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## Effect of PMSG Hormone and Essential Fatty Acids on Gonadal Maturation of Female Bileh Fish (*Rasbora maninjau*)

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## ABSTRACT

The maturation of fish gonads in aquaculture containers requires strategic measures so that fish can reproduce. Reproduction of bileh fish in aquaculture containers experiences obstacles, namely slow gonad maturation. Gonad maturation is influenced by internal factors such as the hormonal system and external factors including nutrition. This study aimed to determine the effect of PMSG hormone administration and essential fatty acids on the maturation of female bileh fish gonads. The experimental research design used a complete randomized design, with four treatments and three replicates. The treatment groups were: T1= control, T2= PMSG hormone 1 ml/kg, T3= corn oil 2% + fish oil 1.5%, and T4= PMSG 1 ml/kg+ corn oil 2% and fish oil 1.5%. The research stages included preparing materials and tools, mixing treatment ingredients into feed, proximate analysis of feed, fish rearing and treatment, observation, and data collection. The research parameters evaluated were the gonadosomatic index (GSI), hepatosomatic index (HSI), brood weight gain (Wg), gonad maturity level (GML), fecundity, and egg diameter. Data were analyzed using variance analysis and Duncan's test. The results showed that administering PMSG hormone and essential fatty acids through feed significantly affected (P < 0.05) IGS, IHS, Wg, and fecundity of bileh fish. The best treatment was T4.

#### INTRODUCTION

Indexed in Scopus

Bileh fish (*Rasbora maninjau*) is a freshwater fish whose habitat is in the waters of lakes, rivers, and swamps in Aceh Province, Indonesia (**Zulfadhli** *et al.*, 2024). The existence of bileh fish in nature is increasingly limited due to overfishing, hence aquaculture efforts are needed to preserve bileh fish and fulfill market demand (**Zulfadhli** *et al.*, 2023). Maturation of fish gonads in culture containers or controlled environments requires strategic steps so that fish can reproduce. The reproduction of bileh fish in aquaculture containers still experiences obstacles, such as slow gonad maturation. Gonad

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maturation is influenced by internal factors such as the hormonal system and external factors, viz. nutrition and the environment. Conditioning fish environmental parameters such as natural habitat is difficult and impractical. Using exogenous hormones is a practical and effective way to stimulate fish gonad maturation (**Mylonas** *et al.*, **2010**).

Maturation of fish gonads using the hormone Pregnant mare serum gonadotropin (PMSG) has been widely used in several fish species (**Nagahama** *et al.*, **1991; Putra** *et al.*, **2017; Pamungkas** *et al.*, **2019; Ath-Thar** *et al.*, **2021**). PMSG hormone contains follicle-stimulating hormone (FSH) and luteinizing hormone (LH); both molecules are important in fish's reproductive system. FSH molecules initiate gonad development, and LH molecules play a role in the final maturation of the gonads and encourage the ovulation process (Mateos *et al.*, **2002**). An increased FSH in the fish body causes the aromatase enzyme to synthesize testosterone into estradiol-17 $\beta$ . Estradiol-17 $\beta$  is the primary steroid that induces vitellogenin production in fish liver and acts as a precursor for oocyte formation, ovarian growth, and steroidogenesis (Clelland & Peng, 2009).

In addition to hormonal factors, dietary nutritional factors such as lipids play an important role in the reproductive success of fish (**Izquierdo** *et al.*, **2001**). Lipids act as a source of metabolic energy for somatic growth and essential fatty acids needed to form cell membranes for gonad development and maturation (**Parpoura & Alexis, 2001**; **Dunning** *et al.*, **2014**). Differences in lipid content in *Channa striatus* diets affect fecundity, egg diameter, hatching rate, and larval length (**Ghaedi** *et al.*, **2016**). Essential fatty acids such as linoleic (n-6) and linolenic (n-3) influence ovarian development and egg quality (**Furuita** *et al.*, **2007**).

Based on the literature description above, the PMSG hormone will accelerate the gonadal maturation process, and adding essential fatty acids will improve the quality of bileh fish eggs. The combination of the two treatment factors is expected to facilitate the maturation of the gonads of bileh fish in aquaculture containers. This study aimed to evaluate the effect of PMSG hormone and essential fatty acids in the gonad maturation of female bileh fish (*Rasbora maninjau*).

## MATERIALS AND METHODS

#### **Research design**

This research was conducted at the Aquaculture Laboratory of the Faculty of Fisheries and Marine Sciences, Teuku Umar University, and it was implemented from September to November 2024.

The experimental design used a completely randomized design (CRD), with four treatments (T) and three replicates (R). The treatments were the administration of PMSG hormone with the Oodev trademark and adding essential fatty acids (n-6 and n-3) in commercial feed. The source of n-6 fatty acid was corn oil (CO), and n-3 was fish oil (FO). The treatment groups were as follows:

T1= Commercial feed (control) T2= Commercial feed + PMSG 1 ml/kg T3= Commercial feed + CO 2% + FO 1.5% T4= Commercial feed + PMSG 1 ml/kg + CO 2% and FO 1.5%

### **Research procedures**

The bileh fish were taken from the Mina Mandiri Hatchery Unit, Nagan Raya District, Aceh Province. The fish had an average length of  $6.61 \pm 0.57$  cm/ head and an average weight of  $2.15 \pm 0.48$  grams/head. The bileh fish were adapted for 2 weeks before treatment. Fish rearing was carried out in 12 aquarium containers of 80x50x40 cm, and fish were filled with 10 fish/container. PMSG hormone and fatty acids (CO and FO) were mixed into commercial feed. Mixing using the repeletting method, the commercial feed was mashed, then the treatment ingredients were added and remolded. The ovendried feed was then subjected to proximate testing according to **AOAC** (2005). The feeding frequency of fish during the study was 3 times in the morning, afternoon, and evening at satiation. The study was conducted for 6 weeks. Observations and data collection were taken on the first day of the research and the middle and last days of the study.

Nutritional composition (9/)	Treatment			
Nutritional composition (%) -	<b>T1</b>	T2	T3	<b>T4</b>
Protein	38.94	39.06	38.90	38.81
Fat	4.03	4.00	6.06	6.00
Carbohydrates	26.91	27.63	26.68	26.42
Crude fiber	4.26	4.29	4.21	4.18
Water content	8.14	8.38	8.35	8.47
Ash	6.51	6.52	6.51	6.51

Table 1. Proximate results of feed treatments

#### **Research parameters**

- Gonadosomatik Index (GSI) (Zulfadhli et al., 2024) GSI (%) = gonad weight / total weight of fish × 100
- Hepatosomatik Index (HSI) (Syarif *et al.*, 2021) HSI (%) = hepar weight / total weight of fish × 100
- Broodstock weight gain (Wg) (Zulfadhli *et al.*, 2024)
   Wg (gram) = final research weight initial research weight
- 4. Gonad maturity level (GML) Visual observation based on TKG criteria (Table 2), and microscopic observation through histological preparations of gonads using Hematoxylin Eosin staining. The

procedure for making histological preparations refers to the research of **Zulfadhli** *et al.* (2016). The gonadal preparation slides were studied under a microscope connected to a computer monitor and documented with a camera (Optical Microscopes, Italy).

## 5. Fecundity (F) (Syarif et al., 2021)

 $F = gonad weight / sample gonad weight \times gonad eggs sampled$ 

6. Egg diameter

Bileh fish eggs were measured with a micrometer mounted under a microscope.

al. (2021)		
GML	Stage	Visual criteria
Ι	Immature	The small ovary occupies one-third of the body cavity, is
		oval in shape, and appears transparent.
II	Maturing	The ovary is elongated and occupies half of the body
		cavity, which is pink and transparent
III	Maturing Ripe	The small ovaries occupy 1/2 to 2/3 of the body cavity,
		and the right and left gonads appear asymmetrical. The
		ovaries appear yellow and mottled, with blood vessels
		visible on their surface
IV	Ripe	The ovary occupies two-thirds of the entire body cavity.
		It is yellow and has blood vessels visible on its surface.

 Table 2. Criteria for the gonad maturity level (GML) of fish in the research of Syarif et al. (2021)

#### Data analysis

Statistical analysis of research data was carried out through one-way ANOVA. The Duncan test was conducted if the ANOVA results showed a significant effect (P<0.05). The analysis used SPSS 21.0 software.

#### RESULTS

The experimental results of the addition of PMSG hormone and essential fatty acids in the diet of bileh broodstock are shown in Table (3). The treatments had a significant effect (P < 0.05) on the reproductive performance of bileh broodstock based on analysis of variance on the parameters of gonad somatic index (GSI), hepatosomatic index (HSI), broodstock weight gain (Wg), and fecundity. The treatment did not affect the diameter of bileh eggs. Adding PMSG hormone and essential fatty acids (T2, T3, T4) gave higher values in all parameters than without addition (T1). The combination of

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hormones and fatty acids (T4) has the best impact on the gonads of female bileh fish and is significantly different between treatments. The results of Duncan's further test can be seen in Table (3). Different superscript letters on the same table row indicate differences between treatments.

Parameter	Treatment				
I al alletel	T1	T2	<b>T3</b>	<b>T4</b>	
GSI (%)	$5.68\pm0.31^{a}$	$10.32\pm0.29^{\rm c}$	$8.55\pm0.46^{b}$	$13.81\pm0.43^d$	
HSI (%)	$0.28\pm0.03^{a}$	$0.61\pm0.04^{b}$	$0.57\pm0.03^{b}$	$0.64\pm0.05^{b}$	
Wg (gram)	$0.15\pm0.02^{a}$	$0.24\pm0.02^{bc}$	$0.21\pm0.02^{\text{b}}$	$0.28\pm0.03^{c}$	
Fecundity (egg)	$2113\pm210^a$	$3164\pm266^c$	$2642\pm248^{b}$	$3340\pm233^c$	
Egg diameter (mm)	$0.74\pm0.03^{a}$	$0.79\pm0.04^{a}$	$0.79\pm0.04^{a}$	$0.82\pm0.03^{a}$	

 Table 3. Evaluation results of research parameters after treatment

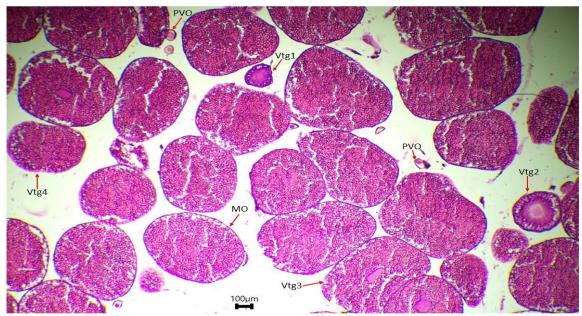
Visual observation of gonad maturity level (GML) of bileh fish after treatment resulted in different levels and percentages of GML between treatment groups. GML observations refer to the criteria of Table (2). The most mature gonads were found in T4 at GML IV, as much as 80%. Followed by T2 at GML IV at as much as 70%, T3 at GML IV at 50%, and T1 at GML III at as much as 30%. The T1 group, without the addition of hormones and fatty acids, was not found to be at GML IV. Ikan bileh, fed with the addition of hormones and fatty acids, can increase the percentage of fish gonad maturity level. Detailed GML results are presented in Table (3) below.

Treatment	Number of individual fish according to GML criteria			
	GML I	GML II	GML III	GML IV
T1	30%	40%	30%	0%
T2	0%	10%	20%	70%
Т3	0%	10%	30%	50%
T4	0%	0%	20%	80%

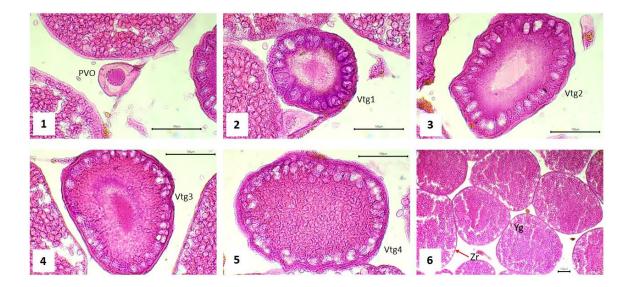
Table 3. Gonad maturity level (GML) of female bileh after being treated for 6 weeks

n = 10 individuals.

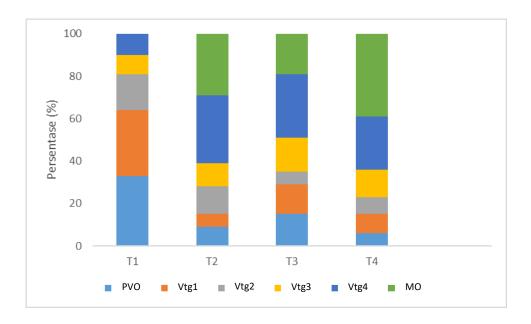
The observations of histological preparations of bileh fish gonads showed that in all treatment groups, there were differences in the stage of oocyte development (Fig. 1). The stages of oocytes seen are pre-vitellogenic oocyte, primary vitellogenesis, secondary vitellogenesis, tertiary vitellogenesis, quaternary vitellogenesis, and mature oocytes (Fig. 2).



**Fig. 1.** Female bileh fish gonad section after treatment. Pre-vitellogenic oocyte (PVO); Vitellogenic oocyte growth; primary vitellogenesis (Vtg1); Secondary vitellogenesis (Vtg2); Tertiary vitellogenesis (Vtg3); Quaternary vitellogenesis (Vtg4); Mature oocytes (MO). Hematoxylin-Eosin staining. 4x magnification



**Fig. 2.** Developmental stages of bileh fish oocytes: 1. Pre-vitellogenic oocyte (PVO); 2. Primary vitellogenesis (Vtg1); 3. Secondary vitellogenesis (Vtg2); 4. Tertiary vitellogenesis (Vtg3); 5. Quaternary vitellogenesis (Vtg4); 6. Mature oocytes (MO); Yolk granules (Yg); Zona radiata (Zr). Hematoxylin-Eosin staining. 40x magnification



# Fig. 3. Percentage of oocyte developmental stages of bileh fish based on histological preparation observation

There are differences in the percentage of the number of oocyte development stages in each treatment group (Fig. 3). Group T1 was dominated by the Vtg1 stage by 33%, and no MO stage was found. Group T2 is dominated by the Vtg4 stage by 32% and the MO stage by 29%. The T3 group was dominated by the Vtg4 stage by 30%, and the T4 group was dominated by the MO stage by as much as 39%. The percentage difference is caused by the influence of hormones and fatty acids that trigger oocytes to develop faster than without adding fatty acids.

## DISCUSSION

The gonadal maturation of female bileh fish is characterized by an increase in the gonadosomatic index (GSI) value. GSI is one of the indicators in assessing the development of fish gonads and the GSI value is obtained from the ratio between gonad weight and total fish weight. The lowest IGS value was 5.68% in group T1 without hormone and fatty acid administration. The T2 group with PMSG hormone administration produced a GSI value of 10.32%, and the T3 group with fatty acid administration produced a GSI value of 8.55%. At the same time, T4 with PMSG hormone + fatty acids produced a higher GSI value of 13.81%. The results of the ANOVA test of the GSI value had a significant effect (P < 0.05). The effect of PMSG hormone in the feed caused the difference in GSI value. PMSG hormone contains follicle-stimulating hormone or FSH (Ath-thar *et al.*, 2021). The FSH acts as an initiation in gonadal development (Nagahama & Yamashita, 2008). FSH signals to the

gonads to synthesize estradiol-17 $\beta$ , which then affects the liver to produce vitellogenin (**Mylonas** *et al.*, **2010**). During vitellogenesis, the volume of the oocyte enlarges as it fills with yolk, and the size of the gonad increases.

Vitellogenesis that takes place in the liver makes the liver larger in size (Akhdiana *et al.*, 2021). The hepatosomatic index (HSI) measures liver volume during vitellogenin synthesis. The lowest HSI in the T1 group was 0.28%, and the highest HSI was in T4 at 0.64%, followed by T2 at 0.61% and T3 at 0.57%. The results of the HSI Anova test had a significant effect (P < 0.05). The increase in HSI value indicates an increase in vitellogenin activity which causes the weight and volume of the liver to increase. The HSI value correlated with the GSI value in this study. The vitellogenesis process also affects the weight gain of fish due to the growth of oocytes in yolk absorption. The development of gonads causes the body weight of fish broodstock to increase (Sudrajat & Sugati, 2014). The highest Wg value was in T4, with a value of 0.28 grams. ANOVA test results were significantly influenced (P < 0.05). Weight gain of bileh fish goes hand in hand with the value of GSI.

Fecundity and egg diameter values increased after PMSG and essential fatty acid treatment. Fecundity and egg diameter values were the highest in T4 and lowest in T1. The difference in the number of eggs is due to the increase in ovarian ovum cells filled with vitellogenin. The treatment had a significant effect on fecundity parameters but no significant effect on egg diameter (P > 0.05).

PMSG hormone stimulates the gonads to mature quickly. A total of 70 and 80% of bileh fish mature gonads in groups T2 and T4 (Table 3). GML visual observation conditions are also depicted on histological preparations. The histological picture of the gonads showed differences in the percentage of oocyte stages (Fig. 3). The percentage of MO stage oocytes was found in the T4 group as much as 39%, while by visual observations it was at GML IV. The combination of hormones and essential fatty acids produces maximum fish maturation compared to the administration of hormones or fatty acids alone. Essential fatty acids are important for broodstock needs in determining the success of fish reproduction (Izquierdo et al., 2001). The maturation of fish gonads is maximized in the T4 group because the feed contains PMSG hormone and essential fatty acids. The T3 group contains fatty acids in the feed but does not contain PMSG hormone, so it is slow in initiating gonad development. While in T2 the feed contains PMSG hormone but low fatty acids. The results of the feed proximate test are presented in Table (1). The fat composition of feed in groups T3 and T4 was 2% higher than that recorded in T1 and T2. The fatty acid requirement needed by brood fish is between 0.5-2.5% (Sargent et al., 2003).

Hormones and essential fatty acids in feed facilitate the gonadal maturation of bileh fish in the formation and development of gonads. In the T4 group, adding 1ml/ kg PMSG and 2% CO + 1.5% FO in feed resulted in optimal gonad maturation of bileh fish in this study. PMSG plays a role in stimulating faster gonadal maturation by activating

the hormone estradiol-17 $\beta$  and inducing vitellogenin production in the fish liver (Aththar *et al.*, 2021). Increased fat content in the diet will stimulate gonadal development, resulting in increased fecundity values and a more significant number of mature oocytes (Ghaedi *et al.*, 2016; Du *et al.*, 2018). According to Hutagalung *et al.* (2015), giving PMSG at a 1.25ml/ kg dose can increase the gonadosomatic and hepatosomatic indices in *Channa striata* fish. Tor tambroides fish given a mixture of FO and CO in a ratio of 1:1 showed a more significant percentage of GSI and faster oocyte development than other treatments (Abduh *et al.*, 2021). Essential fatty acids are the primary nutrient that supplies the metabolic energy required for growth and reproduction (Bhat *et al.*, 2022).

# CONCLUSION

The administration of PMSG hormone combined with essential fatty acids through feed had a significant effect (P < 0.05) on the gonadosomatic index (GSI), hepatosomatic index (HSI), brood weight gain (Wg), and fecundity of bileh fish. Among the treatments, T4 produced the best results, with a GSI of 13.81%, HSI of 0.64%, brood weight gain of 0.28 grams, and fecundity of 3,340 eggs.

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## REFERENCES

- Abduh, M. Y.; Koh, I. C. C.; Abol-Munafi, A. B.; Norazmi-Lokman, N. H. and Mat Noordin, N. (2021). Effects of dietary fish oil and corn oil on gonadosomatic and hepatosomatic index, gonadal histology, 17β-oestradiol level and fatty acids profile of mahseer (*Tor tambroides*) broodstock in captivity. *Aquaculture Nutrition* 27(5): 1448–1459.
- Akhdiana, I.; Zairin Jr, M.; Haryani, G. S. and Suprayudi, M. A. (2021). Kinerja reproduksi induk ikan bada *Rasbora argyrotaenia* (Bleeker, 1849) melalui pemberian kombinasi estradiol dan spirulina dalam pakan. *Limnotek: Perairan Darat Tropis di Indonesia* 28(2).
- AOAC. (2005). Official Method of Analysis of the Association of Official Analytical Chemist. Washington, Virginia, USA: Association of Official Analytical Chemists, Inc. 1786 p.

- Ath-Thar, M. H.; Gustiano, R.; Sundari, S.; Kurniawan, K.; Prakoso, V. A. and Kusmini, I. I. (2021). Pregnant mare's serum gonad hormones and antidopamine treatments on the maturation of snakehead (*Channa striata*). Aquaculture, Aquarium, Conservation and Legislation 14(1): 173–180.
- Bhat, R. A.; Saini, S.; Saoca, C.; Maricchiolo, G. and Fazio, F. (2022). Analysis of fatty acids and sex steroid hormones in rainbow trout testes (*Oncorhynchus mykiss*) during the reproductive process. Aquaculture Research 53(12): 4426– 4436.
- Clelland, E. and Peng, C. (2009). Endocrine/paracrine control of zebrafish ovarian development. *Molecular and Cellular Endocrinology* **312**(1–2): **42–52**.
- Du, H.; Yao, J.; Zhou, H.; Leng, X.; Wu, J.; He, S. and Tan, Q. (2018). Optimal dietary lipid level promoted ovary development of Chinese sturgeon (*Acipenser sinensis*) broodstocks. *Aquaculture* 495: 288–294.
- Dunning, K. R.; Russell, D. L. and Robker, R. L. (2014). Lipids and oocyte developmental competence: the role of fatty acids and β-oxidation. *Reproduction* 148(1): R15–R27.
- Furuita, H.; Hori, K.; Sugita, T. and Yamamoto, T. (2007). Effect of n-3 and n-6 fatty acids in broodstock diet on reproduction and fatty acid composition of broodstock and eggs in the Japanese eel *Anguilla japonica*. *Aquaculture* 267(1–4): 55–61.
- Ghaedi, A.; Kabir, M. A. and Hashim, R. (2016). Effect of lipid levels on the reproductive performance of snakehead murrel, *Channa striatus*. Aquaculture Research 47(3): 983–991.
- Hutagalung, R. A.; Widodo, M. S. and Faqih, A. R. (2015). Evaluation of PMSG (Oodev®) application on hepatosomatic and gonadosomatic index of snakehead fish. *Jurnal Akuakultur Indonesia* 14(1): 24–29.
- Izquierdo, M. S.; Fernandez-Palacios, H. and Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* 197(1–4): 25–42.
- Mateos, J.; Mananos, E.; Carrillo, M. and Zanuy, S. (2002). Regulation of folliclestimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin-releasing hormone (GnRH) and sexual steroids in the Mediterranean Sea bass. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 132(1): 75–86.
- Mylonas, C. C.; Fostier, A. and Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology* 165(3): 516–534.
- Nagahama, Y. and Yamashita, M. (2008). Regulation of oocyte maturation in fish. *Development, Growth and Differentiation* 50: S195–S219.
- Nagahama, Y.; Matsuhisa, A.; Iwamatsu, T.; Sakai, N. and Fukada, S. (1991). A mechanism for the action of pregnant mare serum gonadotropin on aromatase

activity in the ovarian follicle of the medaka, *Oryzias latipes*. Journal of *Experimental Zoology* **259(1)**: **53–58**.

- Pamungkas, W.; Jusadi, D.; Zairin Jr, M.; Setiawati, M.; Supriyono, E. and Imron,
  I. (2019). Induction of ovarian rematuration in striped catfish (*Pangasianodon hypophthalmus*) using pregnant mare serum gonadotropin hormone in out-of-spawning season. *Aquaculture, Aquarium, Conservation and Legislation* 12(3): 767–776.
- Parpoura, A. C. and Alexis, M. N. (2001). Effects of different dietary oils in sea bass (*Dicentrarchus labrax*) nutrition. *Aquaculture International* 9(6): 463–476.
- Putra, W. K. A. and Razai, T. S. (2017). Effect of pure and combined hormone of pregnant mare serum (PMSG) on gonadosomatic index, hepatosomatic index of silver pompano fish (*Trachinotus blochii*). *Journal of Aquaculture Science* 2(1): 61–71.
- Sargent, J. R.; Tocher, D. R. and Bell, J. G. (2003). The lipids. In: Fish Nutrition. 181– 257.
- Sudrajat, A. O. and Sugati, A. (2014). Induced maturation of eel Anguilla bicolor using different hormone combination. Jurnal Akuakultur Indonesia 13(2): 189–201.
- Syarif, A. F.; Putri, D. F. A. and Robin, R. (2021). Induksi maturasi ikan seluang (*Rasbora einthovenii*) betina menggunakan hormon GnRH analog + anti dopamin melalui pakan. Sains Akuakultur Tropis: Indonesian Journal of Tropical Aquaculture 5(1): 22–33.
- Zulfadhli, Z.; Fadhillah, R.; Jusadi, D. and Fujaya, Y. (2023). Domestication of bileh fish *Rasbora* sp. origin of Aceh waters through different feed therapy. In: *IOP Conference Series: Earth and Environmental Science* 1147(1): 012016.
- Zulfadhli, Z.; Saputra, F.; Fujaya, Y. and Burhanis, B. (2024). Effect of pregnant mare serum gonadotropin and antidopamine on the gonads of female bileh fish (*Rasbora maninjau*). In: *IOP Conference Series: Earth and Environmental Science* 1329(1): 012009.
- Zulfadhli, Z.; Wijayanti, N. and Retnoaji, B. (2016). Perkembangan ovarium ikan wader pari (*Rasbora lateristriata* Bleeker, 1854): Pendekatan histologi. *Jurnal Perikanan Tropis* 3(1).