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Survival, growth, and biomass of brine shrimp (*Artemia franciscana*) fed with spirulina powder and soybean flour

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Abstract
Currently, on-grown Artemia is widely needed as live food in aquaculture, especially for ornamental fish. The aim of this study was to determine and compare the survival, growth and biomass of Artemia fed with spirulina powder and soybean flour. The Artemia cyst used in this study was *Artemia franciscana* obtained from the commercial brand Golden West Artemia (Great Salt Lake Artemia). The study was conducted by completely randomized design with three feeding treatments: 100% spirulina powder (S-100%), 100% soybean flour (SB-100%), and 50% spirulina powder + 50% soybean flour (S-50%+SB-50%). The results show that survival, body length and biomass of Artemia were very significantly affected by different feeding. The highest survival rate was obtained by feeding with S-100% (25.07±1.43%), while feeding with SB-100% (19.09±1.36%) and S-50% + SB-50% (19.49±2.03%) were not significantly different. The average body lengths of Artemia measured were 9.00±0.15 mm (S-100%), 7.24±0.44 mm (SB-100%), and 8.89±0.44 mm (S-50% + SB-50%), respectively. The highest production of Artemia biomass was by feeding S-100% (17,667±0.322 g), followed by S-50%+SB-50% (13,622±2,183 g), and SB -100% (10.858±1.428 g). Thus, spirulina powder is a very suitable feed for on-grown Artemia production.

Keywords: On-grown artemia, artemia feed, live food, length growth, survival, biomass

Introduction

Artemia, commonly known as brine shrimp, is a zooplanktonic organism, belongs to the family Artemiidae, order Anostraca, class Brachiopoda, subphylum Crustacea, phylum Arthropoda. Artemia found in hypersaline habitats such as inland salt lakes, coastal salt pans and man-managed saltworks. Artemia are extremely euryhaline, they survive in environments with salinities ranging between approximately 10 and 340 ppt, and can even survive short periods of time in freshwater. He first larval stage (nauplius instar 1) has a body length of 400 to 500 µm, while adult Artemia is 8–10 mm for male and 10-12 mm for female. Under optimal conditions Artemia can live for several months [1, 2].

Artemia is suitable live food for many fish and crustacea. The popularity of Artemia as a live food item is largely due to its convenience in use as it can be hatched within 24 hours from dormant cysts which can be easily stored for prolonged periods of time [2]. All the life stages of Artemia are used as feed in aquaculture according to the feed size requirements. Nauplii of Artemia are the most widely used as live food in the larviculture of fish, ornamental fish and crustacean [2, 3, 4, 5, 6, 7]. Currently, on-grown Artemia is widely needed as live food in aquaculture, especially for ornamental fish. According to Dhont & Sorgeloos [8], the protein and lipid content of on-grown Artemia, were 49.7 - 62.5%, and 9.4 - 19.5%. The nutrition value of on-grown Artemia fed rice bran and microalgae were 53.1% protein, and 10.6% lipid. [9]. On-grown artemia is the preferred live food for ornamental fish [10]. Ornamental fishes were more colorful when they were fed on Artemia [11, 12, 13, 14]. On-grown Artemia has also been used as a maturation diet for Penaeus shrimp [15], as food for Penaeus post larvae [16], and Coho salmon [17]. Artemia is an obligatory nonselective filter feeder zooplankton, so, it can be fed on different diets [5].

Fernández [18] showed that Artemia filtered particles range from 1 - 50 µ and the optimal food particle size is about 16 µ. In natural habitats, microalgae is the main food source of Artemia [19]. The food sources used for rearing artemia include microalgae, dried algae, bacteria, yeasts, microcapsules and waste products from the food industry [8, 20]. However, the best food for growing artemia is phytoplankton, although it is expensive [21].

Spirulina (*Arthrospira platensis*) is a blue green algae, multicellular, and filamentous spiral shape with a diameter of 12 microns. It naturally occurring in tropical and subtropical water bodies that are shallow water environments with high salinity such as alkaline, saline, brackish and freshwater lakes. It has balanced nutritional components that make it desirable as an animal dietary feed supplement [22]. Currently, spirulina which is dried into powder has been produced commercially in Indonesia and is sold as an aqua feed ingredient. Spirulina powder has a high protein concentration in the range of 55-70%, 12-25% Carbohydrate, 4.0 – 8.2% lipid, vitamin, and mineral. Spirulina is rich in phytonutrients that exhibit potent antioxidant properties and contains high number of natural pigments as bioactive component [23]. According to Saharan & Jood [24], spirulina powder contains 71.90% protein, 1.27% lipid, and 13.63% carbohydrates, whereas Qaranjiki and Kırkağaç [25] found that nutrition content of spirulina powder including protein, lipid, and carbohydrate were 60.94%, 4.57%, and 28.07% respectively. Spirulina powder was also used as feed ingredients in several Artemia rearing studies [26, 27, 28].

Soybeans (*Glycine max*) belonging to the family leguminosae that grows in tropical and subtropical regions, and one of the world's most important sources of protein [29]. Soybean is a cheap commodity that is readily available in Indonesia market. Soybeans contain high protein, so it is commonly used as substitute and complement to fish meal in feed (30). According to Adelakun *et al.* [29], soybeans contain 36.49% protein, 30.16% carbohydrate and 19.94% lipid. Alfred *et al.* [30] stated that soybean seeds contain 32 - 43.6% crude protein, 15.5 - 24.7% lipid, and 31.7 - 31.85% carbohydrate on a dry matter basis. Conventional soybean meal has higher content of crude protein (47.5%) [31]. Amin *et al.* [21], stated that soybean meal can used as protein source for Artemia feed. Other studies were also used soybean meal as Artemia feed ingredient [26, 28, 32]. The aim of this study was to determine and compare the survival, length growth and biomass of Artemia fed spirulina powder and soybean flour. These results can be useful for providing alternative culture systems for Artemia biomass production.

Materials and Methods

The study was carried out in the aquaculture laboratory, Palangka Raya Christian University, Indonesia from June to August 2022. The Artemia cyst used in this study was *Artemia franciscana* obtained from the commercial brand Golden West Artemia (Great Salt Lake Artemia). Salt water for hatching cysts and rearing Artemia were made by mixing coarse salt with fresh water according to Islam *et al.* (2015). Three glass hatching jars containing 2 L of salt water (30 ppt) were used for hatching Artemia cysts. One gram of cysts was administered to each hatching jar that received continuous and vigorous aeration for 48 hours. Artemia rearing studies were conducted in nine aquariums (40 x 30 x 30 cm) filled with 20 L of 35 ppt salt water. After hatching, 2000 nauplii/L were stocked into each aquarium and reared for 21 days in 12/12 hour light/dark conditions. Moderate aeration was performed during the rearing period to keep food suspended and to increase dissolved oxygen.

The study was conducted in completely randomized design (CRD) with three treatments and three replications. The feeding treatments applied were 100% spirulina powder (S-100%), 100% soybean flour (SB-100%), and 50% spirulina powder + 50% soybean flour (S-50%+SB-50%). Spirulina powder was obtained from a local producer (PT Polaris Sinar Intan, Tangerang, Indonesia) which was produced as additional feed for shrimp and fish. Soybean flour which made by grinding soybeans into a fine powder was obtained from local market. The feeds that have been prepared according to the treatments were homogenized in salt water, and passed through a 50 µm mesh size sieve to obtained feed size particles suitable for Artemia. Feed was provided twice a day at morning and evening by 2% of Artemia body weight [28].

At the end of rearing, the survival rate, length growth, and biomass of Artemia were calculated. Survival rate (SR) was calculated by counting the number of live brine shrimp in each aquarium. The total length of Artemia was measured by taking pictures using a digital microscope equipped with a length scale. Ten samples of Artemia were sampled in each aquarium using a small siphon tube and their body length was measured. Wet Artemia biomass was obtained by harvesting all Artemia from each aquarium using a 250 µm mesh size net and the remaining water was removed using absorbent paper, then weighed using a digital electric balance. Water quality parameters of rearing aquarium including salinity, temperature, pH, and dissolved oxygen (DO) were observed on day 1, 7, 14, and 21. Water quality parameters were measured by using Lutron digital measuring instrument. Data on survival rate, length growth and biomass of Artemia were analyzed using one-way analysis of variance (ANOVA) and LSD (Least Significant Difference) test, to determine differences between treatments.

Results

The number of Artemia nauplii stocked in the experimental aquarium was 2000 nauplii/L. During the maintenance period (21 days), the density of Artemia decreased due to some dying. The average number of live Artemia after 21 days of rearing were 501.33±28.56 individuals (S-100%), 381.89±37.26 individuals (SB-100%), and 389.78±40.51 individuals (S-50%+SB-50%). The survival rates of Artemia fed with S-100%, SB-100%, and S-50% + SB-50%, were 25.07±1.43%, 19.09±1.36%, and 19.49±2.03% respectively (Fig.1).

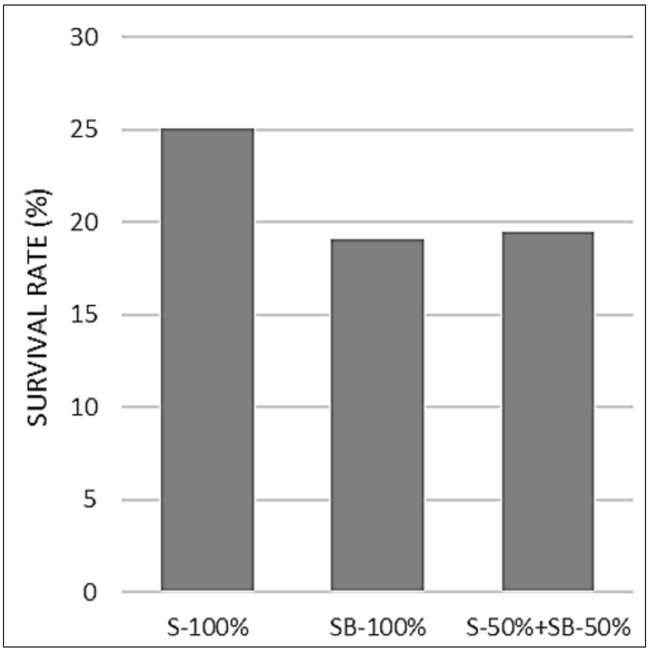

Fig 1: The survival rate of Artemia fed with different feeds at 21 days of rearing

The results of analysis of variance (ANOVA) showed that the survival rate of Artemia was very significantly affected by different feeding (*p*<0.01). The LSD test showed that the highest survival rate was obtained by feeding with S-100%, while fed with SB-100% and S-50% + SB-50% were not significantly different (*p*<0.05).

The 1-day-old Artemia nauplii that were stocked had a body length of 0.94±0.08 mm. Artemia grew and reached a body length of 5.05 – 11.40 mm in 21 days of rearing, depending on the feed treatment given. The body length of Artemia fed with S-100%, SB-100%, and S-50% + SB-50%, were 9.00±0.15 mm, 7.24±0.44 mm, and 8.89±0.44 mm respectively (Fig. 2). The results of analysis of variance (ANOVA) showed that the Artemia body length was very significantly affected by different feeding (*p*<0.01). The LSD test showed that fed with SB-100% resulted in a lower Artemia body length compared to S-100% and S-50%+SB-50%, while S-100% were not significantly different with S-50%+SB-50% (*p*<0.05).

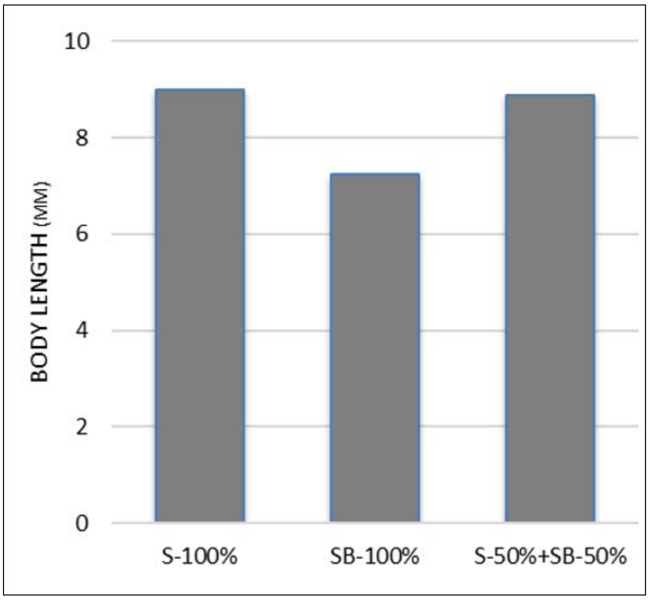

Fig 2: The body length of Artemia fed with different feeds at 21 days of rearing

After 21 days of rearing, Artemia in each aquarium were harvested and the yields were weighed by wet weight as Artemia biomass. The Artemia biomass production from each treatment are presented in Figure 3. The one way ANOVA analysis showed that the Artemia biomass was very significantly affected by different feeding (*p*<0.01). Based on the LSD test results, the highest Artemia biomass was produced by feeding S-100% (17,667±0.322 gm), followed by feeding S-50%+SB-50% (13,622±2,183 gm), and the lowest by feeding SB -100% (10.858±1.428 grams) (*p*<0.05).

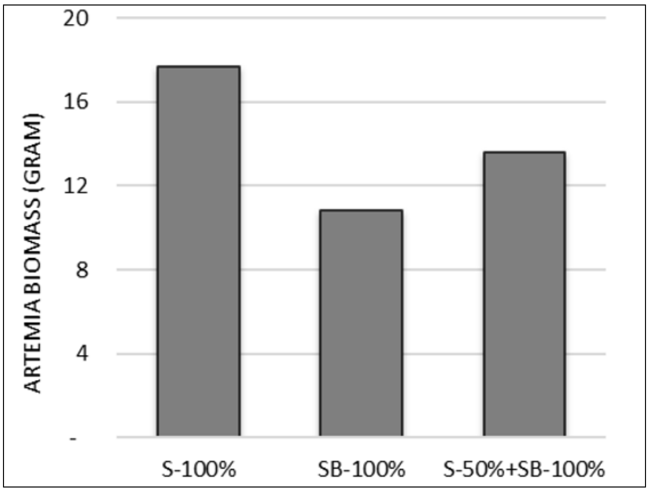

Fig 3: The biomass of Artemia fed with different feeds at 21 days of rearing

Water quality conditions of the Artemia rearing media during 21 days of rearing are presented in Table 1. The salinity of Artemia rearing media ranged from 34.80 - 36.90 ppt. The evaporation process caused salinity of media to increase over time. Therefore, the volume of aquarium water was always controlled. If it decreased due to evaporation, fresh water was added, so that salinity could be maintained. The oxygen content of the rearing media was supported by continuous aeration which produced dissolved oxygen ranging from 4.70 - 6.80 mg L^{-1}. The pH level of the rearing media was recorded in the range 7.02 - 7.42. The water temperature was quite stable, recorded in the range of 27.00 - 28.80 °C. The results of variance analysis (ANOVA) showed that all parameters were no significant differences among the treatments (p>0.05).

Table 1: Mean ± SD value of the water quality parameters

Parameters	Feed		
	S-100%	**SB-100%**	**S-50%+SB-50%**
Salinity (ppt)	35.76±0.64	35.72±0.79	35.96±0.64
Temperature (ºC)	27.53±0.32	27.48±0.56	27.51±0.57
pH	7.30±0.06	7.25±0.13	7.24±0.13
Dissolved Oxygen (mg L^{-1})	5.47±0.94	5.69±0.76	5.66±0.75

Discussions

The success of Artemia biomass production is due to the high survival and growth of Artemia. The survival of Artemia is greatly influenced by the food source provided and how water quality conditions can be maintained at optimal conditions. In this study, nauplii were stocked at moderately densities (2,000 larvae per liter), so the survival was expected to be high, whereas Dhont & Sorgeloos [8] recommended up to 5,000 larvae per liter for batch culture. The survival rates of Artemia fed with S-100%, SB-100%, and S-50% + SB-50%, were 25.07±1.43%, 19.09±1.36%, and 19.49±2.03% respectively. These results indicate that the survival rate of Artemia fed with soybean flour tends to be lower than those fed with spirulina powder. Other studies also obtained this fact [33, 28]. Amin *et al.* [33] obtained a quite low survival rate of the Artemia fed soybean flour (14.70%) compared to those fed spirulina powder (47.29%). Feeding with soybean flour causes a low survival rate, because the flour particle size is too large, so it settles at the bottom of the tank, which pollutes the water [34]. Feed particles that attach to the tank will deteriorate the water quality due to accumulation of the hazardous gases such as nitrite [26, 35].

Factors that influence the growth of Artemia include Artemia strain, nutritional content of feed and water quality, especially temperature and salinity [1, 36, 37]. In this study, the body length of Artemia fed with S-100%, SB-100%, and S-50% + SB-50%, were 9.00±0.15 mm, 7.24±0.44 mm, and 8.89±0.44 mm respectively. Artemia fed spirulina powder showed better length growth compared to feed containing soybeans. Some research also showed that feeding spirulina powder provided the best growth performance compared to other comparison diets [25, 27, 28]. Arumugam *et al.* [27] obtained length growth of Artemia fed with spirulina powder of 1.1±0.6 cm at 20 days of rearing, and Khadka *et al.* [28] obtained 8.7 mm at 21 days of rearing. The length growth of Artemia fed soybean meal at 15 days of rearing was recorded at 7.26 mm [34] and 8.04 mm [32]. The best results from feeding with spirulina powder may be due to the fact that spirulina has higher nutrients compared to soybean flour. Soybeans contain 32 - 43.6% crude protein [30], while Spirulina powder has a high protein concentration in the range of 55-70%. Spirulina is rich in phytonutrients that exhibit potent antioxidant properties and contains very high amounts of natural pigments as bioactive components. [23].

High Artemia biomass is produced by a combination of high survival and growth rates. The Artemia biomass produced by feeding S-100%, SB-100%, and S-50% + SB-50%, were 17,667±0.322 g, 10.858±1.428 g, and 13,622±2,183 g respectively. The highest biomass was obtained by feeding with spirulina powder. The harvest time affects the biomass obtained, because if it is too early, the artemia will still be too small, so the biomass will be lower. In this study, Artemia biomass was harvested after 21 days of rearing, when the Artemia were mature but had not yet produced offspring. According to Saygı and Demirkalp [36], complete development from the nauplius stage to the adult stage required 30 days at 18 °C, 20 days at 24 °C and 15 days at 30 °C. Adult Artemia biomass can be obtained from 15 days of rearing [9, 38]. The highest biomass production was obtained by harvested at 22 to 24 days of rearing [39].

The water quality conditions greatly determine the survival and growth of Artemia or other aquatic organisms. Artemia cannot grow optimally and even die if water quality conditions are beyond its tolerance. In order for Artemia production to be successful under controlled conditions, the water quality conditions must be maintained in the optimal range. The range of salinity, temperature, pH and dissolved oxygen of the Artemia rearing media were 34.80 – 36.90 ppt, 27.00 – 28.80 °C, 7.02 – 7.42, and 4.70 – 6.80 mg L^{-1} respectively. Artemia has a wide tolerance to salinity (euryhaline) ranging from 10 to 340 ppt [37]. The salinity of the Artemia rearing media were maintained at around 35 ppt, by adding fresh water as a substitute for evaporated water. The evaporation process caused the salinity increase over time. The salinity in this study was still in the optimal range for Artemia production, which according to Dhont & Sorgeloos [8], ranged from 32 to 65 ppt. Temperature is a water quality parameter that greatly influences the growth of Artemia (1). Artemia generally grows well in the temperature range of 19 – 25 °C [8]. Artemia culture in tropical areas such as Indonesia, the temperature tends to be higher than the optimal range. According to Lim *et al.* [10], the high temperature (25 – 30 °C) might have contributed to the low survival rate of the on-grown Artemia. The pH of the rearing media during study conducted was in the optimal range for the survival and growth of Artemia. The optimal pH range for artemia production is 6.5 – 8 [8]. Artemia from hatching until 18 days old is tolerance to pH 6 - 8, but after 18 days old, it becomes pH 7 - 8 [40]. Dhont & Sorgeloos [8] stated that dissolved oxygen must be above 2.5 mg L^{-1} for optimal Artemia production. If the concentration of less than 2 mg L^{-1} will limit the production of biomass. The dissolved oxygen was supported by continuous aeration, so that it was always maintained at the optimal range to supported the survival and growth of Artemia.

Conclusion

The success of rearing Artemia until they become adults is influenced by providing appropriate feed and controlling the water quality parameters of the rearing media. Spirulina powder and soybean flour are actually potential Artemia feed ingredients to provide optimal growth, because they contain high protein. The results of this research show that the use of 100% spirulina for artemia feed provided the best performance for survival, growth and biomass production of Artemia. Artemia rearing for 21 days in 20L of 35ppt salt water, fed with spirulina powder had an average body length of 9.00±0.15 mm, survival rate of 25.07±1.43%, and wet biomass production of 17,667±0.322 g. Thus, spirulina powder is a very suitable feed for on-grown Artemia production.

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