The effect additional omega-3 in feed on the gonadal maturity level and fecundity of female silver rasbora, *Rasbora argyrotaenia* 

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Abstract. Silver rasbora (*Rasbora argyrotaenia*) is a high-value commodity. The problem in cultivation of silver rasbora is long gonadal maturation period. The gonadal maturation period can be accelerated by manipulation of feed using omega-3. Omega-3 can affect reproductive performance, egg development, and fish embryogenesis. The purpose of this study was to determine effect of addition of omega-3 in feed on gonadal maturity level and fecundity of silver rasbora. The observed parameters are gonadal maturity level, gonado somatic index, gonado index and fecundity. This research applied a completely randomized design (CRD) method which consist of four treatments, namely control (0 mg/kg feed), P1 (2500 mg/kg feed), P2 (5000 mg/kg feed), and P3 (7500 mg/kg feed). The result of this study was indicate addition of omega-3 to feed increased gonadal maturity at GML IV stage and had a significantly effect on the gonado somatic index, gonado index and fecundity of female silver rasbora. The optimal dose of omega-3 for silver rasbora gonads is 7500 mg/kg of feed, which results in an increase in gonadal maturity to GML IV with a GSI of 18.89 ± 2.24%, a GI of 143.63 ± 6.66 and fecundity of 924.76 ± 9.21 eggs/parent.
1. Introduction
Silver rasbora (*Rasbora argyrotaenia*) is a high-value commodity that has potential to be cultivated as a consumption fish. Silver rasbora cultivation is encouraged by increased demand and price [1]. The problem in cultivation of silver rasbora are the difficulty of obtaining seeds due to the limited number of broodstock [2] and long gonadal maturation period [3]. The gonadal maturation period can be accelerated by feed manipulation. Feed manipulation can be accomplished by adding components in the feed that can accelerated gonad development, such as omega-3 fatty acids [4].

Omega-3 fatty acids, such as linolenic acid (LNA), Eicosapentaenois Acid (EPA) and Docosahexapentaenoid Acid (DHA) in feed can affect reproductive performance, egg development, and fish embryogenesis [5]. Essential fatty acids are precursors to prostaglandins and other steroid hormones, which play a role in regulating spawning behavior and accelerating ovulation [6]. The success of oocyte maturation is influenced by prostaglandins made from essensial fatty acids [6]. The role of essential fatty acids, especially omega-3, is one of the nutritional factors that greatly affects egg and larval quality. Some studies have found that dietary omega-3 has an impact on the reproductive performance, egg quality, and larval quality of several fish [7].

The purpose of this study to acknowledge the effect of omega-3 addition in feed towards gonadal maturity level and fecundity of silver rasbora. This study also ascertains the optimal concentration that results in the gonadal maturity level and fecundity for silver rasbora.

2. Materials and methods
This study was conducted in the Laboratory of the Faculty of Fisheries and Marine, Banyuwangi campus, Universitas Airlangga.

2.1. Test organisms
In this study, the observed fish were silver rasbora 5.24 ± 0.19 cm in average size with a 1.65 ± 0.19 g in average weight, from the Installation of Freshwater Aquaculture Umbulan, Pasuruan, East Java, Indonesia.

2.2. Test feed production
Omega-3 commercial was added in the decided amount of concentration namely control (0 mg/kg feed), P1 (2500 mg/kg feed), P2 (5000 mg/kg feed), and P3 (7500 mg/kg feed). The manufacture of feed refers to Agustin’s [3], first step is mixing omega-3 with egg whites at 2 ml/100 grams of feed, then added with 100 ml of water for every 100 g of feed. Mixed commercial fish feed and omega-3 evenly by spraying. The mixed feed was dried at room temperature.

2.3. Fish rearing
The silver rasbora was reared in a 30x40x30 cm³ aquarium at a density of 10 fish/aquarium for each treatment. The fish were fasted before feeding treatment.

2.4. Treatment
This study employed a complete randomized design (CRD) as an experimental method with four treatments of omega-3 doses and four replications respectively. The dose of omega-3 addition in the fish feed based on fish omega-3 requirement is 0.5% or 5000 mg/kg feed [8]. The treatments were done through the addition of omega-3 in the feed (control (0 mg/kg feed), P1 (2500 mg/kg feed), P2 (5000 mg/kg feed), and P3 (7500 mg/kg feed)). Feeding was done using a Feeding rate (FR) of 3% of the total biomass twice a day. Maintenance was carried out for 40 days.

2.5. Observation Result
Weight and length of fish were measured then underwent surgery using section set to observe the gonad morphologically and weight the gonad. Gonadal maturity level was measurement macroscopically based on morphological characteristics of gonadal maturity level of silver rasbora.
according to Salmatin’s [9]. Gonado somatic index, gonado index and fecundity calculation applied Effendie formula [10].

Table 1. Gonadal maturity level of female silver rasbora [9]

| Maturity stage          | Morphological characteristics                                                                 |
|-------------------------|------------------------------------------------------------------------------------------------|
| Immature (I)            | Ovaries were very thin semi-transparent tubes                                                  |
| Maturing (II)           | The ovaries increase in size. The coloration is darker yellowish, the eggs are not clearly visible |
| Nearly ripe (III)       | Ovary becomes yellow, the egg granules are visible                                              |
| Ripe and running (IV)   | The larger ovary fills ½-2/3 of the abdominal cavity, the egg is yellow and can be separated.   |
| Spent (V)               | Ovaries wrinkled. The remaining eggs are found in the genital opening.                           |

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GSI = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100\% \\
GI = \frac{\text{Gonad weight (g)}}{\text{Body Length}^3 (m)} \times 10^8 \\
\text{Fecundity} = \frac{\text{Gonad weight (g)} \times \text{number of egg from subsample gonad (eggs)}}{\text{Subsample gonad weight (g)}}
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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 version24.0 software.

3. Results and discussion

3.1. Results
Based on the observation, female silver rasbora fed with the addition of omega-3 at a dose of 7500 mg/kg produced fish that developed gonads into TKG IV by 70% of the number of female silver rasbora. Gonadal maturity level of female silver rasbora after being fed with omega-3 supplementation for 40 days is presented in table 2 and Figure 1.

Table 2. Gonadal maturity level (GML) of female silver rasbora after being fed with omega-3 supplementation for 40 days. n = 10 individu

| Treatment | Number of individuals based on GML criteria |
|-----------|--------------------------------------------|
|           | GML I | GML II | GML III | GML IV |
| P0        | 50%   | 20%    | 30%     | 0%     |
| P1        | 10%   | 10%    | 40%     | 40%    |
| P2        | 0%    | 0%     | 60%     | 40%    |
| P3        | 0%    | 0%     | 30%     | 70%    |
ANOVA test resulted that the addition of omega-3 does influence gonado somatic index, gonado index and fecundity of silver rasbora (P<0.05). The average of the gonado somatic index, gonado index and fecundity are presented in Table 3.

Duncan’s multiple ranges test (Table 3.) reveals omega-3 addition in P3 obtains the highest gonado somatic index, gonado index and fecundity of female silver rasbora at 18.89 ± 2.24%, 143.63 ± 6.66 and 924.76 ± 9.21 eggs/parent. Whereas female silver rasbora without omega-3 addition resulted lower gonado somatic index, gonado index and fecundity at 5.84 ± 0.34%, 51.87 ± 1.17 and 305.85 ± 1.97 eggs/parent.
3.2. Discussion

The addition of omega3 to the feed at different doses for 40 days had a significant effect on the gonado somatic index, gonado index, and fecundity of female silver rasbora (P < 0.05). Silver rasbora fed with omega-3 at P3 (7500 mg/kg) grew gonads to GML IV at a rate of 70% of female silver rasbora. Omega-3 fatty acids play a role as a constituent of egg yolks so that the larger the egg means the level of gonad development is also getting to stage IV. This is in agreement with Wiegand’s [11] eggs are primarily composed of lipoprotein, which is high in polyunsaturated fatty acids (n-3), especially DHA. During gonadal development, fatty acids are stored in adipose tissue and subsequently converted into lipoproteins, which are then transported to the oosit [12].

The gonadal maturity level is directly proportional to the GSI and GI values [13]. The addition of omega-3 treatment of 7500 mg/kg of feed indicated the value of somatic gonado somatic index and the greatest gonado index, namely 18.89 ± 2.24% and 143.63 ± 6.66, respectively. The vitellogenesis process in fish is responsible for the rise in GSI values [14]. The vitellogenesis process will continue until the fish spawns, at which point the gonads will develop in weight and size in tandem with the growth of the gonads, reaching their maximum size when the fish spawns [15]. The gonado index is computed by comparing body length, which is linked to fish growth. There are numerous elements that influence fish growth, resulting in a wide range of growth patterns. All of these growth parameters have a direct relationship with fish gonadal weight [16]. One of the dietary elements that has a significant impact on egg and larval quality is the involvement of essential fatty acids, especially omega-3. Omega-3 fatty acids can affect the fluidity of cell membranes [17]. Changes in membrane fluidity will affect cell metabolism by changing the activity of enzymes in the cell membrane, allowing vitellogenin to enter and be absorbed by oocytes more easily [18].

The greatest fecundity value was indicated by the addition of omega-3 in the feed of 7500 mg/kg, namely 924.76 ± 9.21 eggs/parent. According to Salmatin [19] the fecundity of silver rasbora ranges from 22.78-101.36 eggs/parent. Ningrum’s [2] stated that the fecundity of silver rasbora ranged from 439.20 to 613.20 eggs/parent [20]. According to Nikolsky [21] factors that affect fecundity include age, food supply, population density, water temperature, dissolved oxygen and body physiology factors. Research conducted by Furuita’s [22] showed that the use of omega-3 diets in feed can increase fecundity. Hilbig’s [23] stated that the fecundity of female south american catfish (Rhamdia quelen) increased with increasing levels of omega-3 in the feed.

4. Conclusion

The addition of omega-3 in the feed had a significant effect on increasing the gonadal maturity level, somatic gonado index and gonado index, as well as the fecundity of female silver rasbora. Omega-3 that can optimally affect the gonads of female silver rasbora is a P3 (7500 mg/kg feed).

5. References

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