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Addition of lemuru fish oil in feed on the gonadal maturity level of female silver barb, *Rasbora argyrotænia*

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Abstract. The development of aquaculture technology to produce silver barb is constrained by the low fecundity and long space of reproduction periodicity. As a consequence, feed manipulation is necessary to improve fecundity and gonadal maturity. Fecundity and gonadal maturity could be developed through additional fat from lemuru fish oil. Lemuru fish oil contains high Omega-3 that frequently used in the vitellogenesis process. The objectives of this study were adding lemuru fish oil in feed to increase the gonadal maturity level of the female silver barb. This research applied a completely randomized design (CRD) method which consist of five treatments (0%,2%,4%,6%,8%) in adding the different concentration of lemuru fish oil in feed and four replications for each. The feeding was done three times a day, at 4% biomass in 42 days of treatment. The parameters observed in this study were the gonadal maturity level in morphology as well as histology, gonad somatic index (GSI), gonad index (GI) of the female silver barb. The results of this study indicated that the addition of lemuru fish oil at 4% concentration in feed was able to accelerate 60% of the population of female silver barb gonadal maturity in the level of III and IV.

1. Introduction

Silver barb has high demand both in the market and in consumption. To fulfill the demand, the silver barb is caught in common freshwater such as rivers, lakes, and swamps which causes its decreasing productivity [1]. Efforts to increase silver barb productivity have been done such as developing aquaculture technology. Silver barb cultivation faces some obstacles include its low fecundity that ranges 739-1831 seed/g gonad compare to its family, palu fish, that reach 11622 seed/g gonad [2]. As a consequence, availability of silver barb is low. Also, reproduction periodicity that considered long has slow down gonadal development and inhibited the availability of silver barb seeds.

This acceleration of gonad development could be done through improving feed quality, however, the feed is only contained about 31-33% of protein and 3-5% of fats [2]. The nutrient contents (fat) are not specifically for silver barb, the level of fat for silver barb enables to raise to 12% [3], while the fat requirement for cyprinid fish ranges between 5-15% to support reproduction process [4]. Nutrient factors influence the maturation process in gonad development are fat and protein contents in feed [5]. Protein and fat (lipoprotein) help the vitellogenesis process of fish [6]. Fat becomes a limiting factor in gonadal development such as essential fatty acids *eicosapentaenoic acid* (EPA) and *docosahexapentaenoid acid* (DHA) [7].



Fatty acids manage to influence the maturation process in oocyte maturity [7]. The addition of fatty acids and lemuru fish oil to tilapia larvae feed is capable to accelerate gonadal maturity level [8] and gonads somatic index reaches optimally at 2,29% [9]. The utilization of lemuru fish oil that contains rich in omega-3 and omega-6 is not only influence the growth, but also increase gonadal development [9]. Fecundity and gonadal development acceleration which are low in their cultivation are able to be increased using lemuru fish oil in feed. These attempts to accelerate the gonadal development of silver barb are the solution to increase fecundity and seed supply.

2. Materials and Methods

This research was conducted at the Anatomy and Aquaculture Laboratory in the Faculty of Fisheries and Marine, Universitas Airlangga.

2.1. Test organism

In this study, the fish observed were female Silver Barb with 5-6 cm in size with a $\pm 2,5$ g average weight came from the installation of Freshwater Aquaculture Umbulan, Pasuruan, East Java, Indonesia.

2.2. Feed mixing

The required fish feed contained 36,95% of protein and 0,7-1 mm in size. Lemuru fish oil added in feed has five different concentrations; 0%, 2%, 4%, 6%, and 8%. Lemuru fish oil was added according to concentration after mixing it with 2 ml/100 g eggwhite in feed [10]. The mixture of eggwhite and lemuru fish oil then added with 100 ml of water. Afterwards, the mixture was sprayed to the feed. The ready-made fish feed was dried-up in the oven at 60⁰ C for 48 hours [11].

2.3. Fish rearing

The female silver barbs were reared in a 20 L of volume aquarium at a density of 1 fish/3L for each treatment. The fish were fasted before feeding treatment.

2.4. Treatment

This study employed a complete randomized design for the experimental method with five treatment of lemuru fish oil concentration and four replication respectively. Feeding treatment was done three times a day at 4% biomass weight in 42 days of treatment. After 42 days, weight and length of fish were measured then underwent surgery using section set to observe the gonad morphologically and weight the gonad. Gonado somatic index and gonado index calculation applied Effendie formula (1979) [12]. Afterward, the gonad was fixed in BNF solution to be histological preparation which is stained in hematoxylin-eosin [13]. The histological observation was performed with a binocular microscope under 400x magnification to strengthen the analysis of gonadal maturity level (GML) morphologically.

2.5. Data analysis

The observation data were analyzed using ANOVA, whether or not the results from the analysis of variant showed significant different results, then it was followed by Duncan's multiple range test to determine the treatment with the best response at 95% degree.

3. Results and discussions

3.1. Results

Based on the observation, the gonadal maturity level of female silver barb changed morphologically after added 4% concentration of lemuru fish oil. In results, the addition accelerated the gonadal maturity level of female silver barb as proven 60% of the population entered GML III and GML IV. Results of the observation morphologically presented in Figure 1.

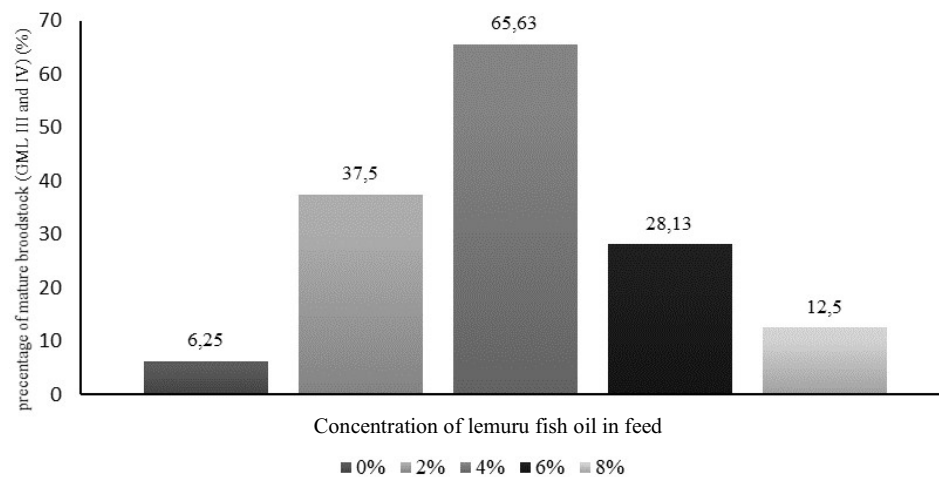


Figure 1. Gonadal maturity level percentage of female silver barb with different concentrations of lemuru fish oil in 42 days of treatment.

Observation of gonadal maturity level showed in gonadal histology in Figure 2.

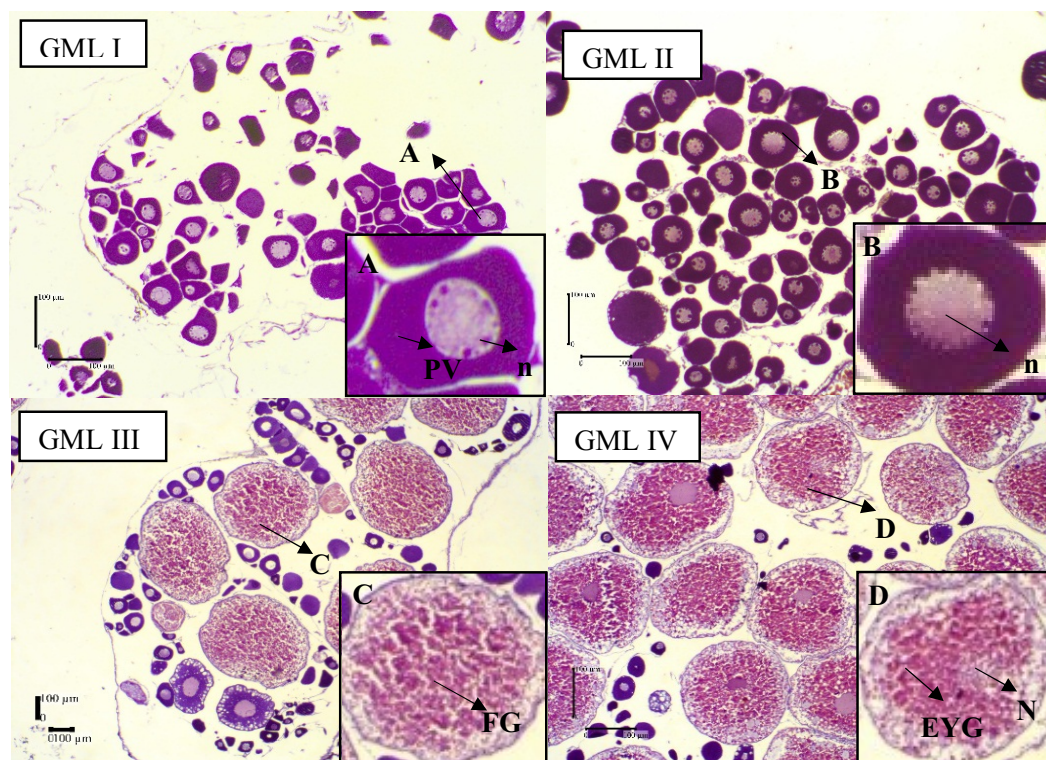


Figure 2. Histological development of female silver barb gonad in 42 days. A: oogonium (PV: Previtellogenic, n: nucleolus), B: oocyte (n: nucleolus), C: oocyte (FG: Fat Granules), D: oocyte (N: Nucleus, EYG: Egg Yolk Granules). Histology was stained with *Hematoxylin-Eosin*. Magnification 400x.

ANOVA test resulted that the addition of lemuru fish oil does influence gonado somatic index and gonado index of silver barb ($P < 0,05$). The average of the gonado somatic index and gonado index are presented in Table 1.

Table 1. Average of gonado somatic index and gonado index.

Concentration of lemuru fish oil in feed	Gonado somatic index (%) $\pm$ SD	Gonado index $\pm$ SD
0%	$2.23^d \pm 0.32$	$23.47^d \pm 5.56$
2%	$4.20^b \pm 0.59$	$43.76^b \pm 4.44$
4%	$5.60^a \pm 0.49$	$59.21^a \pm 6.51$
6%	$3.50^{bc} \pm 0.81$	$34.73^c \pm 6.75$
8%	$2.92^{cd} \pm 0.34$	$30.10^{cd} \pm 4.86$

Duncan's multiple ranges test (Table 1.) reveals lemuru fish oil addition in 4% of concentration obtains the highest gonado somatic index and gonado index at $5.60 \pm 0.49\%$ and $59.21 \pm 6.51\%$. Whereas female silver barb with 8% addition of lemuru fish oil and without the fish oil addition resulted lower gonado somatic index ($2.92 \pm 0.34\%$ and $2.23 \pm 0.32\%$) and gonado index (30.10 ± 4.86 and 23.47 ± 5.56).

3.2. Discussion

The addition of lemuru fish oil with different concentrations significantly affected gonadal somatic index, gonadal index, and hepato somatic index of the silver barb. 4% of fat addition in feed has been able to increase the amount of fish which gonad has been developing. The gonadal maturity level is the gonadal development stage. The increase of GML is followed by the raise of GSI, and GI, while oocyte diameter and HIS decrease [8]. Nutrient factor, prominently fat and protein have a main role in gonadal development. In the vitellogenesis process, synthesized lipoprotein is composed of fat and protein, thus vitellogenesis rate depends on protein and fat concentration given to fish. Nutrient concentration, especially, protein and fat (lipoprotein) affects gonadal development and vitellogenin formation [8]. The type of fats required in the vitellogenesis process is omega 3 (EPA dan DHA). Lemuru fish oil that has been added in fish feed contains 13.45% of omega 3 (EPA 7.19% and DHA 5.68%) which caused the increase of gonadal development of female silver barb. EPA and DHA are one of the factors that influence gonadal maturity level and oocyte development [14]. Fat in the form of cholesterol has affected steroid formation from gonadotropin [15] that works in hypophysis to stimulate LH (*Luteinizing Hormone*) and FSH (*Follicle Stimulating Hormone*) [19]. FSH then induces theca cell in gonad to synthesize estrogen in stimulating the vitellogenesis process in the liver which affects the rate of gonadal development [16].

The addition of 4% concentration of lemuru fish oil in feed has driven the population of the female silver barb to develop their gonad, 60% of the population are in GML III and GML IV as supported by the raise of GSI, GI and oocyte measure. The increase of gonadal maturity level is positively linear with GSI and GI [8]. The raise of GSI and GI are caused by vitellogenin synthesis in the vitellogenesis process. The formation and growth of gonad gain more weight [8]. Female silver barb ovary is dominated by oocytes which have yolk granules and the nucleus are gathering along the side while entering GML III and GML IV. The raising weight of gonad since there is yolk accumulation which multiplies oocytes diameter about $160 \mu\text{m}$ in phase III [17]. This condition happens due to vitellogenesis process which causes an increase of gonad volume. The increase of gonad weight causes the liver to lose weight because of vitellogenesis process in the liver was done and allocated to gonad [8].

Excessive addition of lemuru fish oil as in 8% of concentration (5.29% to 12.64%) results the lowest gonado somatic index and gonado index (Table 1.). It reveals that this excessive addition inhibits the vitellogenesis process in the liver and inhibits gonadal development. High availability of fat is capable to inhibit the beta-oxidation process thereby reducing fatty acids absorption [18]. A diet containing 7-12% of fats is considered as an optimal level for *Chinese perch* fish metabolism, while feed contains fat more than 12% is not useful for lipid formation process in liver and metabolism [18]. Excessive fat intake is able to accelerate the acid esterification in the liver as the absence of the beta-oxidation process. Adding fatty acid gives more acetyl-CoA for lipogenesis. Increased excess lipogenesis is capable to inhibit gluconeogenesis and worsen the accumulation of triglycerides in the liver [19] which causes fat accumulation in the liver. Fat accumulation in the liver then transfers to gonad excessively by lipoprotein through the blood which causes oogonium is filled by fat. Thus it is not capable to create new oogonium [20] and inhibit gonadal development.

4. Conclusion

The addition of lemuru fish oil at 4% concentration in commercial feed is the exact as well as optimum concentration to increase mature gonad of the female silver barb.

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