Effect of PMSG+AD hormone variation on the gonadal maturation of Pedih fish (Tor douronensis)

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Abstract. Pedih (Tor douronensis) is a freshwater fish that live in mountainous areas and swift rivers in the interior of Aceh. The aim of this research was to analyze the dosage of PMSG+AD hormone orally for the gonadal maturation of pedih (Tor douronensis). This research was conducted at technical implementation unit of fish breeding center Lukup Badak, Pegasing district, Aceh Tengah on February to March 2018. The method used was Completely Randomized Design (CRD) with 4 level of treatment and three replications: 0 ml/kg feed, 0.3 ml/kg feed, 0.5 ml/kg feed and 0.7 ml/kg feed. The result of ANOVA test showed that different dosage of PMSG+AD hormone orally showed significant effect on the weight of broodstock, relative weight, gonad maturations index, eggs diameter, and relative eggs diameter. The result of Duncan test showed that the best dosage was found 0.7 ml/kg feed, but this treatment was not significantly different with the dosage of 0.5 ml/kg feed.

1. Introduction
The abundant of fish resources is common in Indonesia as equatorial country [1]. Pedih fish (Tor douronensis) are freshwater fish that live in mountainous areas and swift rivers in the interior of Aceh. The poem comes from the Gayo language, the Acehnese know this fish as the kerling fish. Pedih fish on the international market are called Mahseer. Pedih fish are among the leading freshwater commodity fish that have very high economic value. So far the fulfillment of the demand for Pedih fish still relies on massive capture from nature. In 2014 the Pedih fish breeding technology of the UPTD BBI Lukup Badak Central Aceh was declared successful. However, larvae production is still not optimal because the broodstock maturation of fish is still constrained by the season.

According to [2] in producing large quantities of Tor fish species is not easy to do by relying on natural spawning or capture from nature. One alternative method in an effort to produce large amounts of Tor fish is through artificial spawning using the hormone induction method. One of the hormones that can accelerate the process of gonadal maturation is the hormone PMSG + AD.

Pregnant mare Serum Gonadotropin (PMSG) is a unique group of gonadotropins, this hormone has dual characteristics such as Follicle Stimulating Hormone (FSH) and Luetinizing Hormone (LH) [3]. PMSG contains FSH if added to commercial feed containing high protein will accelerate the maturation of gonadal fish. Meanwhile AD (Antidapamine) is a chemical compound and has a function to inhibit the work of dopamine, so it can stimulate gonadotropin secretion. AD will block dopamine so that it helps PMSG to accelerate gonadal maturity [4]. To meet market and community demand for Pedih fish and very minimal population in nature due to environmental influences and over-fishing (over fishing), it is therefore necessary to study the variation of PMSG + AD hormones
that are applied orally to Pedih fish and are expected to be able accelerate the maturation of the gonad of Pedih fish.

The purpose of this study was to analyze the effect of PMSG + AD hormone on gonad maturation of Pedih fish (*T. douronensis*) including broodstock weight gain, relative broodstock weight gain, percentage of mature gonad mature broodstock, gonad maturity index (IKG), egg diameter, and egg diameter relative.

2. Materials and Methods

2.1 Time and Place

This study was conducted at the Fish Seed Center (BBI) Lukup Badak, District. Pegasing, Central Aceh Regency. The time of the study was carried out in February 2018 to March 2018.

2.2 Experimental Design

The method used in this study was the experimental method with Completely Randomized Design (CRD) which consists of 4 treatments and 3 replications. The treatments tested in this study are as follows:

- Treatment A: not hormon PMSG+AD (control)
- Treatment B: hormon PMSG+AD dose 0.3 ml/kg feed
- Treatment B: hormon PMSG+AD dose 0.5 ml/kg feed
- Treatment B: hormon PMSG+AD 0.7 ml/kg feed

2.3 Preparation of test containers

The container used in this study was floating net cage (FNC) container size 3x2 m, 3 m depth and 2 m width. Then FNC was placed in a water column and given a weight. Then FNC was tied using a rope so that it was not carried away. Then the cover was placed so that the fish do not jump into another container.

2.4 Broodstock preparation

The Pedih broodstock used in this study was the broodstock collection of UPT BBI Lukup Badak. Brood fish taken in the water column and then inserted into the steropheme and then anesthetized. After being anesthetized, let standed for several minutes and then the anesthetized fish was examined for gonadal maturity using a catheter and then weighed the fish weight. Broodstock was moved into a container that has been prepared for gonad maturity will be given a microchip as a marker of the treatment given.

2.5 Mixing of Hormonal feed

Making a hormone began with mixing the hormone PMSG + AD with distilled water in a sprayer. The PMSG + AD hormone given was adjusted to the treatment dose. The mixture was stirred evenly, then the feed was sprayed with hormones while stirring evenly. After that the egg white was taken into the pan and stirred until foamy. Next, the egg whites were put into the sprayer and sprayed evenly into the feed mixed with hormones. Next, the food was aerated until dry and stored until the time of administration.

2.6 Feeding

Hormonal feed was given for 14 days of study period. Feed was given 2 times a day, namely in the morning (at 07.00 WIB) and in the afternoon (at 17.00 WIB). Feed given during the maintenance period was 2-3% by weight of fish.
2.7 Research parameters

Broodstock weight gain [5, 20].

Information:
W: Weight gain, Wt: average weight of the final fish of the study, W0: average weight of the initial fish of the study.

Relative Growth Rate
Measurement of relative weight gain using equations [5, 20].

\[ RGR = \frac{Wt-W0}{t} \times 100\% \]

Information:
RGR = Relative main weight gain (%), Wt = Final weight (gr), W0 = Initial weight (gr), t = Maintenance time (days).

Gonad Maturity Index
The measurement of the gonad maturity index (IKG) used the equation

\[ IKG = \frac{Bg}{Bt} \times 100\% \]

Information:
IKG = gonad maturity index (%), Bg = gonad weight, Bt = body weight

Egg diameter
The diameter of fish eggs was measured as much as one egg for one sample fish. Tool to measure egg diameter in the form of a binocular microscope equipped with a micrometer. The measurement results were observed under a microscope using the formula Cindelas (2005) in [7] using the formula:

\[ A = \frac{B}{0.4} \times 0.1 \text{ mm} \]

Information:
A = Actual size (mm), B = Readable number on the micrometer, 0.4 = Objective lens enlargement 40x, 0.1 = Value of unit in the preparation.

Relative egg diameter
to measure the relative increase in egg diameter using equations:

\[ DS = \frac{Dt-D0}{t} \times 100\% \]

Information:
Ds = Actual egg diameter%, Dt = Final egg diameter, D0 = Initial egg diameter

3. Results and Discussions
The ANOVA test results showed that the administration of PMSG + AD 0.7 ml / kg feed had a significant effect (P <0.05) on broodstock weight gain, relative broodstock weight gain, egg diameter, relative egg diameter. But it did not have a significant effect on the gonadal maturity index.

The results of the follow up test in Table 1 showed that the broodstock weight gain, relative broodstock weight gain, gonad maturity index, egg diameter and relative egg diameter in the treatment of PMSG + AD 0.7 ml / kg feed were significantly different from the control of 0 ml / kg and PMSG treatment + AD 0.3 ml / kg but not significantly different from the treatment of PMSG + AD 0.5 ml / kg of feed.
Table 1. ANOVA test values for broodstock weight, relative broodstock weight, gonad maturity index, egg diameter, relative egg diameter.

| Treatment          | Broodstock weight gain (gr) | Relative broodstock weight increase (%) | IKG (%)  | Egg diameter (mm) | Egg diameter relatively (%) |
|--------------------|-----------------------------|----------------------------------------|----------|------------------|-----------------------------|
| 0.0 ml/kg feed     | 35.00<sup>a</sup>          | 1.67<sup>a</sup>                      | 2.76<sup>a</sup> | 0.71<sup>a</sup> | 0.71<sup>a</sup>           |
| 0.3 ml/kg feed     | 61.70<sup>a</sup>          | 2.94<sup>a</sup>                      | 4.17<sup>a</sup> | 0.99<sup>ab</sup> | 1.84<sup>a</sup>           |
| 0.5 ml/kg feed     | 98.30<sup>ab</sup>         | 4.68<sup>ab</sup>                     | 6.44<sup>ab</sup> | 1.18<sup>bc</sup> | 2.08<sup>ab</sup>          |
| 0.7 ml/kg feed     | 156.70<sup>b</sup>         | 7.46<sup>b</sup>                      | 11.45<sup>b</sup> | 1.56<sup>c</sup>  | 3.11<sup>b</sup>           |

Description: Different superscript letters in the same column show significantly different (P <0.05).

The best broodstock weight gain was 156.70 gr, namely the treatment of the dose of PMSG + AD 0.7 ml / kg of feed while the lowest yield was 35.00 gr in the control treatment. On the relative weight gain there is the best treatment, namely at the treatment of PMSG + AD 0.7 ml / kg of feed at 7.46% and the lowest yield on the control treatment 1.67%. The best gonad maturity index value of fish is 11.45% in the treatment of 0.7 ml / kg of feed and the lowest in the control treatment of 2.76% and 0.3 ml / kg of feed (table 1). The best egg diameter was found in the treatment of PMSG + AD 0.7 ml / kg feed of 1.56 mm and the lowest was 0 mm in the control treatment, the dose of PMSG + AD 0.3 ml / kg of feed was 0.99 mm and the dose 0.5 PMSG + AD / kg feed 1.18 mm (Table 4.2 and Appendix 4). The egg diameter showed relatively similar results, namely in the treatment of PMSG + AD 0.7 ml / kg of feed at 3.11%.

PMSG hormone is a complex glycoprotein obtained from serum of pregnant horses and plays a role such as Luteneizing hormone (LH) and Follicle Stimulating Hormone (FSH) but the effect of FSH is greater than LH for maturation of the initial gonad [8]. PMSG has a role in stimulating follicle formation because it contains a lot of the working power of FSH and a little LH. Follice stimulating hormone (FSH) or GTH I will stimulate a surge in GnRH levels which then affects the pituitary gland to produce gonadotropin, while LH is involved in the development phase so that the fish will ovulate [9]. Antidopamin (AD) is a chemical that can inhibit the work of dopamine. Dopamine inhibits gonadal maturation by stimulating the secretion of hormones that can inhibit gonadal development (GHI). Dopamine also inhibits the synthesis and secretion of luteinizing hormone (LH) [10]. The research [11] reported that PMSG + AD with the injection method was effective in inducing the maturation of gonad fish Tor soro. [12] also reported the administration of PMSG + AD ml / kg for 7 days in 4 weeks with commercial injection and feeding methods capable of accelerating vitelogenesis and maturation of gonads of catfish broodstock (Pangasius hypophthalmus).

The induction of the PMSG + AD hormone that was applied orally had a significant effect (P <0.05) on broodstock weight gain. PMSG + AD which is mixed into feed at a dose of 0.7 ml / kg is the effective dose of the broodstock weight gain of 156.70 gr and the lowest dose of PMSG + AD 0 ml / kg of feed. This is due to the higher dose of PMSG + AD which is associated with an effect on weight gain due to the growing part of the gonad. The effect of gonadal development causes an increase in the growth rate of the broodstock weight because FSH contained in the hormone PMSG + AD is known to stimulate early gonadal development or vitelogenesis [13], whereas Antidopamine (AD) contained in PMSG + AD can block dopamine which can inhibit the maturation of gonads of fish [10].

Based on research, it can be seen that the broodstock weight gain is relatively increased in each treatment. The relative effective weight gain of the broodstock was at the treatment dose of 0.7 ml / kg of feed at 7.46% while the lowest in the control treatment of 0 ml / kg of feed was 1.67% (Table 1). This is presumably due to adequate feeding of protein and also due to the development of vitelogenin-filled oocytes. Vitelogenin is an egg yolk which is the main component of oocytes that have grown and produced in the liver. This is in accordance with [2] statement that PMSG hormone contains a lot
of work power (FSH) which plays a role in the maturation of the initial gonad or vitelogenesis. The absorption of vitelogenin will make oocytes reach a certain size which is then ready to be ovulated.

The synthesis of vitelogenin (the precursor of yolk) in the liver is called vitelogenesis. Vitelogenin is transported in the blood to the oocytes, then selectively absorbed and stored as egg yolk [14]. The vitelogenesis process involves several types of hormones, and in fish there are two kinds of gonadotropin hormones produced by adenohypophysis which act as FSH and LH. The hormone is FSH (GTH I), which works to stimulate follicle development through the secretion of estradiol 17β in the ovaries and LH (GTH II) needed for the final oocyte maturation process [15] Estradiol 17β is a stimulant in the biosynthesis of vitelogenin liver. Estradiol 17β which is present in the blood gives a back stimulation to the fish pituitary is stimulation in the process of gonadotropin formation [16].

Variation of the hormone PMSG + AD ml / kg of feed applied orally gave a positive response to the IKG value. The results showed that the highest gonad maturity index value of PMSG + AD 0.7 ml / kg feed was 11.45% and the lowest was in the control treatment. This is due to a balance between the nutrient content in the feed and the dose of PMSG + AD given. PMSG has a role in stimulating follicle formation because it contains a lot of the working power of FSH and a little LH. FSH or GTH I will stimulate a surge in GnRH levels which then affects the pituitary gland to produce gonadotropin, while LH is involved in the development phase so that the fish will ovulate. This is in accordance with the results of the [17] which stated that the potential for FSH which is more dominant in PMSG is a source of the addition of FSH hormone in the blood and can stimulate the maturation process of gonad Siamese catfish (Pangasianodon hypophthalmus).

The administration of PMSG + AD hormone mixed in feed affects the egg diameter and relative diameter of the poached fish. Based on the results of the duncan advanced test the highest egg diameter results obtained at the dose of 0.7 ml / kg feed PMSG + AD while the lowest results in the control treatment 0 ml PMSG + AD / kg feed and treatment PMSG + AD 0.3 ml / kg feed (table 1). This is in accordance with the results of research [12] Giving a dose of 0.7 ml PMSG + AD was able to accelerate the increase in egg diameter compared to the dose of 0.3 ml and 0.5 ml.

An increase in the size of the egg diameter is caused by a large number of glycolipoproteins called vitelogenesis. Glycolipoproteins are made in the liver under the control of steroid hormones found in ovarian follicles. Glycolipoprotein plays a role in the development of eggs, in this situation the eggs are in the secondary oocyte stage and can be seen with various sizes. This is in accordance with the statement of [18] that the diameter of fish eggs varied, both between species and between individuals in the same species.

Water quality parameters measured in the study were temperature, pH, and dissolved oxygen (DO). The value of measuring water quality the temperature ranges between 24-25 C, pH 7.3-7.5, and dissolved oxygen (DO) 7.7-7.8. The range of water quality during the study was still in optimal condition for the maturation of gonads of Pedih fish [19]. The range of water quality gonad maturation of Kancera fish (Tor soro) similar to Pedih fish (T. douronensis) temperatures ranging from 24°C-29°C, pH ranges from 7.0 to 7.5 and dissolved oxygen (DO) ranges 4.85-8.26 ppm.

Conclusion
It can be concluded that variations in the PMSG + AD hormones applied orally have a significant effect on broodstock weight gain, relative broodstock weight gain, gonad maturity index (IKG), egg diameter, and relative egg diameter p <0.05. The dose of 0.7 PMSG + AD that is applied orally can provide stimulation to the broodstock of the Pedih fish so that it can accelerate the maturation of the gonad of Pedih fish.

References
[1] Rizwan T, T K Nasution, I Dewiyanti, S A E Rahimi, D F Putra 2017 AACL Bioflux, 10(5):1180-1185
[2] Farastuti E R, O S Agus, S Rudhy 2014 LIMNOTEK 21(1): 87-94
[3] Stewart S L, T D Querec, B N Gruver, B O’Hare, J S Babb, C Patriotis 2004 Journal of Cellular
Physiology 198: 119–124

[4] Sarojini R, R Nagabhushanam, M Fingerman 1995 Biological Bulletin 189: 340-346
[5] Effendie M I 1997 Yayasan Pustaka Nusatama Yogyakarta
[6] Johnson J E 1971 Trans. Am. Fish. Soc. 100(1): 74-85
[7] Zultamin Z, M Muslim, Y Yulisman 2014 Jurnal Akuakultur Rawa Indonesia 2(2): 162-174
[8] Gallego V, I Mazzeo, M C Vilchez, D S Peñaranda, P C F Carneiro, L Pérez, J F Asturiano 2012 Aquaculture 354–355: 7-16.
[9] Bolamba D, P Matton, R Estrada, J J Dufour 1992 Journal of Animal Science 70: 1916-1922
[10] Weltzien F A, C Pasqualini, M E Sébert, B Vidal, N Le Belle, O Kah, P Vernier, S Dufour 2006 Endocrinology 147(6):2964–2973
[11] Farastuti E R, O S Agus, S Rudhy 2014 LIMNOTEK 21(1): 87-94
[12] Tinus A 2013 Fish Scientiae 3(5): 10-16
[13] Gallego V, I Mazzeo, M C Vilchez, D S Peñaranda, P C F Carneiro, L Pérez, J F Asturiano 2012 Aquaculture 354-355: 7-16
[14] Komatsu M, S Hayashi 1997 Fisheries Sciences 63: 98-994
[15] Nagahama Y, M Yoshikuni, M Yamashita, T Takumoto, Y Katsu 1995 Academic Press, Inc. 103-145
[16] Yaron Z 1995 Aquaculture 129: 49-73
[17] Tahapari E, R Roro, S P S D 2013 Biology News 12(2): 203-209
[18] Unus F, S Bin, A Omar 2010 Torani (Jurnal Ilmu Kelautan dan Perikanan) 20: 37-43
[19] Subagja J, Sulhi, M Asih, S Haryono 2009 Indonesian Biology Journal 5(3): 259-267
[20] Putra D F, L Armaya, S A E Rahimi, N Othman 2019 BIOTROPICA, 26(2): 136-142