular microscope. Thereafter, eggs were dissected out and counted individually using a hand counter. Meanwhile, nauplius production was monitored by counting nauplii produced by each *Artemia* broodstock daily after day 15 according to Sleet and Brendel (1983). In brief, aeration in the rearing tank was the turn-off and a light source was placed on of rearing tank side to attract *Artemia* nauplii. Then, the nauplius number was counted using a hand counter.

Feed utilization

Feed utilization was analyzed by measuring total feed intake/feed consumption, feed conversion ratio, and feed efficiency. Feed consumption was measured by weighing feed given and uneaten feed. Uneaten feed was collected by siphoning before feeding time daily. Then, the level of feed consumption was calculated using the formula (Amalia et al. 2019). While feed conversion ratio (FCR) was monitored by measuring total feed consumption and total biomass gain, and calculated by a formula of Méndez-Martínez et al. (2018). In addition, feed efficiency was calculated using the formula below.

FC = F1 - F2

$$FCR = \frac{F}{W_t - W_0}$$

$$FE\ (\%) = \frac{W_t - W_0}{F}\ x\ 100\%$$

Where: FC is feed consumption (g); F1 is the amount of initial feed (g); F2 is the amount of feed left (g). FCR is Feed Conversion Ratio; F is the amount of feed given (g); W_t is *Artemia* biomass at the end of the study (g), and W_0 is the biomass of *Artemia* at the start of the study (g). FE: feed efficiency (%); W_t is the final *Artemia* biomass weight (g), W_0 is the biomass weight of *Artemia* at the beginning of the study (g); F is the total weight of *Artemia* feed given during the study (g).

Nutrient retention

Nutrient retentions including protein retention, fat retention, and energy retention were monitored by measuring protein, fat and energy contents of diets and artemia at nauplii (in the beginning) and adult (at the end of experimental period). The proximate analysis was performed according to the protocol of the Association of Official Analytical Chemists (AOAC) (Cunniff and Washington 1997). Thereafter, protein retention, fat retention and energy retention were calculated as follows (Amin et al. 2022a):

$$PR = \frac{(W_t\ x\ P_t) - (W_0\ x\ P_0)}{Pp}\ x\ 100$$

$$FR = \frac{(W_t\ x\ F_t) - (W_0\ x\ F_0)}{Fp}\ x\ 100$$

$$ER = \frac{(W_t\ x\ E_t) - (W_0\ x\ E_0)}{Ep}\ x\ 100$$

Where: PR is Protein Retention (%), W_t is The final wet weight of Artemia (g); W_0 is the initial wet weight of *Artemia* (g); P_t is body protein at the end of *Artemia* (g); P_0 is body protein at the beginning of *Artemia* (g), Pp is feed protein (The amount of protein given (g)). FR is Fat Retention (%); F_t is Body fat at the end of the study (g); F_0 is Body fat at the beginning of the study (g); Fp is Feed fat (The amount of fat given (g)). ER: Energy Retention (%); E_t is body energy at the end of the study (kcal.g^{-1}); E_0 is Body energy at the beginning of the study (k.cal.g^{-1}); E_p is feed energy (amount of feed consumed × energy value (k.cal.g^{-1})).

Water quality

Water quality parameters including temperature, dissolved oxygen (DO), pH, salinity, ammonia, and nitrite were monitored on day 0, 3, 6, 9, 12, and day 15 to assure that the *Artemia* lived under optimal culture conditions (Amin et al. 2022b). Temperature, pH, and DO were measured with a DO meter probe (HI98193 - Waterproof Portable Dissolved Oxygen Meter). Salinity was monitored with a refractometer (ATAGO 20M). While ammonia and nitrite concentrations were measured with commercial kits (HANNA instrument). All measurement were performed as previously described by Amin et al. (2022b).

Data analysis

All data obtained in the present study including specific growth rate, survival rate, protein retention, fat retention, energy retention, feed consumption, feed conversion ratio, feed efficiency, fecundity, and nauplius production were analysed using analysis of variance (ANOVA), followed by Duncan tests for any significant differences among the treatments. All statistical analyses were performed with a statistical product and service solution (SPSS) version 13.0**.**

Results

Specific growth rate

Protein sources in the formulated diets had a significant effect on the specific growth rate of *A. fransciscana* ($p < 0.05$). The highest specific growth rate in terms of length (SGR_L) was obtained from those *Artemia* fed on the soybean meal-based formulated diet (T4; 17.78 ± 0.41 % BL.day^{-1}), but not significantly different from those Artemia fed on sergestid meal–based formulated diet (T3) and *T. chuii*-fed group (T1). Meanwhile, the lowest SGR_L was obtained from *Artemia* receiving a formulated diet with protein sources of BSF meal (T5; 13.63 ± 1.63 % BL.day^{-1}), but not significantly different from SGR_L of *Artemia* in the fish meal-fed group, Fig. 1.

Similarly, the protein sources in the formulated diet gave a significant effect on the specific growth rate of *A. fransciscana* in terms of weight ($p < 0.05$). The best specific growth rate of weight (SGR_W) was obtained from those *Artemia* fed in the BSF meal-fed group (T5), 45.41 ± 10.16 % BW.day^{-1}, but no significant differences from those *Artemia* in the soybean meal-fed group (T2). Meanwhile, the lowest SGR_W was obtained from *Artemia* receiving a formulated diet with protein sources of fish meal meal (T2), 33.28 ± 0.52 % BW.day^{-1}, Fig. 2.

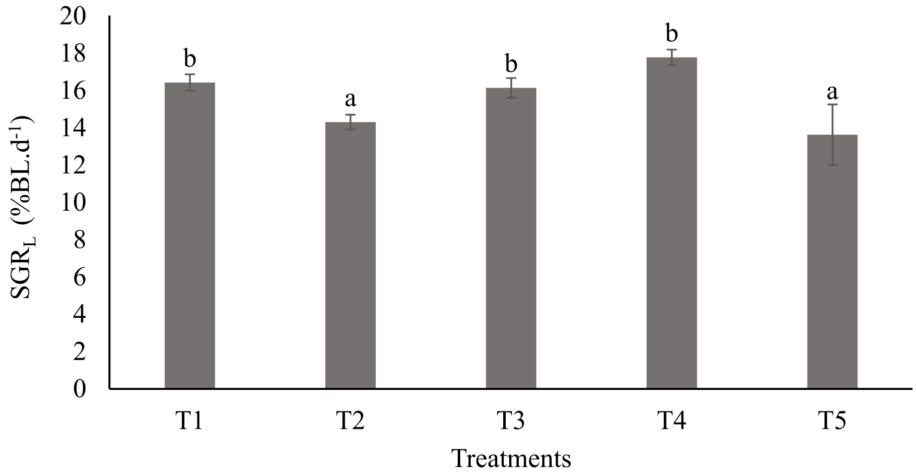

Fig. 1 Specific growth rate of *A. franciscana* in terms of length calculated after 15-day-culturing period; SGR_L = specific growth rate in terms of length; BL = total body length; T1 = *T. chuii*-fed group; T2 = fish meal-fed group; T3 = sergestid shrimp meal-fed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group. Superscripts with different letters indicate the SGR_L was significantly different at $p < 0.05$

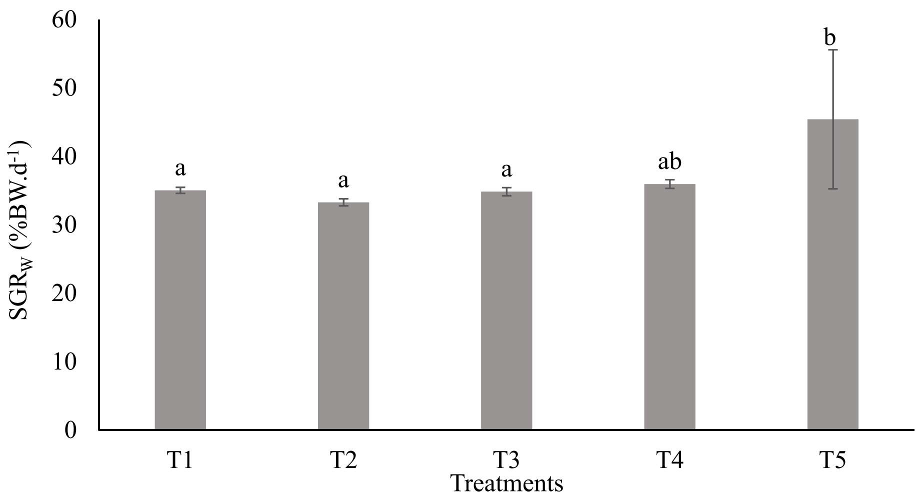

Fig. 2 Specific growth rate of *A. franciscana* calculated based on weight after being cultured for 15 days. SGR_W = specific growth rate in terms of weight; BW = Body Weight; T1= *T. chuii*-fed group; T2 = fish meal-fed group; T3 = sergestid shrimp meal-fed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group

Feed consumption rate

The formulated diets with different protein sources had a significant effect on the level of feed consumption of *A. franciscana* ($p < 0.05$). Feed consumption of *Artemia* fed with five diet groups (T1, T2, T3, T4, and T5) was not significantly different, with $P > 0.05$. While the lowest feed consumption was obtained from *Artemia* fed on a formulated diet with BSF meal (T5), 0.69 ± 0.201, Table 2.

Feed conversion ratio

The formulated diets with different protein sources had a significant effect on the specific growth rate of *A. fransciscana* ($p < 0.05$). The best feed conversion ratio was obtained from *Artemia* fed on formulated diet with a protein source of soybean meal (T4: 1.37 ± 0.12). Meanwhile, the highest feed conversion ratio was obtained from *Artemia* which received fishmeal-based formulated diet (T2: 2.33 ± 0.07), Table 2.

Feed efficiency

The different protein sources had a significant effect on the feed efficiency of *A. franciscana* ($p < 0,05$). The highest feed efficiency was obtained from *Artemia* receiving soybean meal-based formulated diet (T4: 74%), and was not significantly different from the control (T1: 70%). While the lowest feed efficiency was obtained from those *Artemia* receiving fish meal-based formulated diet (T2: 43%).

Protein, fat, and energy retentions

Protein sources in the formulated diets had a significant effect on nutrient retention in Artemia, $p < 0.05$. The highest protein retention was recorded from the formulated diet with soy meal protein source (T4: 64.07%), followed by *T. chuii* (T1: 36.08%), and formulated diet with a sergestid shrimp meal protein source (T3: 30.16%). While, the lowest protein retention was recorded from the formulated diet with the fish meal (T2: 20.65 %), Fig. 3.

Fat retention of formulated diet in *A. franciscana* was significantly affected by protein sources in the formulated diet after being culture for 15 days, $p < 0.05$. The highest fat retention was obtained from the soybean meal-fed group (T4), 55.93%, followed by *Artemia* in the fish meal-fed group (T2: 47.52%), and *Artemia* in the *T. chuii*-fed group (T1: 41.41%). Meanwhile, the lowest fat retention was obtained from those *Artemia* in the sergested shrimp meal-fed group (T3: 33.82%), Fig. 4.

In addition, protein sources in the formulated diets had a significant effect on the energy retention of *A. franciscana* after being cultured for 15 days. The highest energy

Table 2 The average level of feed consumption, feed conversion ratio, and feed efficiency of *A. franciscana* with artificial feeding of different protein sources

Treatment	FC ± SD (g)	FCR± SD	FE ± SD (%)
T1	1.99 ± 0.00 ^{b}	1.42 ± 0.13 ^{a}	70 ± 6.02 ^{c}
T2	1.24 ± 0.023 ^{b}	2.33 ± 0.07 ^{c}	43 ± 1.19 ^{a}
T3	1.29 ± 0.019 ^{b}	1.79 ± 0.17 ^{b}	56 ± 5.45 ^{b}
T4	1.33 ± 0.004 ^{b}	1.37 ± 0.12 ^{a}	74 ± 6.47 ^{c}
T5	0.69 ± 0.201 ^{a}	-	-

Note: Different superscripts show significant differences between treatments ($p < 0.05$); *SD*, standard deviation; *T1*, *T. chuii*-fed group; *T2*, fish meal-fed group; *T3*, sergestid shrimp meal-fed group; *T4*, soybean meal-fed group; *T5*, BSF meal-fed group

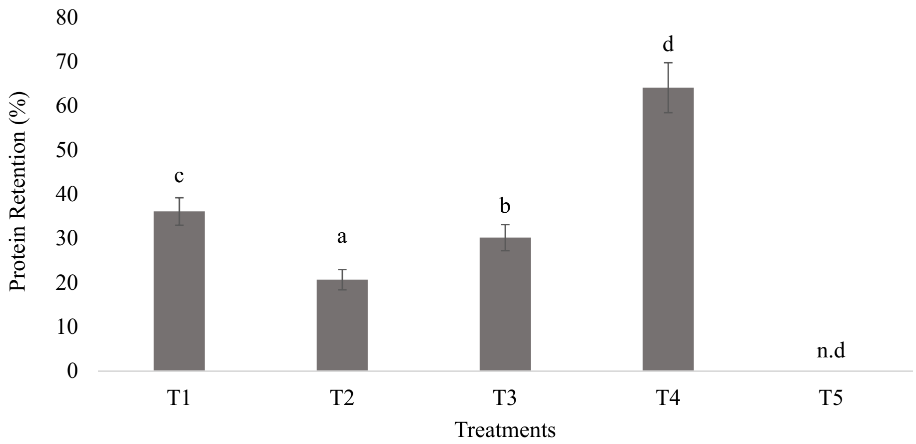

Fig. 3 The average values of protein retention in *Artemia franciscana* fed on formulated diets with different protein sources. Superscripts with different letters indicate significant differents at $p < 0.05$; T1 = *T. chuii*-fed group; T2 = fish meal–fed group; T3 = sergestid shrimp meal-fed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group. "n.d" means no data since no survival rate

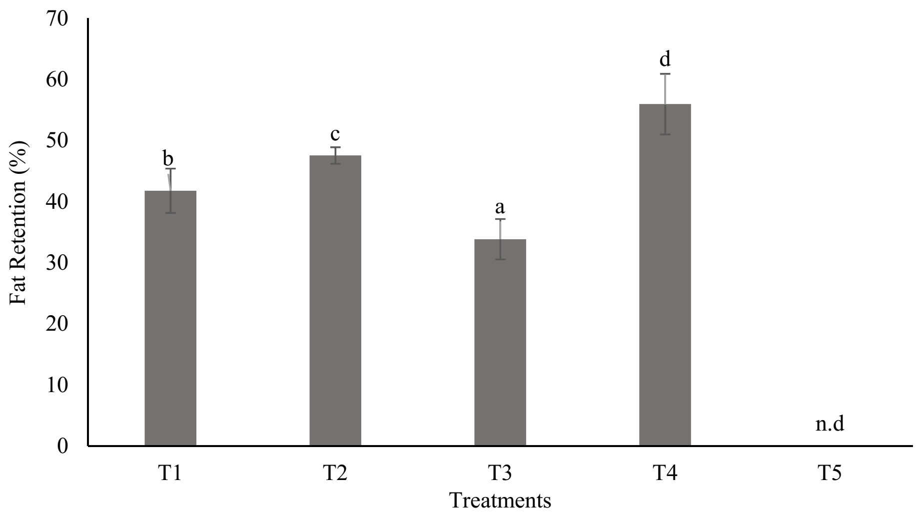

Fig. 4 Graph of the average value of fat retention in *Artemia franciscana*. Different superscripts showed significant differences between treatments ($p < 0.05$); T1 = *T.chuii*; T2 = fish meal formulation feed; T3 = wild-shrimp meal formulation feed; T4 = soybean meal-based formulated diet; T5 = BSF meal-based formulated diet. "n.d" is no data since no survival rate

retention was obtained from those *Artemia* in *T. chuii*-fed group (T1: 60.90%), followed by those *Artemia* in soybean meal-fed group (T4: 48.04%). While the lowest energy retention was obtained from those *Artemia* in the fish meal-fed group (T2: 32.69%), but no significant difference from those *Artemia* in sergestid shrimp meal-fed group(T3: 37.69%), Fig. 5.

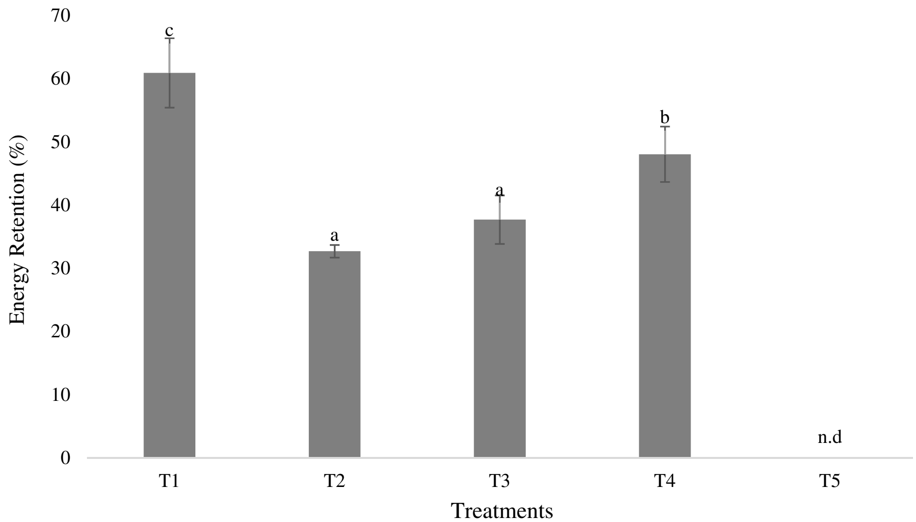

Fig. 5 Energy retention of *Artemia franciscana* fed with formulated diets with different protein sources for 15 days. Superscripts with different letters showed significant differences in the survival rates among treatments ($p < 0.05$); T1 = *T.chuii*-fed group; T2 = fish meal-fed group; T3 = sergestid shrimp meal-fed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group. "n.d" is no data since no survival rate

Fecundity and naupli productions

The results showed that protein sources in formulated diet had a significant effect on fecundity and nauplius production of Artemia ($p < 0.05$). The highest fecundity was obtained from those Artemia in the soybean meal-fed group (T4: 44.13 ± 2.89 eggs.broodstock^{-1}), followed by those *Artemia* in the fish meal-fed group (T2: 15.40 ± 2.64 eggs.broodstock^{-1}), and those *Artemia* in sergestid shrimp meal-fed group (T3: 33.73 ± 2.21 eggs.broodstock^{-1}). While, the lowest fecundity was obtained from those *Artemia* fed with *T. chuii* (T1: 29.95 ± 4.37 eggs.broodstock^{-1}), although not significantly different from those *Artemia* in T3 Fig. 6.

Furthermore, protein sources in the formulated diets had a significant effect on the capacity of *A. fransciscana* broodstock to produce nauplii, $p < 0.05$. The highest number of nauplius production was obtained from those *Artemia* in the soybean meal-fed group (T4: 35.28 ± 1.78 nauplii.broodstock^{-1}), followed by those *Artemia* in the fish meal-fed group (T2: 12.53 ± 2.40 nauplii.broodstock^{-1}), and those *Artemia* in the sergestid shrimp meal-fed group (T3: 28.45 ± 2.87 nauplii.broodstock^{-1}). While, the lowest Nauplius production was obtained from those *Artemia* fed with *T. chuii* (T1: 25.83 ± 4.04 nauplii.broodstock^{-1}), Fig. 7.

Survival rate (SR)

Protein sources in formulated diets had a significant effect on the survival rate of *A. franciscana* ($p > 0.05$). A formulated diet with soybean meal had the highest survival rate (T4: 92.00 ± 2.58%), although not significantly different from those *Artemia* in *T. chuii*-fed

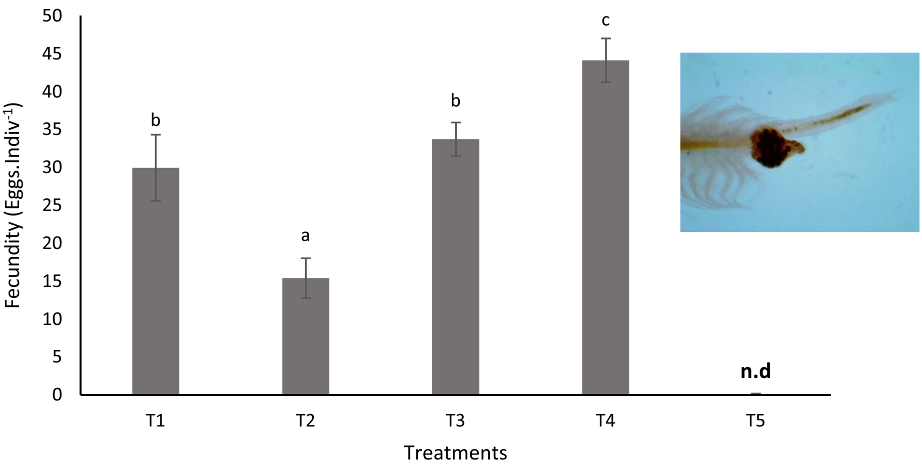

Fig. 6 Fecundity of *A. franciscana* fed with formulated diets with different protein sources for 15 days. Superscripts with different letters indicate significantly different at $p < 0.05$); T1 = Artemia with *T. chuii*-fed group; T2 = Artemia with fish meal-fed group; T3 = Artemia with the sergested shrimp meal-fed group; T4 = Soyabean meal-fed; T5 = BSF meal-fed group.“n.d” is no data since no survival rate

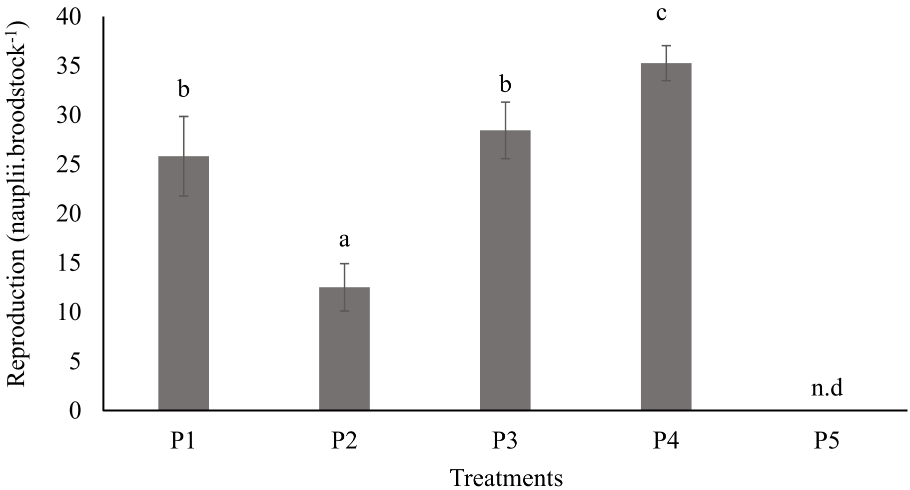

Fig. 7 Nauplii production of *A. franciscana* fed with formulated diets with different protein sources. Superscripts with different letters indicate significantly differences in nauplius production at $p < 0.05$. T1 = Artemia with *T.chuii*-fed group; T2 = Artemia with fish meal-fed group; T3 = Artemia with sergested shrimp meal-feed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group. “n.d” is no data since no survival rate

group (T1:89.50 ± 3.42 %). Meanwhile, the second lowest survival rate was obtained from those *Artemia* fed with sergested shrimp meal-fed group (T3: 81.50 ± 3.11%), but not significantly different from those *Artemia* fed with a fishmeal-fed group (T2: 79.25 ± 2.75%), Fig. 8. Meanwhile, no survival rate was observed from those *Artemia* fed with the formulated diet with BSF meal-fed group (T5).

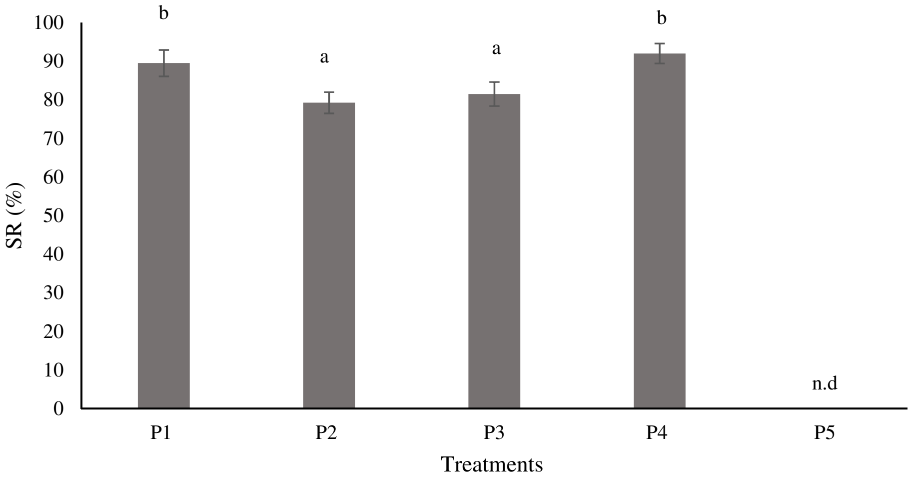

Fig. 8 Survival rate of *A. franciscana* fed with formulated diets with different protein sources for 15 days: Superscripts with different letters showed significantly different survival rates ($p < 0.05$); SR = survival rate; T1 = *T.chuii*-fed group; T2 = fish meal–fed group; T3 = sergested shrimp meal-fed group; T4 = soybean meal-fed group; T5 = BSF meal-fed group. "n.d" is no data since no survival rate

Discussion

The present study reported the effect of different protein sources (fish meal, sergested shrimp meal, BSF meal, and soybean meal) in formulated diets on growth performance, feed utilization, survival rate, fecundity, and nauplius production of *A. franciscana*. The results in general indicated that soybean meal had the best results over the other three protein sources (fish meal, BSF meal, and sergestid shrimp meal). In addition, the *Artemia* receiving soybean meal–based formulated diet had even better performances in some parameters including nutrient utilization (protein and fat retentions), fecundity, and nauplius production, than those *Artemia* receiving live diet (*T. chuii*).

Artemia fed on soybean meal–based formulated diet had a significantly higher growth rate (SGRw and SGR_L) compared to the other formulated diets (fish meal, and BSF meal). The high growth of a soybean-based formulated diet could be due to better nutritional contents and suitable amino acid compositions. Soybean meal, for instance, had been documented to have higher four essential amino acids compared to fishmeal including phenylalanine, histidine, and isoleucine (Barone et al. 2018), and lysin (Muktiani and Prastiwi 2014). In addition, several authors have also confirmed that some non-essential amino acids such as cysteine, glutamine and aspartic acids have also been reported to be higher in soybean meal compared to that of fish meal (Kim et al. 2012); Pongmaneerat (1992). These amino acids might play critical roles in growth, nutrient utilization, fecundity, and nauplius production of *A. fransciscana*. While lower growth of *A. franciscana* fed with the other three formulated diets such as (T2, T3, and T5) could be due to the lack of a few essential amino acids such as lysine and methionine especially in BSF meal or sergested shrimp meal (Tampubolon et al. 2020). The lowest growth that was observed from those *Artemia* fed with a BSF-based formulated diet (T5) might be because BSF contains indigestible fibre such as chitin (33.7%) (Harefa et al. 2018). In addition, the low growth of *Artemia* receiving BSF-based formulated diet might be because of the high-fat content (17.84%), and soybean meal (8.81%). Azir et al. (2017) explained that a high-fat content in a diet can cause fat accumulation in the liver which later interferes with the metabolic function of cultured animals, and lead to the reduction of feed intake and growth.

In terms of feed utilization efficiency, *A. franciscana* fed on the soybean-based formulated diet (T4) had the best feed conversion ratio (1.37), and was not significantly different from the control (Artemia fed on *T. chuii*). This result suggests that *A. franciscana* can utilize nutrients in the formulated diet optimally. Zainuddin et al. (2019) reviewed that the feed conversion ratio can be influenced by several factors including the quality and quantity of feed, species, and size of the cultured animal, as well as water quality. Among these factors, the species and size of the cultured animal, as well as water quality were quite similar in the present study. Thus, the only difference was in the quality of the diet which might be highly affected by the different raw materials used in the formulated diets. According to Sharifi et al. (2021), soybean meal was rich in a variety of essential amino acids as well as high protein content (35–40%) which is comparable to the protein content of *Artemia* carcass. In addition, the composition of nutrients in soybean meal was quite similar to *T. chuii* (best live diet of *A. franciscana*) (Martin et al. 2010). Accordingly, the present study results showed that the highest feed efficiency occurred in *Artemia* fed on a soybean-based formulated diet (74%), although not significantly different from the feed efficiency of *Artemia* in the control (T1), which was 70 %. According to De Verdal et al. (2018), values of feed efficiency can be determined by the nutritional quality of feed. High feed efficiency means that the feed is efficiently absorbed by the cultured animals, therefore, increasing the growth and weight of cultured organisms. Amalia et al. (2019) added that high feed efficiency means that a small amount of nutrients is effectively used for growth. Additionally, nutrient retention (protein, fat, and energy) in the body of *A. franciscana* was significantly affected by protein sources of formulated diets. The highest protein and fat retention were obtained from those *Artemia* fed with soybean-based formulated diet (~65% and 55.93 respectively). While the lowest protein retention was obtained from those *Artemia* fed on sergestid shrimp–based formulated diet (33.82%). The low lipid retention value might be due to the high amount of crude fibre in the raw material of sergestid shrimp which results in decreased feed consumption. Accordingly, Arief et al. (2012) reported that the more fibre consumption, the higher the proportion of fat that is wasted and causes less fat to be absorbed. Furthermore, the highest energy retention was obtained from *Artemia* fed on *T. chuii* (60.90%) followed by those *Artemia* fed on a soybean meal–based formulated diet (48.04%). These results might be explained by the higher digestibility of protein and amino acids in soybean meal compared to that of fish meal, 94% and 90% respectively (Barone et al. 2018; Kim, E et al. 2012). The high energy utilization in the control might indicate that the energy required for nutrient digestion was lower compared to formulated diets (Nankervis et al. 2000). While *Artemia* appeared to spend more energy digesting nutrient content in the formulated diets.

In addition, the present study results showed that diets had a significant effect on the fecundity and nauplius productions of *A. fanciscana*. The highest nauplius production was obtained from those *Artemia* fed on the soybean-based formulated diet (T4), which was 44.13 ± 3 eggs.broodstock^{-1} and produced average nauplii of 35 nauplii/broodstock. These numbers were higher than fecundity and nauplius production reported in previous studies (Naegel 1999). The high fecundity and nauplius production might be because the quantity and quality of protein content in the soybean meal are better than the other raw materials used in the present study. According to Mubarak et al. (2017), protein and amino acids in feed can affect fecundity, protein is also the dominant component of egg yolk. Similarly, Fink et al. (2011) added that amino acid contents including arginine and histidine affected the fecundity value of the culture organism. Soybean meal contains the essential lysine which affects the development of *Artemia* embryos (Li et al. 2009). Soybean meal also contains high vitamin E (Winarsi 2010), and may also contribute to fecundity and nauplius production (Koch et al. 2011; Lee 2012). While vitamin E has been reported to accelerate the secretion of reproductive hormones and protecting eggs during development taking place (Napitu and Santoso 2013). All these results suggested that soybean meal can be used as protein source in formulated diet for *Artemia* culture. The use of such formulated diet may significantly reduce operational cost of *Artemia* culture and contribute to higher economic benefits.

Conclusion

Formulated diets with different protein sources had significant effects on growth performance, nutrient utilization, survival rate, fecundity, and nauplius production of *Artemia franciscana.* The best results were obtained from those Artemia fed on a soybean-based formulated diet, followed by the fish meal–based formulated diet, and sergestid shrimp–based formulated diet. The results of the study indicate that formulated diet with soybean meal as a protein source could replace the use of phytoplankton as the diet of *Artemia* culture and produce nauplii for fish or shrimp hatcheries.

Acknowledgements The author expresses his gratitude to the Faculty of Fisheries and Marine Affairs, Airlangga University, for providing laboratory facilities as a place for research to take place. The authors would like to thank the supervisors and research colleagues who have always helped the research process to the end.

Authors' contributions M.A. Experimental design, data collection, data analysis, writing draft, data validation, submission, funding acquisition. B.I.Experimental design, data collection, data analysis, writing the draft, data validation. M.A.R.P. Experimental design, data collection, data analysis, writing the draft, data validation. A.T.M. Experimental design, data collection, data analysis, writing the draft, data validation. M.A.A. Experimental design, data collection, data analysis, writing a draft, data validation, and funding acquisition. The authors read and approved the final manuscript.

Funding This reseach was funded by Universitas Airlangga and Ministry of Finance, the Republic of Indonesia.

Declarations

Competing interests The authors declare no competing interests.

References

Amalia R, Hastuti S, Sudaryono A (2019) Pengaruh pemberian tepung cacing tanah (*Lumbricus* sp.) sebagai atraktan dalam pakan terhadap tingkat konsumsi pakan, efisiensi pakan dan pertumbuhan ikan patin (*Pangasius* sp.). Indo J of Trop Aqua3(1):27–35. https://doi.org/10.14710/sat.v3i1.3901

Amin M, Musdalifah L, Ali M (2020) Growth performances of Nile Tilapia, Oreochromis niloticus, reared in recirculating aquaculture and active suspension systems. IOP Conf Ser: Earth Environ Sci 441(1):012135. https://doi.org/10.1088/1755-1315/441/1/012135

Amin M, Agustono A, Ali M, Prayugo P, Hum NNMF (2022a) Apparent nutrient utilization and metabolic growth rate of Nile tilapia, Oreochromis niloticus, cultured in recirculating aquaculture and biofloc systems. Open Agric 7(1):445–454. https://doi.org/10.1515/opag-2022-0109

Amin M, Erwinda M, Nissa M, Nindarwi DD, Setyantini WH, Alamsjah MA (2022b) Fatty acids profiles and growth performances of *Artemia franciscana* fed with different types of microalgae. Sains Malays 51(8):2449–2459. https://doi.org/10.17576/jsm-2022-5108-09
Amin M, Taha H, Samara SH, Fitria A, Muslichah NA, Musdalifah L, Odeyemi OA, Alimuddin A, Arai T (2022c) Revealing diets of wild-caught ornate spiny lobster, Panulirus ornatus, at puerulus, post-puerulus and juvenile stages using environmental DNA (eDNA) metabarcoding. Aquac Res 27:101361. https://doi.org/10.1016/j.aqrep.2022.101361
Arief M, Yudiarto S, Agustono A (2012) Pengaruh Penambahan Atraktan yang Berbeda dalam Pakan Pasta Terhadap Retensi Protein, Lemak dan Energi Benih Ikan Sidat (Anguilla bicolor) Stadia Elver [Effects of addition different attractants in pasta feed against retention of protein, fat and energy eel fish seed (Anguilla bicolor) Stadia Elver]. JIPK 4(2):135–140. https://doi.org/10.20473/jipk.v4i2.11564
Azir A, Harris H, Haris R (2017) Production and nutritional content of maggots (*Chrysomya megacephala*) using different culture media composition. JIIPP 12(1):34–40
Balachandar S, Rajaram R (2019) Influence of different diets on the growth, survival, fecundity and proximate composition of brine shrimp *Artemia franciscana* (Kellog, 1906). Aquac Res 50(2):376–389. https://doi.org/10.1111/are.13882
Barone RSC, Sonoda DY, Lorenz EK, Cyrino JEP (2018) Digestibility and pricing of Chlorella sorokiniana meal for use in tilapia feeds. Sci Agric 75:184–190. https://doi.org/10.1590/1678-992X-2016-0457
Budaarsa K, Stradivari G, IPGASK J, Mahardika I, Puger A, Suasta I, Astawa IPA. (2015) Pemanfaatan ampas tahu untuk mengganti sebagian ransum komersial ternak babi. JIP Univ Udayana 18(1)
Bwala R (2019) Development of a cost-effective system for ovoviviparous production of *Artemia nauplii* at low-salinity as live food for the larvae of the African catfish (*Clarias gariepinus*: Burchell, 1822) Ghent University
Cunniff P, Washington D (1997) Official methods of analysis of AOAC international. J AOAC Int 80(6):127A
Dan S, Yamashita K, Matsunari H, Nagakura K, Hamasaki K (2022) Capability of *Artemia* to digest Chlorella vulgaris and Nannochloropsis oculata under stagnant culture conditions. Aquac Res 53(12):4316–4326. https://doi.org/10.1111/are.15929
De Verdal H, Komen H, Quillet E, Chatain B, Allal F, Benzie JA, Vandeputte M (2018) Improving feed efficiency in fish using selective breeding: a review. Rev Aquac10(4):833–851. https://doi.org/10.1111/raq.12202
Djunaedi A (2016) Pertumbuhan *Artemia* sp. Dengan pemberian ransum pakan buatan berbeda. J Kelaut Trop 18(3):133–138. https://doi.org/10.14710/jkt.v18i3.525
El-Sayed HS, Mohamed LaE-F, Aly-Eldeen MA-E-N, Khairy HM (2020) Purification of petroleum wastewater and biodiesel production by Prasinophyte alga *Tetraselmis chuii*. Rom Biotechnol Lett 25(4):1790–1801. https://doi.org/10.25083/rbl/25.4/1790.1801
Fink P, Pflitsch C, Marin K (2011) Dietary essential amino acids affect the reproduction of the keystone herbivore Daphnia pulex. PLoS One6(12):e28498. https://doi.org/10.1371/journal.pone.0028498
Harefa D, Adelina A, Suharman I (2018) Pemanfaatan fermentasi tepung maggot (Hermetia illucens) sebagai substitusi tepung ikan dalam pakan buatan untuk benih ikan baung (Hemibagrus nemurus). JOM BPIK 5(1):1–15
Kim E, Utterback P, Parsons C (2012) Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult Sci91(6):1350–1355. https://doi.org/10.3382/ps.2011-01861
Koch U, Martin-Creuzburg D, Grossart H-P, Straile D (2011) Single dietary amino acids control resting egg production and affect population growth of a key freshwater herbivore. Oecologia 167(4):981–989. https://doi.org/10.1007/s00442-011-2047-4
Le TH, Hoa NV, Sorgeloos P, Van Stappen G (2019) *Artemia* feeds: a review of brine shrimp production in the Mekong Delta, Vietnam. Rev Aquac11(4):1169–1175. https://doi.org/10.1111/raq.12285
Lee KB (2012) Effect of culture density and antioxidants on naupliar production and gene expression of the cyclopoid copepod. Paracyclopina Nana Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. Comp Biochem Phys A161(2):145–152. https://doi.org/10.1016/j.cbpa.2011.10.019
Lestari SF, Yuniarti S, Abidin Z (2013) Pengaruh formulasi pakan berbahan baku tepung ikan, tepung jagung, dedak halus dan ampas tahu terhadap pertumbuhan Ikan Nila (*Oreochromis* sp.). Indo J Mar Sci Tech (1):36–46
Li P, Mai K, Trushenski J, Wu G (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. J Amino Acids 37(1):43–53. https://doi.org/10.1007/s00726-008-0171-1
Lu L, Wang J, Yang G, Zhu B, Pan K (2017) Biomass and nutrient productivities of Tetraselmis chuii under mixotrophic culture conditions with various C: N ratios. Chin J Oceanol. Limnol 35(2):303–312. https://doi.org/10.1007/s00343-016-5299-3

Martin H, Laswai H, Kulwa K (2010) Nutrient content and acceptability of soybean based complementary food. African J Food, Agric Nutr Dev 10(1). https://doi.org/10.4314/ajfand.v10i1.51482

Méndez-Martínez Y, García-Guerrero MU, Lora-Vilchis MC, Martínez-Córdova LR, Arcos-Ortega FG, Alpuche JJ, Cortés-Jacinto E (2018) Nutritional effect of *Artemia nauplii* enriched with Tetraselmis suecica and Chaetoceros calcitrans microalgae on growth and survival on the river prawn Macrobrachium americanum larvae. Aquac Int 26(4):1001–1015. https://doi.org/10.1007/s10499-018-0264-0

Mubarak AS, Jusadi D, Junior MZ, Suprayudi MA (2017) The population growth and the nutritional status of Moina macrocopa feed with rice bran and cassava bran suspensions. J Akua Indo 16(2):223–233. https://doi.org/10.19027/jai.16.2.223-233

Muktiani A, Prastiwi WD (2014) Correlation protein and amino acid content in feed ingredients with zinc binding protein. *Anim Prod 16*(2):114–120

Naegel LC (1999) Controlled production of *Artemia* biomass using an inert commercial diet, compared with the microalgae Chaetoceros. *Aquac Eng 21*(1):49–59. https://doi.org/10.1016/S0144-8609(99)00023-0

Nankervis L, Matthews S, Appleford P (2000) Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, Lates calcarifer. Aquac 191(4):323–335. https://doi.org/10.1016/S0044-8486(00)00436-1

Napitu R, Santoso L, Suparmono S (2013) Pengaruh penambahan vitamin E pada pakan berbasis tepung ikan rucah terhadap kematangan gonad ikan nila merah (Oreochromis niloticus). Aqua Eng Tech J 1(2)

Nascimento MDP, Schorer M, Dos Santos JC, Rocha MS, Pedreira MM (2020) Live and frozen *Artemia nauplii* for catfish Lophiosilurus alexandri (Steindachner, 1876) larvae in different salinities. Trop Anim Health Prod 52(2):653–659. https://doi.org/10.1007/s11250-019-02053-3

Nieves-Soto M, Lozano-Huerta R, López-Peraza DJ, Medina-Jasso MA, Hurtado-Oliva MA, Bermudes-Lizárraga JF (2021) Effect of the enrichment time with the tuna orbital oil emulsion on the fatty acids profile of juveniles of *Artemia franciscana*. Aquac Fish 6(1):69–74. https://doi.org/10.1016/j.aaf.2020.03.008

Perera E, Simon C (2015) Digestive physiology of spiny lobsters: implications for formulated diet development. Rev Aquac 7(4):243–261. https://doi.org/10.1111/raq.12066

Pongmaneerat J (1992) Utilization of soybean meal as protein source in diets for rainbow trout. Jpn Soc Fish Sci 58(10):1983–1990. https://doi.org/10.2331/suisan.58.1983

Sharifi S, Majzoobi M, Farahnaky A (2021) Development of healthy extruded maize snacks; effects of soybean flour and feed moisture content. Int J Food Sci 56(7):3179–3187. https://doi.org/10.1111/ijfs.14842

Sleet R, Brendel K (1983) Improved methods for harvesting and counting synchronous populations of *Artemia nauplii* for use in developmental toxicology. Ecotoxicol Environ Saf 7(5):435–446. https://doi.org/10.1016/0147-6513(83)90082-9

Sorgeloos P, Coutteau P, Dhert P, Merchie G, Lavens P (1998) Use of brine shrimp, Artemia spp., in larval crustacean nutrition: a review. Rev Fish Sci 6(1-2):55–68. https://doi.org/10.1080/10641269891314195

Tampubolon K, Effendi I, Tanjung A (2020) The effect of different feed on the growth rate of *Artemia salina*. Asi J Aqua Sci3(1):77–83

Turcihan G, Turgay E, Yardımcı RE, Eryalçın KM (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. Aquac Int 29(5):2223–2241. https://doi.org/10.1007/s10499-021-00745-y

Winarsi H, Purwanto A, Dwiyanti H (2010) Kandungan protein dan isoflavon pada kedelai dan kecambah kedelai. Biota: J Ilmi Ilmu-Ilmu Hayat 181–187. https://doi.org/10.24002/biota.v15i2.2696

Zainuddin Z, Aslamyah S, Azis HY, Hadijah H (2019) Pengaruh kombinasi dosis dan frekuensi pemberian pakan terhadap rasio konversi pakan juvenil udang vaname di tambak. Pros Simp Nas KP 6. https://doi.org/10.14710/sat.v3i1.3901

Authors and Affiliations

Muhamad Amin[1] · Bunga Intan[2] · Mashielda Arbias Ridwan Putri[2] · Akhmad Taufiq Mukti[1] · Mochammad Amin Alamsjah[3]

[1] Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR Mulyorejo, Jl. Mulyorejo, Surabaya 60115, Indonesia

[2] The aquaculture Study Program, Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR Mulyorejo, Jl. Mulyorejo, Surabaya 60115, Indonesia

[3] Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR Mulyorejo, Jl. Mulyorejo, Surabaya 60115, Indonesia