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Research Article

Maturation of Female Yellow Rasbora (*Rasbora Lateristriata* Bleeker, 1854) Using Oodev at Different Doses in Feed

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ABSTRACT
The current high demand for Yellow rasbora (*Rasbora lateristriata*) is not supported by the availability of captured Yellow rasbora in nature. Aquaculture is the most rational way of utilizing biological natural resources. In intensive aquaculture, it is necessary to optimize all processes that occur in aquaculture, including hatchery. However, the common problem that often happens in hatchery activities is spawning which depends on the season. The hormonal manipulation technique is an appropriate way to stimulate gonadal maturation. Oodev is a hormonal combination of pregnant mare serum gonadotropin and anti-dopamine to stimulate gonadal maturation. The purpose of this study was to determine the effectiveness of using the Oodev with different doses in feed to accelerate gonad maturation of female Yellow rasbora. The study was carried out with four treatments and three replications in 21 days with different doses of Oodev, such as; A (Feed without Oodev), B (0.5 mL/kg feed), C (1 mL/kg feed) and D (2 mL/kg feed). The parameters observed in this study were gonad maturity level, histological structure of ovary, gonadosomatic index, fecundity, and diameter of eggs. The results showed that the dose of Oodev at 1.0 mL/kg feed was an effective dose to optimize the gonad maturity of female Yellow rasbora. This is proven by the highest results shown on all parameters, such as; the maturity level in the IV phase, histological structure of the ovary which showed the dominance of the oocyte maturation phase, gonadosomatic index of 14.014%, the fecundity of 721 eggs, and egg diameter of 0.865 mm. In conclusion, using Oodev in feed at a dose of 1.0 ml/kg of feed for 21 days is an effective dose to optimize the maturation of female Yellow rasbora.

INTRODUCTION
Yellow rasbora (*Rasbora lateristriata*) is a fish favored by consumers because it has a distinctive taste. The high demand for Yellow rasbora fish is not supported by their availability due to the catches from nature are still limited (Zulfadhli 2015; Puspitasari 2016). Aquaculture is the most rational way of utilizing biological natural resources. Retnoaji et al. (2017) succeeded in aquaculture and conducting conservation through empowering aquaculture groups in Yogyakarta, Indonesia. In intensive aquaculture, it is necessary to optimize all processes that occur in aquaculture, including hatchery breeding which highly determines the number of productions. However, a common problem that is often occurred in

hatchery activities is the aquaculture environment which is sometimes not suitable for stimulating spawning, and the spawning depends on the season (Alavi et al. 2009). In the tropics, changes in water temperature and the amplitude of the air surface elevation caused by changing seasons can be a trigger for spawning (Hutagalung 2015).

The hormonal manipulation technique is an appropriate way to stimulate gonadal maturation in fish. Hormonal engineering to induce gonadal maturation uses a combination of several hormones (Putra et al. 2017). One of the commercial products that contain many alternative hormones is Oodev. Oodev is an innovation trademark developed by the Fish Reproduction and Genetics Laboratory of IPB University, Indonesia. Oodev is a hormonal induction material is that able to accelerate the maturation and re-maturation of the gonad in fish. Oodev is a combination of Pregnant Mare Serum Gonadotropin (PMSG) and anti-dopamine (AD) (Nugraha 2014).

PMSG is a complex glycoprotein obtained from the serum of pregnant horses and, acts similarly like luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Gallego et al. 2012). The effect of PMSG as FSH is more dominant than LH. The half-life of PMSG is quite long when compared to other gonadotropin hormones. This is because PMSG has a high carbohydrate content, especially in the cyclic acid group. The function of AD in the Oodev hormone is to block dopamine to prevent pituitary gonadotropin secretion from being inhibited (Arfah 2018). Inhibition of dopamine activity can stimulate LH synthesis and its secretion in the hypothalamus (Natalia 2018). LH plays a role in the final egg maturity process. Therefore, dopamine needs to be inhibited using anti-dopamine (Pamungkas et al. 2019).

The use of hormones in fish is usually done by intramuscular injection however, due to several considerations, such as the size of the fish are the reason for the ineffectiveness and inefficient use of hormones by injection. Another alternative method is the use of hormones through oral administration in feed. Research on the use of Oodev hormone through oral administration for gonad maturation of cultured fish has been carried out in several species, including *Chromobotia macracanthus* (Setiowibowo 2019), (*Helostoma teminkii*) (Farida et al. 2019), *Amphiprion clarkia* (Tomasoa et al. 2018), *Pangasianodon hypophthalmus* (Nugraha 2014.; Arfah 2018) but never been done in Yellow rasbora. The purpose of this study was to determine the effectiveness of the Oodev through oral administration in feed to accelerate gonad maturation of female Yellow rasbora.

MATERIALS AND METHODS

Materials

Yellow rasbora broodstocks used in this study were 6-month-old with an average body weight of 5.6 grams obtained from Cangkringan Fisheries Technology Development Center, Sleman, Yogyakarta. This study used a completely randomized design (CRD) with four treatments and three replications with different doses of Oodev, such as; A (Feed without Oodev), B (0.5 mL/kg feed), C (1.0 mL/kg feed), and D (2.0 mL/kg feed).

The tank used in this study was 2 fiber cubes (1.0 m x 0.80 m x 0.60 m). Each cube contains 6 happa nets with a size of 40 cm^3. To support growth and maintenance feasibility according to the research by Zulfandi (2015), the appropriate stocking density of broodstock is 10-15 fish/liter. Furthermore, the broodstocks were acclimatized for one week and then selected. Broodstock selection is the main key before spawning. The selection of broodstock aims to ensure uniformity of brood fish, re-

lated to uniform body weight, body shape is not deformed, and in the same reproductive phase that is not present in the gonad maturity phase.

Methods

The supplementation of feed with Oodev uses a coating method that begins with mixing Oodev in feed with a dose according to the treatment applied in this study (Nugraha 2014). The feed coating method begins with the addition of egg white as a binder into the aquades which is then sprayed onto the feed. The feed used was the floating-type pellet (crude protein 31-33%). The frequency of feeding was three times a day for 21 days with a feeding ratio of 3-5% of body weight. The parameters used in this study are described as follows:

Gonad Maturity Level (GML)

After 21 days of treatment, 2-3 fish in each treatment group will be dissected to obtain gonads for determination of GML based on the morphological characteristics of the gonads according to Effendie (1997) which are listed in Table 1.

Table 1. The Characteristics of Gonad Maturity Level.

GML	Female Gonad Morphology
I Immature	The ovary is like a thread. The eggs are not yet distinguishable. The length of the gonads varies between 1/3-1/2 on the length of the body cavity.
II Maturing	There is a milky white tissue, the eggs are still fused and cannot be separated. The length of the gonads varies between 1/3–2/3 on the length of the body cavity.
III Maturing ripe	Larger size, widened anteriorly, and tapered posteriorly, the eggs can be separated, and darker in color. The length of the gonads varies between1/3–2/3 of the length of the body cavity.
IV Ripe	The egg diameter is getting bigger and visible under the microscope. The eggs are yellow. The length of the gonads varies between 2/3–3/4 of the length of the body cavity.
V Spent	Crimped ovaries, leftover eggs in the posterior. Ovaries are reddish.

Histological structure of the ovary

Development of fish reproductive organs identified morphologically and histologically. Histological observation is the most accurate method and yields the most detailed information because the observations are carried out at the tissue level (Zulfadhli 2015; Suryanti et al. 2015). GML is determined based on morphological characteristics, then it is continued with the histological preparation using the paraffin method. The thickness of the slice on the microtome was adjusted to 5 µm and then Hematoxylin Eosin (HE) staining was performed to identify the different stages of the oocyte formation histologically more accurately. The development of fish reproductive organs can be identified by morphological and histological structures. The terminology in determining the phase of each oocyte development refers to Costa (2015) who suggests an analysis of the histological sections on the ovaries of all species into ten stages of oocyte development with three main phases, namely pre-vitellogenic oocyte growth, vitellogenic oocyte growth, and oocyte maturation.

Gonadosomatic Index (GSI)

Gonadosomatic index is calculated by the following formula (Dadzie & Wangila 1980):

$$GSI = \frac{Weight\ of\ Gonad}{Weight\ of\ Fish} x100\%$$

Fecundity

Fecundity is assumed as the number of eggs contained in the ovaries that have reached GML III and IV. The total fecundity was calculated using the sub-sample method of gonadal weight or the gravimetric method with the following formula (Effendie 1997):

$$F = \frac{Total\ Weight\ of\ Gonad}{Sample\ Weight\ of\ Gonad} x\ total\ of\ gonad\ in\ sample$$

Eggs Diameter

The measurement of egg diameter was carried out using ImageJ software developed by the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation. The number of eggs measured was 20 eggs for each treatment group.

Data analysis

Data on gonadosomatic index, fecundity, and egg diameter were analyzed by one-way ANOVA and followed by Tukey test using IBM SPSS version 25. The differences between each sample were considered significant at $p < 0.05$ (Gomez & Gomez 1995). Histological observation of the stages of gonad development was done microscopically using the descriptive comparative method. The advantages of histological observations can provide accurate and detailed information at the tissue level (Zulfadhli 2015).

RESULTS AND DISCUSSION

Gonad Maturation Level (GML)

The level of gonad maturity in broodstock can be seen morphologically with several characteristics that indicate that the broodstock has matured gonads, the most common characteristic of which is the enlarged abdomen towards the anus (Susilo et al. 2019). In female Yellow rasbora, it could be seen that there were differences between the treatments from the morphological characteristics based on Figure 1)

In treatment A (Feed without Oodev) and D (2 mL/kg feed), it's seen that the abdomen of fish was smaller when compared to treatment B (0.5 mL/kg feed) and C (1 mL/kg feed). In treatment C, the abdomen is bigger and fulfilled the abdominal cavity more than in treatment B. Treatment C has shown a different morphological characteristic than the other treatments. The abdominal cavity in treatment C seems to have a lot of volumes. After the morphological characteristics of the fish body shows a difference, surgery was carried out to identify the morphology of the gonads.

Figure 1 shows that treatment C is clearly different from the other treatments. Based on the morphological characteristics of the gonads, according to Effendie (1997), treatment C was in the GML IV category, the which the eggs are yellowish-orange color and occupy 2/3 to 3/4 part of the abdominal cavity. On the other hand, the other treatments did not show the same gonadal morphology as shown by the treatment C. This shows that the treatment C with a dose of Oodev hormone of 1.0 mL/kg of feed is the best treatment. The accuracy of the GML will then be confirmed by the histology of the ovary and GSI results.

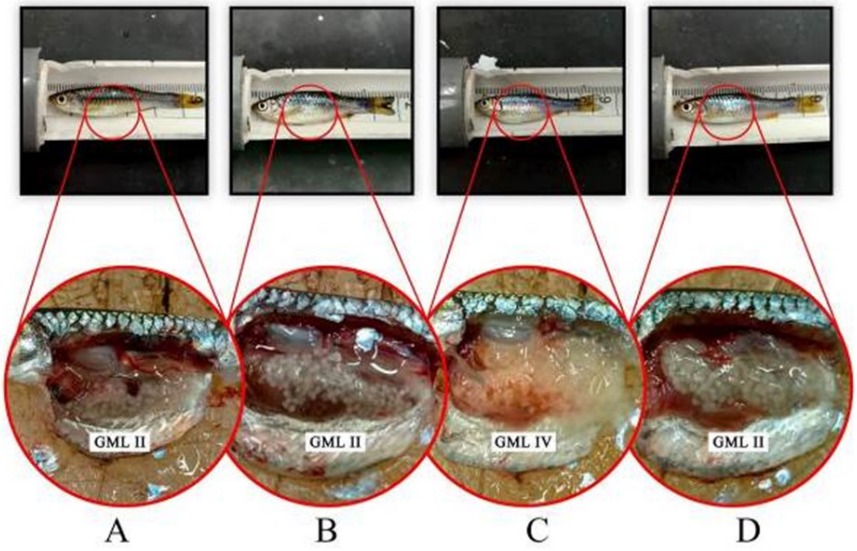

Figure 1. Gonad maturity level (GML) of Yellow rasbora, *Rasbora lateristriata* in Oodev treatments: A. Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed.

Histological Structure of Ovary

According to Zulfadhli et al. (2016), the terminology and features used to differentiate and identify the different stages of oocyte formation histologically may vary according to the researcher and the species. The analysis of the histological section of the ovary according to Costa (2015) is the basis for the terminology guidelines to identify and classify the oocyte phase based on the histological results of the ovaries in each treatment. The results of this histological structure also support a more accurate GML by determining each oocyte phase. Figure 2 shows the histological results of the Yellow rasbora ovary in each treatment and gives a clear illustration that each treatment has different phase dominance.

It is seen in treatment A that the histological results showed several phases of oocyte development including pre-vitellogenic and vitellogenic phases. Treatment A was dominated by pre-vitellogenic, especially in the early primary growth phase with a percentage of 1.75%, late primary growth of 45.61%, and cortical alveolar of 14.04%. The vitellogenic phase was also identified in treatment A with the percentage of primary vitellogenesis was 22.81%, secondary vitellogenesis at 3.51%, and quaternary vitellogenesis at 12.28% (Figure 4).

The late primary growth phase that dominated treatment A was characterized by basophilic cytoplasm stained with hematoxylin and a nucleus showing nucleoli arranged on the periphery. Based on research by Johnson and Braunbeck (2009) and Erkmen and Kirankaya (2016), this phase is also called the perinucleolar oocyte phase. This phase is characterized by the growth of the oocyte, the nucleus (germinal vesicle) increases in size and many nucleoli appear at the periphery of the nucleus. The cytoplasm is darker in color, although late perinucleolar oocytes may have small well-defined, or amphophilic vacuoles in the cytoplasm (Figure 3).

Based on Figure 4, treatment D is like treatment A and B, which were dominated by the pre-vitellogenic phase with a larger portion, namely in the late primary growth phase of 79.75% and cortical alveolar of 10.13%. In the cortical alveolar phase, the diameter is generally larger than the perinuclear phase and characterized by the appearance of cortical alveoli (yolk vesicles) inside the ooplasm.

Treatment B was also identified as being in the vitellogenic phase.

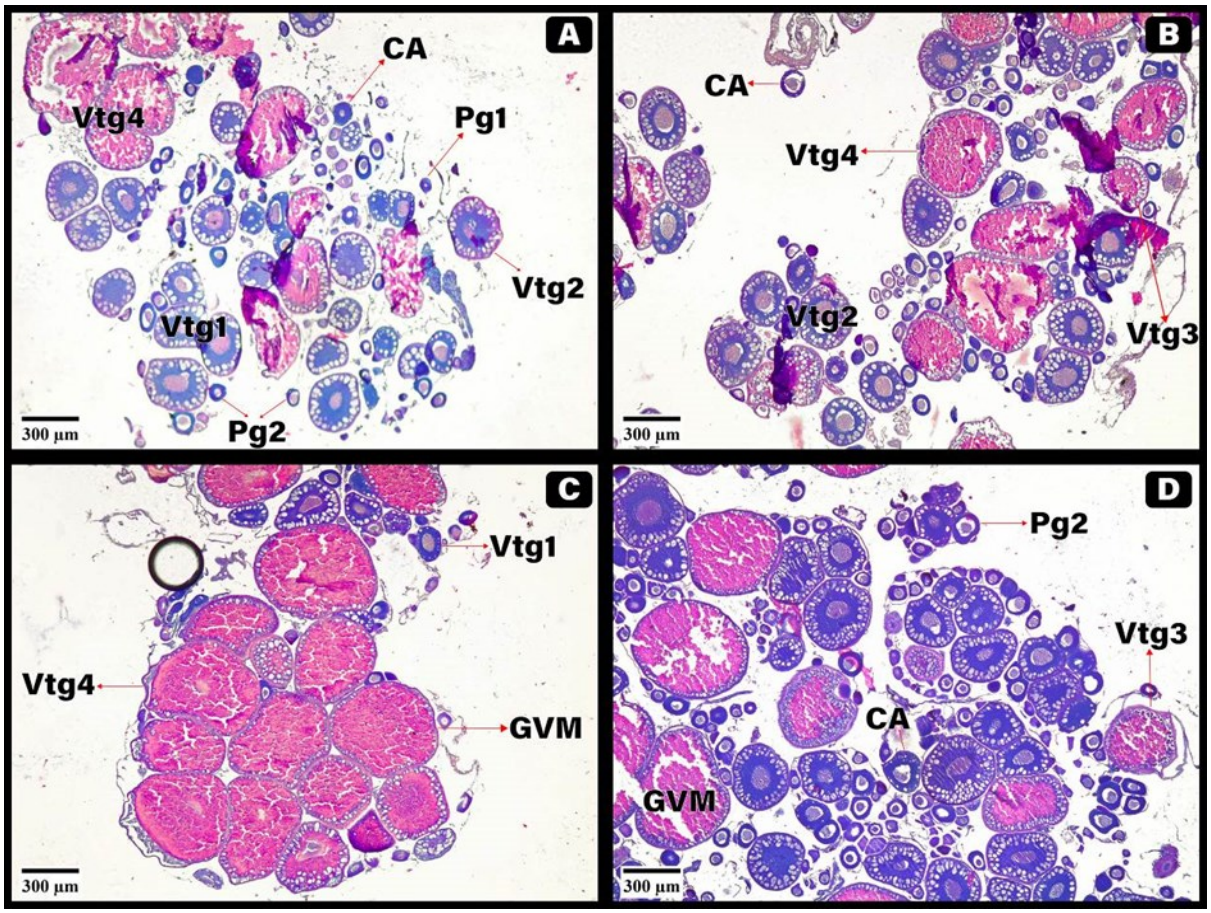

Figure 2. Ovary's histological structure of Yellow rasbora (*Rasbora lateristriata*) in Oodev treatments: A.Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed. *Pre-vitellogenic oocyte growth*: early primary growth (Pg1), late primary growth (Pg2), cortical alveolar (CA); *Vitellogenic oocyte growth*; primary vitellogenesis (Vtg1), secondary vitellogenesis (Vtg2), tertiary vitellogenesis (Vtg3), quaternary vitellogenesis (Vtg4); *Oocyte maturation*: germinal vesicle migration (GVM). Hematoxylin-Eosin staining; 4×10 magnification.

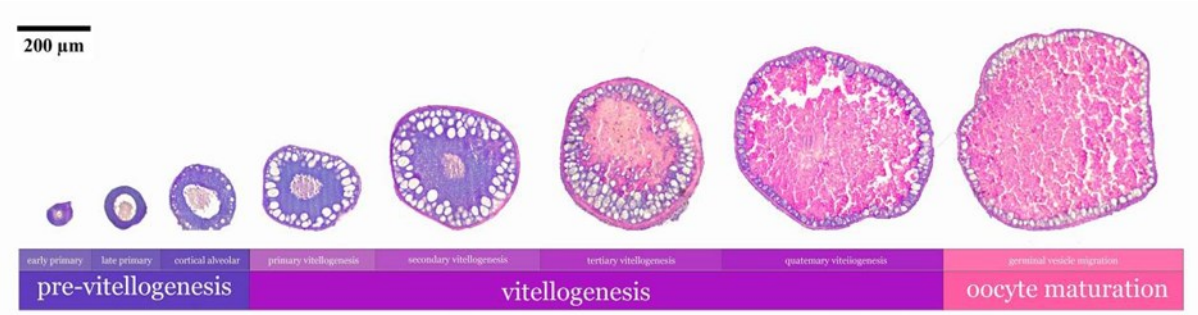

Figure 3. Oocyte stages of development of Yellow rasbora (*Rasbora lateristriata*); *Pre-vitellogenic oocyte growth*: early primary growth (Pg1), late primary growth (Pg2), cortical alveolar (CA); *Vitellogenic oocyte growth*; primary vitellogenesis (Vtg1), secondary vitellogenesis (Vtg2), tertiary vitellogenesis (Vtg3), quaternary vitellogenesis (Vtg4); *Oocyte maturation*: germinal vesicle migration (GVM). Hematoxylin-Eosin Staining; 10×10 magnification.

According to Costa (2015), this phase is divided into; primary vitellogenesis, secondary vitellogenesis, tertiary vitellogenesis, and quaternary vitellogenesis. Primary vitellogenesis is eosinophilic protein granules that are stained pink in hematoxylin-eosin staining, starting to slightly fill the cytoplasm, and more small-sized oil droplets and cortical alveoli can be seen arranged in the periphery of the oocyte. Secondary vitellogenesis (Vtg2) showed regular granules surrounding the cytoplasm and oil droplets began to appear and were located around the nucleus. In tertiary vitellogenesis (Vtg3), yolk granules multiply and fill the cytoplasm. Oil droplets increase in size and are distributed around the nucleus. In qua-

ternary vitellogenesis (Vtg4), the cytoplasm is filled with yolk granules and many oil droplets arranged around the nucleus.

Treatment C as shown in Figure 4, resulted in quaternary vitellogenesis of 20% and an oocyte maturation phase of 32%. This shows that treatment C is the best treatment with oocyte dominance in the late vitellogenesis and oocyte maturation phase. According to Johnson and Braunbeck (2009), in the oocyte maturation phase the cells become larger and hydrated, the nucleus has migrated towards the cell periphery and its size decreased in the periphery making it difficult to identify. The zona radiata will be divided into two layers, including a thick and hollow internal layer and a thinner, noncellular external layer. (Erkmen & Kirankaya 2016) explained that there is a vitelline layer composed of follicular cells that are cuboidal in shape with a large, rounded nucleus which is clearly visible as the outer layer. On the outside, the follicular layer contains a basal lamina and a theca layer (Menke et al. 2011). Histological results in treatment C also supported the previous parameters in this study regarding GML, namely the GML IV phase. This result is like (Prakasa 2020) which states that female Yellow rasbora with GML IV will be dominated by the maturated oocyte. This shows that the administration of Oodev at a dose of 1.0 ml/kg of feed is the best dose to make maturated oocyte dominated the ovaries.

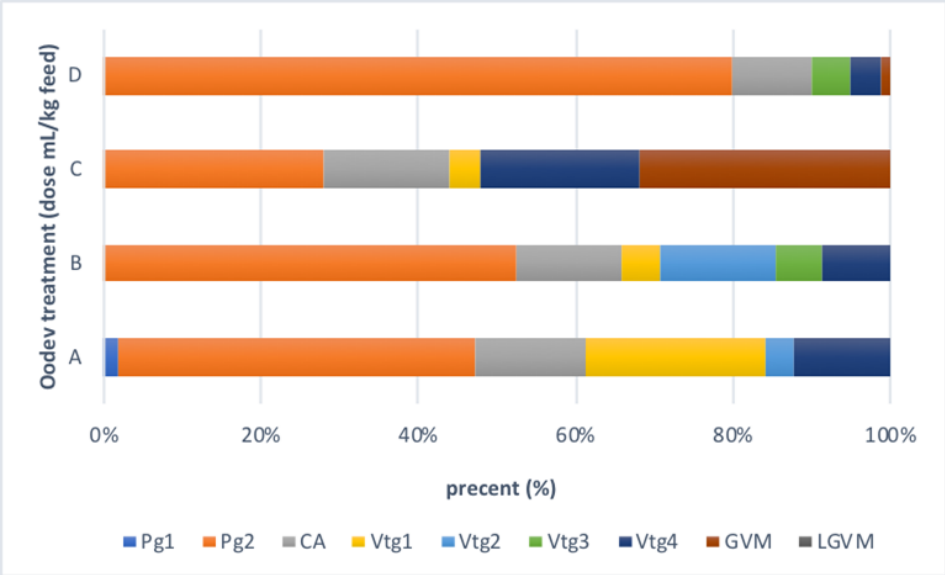

Figure 4. Percentage oocyte stages of development of Yellow rasbora, *Rasbora lateristriata*; *Pre-vitellogenic oocyte growth*: early primary growth (Pg1), late primary growth (Pg2), cortical alveolar (CA); *Vitellogenic oocyte growth*; primary vitellogenesis (Vtg1), secondary vitellogenesis (Vtg2), tertiary vitellogenesis (Vtg3), quaternary vitellogenesis (Vtg4); *Oocyte maturation*: germinal vesicle migration (GVM). Oodev treatments: A.Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed.

Gonadosomatic Index (GSI)

Figure 5 shows the results of the analysis of variance (ANOVA) test on the administration of Oodev through oral administration with different doses on female Yellow rasbora fish which showed significant differences between treatments. The highest average GSI value was obtained from treatment C (1.0 mL/kg feed), which shows values of 14.01%, followed by treatment B (7.84%) and treatment D (7.77%), The lowest GSI value was obtained from treatment A which showed a value of 7.22%.

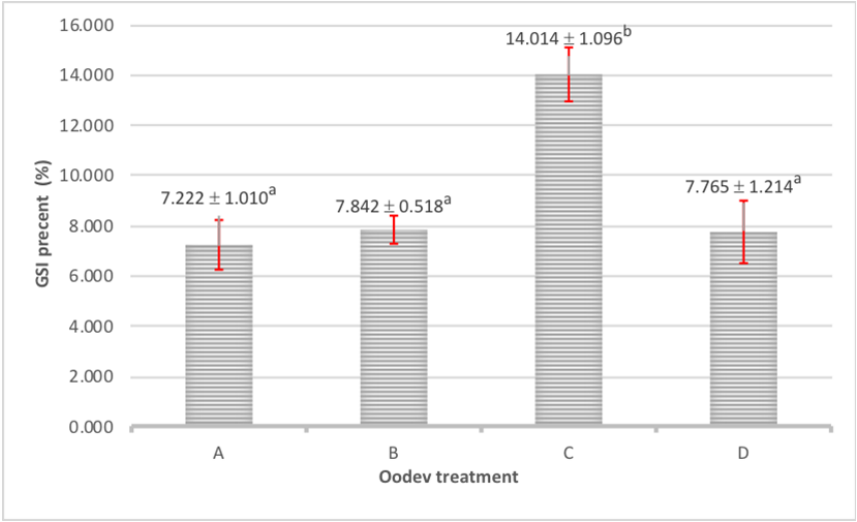

Figure 5. Gonadosomatic index (GSI) value of Yellow rasbora (*Rasbora lateristriata*) in Oodev treatments: A.Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed. Notes: The means ± SD on the bars graph with different superscripts letters are significantly different ($p < 0.05$).

It is assumed that the supplementation of Oodev with a higher dose of more than 1.0 mL/kg feed does not affect the increase in gonadal development. This is due to an excess concentration of FSH in the blood because of the large doses of PMSG given (Nur et al. 2017). The increase in GSI values was related to the increase in gonad size due to the increase in oocyte size and the number of yolk granules during the vitellogenesis process (Susilo et al. 2019; Mellisa et al. 2022).

The Fecundity

The results of the analysis of variance (ANOVA) test on Oodev hormone through oral administration with different doses in female Yellow rasbora showed no significant difference in fecundity values. The value of fecundity obtained varied between each treatment. Figure 6 shows the difference in the average fecundity of female Yellow rasbora in each treatment due to different doses of Oodev administration. Administration of Oodev at a dose of 1.0 mL/kg of feed-in treatment C resulted in the highest average value of fecundity of 721 eggs. In treatments A, B, and D, the fecundity values were 465, 496, and 476 eggs, respectively. Therefore, treatment C is said as the best dose for the effectiveness of the Oodev on fecundity, compared to other treatments. Treatment A, B, and D the difference is not significantly different from each other in the average value. There was a difference of 31 eggs between treatments A and B, 20 eggs between treatments B and D and a difference of only 11 eggs between treatment A and D. Treatment A (Feed without Oodev) was the treatment with the lowest fecundity value.

According to Hutagalung (2015), the administration of Oodev containing PMSG in fish influences earlier egg maturation. Administration of Oodev will increase the accumulation of GtH in fish so that the gonads of fish are stimulated to carry out a faster egg formation process even though environmental conditions are not suitable. In another study, it was explained that the administration of Oodev to improve reproductive performance in fish through oral administration in feed or the intramuscular injection method influenced fish fecundity (Darliansyah et al. 2017).

Egg Diameter

The effect of Oodev in feed through oral administration on Yellow rasbora egg diameter is seen in Figure 7. In this study, the results of the analy-

sis of variance (ANOVA) test showed significantly different results between treatments.

Treatment C showed a significantly different from other treatments with an average egg diameter 0.865 mm. While treatment A, B, and D are not significantly different with an average egg diameter where the treatment are A 0.349 mm, treatment B 0.485 mm, and treatment D 0.416 mm, respectively. It is seen that treatment A has the lowest egg diameter and is assumed due to no PMSG administration.

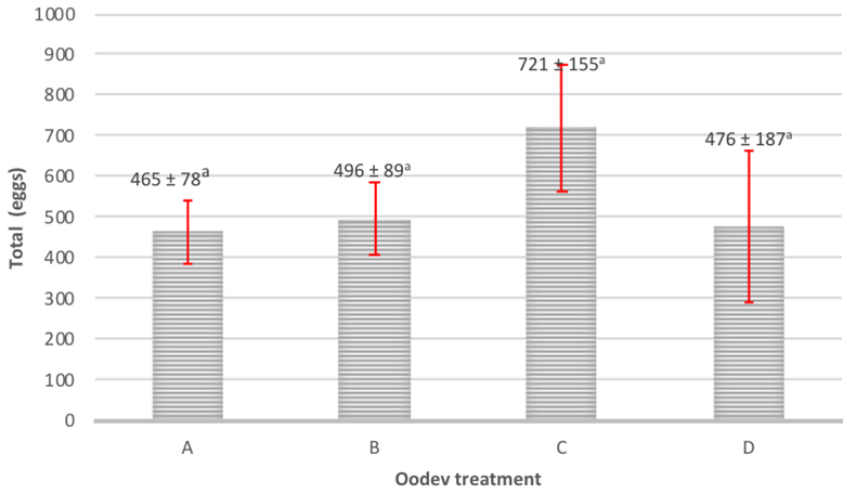

Figure 6. The fecundity of Yellow rasbora (*Rasbora lateristriata*) in Oodev treatments: A. Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed. Notes: The means ± SD on the bars graph with different superscripts letter were significantly different ($p < 0.05$).

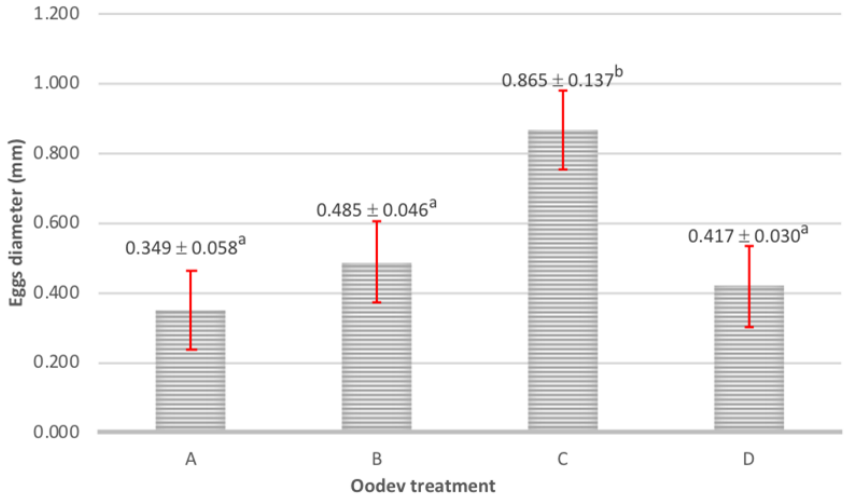

Figure 7. Diameter of Yellow rasbora (*Rasbora lateristriata*) eggs in Oodev treatments: A. Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed. Notes: The means ± SD on the bars graph with different superscripts letter were significantly different ($p < 0.05$).

Treatment D with a higher dose than other treatments also did not show a higher mean diameter of egg. It was assumed that the administration of Oodev with a higher dose did not affect the higher increase in gonad development. This may be due to an excess concentration of FSH in the blood because of high doses of PMSG (Nur et al. 2017). Dhewantara and Rahmatia (2017) revealed that the PMSG hormone affects the development of fish egg diameter due to the increase of the vitellogenin content in eggs. PMSG has a role in stimulating the formation of follicles

because it contains a higher level of FSH and a little bit of LH. The role of FSH in the gonadotropin hormone will stimulate the egg maturation process in fish. Figure 8, it is seen that the variation in egg diameter was obtained in female Yellow rasbora administered with Oodev in feed with different doses.

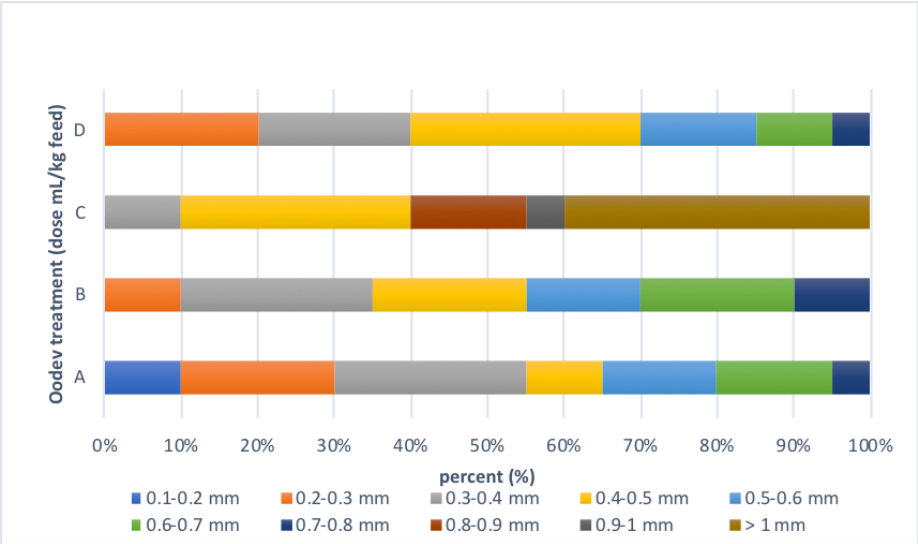

Figure 8. Percentage diameter of Yellow rasbora (*Rasbora lateristriata*) eggs in Oodev treatments: A. Feed without Oodev; B. 0.5 mL/kg feed; C. 1.0 mL/kg feed; and D. 2.0 mL/kg feed.

Figure 8 shows the percentage of egg diameter measurement in treatment C with a diameter of > 0.8 mm which is 60% and the percentage of egg diameter < 0.5 mm is 40%. In treatment B there were no eggs measuring > 0.8 mm, only 10% in this treatment had egg diameters ranging from 0.7 to 0.8 mm, and 55% with a measured diameter of < 0.5 mm. The same results were shown in treatments A and D, that is, there were no eggs with a size > 0.8 mm and only 5% of eggs with a diameter of 0.7–0.8 mm. It is shown that egg diameter in treatment C is proportional to the previously confirmed GML, GSI, and fecundity values obtained in this study. This is also relevant to Farida et al. (2019) that confirm the higher the level of gonad maturity, the larger the diameter of the eggs in the ovaries of *Helostoma temminckii*.

CONCLUSIONS

Administration of Oodev can accelerate the gonad maturation process for female Yellow rasbora (*Rasbora lateristriata*) and improve reproductive performance. Administration of Oodev at a dose of 1.0 mL/kg of feed is the most effective dose for optimizing gonadal maturity, this is evidenced by the highest results for all parameters, namely: gonads in GML IV phase, histological structure of the ovary showing dominance in the oocyte maturation phase, gonadosomatic index (GSI) 14.01%, 721 egg fecundity and an average egg diameter of 0.865 mm.

AUTHOR CONTRIBUTION

All authors have equal contributions to the research and publication. J.R., S.W., and B.R. wrote the manuscript. J.R. designed the study, observed, and collected broodstock of the research samples from the Center Development of Fisheries Technology, Cangkringan, Sleman Jogjakarta. S.W. and B.R. supervised all the processes from the field work to laboratory analysis.

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CONFLICT OF INTEREST

The authors state that they do not have any conflicts of interest from this manuscript. The authors are solely responsible for the article content and writing.

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