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SURVIVAL AND GROWTH OF SILVER RASBORA (*RASBORA ARGYROTAENIA*) FED ARTEMIA ENRICHED WITH *SARDINELLA LEMURU* FISH OIL

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Abstract

Silver rasbora (*Rasbora argyrotaenia*) is a new fish species cultured economically for consumption and aquascape. The purpose of this study was to know the effect and determine the optimal concentration of *Sardinella lemuru* fish oil enrichment on artemia to the survival rate and growth of silver rasbora larvae. Fish larvae were feed by enriched artemia nauplii with different doses per liter water. Larvae feeding artemia enrichment with *Sardinella lemuru* fish oil in different doses not affect ($P > 0.05$) on survival, but all growth parameters significantly affected ($P < 0.05$). Optimal artemia enrichment dose for feeding practical of silver rasbora larvae is 0.5 ml fish oil + 0.5 ml egg yolk. Estimate doses that leads to the maximum length gain is 0.6035 ml fish oil + 0.6035 ml egg yolk.

Introduction

Silver rasbora (*Rasbora argyrotaenia*) is a tropical freshwater fish spread in Asian region (Mekong, Chao Phraya and Mae Khlong basins, Malay Peninsula to Borneo, Java and Sumatra in Indonesia), and occurs in rivers and enters flooded fields mainly (CAPULI and BAILLY 2019). The decline in silver rasbora wild population due to high capture for consumption (ROSADI et al. 2014) needs aquaculture production to overcome this problem. As newly cultured species (ADAWIYAH et al. 2019), silver rasbora farming continues to grow and several studies on hatcheries and grow-ups have been carried out.

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The fry availability affects the reliability of silver rasbora farming as a limiting factor (BUDI et al. 2020). Thus, increasing survival and growth must be one of the study goals for many species during the larval phase (SLATER et al. 2018). The quality and quantity of suitable feed in the early larval life can affect the growth and survival of fish (QIN et al. 1997). As the first feed for larvae, many hatcheries still depend on the zooplankton in spite of little or absence of nutrients (KOTANI 2017). Although deficient in certain important nutrients, artemia is important zooplankton as early feed used in larviculture, and enrichment is thus common with nutrients and other functional additives (LANES et al. 2012).

Fish oil is one of additives used in artemia enrichment as life food (LANES et al. 2012). As the major lipid source, the fish oil used to increase energy content and essential fatty acids (EFA) in fish diets (HOSSEINI et al. 2010). EFA are important for early development and survival of fish larvae (IZQUIERDO 1996). *Sardinella lemuru* fish oil is a by-product produced in the fish canning industry located in Banyuwangi, East Java, Indonesia. It has been used for feed supplementation in silver rasbora juvenile (AGUSTIN et al. 2020, AYUNDA et al. 2020, DEWI et al. 2020, MARINI et al. 2020), and potentially use for silver rasbora larvae. The purpose of this study was to know the effect and determine the optimal concentration of *Sardinella lemuru* fish oil enrichment on Artemia to the survival rate and growth of larvae of silver rasbora.

Materials and Methods

Larvae origin

This study was conducted from January to February 2019 in Universitas Airlangga, Banyuwangi Campus. The research was carried out in accordance with the ethical standards of the Law of the Republic of Indonesia Number 18 of 2002 about the National System of Research, Development, and Application of Science and Technology.

Approximately 1-year old brood fish of silver rasbora were imported from Technical Implementation of Development Unit of Freshwater Aquaculture of Umbulan (Pasuruan, East Java, Indonesia). Breeding of brood fish were done using 6 males (4.62 ±0.07 g of body weight and 7.50 ±0.40 cm of total length) and 2 females (5.20 ±0.14 g body weight and 8.60 ±0.56 cm total length). Palm tree fibers were used as a substrate of brood fish natural mating and spawning (BUDI et al. 2020) in a glass aquarium (40 x 50 x 50 cm^3). Brood fish were transferred from the aquarium and the eggs stick to the substrate incubated in this media approximately

2 days for hatching. The dissolved oxygen (DO), temperature, pH, and total ammonia nitrogen (TAN) measured were 6.82 ±0.38 ppm (range 6.2–7.5 ppm), 24.94 ±0.6ºC (range 24–27ºC), 7.24 ±0.32 (range 6.9–7.5), and 0.012 ±0.001 ppm (range 0.010–0.013 ppm), respectively.

Artemia culture and enrichment

A total 1 g of Artemia cysts were cultured in 1.5 l aerated conical plastic bottle using 1 l salt water (30 ppt). After 24 hours, artemia nauplii were transferred to enriched media (100 individual/ml) with various *Sardinella lemuru* fish oil concentrations based on treatments for 6 hours. Before enrichment process, chicken egg yolk was mixed with fish oil (ratio 1:1) to formed emulsion in enrichment media.

Experimental design and rearing

A total of 900 silver rasbora larvae (1.08 ±0.06 mg body weight, 3.14 ±0.17 mm total length) aged 4 days after fertilization (DAF) were assigned to 18-cylinder plastic tank (10 l capacity; 30 cm diameter) with 5 l water volume with maintain dissolved oxygen level using gentle aeration. Fish larvae were feed by enriched artemia nauplii based on the treatments with doses per liter media 0 ml fish oil (P_0), 1 ml egg yolk (P_1), 0.25 ml fish oil + 0.25 ml egg yolk (P_2), 0.5 ml fish oil + 0.5 ml egg yolk (P_3), 0.75 ml fish oil + 0.75 ml egg yolk (P_4), and 1 ml fish oil + 1 ml egg yolk (P_5). There were 3 repetitions for each treatment. Feed was given ad satiation and estimated 20 artemia nauplius per day per larvae with feeding frequency 4 times per day which is 06.00 AM, 12.00 AM, 05.00 PM, and 09.00 PM. Water quality parameters of larvae rearing can be seen in Table 1.

Table 1

Water quality parameters of silver rasbora larvae fed artemia enriched with *Sardinella lemuru* fish oil for 21 days

Treatments*	Dissolved oxygen [mg/l]	Temperature [ºC]	pH	Total ammonia nitrogen [mg/l]
P_0	7.2–8.5	25–27	7–7.4	0–0.029
P_1	7.5–8.5	25–27	6.9–7.4	0–0.002
P_2	7.2–8.4	25.5–27	6.9–7.3	0–0.027
P_3	7.8–8.5	25.5–27	6.9–7.3	0–0.035
P_4	7.8–8.4	25–27	7–7.4	0.038–0.069
P_5	7.8–8.4	25.5–27	6.9–7.3	0–0.073

*Treatments doses per liter media 0 ml fish oil (P_0), 1 ml egg yolk (P_1), 0.25 ml fish oil + 0.25 ml egg yolk (P_2), 0.5 ml fish oil + 0.5 ml egg yolk (P_3), 0.75 ml fish oil + 0.75 ml egg yolk (P_4), and 1 ml fish oil + 1 ml egg yolk (P_5).

Observation and measurements of larvae

At least 10% (n = 5) of larvae number at each treatment were sampled randomly in 4 DAF, 11 DAF, 18 DAF, and 25 DAF. Rapid cooling anesthetic method was used for larvae sampling procedure (CHEN et al. 2013). Larvae total body length (mm) was measured using a micrometer under a stereo-microscope and larvae body weight was measured using a digital scale with 0.1 mg precision.

Observed parameters

The effects of feeding artemia enriched with *Sardinella lemuru* fish oil on survival and growth of silver rasbora larvae were calculated the following parameters. Survival rates (SR, %) was calculated following formula:

$$SR\ [\%] = [(N_f / N_i) \cdot 100],$$

where:

N_i – the initial larvae numbe
N_f – the final larvae number.

Length gain (LG, mm) was calculated based on formula:

$$LG = TL_f - Tl_i,$$

where:
TL_i and TL_f are initial and final average total length [mm].

Weight gain (WG, mm) was calculated based on formula:

$$WG = W_f - W_i,$$

where:
W_i and W_f are initial and final average weight [mg].

Growth rate (GR, mg/days) was calculated following formula:

$$GR = (W_f - W_i)/ D,$$

where:
W_i and W_f are initial and final average weight [mg]
D – rearing times [days].

The specific growth rate (SGR, %/day) was calculated by formula:

$$SGR = [(\ln BW_f - \ln BW_i) / D) \cdot 100],$$

where:
BW_i and BW_f are the initial and final body weights of fish
D – rearing times [days].

Data analysis

The each analyzed data parameters were confirmed normal distribution and homogeneity of variances. Statistically data analysis was used ANOVA test with 95% confidence level and continued with Duncan Multiple Range Test (DMRT) using SPPS 17.0 software. *Sardinela lemuru* fish oil doses that leads to the maximum length gain of silver rasbora (*Rasbora argyrotaenia*) was estimated using polynomial contrast (regression) (YOSSA and VERDEGEM 2015).

Results

The survival and growth of silver rasbora larvae fed artemia enriched with *Sardinella lemuru* fish oil are showed in Table 2. Larvae feeding with artemia enriched with *Sardinella lemuru* fish oil affect significantly ($P < 0.05$) on all growth parameters, but not so with survival. Overall growth data showed the same tendency; where LG, WG, GR, and SGR tends to be increase from P_0 to maksimum in P_3; and then tends to be decrease to P_5 as last treatment. *Sardinela lemuru* fish oil doses that leads to the maximum length gain of silver rasbora (*Rasbora argyrotaenia*) estimated using polynomial contrast (regression) was 0.6035 ml/l.

Table 2

Survival rate (SR), length gain (LG), weight gain (WG), growth rate (GR), and specific growth rate (SGR) of silver rasbora larvae fed artemia enriched with *Sardinella lemuru* fish oil for 21 days

Treatments*	SR [%]	LG [mm]	WG [mg]	GR [mg/days]	SGR [%/days]
P_0	88.3 ±7.8	7.53^{c} ±0.46	13.7^{b} ±1.71	0.65^{b} ±0.08	12.73^{c} ±0.55
P_1	87.3 ±5.8	7.59^{c} ±0.20	15.5^{ab} ±0.68	0.74^{ab} ±0.03	13.29^{abc} ±0.20
P_2	88.7 ±1.3	8.11^{bc} ±0.54	15.7^{ab} ±0.76	0.75^{ab} ±0.04	13.35^{abc} ±0.22
P_3	91.8 ±2.1	8.73^{a} ±0.14	18.0^{a} ±1.80	0.86^{a} ±0.09	13.96^{a} ±0.46
P_4	90.0 ±5.4	8.51^{ab} ±0.10	16.2^{ab} ±1.04	0.77^{ab} ±0.05	13.48^{ab} ±0.29
P_5	89.8 ±3.3	8.14^{abc} ±0.19	13.8^{b} ±1.80	0.66^{b} ±0.09	12.89^{bc} ±0.39

*Treatments doses per liter media 0 ml fish oil (P_0), 1 ml egg yolk (P_1), 0.25 ml fish oil + 0.25 ml egg yolk (P_2), 0.5 ml fish oil + 0.5 ml egg yolk (P_3), 0.75 ml fish oil + 0.75 ml egg yolk (P_4), and 1 ml fish oil + 1 ml egg yolk (P_5). Values are means ± SD. Superscript letters denote significant differences ($P < 0.05$) between treatments.

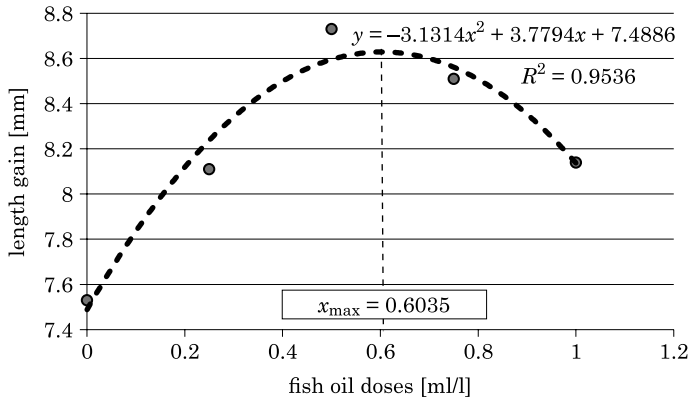

Fig. 1. Estimate *Sardinela lemuru* fish oil doses (0.6035 ml/l) that leads to the maximum length gain of silver rasbora (*Rasbora argyrotaenia*) using polynomial contrast (regression)

Discussion

Enrichment diets have been successfully used in artemia as natural feed for fish larvae (OZUSAGLAM et al. 2013). Fatty acid profiles of sardine oil emulsions increase levels of PUFA in enriched artemia (ARULVASU and MUNISWAMY et al. 2009). Larvae feeding artemia enrichment with *Sardinella lemuru* fish oil in different doses not affect ($P > 0.05$) on survival. Meanwhile, interestingly, all growth parameters significantly affected ($P < 0.05$) and increase compare to control. Similar result was also obtained in previous study with artemia fed on gelatin-acacia microcapsules containing cod liver oil, which supported faster growth rate significantly in post-larval gobies than control (JONES et al. 1984) and using sardine oil in artemia enrichment as natural food was also increased growth in *Poecillia latipinna* fry (ARULVASU and MUNISWAMY et al. 2009). *Sardinella lemuru* fish oil contain essential fatty acids (EFA) including EPA and DHA (MARINI et al. 2020) that have important role in fish growth (IZQUIERDO 1996) and deficiency of EFA causing some non-infectious diseases, low immunity, and increase abnormality rate (NOGA 2010).

In present study, the higher growth was obtained in P_3 (enrichment doses 0.5 ml fish oil + 0.5 ml egg yolk) and tends to be decrease in higher doses. It shows that excessive of *Sardinella lemuru* fish oil in enrichment artemia is not good for larvae growth. *Sardinella lemuru* fish oil as source of fatty acid (lipids) contain high energy that use for growth. Excess of fatty acid means that energy also excessive. Similar case was also obtained in other previous excessive dietary of fatty acid; excessive levels of DHA significantly reduced total weight of summer flounder larvae (BISBAL

and BENGSTON 1991), also growth of African catfish larvae tended to be reduced by excess in cod fish oil and coton seed oil in the diet (LEGENDRE et al. 1995). Based on the study, we recommend 0.5 ml fish oil + 0.5 ml egg yolk as optimal artemia enrichment dose for feeding practical of silver rasbora larvae.

Larvae feeding artemia enrichment with *Sardinella lemuru* fish oil in different doses not affect ($P > 0.05$) on survival, but all growth parameters significantly affected ($P < 0.05$). Optimal artemia enrichment dose for feeding practical of silver rasbora larvae is 0.5 ml fish oil + 0.5 ml egg yolk. Estimate doses that leads to the maximum length gain is 0.6035 ml fish oil + 0.6035 ml egg yolk.

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