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APPLICATION OF DIFFERENT FEED AND FEEDING PERIODS DURING REARING OF MALAY COMBTAIL (*Belontia hasselti*) LARVAE

Danang Yonarta[*], Tanbiyaskur[*)#], I Gede Arya Weda[*], and Fitra Gustiar[**]

[*]Department of Aquaculture, Faculty of Agriculture, Sriwijaya University
Jalan Raya Palembang Prabumulih KM 32, Indralaya 30862, Ogan Ilir, South Sumatra
[**]Department of Agroecotechnology, Faculty of Agriculture, Sriwijaya University
Jalan Raya Palembang Prabumulih KM 32, Indralaya 30862, Ogan Ilir, South Sumatra

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ABSTRACT

Feed type and feeding period play critical roles in growth and survival of fish larvae during rearing period, for which no related studies are available for Malay combtail larvae. This research aimed to determine the best feed type and feeding period for growth and survival of Malay combtail larvae. The research experiment was arranged in a completely randomized design with five treatments of different feed and feeding periods with three replications, namely (P1) nauplii *Artemia* sp. (4-15 days), *Moina* sp. (14-24 days), and *Tubifex* sp. (23-35 days), (P2) nauplii *Artemia* sp. (4-13 days), *Moina* sp. (12-20 days), and *Tubifex* sp. (19-35 days), (P3) nauplii *Artemia* sp. (4-11 days), *Moina* sp. (10-16 days), and *Tubifex* sp. (15-35 days), (P4) nauplii *Artemia* sp. (4-11 days), *Moina* sp. (12-20 days), and artificial feed (19-35 days), and (P5) nauplii *Artemia* sp. (4-11 days), *Moina* sp. (10-16 days), and artificial feed (15-35 days). The results showed that P4 was the best treatment, where larvae had better absolute growth in length and weight and survival of 11.09 ± 0.03 mm, 0.083 ± 0.001 g, and 50.67 ± 1.15%, respectively. Variations of water quality parameters during the experiment in all treatments ranged between 6.0-6.6 for pH, 0.017-0.091 mg L^{-1} for ammonia, and 4.03-4.43 mg L^{-1} for dissolved oxygen. The results of this research that the sequential and early application of live feed and much later artificial feed appication in combination with the timely feeding period and the larval development improve growth and survival of Malay combtail larvae.

KEYWORDS: feeding period; growth; Malay combtail; survival rate; type of feed

ABSTRAK: Aplikasi Jenis Pakan dan Periode Pemberian Pakan yang Berbeda pada Pemeliharaan Larva Ikan Selincah *(Belontia hasselti)*

Jenis pakan dan periode pemberian pakan memainkan peran penting dalam pertumbuhan dan kelangsungan hidup larva ikan selama masa pemeliharaan, hingga saat ini belum ada penelitian terkait mengenai larva ikan selincah. Penelitian ini bertujuan untuk mengetahui jenis pakan dan lama pemberian pakan yang terbaik untuk pertumbuhan dan kelangsungan hidup larva ikan selincah. Penelitian disusun dalam rancangan acak lengkap dengan lima perlakuan pakan dan lama pemberian pakan berbeda dengan tiga ulangan yaitu (P1) nauplii **Artemia** *sp. (4-15 hari),* **Moina** *sp. (14-24 hari), dan* **Tubifex** *sp. (23-35 hari), (P2) nauplii* **Artemia** *sp. (4-13 hari),* **Moina** *sp. (12-20 hari), dan* **Tubifex** *sp. (19-35 hari), (P3) nauplii*

#Korespondensi: Department of Aquaculture, Faculty of Agriculture, Sriwijaya University
Email: tanbiyaskur@unsri.ac.id

***Artemia** sp. (4-11 hari), **Moina** sp. (10-16 hari), dan **Tubifex** sp. (15-35 hari), (P4) nauplii **Artemia** sp. (4-11 hari), **Moina** sp. (12-20 hari), dan pakan buatan (19-35 hari), dan (P5) nauplii **Artemia** sp. (4-11 hari), **Moina** sp. (10-16 hari), dan pakan buatan (15-35 hari). Hasil penelitian menunjukkan bahwa P4 merupakan perlakuan terbaik, di mana larva mempunyai pertumbuhan panjang dan berat absolut yang lebih baik serta kelangsungan hidup masing-masing sebesar 11,09 ± 0,03 mm, 0,083 ± 0,001 g, dan 50,67 ± 1,15%. Variasi parameter kualitas air selama percobaan pada semua perlakuan berkisar antara 6,0-6,6 untuk pH, 0,017-0,091 mg L^{-1} untuk amoniak, dan 4,03-4,43 mg L^{-1} untuk oksigen terlarut. Hasil dari penelitian ini adalah pemberian pakan hidup secara berurutan dan dini serta pemberian pakan buatan yang dikombinasikan dengan periode pemberian pakan yang tepat waktu dan perkembangan larva akan meningkatkan pertumbuhan dan kelangsungan hidup larva ikan selincah.*

KATA KUNCI: ikan selincah; jenis pakan; kelangsungan hidup; periode pemberian pakan; pertumbuhan

INTRODUCTION

During the larval stage in fish, appropriate initial feeding has to be carried out in conjunction with the diminishing egg yolk reserve. According to Lucas *et al*. (2015), a lack of food during this critical phase of larval development frequently causes a high death rate in fish larvae. The critical larval phase occurs mainly from 1 day after hatching to 40 days (Febrianti *et al*., 2015). For example, the survival rate of gourami larvae kept for 15 days only reached 58.33% (Ghofur *et al*., 2014). Furthermore, Lucas *et al*. (2015) reported that gourami larvae survival rate can be as low as 34%.

The digestive system of fish larvae has not yet fully developed, for which they need natural food that is easily digestible to stimulate the activity of their digestive enzymes. One of the advantages of natural foods is that the food particles' sizes mostly fit the mouth openings of fish larvae. Considering that studies related to the feeding behavior and mouth opening of Malay combtail larvae are non-existent, the approach of this research focuses on fish from the same family, namely gourami fish. Based on WoRMS (World of Register Marine Science) (2023), Malay combtail and gourami belong to the same fish family, namely Osphronemidae. The eating habits of Malay combtail and gourami also have similarities, as described by Sari *et al.* (2019). In addition, Agustinus and Minggawati (2021), gourami and Malay combtail are both classified as omnivorous fish (eaters of all animals or plants) but are more inclined towards herbivores (plant eaters).

According to Febrianti *et al*. (2015), gourami larvae at the age of 4 days have a mouth opening size of around 0.39 ± 0.72 mm and feed on *Moina* sp. (0.25-0.40 mm) until the age of 28 days and have an average length of 18.5 ± 1.9 mm. Considering the mouth size of gourami larvae, Mubarak *et al.* (2023) argued that the larvae can be fed on nauplii *Artemia* sp., whose size ranges from 0.22 to 0.25 mm, which is suitable for an incomplete digestive tract in a 4-day-old fish larva. Apart from nauplii *Artemia* sp., natural food that can be given to the fish larvae is *Daphnia* sp. and *Tubifex* sp.

Each natural food has a different nutritional value (Pratama, 2021). Protein contents in *Artemia* sp., *Moina* sp., and *Tubifex* sp. are estimated at around 56.20% (Septian *et al*., 2017), 36.08% (Rozi *et al*., 2017), and 41.79% (Septian *et al.*, 2017), respectively. Besides being given natural food, fish larvae can also be fed artificial feed. According to Hidayat *et al.* (2021), commercial pellets can be formulated to have up to 60% protein, which can theoretically substitute protein content in natural feed.

However, replacing natural feed with artificial feed must be carried out at the right time in the fish larval phase (Suhenda, 2010). Research regarding the period of administration with different types of feed on tambakan fish larvae showed that nauplii *Artemia* sp. given to larvae aged 4-11 days, *Moina* sp. given at 10-16 days of age, and artificial feed given at 15-35 days of age produced the best survival and growth of the fish larvae (Agustina *et al*., 2015). In the climbing perch larvae, the use of *Artemia* sp. as feed for the fish larvae at the age of 3-15 days, *Moina* sp. at the age of 14-24 days, and artificial feed at the age of 21-33 days also produced the best growth and survival of the larvae (Muslim, 2019). Moreover, combining *Artemia* sp. given at the start of larval rearing until day 19 and *Tubifex* sp. given between days 20 to 30 produced the best survival and growth of lais fish larvae (Yurisman & Heltonika, 2010). In general, giving artificial feed to gourami fish larvae starting at 25 days old is considered the optimal application (Suhenda, 2010). However, similar research on Malay combtail larvae has never been carried out. Therefore, there is a need for research regarding the correct type of feed according to the mouth-opening time of Malay combtail larvae to increase their survival and growth. It is expected that the results of this research could increase the production of Malay combtail in a controlled environment and serve as a reference for cultivators.

MATERIALS AND METHODS

Location and Time of Research

This research was carried out at the Basic Fisheries Laboratory, Aquaculture Laboratory and Experimental Ponds, Aquaculture Study Program, Fisheries Department, Faculty of Agriculture, Sriwijaya University. The research was carried out from January to March 2023.

Materials and Tools

The test fish used in this research consisted of Malay combtail broodstock sized 10 ± 1 cm and Malay combtail larvae of 4-day-old. Additionally, NaCl solution, gonadotropin hormones, *Artemia* sp., *Moina* sp., *Tubifex* sp., commercial pellets, distilled water, MnSO4, Chlorox, Fenat, and potassium permanganate were also prepared. The tools used in this research included aquariums, concrete ponds, plastic boxes, aerators, heaters (Roston RSK-50), syringes, digital scales, analytical balances (Ohaus PR-224 E), thermometers, pH meters (EZ- 9909), DO meter (Hanna HI-9146), spectrophotometer (Orion AquaMate 7100 Visible), and caliper.

Experimental Design

The experimental units were arranged in a completely randomized design with five treatments and three replications. The choice of food type for Malay combtail larvae referred to research conducted by Agustina *et al.* (2015), consisting of:

P1: Nauplii *Artemia* sp. (4-15 days old), *Moina* sp. (14-24 days old), and *Tubifex* sp. (23-35 days old).
P2: Nauplii *Artemia* sp. (4-13 days old), *Moina* sp. (12-20 days old), and *Tubifex* sp. (19-35 days old).
P3: Nauplii *Artemia* sp. (4-11 days old), *Moina* sp. (10-16 days old), and *Tubifex* sp. (15-35 days old).
P4: Nauplii *Artemia* sp. (4-11 days old), *Moina* sp. (12-20 days old), and artificial feed (19-35 days old).
P5: Nauplii *Artemia* sp. (4-11 days old), *Moina* sp. (10-16 days old), and artificial feed (15-35 days old)

Test Fish Preparation

The Malay combtail broodstock were initially reared in a concrete pond measuring 4 x 2 x 2 m^3, filled with water to a height of 1.5 m. The pond was used for spawning the broodstock and hatching the produced fish eggs using a plastic box measuring 60 x 40 x 45 cm^3. Afterward, the larval-rearing container used was an aquarium measuring 25 x 25 x 25 cm^3. The plastic box and aquarium used were first cleaned, then filled with water, and 20 mg L^{-1} of potassium permanganate was added and left for one day to sterilize the media (Agustina *et al.*, 2015). The water volume in the plastic box was 84 L, and the aquarium was 12 L.

The broodstock fish were collected from the wild by fishermen in Tanjung Baru Village, Tanjung Batu District, Ogan Ilir Regency, South Sumatra Province. The Malay combtail broodstock used had a length of 9 ± 1 cm (Yonarta *et al.*, 2023c). The male and female brood fish were reared separately for two months. The stocking density in the concrete pond was 150 broodstock which were fed with commercial pellets (protein content 39%) enriched with vitamin E as much as 5% of body weight three times a day (08:00, 12:00, and 17:00 WIB) (Yonarta *et al.*, 2023b). Once every two weeks, water exchange was done by 50% of the total water volume. When the broodstock were ready, the spawning process was carried out semi-naturally. The brood of Malay combtail were given a single injection of gonadotropin hormone at a dose of 0.5 mL kg^{-1} (Yonarta *et al.*, 2023a). After the females produced the eggs, they were carefully collected and placed in the aquarium until they hatch into larvae.

The stocking density of Malay combtail larvae was four ind. L^{-1} (Pranata *et al.*, 2017). The larvae used were 4 days old and kept for 31 days. The larvae were stocked in the morning after 10 minutes of acclimatization. Prior to the stocking and at the end of the rearing period, the length and weight of the Malay combtail larvae were recorded. However, weight measurements were also carried out after the larvae were fed with *Tubifex* sp. and commercial pellets to determine the amount of feed consumed. The survival rate was determined by counting and recording the number of survived larvae at the end of the rearing period. Water quality parameters, consisting of pH, dissolved oxygen, and ammonia, were measured regularly during the rearing period. The temperature was controlled in the range of 30 ± 0.5ºC. Water was siphoned once a week and refilled as needed.

The fish larvae were fed with *Artemia* sp. and *Moina* sp. as many as ten individuals per feeding time (Agustina *et al*., 2015), chopped *Tubifex* sp. as much as 3% of body weight (Mahary *et al*., 2022) and artificial feed as much as 6% of body weight (Bulanin *et al*., 2021). The feeding frequency was five times a day at 07:00, 10:00, 13:00, 16:00 and 19:00 WIB (Sugihartono *et al.*, 2016). The adjustment period was completed first by giving the previous feed 1:1, with the new feed given afterward for a day.

Experimental Parameters

The main parameters observed in this study included growth in absolute length and weight and survival rate. Measured water quality included temperature, pH, dissolved oxygen, and ammonia. Formulas 1, 2, and 3 below were used to measure growth in length and weight and survival (Effendie, 2002):

$$\text{Absolute length growth (mm)} = L_t - L_0 \quad \text{.......... (1)}$$

Where
L_o = Average length of fish at the start of rearing (mm)
L_t = Average length of fish at the end of rearing (mm)

$$\text{Absolute weight growth (g)} = W_t - W_0 \quad \text{..........(2)}$$

Where
W_0 = Average weight of fish at the start of rearing (g)
W_t = Average weight of fish at the end of rearing (g)

$$\text{Survival rate (\%)} = \frac{N_t}{N_0} \times 100\,\% \quad \text{..................(3)}$$

N_0 = Number of fish at the start of rearing (ind)

N_t = Number of fish at the end of rearing (ind)

Data Analysis

The collected data were statistically analyzed using analysis of variance, presented in tabulation form. If the results obtained showed a significant difference, it was continued with the least *significant difference* (LSD) *test* with a 95% confidence interval. Water quality data was analyzed descriptively.

RESULTS AND DISCUSSION

Absolute Growth in Length and Weight of Malay Combtail Larvae

The absolute growth in length and weight of Malay combtail larvae reared for 31 days with different types and periods of feed is presented in Table 1. Analysis of variance showed that different periods and types of feed significantly affected the absolute growth in length and weight of Malay combtail larvae. The results of the LSD test showed that the absolute growth in length and weight of Malay combtail larvae in P4 was higher than in P1, P2, and P3 but was not significantly different from P5, while P1 showed the lowest absolute growth in weight and length compared to the other treatments.

The high growth in P4 compared to the other treatments proves that the right time to change feed can accelerate larval growth, which is in line with the increasingly more developed digestive system of Malay combtail larvae. This means the larval can receive more complex nutritional intake, such as artificial feed. Therefore, feeding management must consider several aspects, such as the sufficient nutrition contained in the feed, the specific nutrient requirement of the fish, and the right time of feed application. The nutrients in fish need sufficient protein, fat, carbohydrates, minerals, and vitamins (Kardana *et al.*, 2019). The protein contained in feed is a primary factor that directly influences fish growth. The role of protein can be maximized to support fish growth if the need for energy sources can supplied by ingredients other than protein (Utomo *et al.*, 2013). Hidayat *et al*. (2021) stated that artificial feed in powder form has 60% protein, while *Artemia* sp. contains 56.2% protein (Septian *et al*., 2017), *Moina* sp. contains 36.08% protein (Rozi *et al*., 2017), and *Tubifex* sp. contains 41.79% protein (Septian *et al*., 2017). Additionally, artificial feed has an advantage over natural feed in that its nutritional content can be tailored to the needs and mouth openings of the fish (Gunawan & Khalil, 2015).

The suppressed growth of Malay combtail larvae, especially in P1, was caused by feeding *Moina* sp. given at 14-24 days, which was relatively more extended than the other

Table 1. Absolute growth in length and weight of Malay combtail larvae reared with different periods of time and types of feed

Treatments	Absolute length growth (mm)	Absolute weight growth (g)
P1	10.20 ± 0.04[a]	0.072 ± 0.002[a]
P2	10.81 ± 0.01[b]	0.077 ± 0.001[b]
P3	10.84 ± 0.03[b]	0.079 ± 0.001[b]
P4	11.09 ± 0.03[c]	0.083 ± 0.001[c]
P5	11.03 ± 0.05[c]	0.082 ± 0.001[c]

Note: Numbers in the same column followed by different superscript letters indicate significantly different results in the LSD test with a 95% confidence interval.

treatments. The lack of protein in *Moina* sp. is believed to be the reason for the stunted growth of Malay combtail larvae. Marzuqi *et al.* (2017) stated that a lack of protein intake from feed can cause fish growth to be hampered and accompanied by a reduction in fish weight because fish utilize energy sources in the body to provide sufficient energy for body maintenance. As a result, the fish will grow longer compared to its body weight.

Survival of Malay Combtail Larvae

The average survival of Malay combtail larvae reared for 31 days with different periods and types of feed is presented in Table 2. Analysis of variance showed that different periods and types of feed had a significant effect ($P<0.05$) on the survival of Malay combtail larvae. The results of the LSD test showed that the survival rate of Malay combtail larvae in P1 was higher than in P2, P3, and P5 but was not significantly different ($P>0.05$) from P4. The lowest survival was in P2.

The larval stage is the most critical phase in the life process of fish, and generally, the highest death rate occurs in this phase (Kelabora, 2010). The high survival rate in P1 is due to the proper feeding in terms of feeding time, quantity, quality, and size of feed (Tjodi *et al*., 2016). More specifically, the high survival rate in P1 is due to the longer food transition period than in the other treatments leading to better adaptation of the fish larvae to the feed. Rahmi *et al*. (2016) suggested that the transition of food types from the larval to the fry stage must consider the correct size and age of the fish. This is related to the availability of digestive enzymes within the intestinal tract of the fish larvae. According to Raharjo *et al*. (2016), the digestive system is not yet fully formed in the larval phase. During this stage, the stomach has not yet developed, and the digestive enzymes do not yet work optimally. Therefore, the fish larvae must feed on natural food containing digestive enzymes. Therefore, providing appropriate food has to match with the larval developmental stages and type of food which supports the survival of Malay combtail larvae.

High mortality observed specifically in P2 was caused by abnormal larvae in each rearing container with abnormal body shape characteristics. This can be identified when the larvae were 2 weeks old, during which period many deaths occur. According to Aidil *et al*. (2016) the digestive system of abnormal larvae has anomalies that will not develop properly. Such abnormality makes the larvae more susceptible to death. In addition, Malay combtail larvae also experienced stress caused by initial sampling and disturbances in rearing containers. Rohaniawan *et al*. (2017) argued that siphoning could cause the remaining feces and feed at the bottom of the rearing container to stir up, which can cause stress to fish larvae. According to Sarimudin *et al.* (2016), stress is a response from the fish's body that will have an impact on decreasing immunity and increasing the possibility of disease and death in fish.

Table 2. Survival rate of Malay combtail larvae reared with different feeding periods and types of feed

Treatments	Survival Rate (%)
P1	51.33 ± 3.06^{c}
P2	30.00 ± 3.46^{a}
P3	44.00 ± 5.29^{b}
P4	50.67 ± 1.15^{bc}
P5	45.33 ± 5.03^{b}

Note: Numbers in the same column followed by different superscript letters indicate significantly different results in the LSD test with a 95% confidence interval

Table 3. Water quality of the medium for rearing Malay combtail larvae with different periods of time and types of feed

Treatments	Parameters		
	pH	Dissolved oxygen (mg L^{-1})	Ammonia (mg L^{-1})
P1	6.1-6.6	4.13-4.37	0.025-0.073
P2	6.2-6.5	4.13-4.43	0.017-0.041
P3	6.1-6.6	4.23-4.41	0.031-0.091
P4	6.2-6.6	4.03-4.43	0.035-0.061
P5	6.0-6.6	4.07-4.47	0.022-0.073

Water Quality of Rearing Media of Malay Combtail Larvae

The ranges of water quality measured in the rearing media of Malay combtail larvae are presented in Table 3. Overall, the water quality during the rearing of Malay combtail larvae was considered relatively stable. Water quality is an important aspect that can influence fish's growth and development, survival, and productivity. Good water quality can support the survival of farmed fish (Fauzia & Suseno, 2020). On the other hand, poor water quality can cause diseases and death of farmed fish Nasir and Khalil (2016).

The temperature in the container for rearing Malay combtail larvae was controlled using a heater at 30 ± 0.5°C. Temperature has an essential effect on fish, such as respiration, reproduction, and growth (Kelabora, 2010). At high temperatures, the activity of digestive enzymes and growth hormones in fish increases, and the fish's metabolic rate runs faster (Ridwantara *et al.*, 2019). On the other hand, at low temperatures, the fish's appetite will decrease, and the possibility of the fish being attacked by disease will increase (Sudrajat & Solang, 2014). The acidity (pH) degree in all treatments ranged from 6.0 to 6.6. According to Hasanah *et al*. (2019), the degree of acidity (pH) is a factor that limits the metabolic rate of fish, which also affects the survival and growth of fish. According to Mulyani *et al*. (2014), a pH that is too high or low can disrupt fish survival.

Dissolved oxygen in all treatments ranged from 4.03 to 4.47 mg L^{-1}. Based on the Ministry of State Secretariat of the Republic of Indonesia through President Regulation Number 22 (2021), the water quality standard for dissolved oxygen is a minimum of 3 mg L^{-1}. Dissolved oxygen is an important aspect that determines fish survival (Mulqan *et al*., 2017). A decrease in the value of dissolved oxygen in a body of water can be dangerous, especially for aquatic organisms. Generally, fish found in several polluted waters die not only because of the direct toxicity of waste materials but also due to a lack of dissolved oxygen in the waters because it is used more to process the degradation of organic materials in the waters (Nanda & Abdullah, 2021). On the other hand, good oxygen levels can support fish metabolic processes so that more energy produced will be allocated for growth (Scabra *et al*., 2022).

Ammonia in all treatments ranged from 0.017 to 0.091 mg L^{-1}. Ammonia comes from leftover feed and feces produced by fish. At certain levels, ammonia can be toxic to fish (Siegers *et al*., 2019). The nitrification process that does not run smoothly is also another factor that contributes to increasing ammonia levels (Rarassari *et al*., 2024). According to Widiastuti & Irawati (2009), excess ammonia can cause death and inhibit fish growth.

CONCLUSION

A treatment P4 consisting of nauplii *Artemia* sp. (4-11 days), *Moina* sp. (12-20 days), and artificial feed (19-35 days) are the most appropriate type and time period of feeding to support the growth and survival rate of

Malay combtail larvae. This can be seen from the research results which found that P4 was higher than other treatments. We recommend that Malay combtail breeders use the findings of this study as an essential reference during the rearing of Malay combtail larvae until they reach seed size for grow-out.

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