Growth and survival rate of silver barb, *Rasbora argyrotaenia* under different concentrations of sardine fish oil addition in fish feed

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Abstract. Sardine fish oil is one of the compositions of the fat source which contains Omega-3 potentially to improve growth performance. The addition of sardine fish oil in fish feed is, therefore, important to do to could boost the growth of the fish. This study aims to determine the effect of adding sardine fish oil towards the growth performance and survival rate of silver barb and also determines the optimum concentration of sardine fish oil in feed formulation that results in the highest growth and survival rate of the silver barb. This research applied a completely randomized design for experimental method, which consisted of five treatments each treatment has four replications in 42 days. The results showed that adding sardine fish oil with different concentrations significantly has affected specific growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR), but did not significantly affect survival rate (SR) and average daily length gain (ADGL) of silver barb. Addition of sardine fish oil by 4% resulted in SR (100%), SGR (2.17%/ day), ADGL (0.85%/ day), highest FI (63.81g/ 50 fish) and lowest FCR (1.73).

1. Introduction
The major problem of silver barb cultivation is its low growth rate even though artificial fish feed has highly developed [1]. Silver barb cultivation for three months shows an absolute growth rate of 0.47 g and absolute length growth 2.56 cm [2]. The available artificial fish feed in the market only contains 5% of fat and still below the fat requirement of silver barb which is about 5-15 % [3]. The fulfillment of the energy needs of silver barb through adding sardine fish oil in fish feed to increase fat requirement. As the results, the fulfillment stimulates silver barb mobility and movement; thus the energy from protein metabolism causes the increasing of growth performance and important to do.

Sardine fish oil is one of the compositions of the fat source. Most of the fish oil contains Omega-3 [4]. Whereas Sardinelle fish oil contains Omega-3 up to 17.79% (EPA 1.84% dan DHA 15.95%) [5]. Omega-3 has an important role to improve the function of the central nervous system and neurogenesis in the brain, cell fission and growth [6]. Some researches about adding fish oil in feed’s trout have been conducted. For instance, the addition of Omega-6 and Omega-3 to selais fish discovers
1.76-2.20% of growth rate [7], whereas the growth of rainbow fish increases up to 0.5-1.5%kg with Omega-3 [8]. While the addition of sardinelle fish oil in 6% of concentration has been able to increase the content of Omega-3 as 2.75% for eel, it consists of 2.87% EPA and 1.29% DHA [9].

This research aims to acknowledge the influence of sardinelle fish oil addition in fish feed towards growth and survival rate of silver barb. This study also ascertains the optimal concentration that results in the highest growth and survival rate for silver barbs.

2. Materials and methods

This study was conducted in the Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga and Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia.

2.1. Test organisms

In this study, the observed fish were Silver barb 30-40 mm in size with a 0.49±0.01 g in average weight, from the Installation of Freshwater Aquaculture Umbulan, Pasuruan, East Java, Indonesia.

2.2. Test feed production

Commercial feed in this research contained 36.95% protein and 5.29% fat, feed diameter was 0.7-1.0 mm. Sardinelle fish oil was added in the decided amount of concentration (0 %/ as control, 2 %, 4 %, 6 %, and 8 %) and mixed with 2 mL/100 g of egg white in feed according to Paratiwi’s method [10]. Next, added 100mL of water to the composition for every 100 g of fish feed like in Mokoginta’s method [11]. Mixed commercial fish feed and sardinelle fish oil evenly by spraying. The mixed feed was dried in the oven at 60 °C in 48 hours according to the Saltin’s method [12]. The approximate analysis of feed results was presented in Table 1.

| Proximate Analysis | The concentration of fish oil addition (%) |
|--------------------|--------------------------------------------|
|                    | 0  | 2  | 4  | 6  | 8  |
| Protein (%)        | 38.37 % | 38.98 % | 38.41 % | 38.26 % | 38.61 % |
| Lipid (%)          | 7.63 % | 7.66 % | 9.46 % | 10.16 % | 12.64 % |

2.3. Fish rearing

The silver barb was reared in a 20 L volume aquarium at a density of 3 fish/L for each treatment. The fish fasted for twenty-four hours before the treatment was done. Water was replaced every 2-3 days about 25% of the volume of the treatment unit.

2.4. Treatment

This study employed a complete randomized design (CRD) as an experimental method with five treatments of sardinelle fish oil concentration and four replications respectively. The concentration of sardinelle fish oil addition in the fish feed based on cyprinid fish fat requirement is 5-15% and the optimum fat level in fish feed is 12% according to Kaushik’s method [13]. The treatments were done through the addition of sardinelle fish oil in the feed (0 %/ as control, 2 %, 4 %, 6 %, and 8 %) for 42 days. Feeding frequency was three times a day as in Masitoh’s method [14] for 4 % biomass weight according to Mukti’s method [15].

2.5. Observation Result

The growth rate is measured by weighing the fresh weight of fish using analytical balance and measuring the fish length with millimeter paper. The growth rate is measured in every 14 days. The growth and survival rate are calculated using this following formula.
SGR (%/ day) = \( \frac{\ln \text{Final body weight} - \ln \text{Initial body weight}}{\text{days}} \times 100 \% \)

ADGL (%/ day) = \( \frac{\ln \text{Final body length} - \ln \text{Initial body length}}{\text{days}} \times 100 \% \)

\( \text{FI (g/ 50 fish)} = \text{Initial feed intake - Residual feed intake} \)

\[ \text{FCR} = \frac{\text{Total feed weight}}{\text{Initial body weight}} \]

\[ \text{SR} = \frac{\text{Final fish count}}{\text{Initial fish count}} \times 100 \% \]

2.6. Data analysis

This research were analyzed by the quantitative approach. The data were analyzed using Analysis of Variance (ANOVA) and continued by Duncan’s Multiple Range Test. The statistical analysis was performed using the IBM SPSS statistics version23.0 software.

3. Results and discussion

3.1. Results

ANOVA statistic test result shows that sardine fish oil addition with different concentrations has absolute influence towards Specific Growth Rate (SGR), Feed Intake (FI) and Feed Conversion Ratio (FCR) (p<0.05). However, this treatment does not show significant influence to silver barb’s Average Daily Length Gain (ADGL) and Survival Rate (SR) (p>0.05) (Table 2.). 4% addition of sardine fish oil (total fat in fish feed 9.46%) results the highest in SR (100%), SGR (2.17%/ day), ADGL (0.85%/ day), FI (63.81g/ 50 fish) and the lowest FCR (1.73%). The addition of sardine fish oil more than 4% reveals a lower result.

**Table 2.** Specific growth rate (SGR), average daily length gain (ADGL), feed intake (FI), feed conversion ratio (FCR), and survival rate (SR) of silver barb.

| Additional fish oil concentration (%) | Specific growth rate (%/days) | Average daily length gain (%/days) | Feed intake (%/days) | Feed conversion ratio | Survival rate (%) |
|--------------------------------------|--------------------------------|-----------------------------------|----------------------|-----------------------|------------------|
| 0                                    | 1.98±0.06                     | 0.81±0.08                         | 57.10±2.47           | 1.88±0.05             | 100±0.00         |
| 2                                    | 2.06±0.07                     | 0.83±0.06                         | 60.03±4.67           | 1.74±0.08             | 100±0.00         |
| 4                                    | 2.17±0.12                     | 0.85±0.07                         | 63.81±1.86           | 1.73±0.14             | 100±0.00         |
| 6                                    | 1.79±0.12                     | 0.76±0.04                         | 54.20±0.95           | 2.08±0.18             | 100±0.00         |
| 8                                    | 1.69±0.15                     | 0.75±0.08                         | 53.75±2.38           | 2.20±0.22             | 100±0.00         |

Note: Different superscript in the same column indicates a significant difference (p<0.05)

The average weight of silver barb has increased during the treatment from 0.49±0.01 g/ fish to 1.00-1.24 g/ fish (Figure 1.). Individual weight with 4% of sardine fish oil concentration is 1.24±0.32 g which is not different with 2% of sardine fish oil addition (1.19±0.30 g) and 0% (1.12±0.27 g). The lowest average of individual weight when fish feed is added 8% of sardine fish oil that results in 1.00±0.22 g.
Average of length gain also increases from 34.28±0.91 mm/fish to 46.65-49.50 mm/fish (Figure 2). The highest average individual length 49.50±0.61 mm after added 4% of sardinelle fish oil in fish feed. Under the addition of 2% of sardinelle fish oil concentration has not much different result which is (47.90±2.42 mm) and in 6% (48.90±1.42 mm). The lowest average length is 46.73±0.99 mm under the addition of 8% sardinelle fish oil.

3.2. Discussion
The addition of sardinelle fish oil in fish feed under different concentration shows the essential effect to specific growth rate, feed intake, feed conversion ratio. However, this treatment does not affect average daily length gain and survival rate of silver barb during the treatment. Fat is capable of producing energy for metabolism, thus the protein is utilized to support growth. Fat is considered as essential fatty acid which is necessary for fish to affect their growth [16].

Sardinelle fish oil regards as the source of fat for silver barb’s feed and contains essential fatty acid, the Omega-3. Research result reveals that sardinelle fish oil contains 13.45% Omega-3 (EPA 7.19% and DHA 5.68%). Omega-3 is essential to affect the cell fission, growth, development, and function of the central nervous system as well as neurogenesis in the brain [17]. The addition of sardinelle fish oil in fish feed increases feed’s fat intake which stimulates energy availability and mobility of silver barb to raise. As a result, the energy from protein metabolism increases growth rate and decreases the feed conversion ratio.
Nutrient content in fish feed influences fish growth rate [18]. Silver barb which is being fed by an additional 4% concentration of sardinelle fish oil has shown the highest growth rate rather than silver barb with additional sardinelle fish less than 4% (0% and 2%) and more than 4% (6% dan 8%). Proximate analysis feed result, the addition of sardinelle fish oil at concentration 4%, has contained 9.46% of fat. This research has a parallel result with another research [19] about fish feed supplements that contains 7-12% fat that has influenced the specific growth rate of Chinese perch fish. On the contrary, fish feed that contains more than 12% of fat is decreasing the specific growth rate of Chinese perch fish.

Adding sardinelle fish oil in fish feed increases fat availability in silver barb. Thus, the level of calories in feed is high which makes the energy from protein metabolism is allocated to support the growth. Fat intake in the body accelerates triglyceride hydrolysis to produce much fatty acid and glycerol in the liver. The increase of fatty acid in liver precipitates beta-oxidation which produces acetyl-CoA. Acetyl Co-A from beta-oxidation enters the citric acid cycle to produce carbon dioxide and chemical energy in the form of ATP which increases growth rate [20].

Further, sardinelle fish oil also influences silver barb’s food intake ratio. As a result, this additional sardinelle fish oil in fish feed stimulates fish’s appetite [21] because fish oil contains some fatty acids which are erucic acid, behenic acid, docosadienoic and lauric that enhances feed’s palatability (flavor and taste) [22]. Analysis result of sardinelle fish oil in this research, contains 0.0596% erucic acid, 0.1819% behenic acid, docosadienoic 0.3415% and lauric 0.1629%. Silver barb under the addition of 4% sardinelle fish oil shows higher feed intake (63.81g/ 50 ) compares to 8% sardinelle fish oil (53.75 g/ 50). Higher the additional fatty acid source causes lower digestibility of organic and energy [23], this results in the disruption of digestive microbial activity and changes in the acidity in the stomach due to additional oil. Because the amount of feed intake is related to the nutrient content and digestibility of the fish [24], thus high-energy feed due to the high-fat causes a low feed intake.

Fish has a good growth rate because of its ability to utilize feed nutrition and convert them to energy. The cultivated silver barb with a 4% concentration of sardinelle fish oil has a lower feed conversion ratio (1.73) rather than with 8% concentration (2.20). Low availability of fat in silver barb fish feed causes low calories which also causes high feed conversion ratio to fulfill the protein metabolism process. Excessive fat concentration raises excessive energy which is capable to damage the liver due to precipitation of triglycerides in the liver [25]. The high concentration of fat inhibits beta-oxidation and accelerates the esterification of fatty acids in the liver [26] which prevents fatty acid to produce energy through the citric acid cycle. Even so, the previous process produces enough Acetyl Co-A to stimulates lipogenesis [27]. The increasing Lipogenesis obstructs gluconeogenesis process which generates excessive triglycerides build up in the liver that slower growth rate of Chinese perch.

Adding sardinelle fish oil in fish feed does not affect the survival rate of silver barb. The survival rate of silver barb in all treatments is 100%. Besides, the addition of sardinelle fish oil does not harm its survival rate. The survival rate of fish is influenced by feed quality and chemical and phyic factors of the water unit. Proximate analysis test of feed in this research 36.95% protein and 5.29% fat that is enough to fulfill protein and fat requirement to support the survival rate of silver barb. The fat intake of cyprinid is between 5-15% and 30-41% protein. The water quality of the water unit during the observation is pH 7.5-7.6, temperature 27-28.8°C, dissolved oxygen 5.2-6.2% and ammonia 0.5 mg / l which is considered as the optimal level for the survival of silver barb. Silver barb is capable to survive in waters with a temperature range of 27.3-29.2 0C, pH 7.4-7.9 [28], dissolved oxygen 5.2-6.3 mg / l and 0.5 mg ammonia / l [29].

4. Conclusion
The addition of sardinelle fish oil affects specific growth rate, feed intake, and feed conversion ratio, however, it does not influence daily length gain and survival rate of silver barb. Under 4% addition of sardinelle fish oil (total fat in fish feed is 9.46 %) results higher specific growth rate (SGR), average daily
length gain (ADGL), feed intake (FI) and lower feed conversion ratio (RKP) compares to less than 4% addition of sardinelle fish oil.

5. References

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