

Enhancement of Ovarian Development in Striped Catfish (*Pangasianodon Hypophthalmus*) Through Supplementation of Katuk Extract (*Sauvopis Androgenus*), Turmeric Powder (*Curcuma Longa*), and Vitamin C

Awaludin^{1,4}, Wasmen Manalu^{2*}, Andriyanto², Agus Oman Sudrajat³, Joni Haryadi⁵

¹Doctoral Program Student, School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia

²School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia

³Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia

⁴Department of Aquaculture, Faculty of Fisheries and Marine Science, Borneo Tarakan University, Tarakan, Indonesia

⁵Center for Research on Ornamental Fish Culture, Depok, Indonesia

*Corresponding author: wasmenmanalu@ymail.com

Received: 17 May 2024, Approved: 20 January 2025

ABSTRACT

The development of fish ovaries influences the quality of the fingerlings. The research aimed to accelerate the maturity of the striped catfish gonads with supplementation of katuk extract (0.6 g/kg of feed), turmeric powder (4.8 g/kg of feed), and vitamin C (599 mg/kg of feed). The study used a completely randomized design with eight treatments and three replications: CON (Control), SA (katuk extract), TU (turmeric powder), VC (vitamin C), SATU (katuk extract and turmeric powder), SAVC (katuk extract and vitamin C), TUVC (turmeric powder and vitamin C), and COM (katuk extract, turmeric powder, and vitamin C). The fish broodstock was maintained for two months, receiving a feed dosage equivalent to 5% of their body weight. The observed parameters included broodstock weight, gonad weight, gonadosomatic index (GSI), ovarian histology, and oocyte diameter during the endogenous vitellogenesis phase. The findings indicated that incorporating katuk extract, turmeric flour, and vitamin C led to increased GSI values and gonad weights relative to the control group. The supplementation of katuk extract, turmeric flour, and vitamin C facilitated the development of oocytes, advancing them to the endogenous vitellogenesis phase. At the same time, in the control treatment, there were still many oocytes in the previtelogenesis phase. The largest oocytes during the highest endogenous vitellogenesis phase were observed in the combination treatment group ($603.60 \pm 99.09 \mu\text{m}$), while the smallest were recorded in the control group ($412.60 \pm 39.76 \mu\text{m}$). The addition of katuk extract, turmeric flour, and vitamin C significantly enhances the development and quality of striped catfish oocytes.

Keywords: katuk extract, ovarian growth and development, striped catfish reproduction, turmeric powder, vitamin C

ABSTRAK

Perkembangan ovarium ikan mempengaruhi kualitas anakan yang dihasilkan. Penelitian bertujuan mempercepat kematangan gonad ikan patin dengan suplementasi ekstrak katuk (0,6 g/kg pakan), tepung kunyit (4,8 g/kg pakan), dan vitamin C (599 mg/kg pakan). Penelitian menggunakan rancangan acak lengkap, 8 perlakuan dan 3 ulangan, yaitu CON (Kontrol), SA (ekstrak katuk), TU (tepung kunyit), VC (vitamin C), SATU (ekstrak katuk dan tepung kunyit), SAVC (ekstrak katuk dan vitamin C), TUVC (tepung kunyit dan vitamin C), dan COM (ekstrak katuk, tepung kunyit, dan vitamin C). Induk ikan dipelihara selama 2 bulan dengan pemberian pakan dosis 5% bobot tubuh. Parameter yang diamati adalah bobot induk, bobot gonad, gonadosomatik indeks (GSI), histologi ovarium, dan diameter oosit fase endogenous vitellogenesis. Hasil penelitian menunjukkan bahwa penambahan ekstrak katuk, tepung kunyit, dan vitamin C menghasilkan nilai GSI dan bobot gonad yang lebih tinggi dibandingkan dengan kontrol. Perkembangan oosit dengan suplementasi ekstrak katuk, tepung kunyit, dan vitamin C mencapai fase endogenous vitellogenesis, sedangkan pada perlakuan kontrol masih banyak oosit pada fase previtelogenesis. Ukuran oosit fase endogenous vitellogenesis tertinggi ditemukan pada perlakuan kombinasi ($603.60 \pm 99.09 \mu\text{m}$) dan terendah pada kontrol ($412.60 \pm 39.76 \mu\text{m}$). Dapat disimpulkan bahwa penambahan ekstrak katuk, tepung kunyit, dan vitamin C mampu mempercepat perkembangan dan kualitas oosit ikan patin.

Kata kunci: ekstrak katuk, perkembangan ovarium, reproduksi ikan, tepung kunyit, vitamin C

INTRODUCTION

Patin or striped catfish is recognized as a superior freshwater fish commodity and is prioritized for development in alignment with Presidential Instruction Number 7 of 2016 due to its manageable requirements for seed provision, growth, and feed management (Inpres, 2016). Enhancing fish seed production can be achieved by preparing high-quality broodstock to yield superior eggs. Egg quality is primarily influenced by the presence of vitellogenin compounds, which supply the essential components required for the growth and development of embryos into larvae.

Multiple factors affect the development of striped catfish ovaries, including gene expression, hormonal levels, dietary supplementation, and environmental conditions. The quality of offspring is primarily influenced by the development of the parent's ovaries, which is affected by vitellogenin synthesis in the liver and the accumulation of vitellogenin in developing follicles. To enhance ovarian development, one can utilize natural ingredients rich in phytoestrogens. Estrogen plays a crucial role in the reproductive process by stimulating the synthesis of vitellogenin in hepatocytes. This compound is subsequently transported via the bloodstream and deposited in the follicles, influencing the maturation rate of both follicles and ovaries in animals.

Estrogen is essential for stimulating hepatocytes, leading to an increase in vitellogenin synthesis. Follicle-stimulating hormone (FSH) promotes the synthesis of estradiol in the ovary. In undeveloped ovaries, estradiol synthesis is absent, resulting in the lack of stimulation for vitellogenin synthesis in hepatocytes. To initiate reproductive activity, vitellogenin synthesis by hepatocytes can be initiated by adding exogenous estrogen or phytoestrogens to stimulate vitellogenin synthesis by hepatocytes. Vitellogenin, produced by hepatocytes, is transported to the ovary for deposition in the follicles, thereby initiating the recruitment of follicle development within the ovary. Follicle recruitment initiates the synthesis of estradiol 17β , which subsequently acts on hepatocytes to stimulate vitellogenin synthesis. Consequently, the initiation of gonadal development can be achieved by incorporating phytoestrogens.

Natural ingredients like katuk (*Sauvages androgynus* L) and turmeric (*Curcuma longa*) offer numerous benefits due to their secondary metabolic compounds, including sterols, tannins, saponins, terpenoids, phenols, and alkaloids (Awaludin et al., 2020; Bunawan et al., 2015). Curcumin, the primary phenolic compound in turmeric rhizomes, exhibits antioxidant and hepatoprotective properties (Zhou et

al., 2012), enhancing the functionality and capacity of hepatocytes to synthesize vitellogenin. Furthermore, curcumin exhibits a phytoestrogen function (Cseke, 2006), promoting vitellogenin production in hepatocyte cells.

Vitamin C is noted for its important role in ovarian development. Abdollahifar et al. (2019) indicated that the administration of vitamin C can enhance the total volume of ovaries and oocytes, as well as the average number of follicle cells. Vitamin C can specifically synthesize carnitine in the mitochondria. Carnitine transports fatty acids from the cytosol to the mitochondria, where they are processed to produce ATP and then used for vitellogenin synthesis, egg growth, and development. Enhancing reproductive activity by increasing the synthesis of essential materials for optimal egg and embryo growth and development necessitates an increased production of ATP. Consequently, incorporating vitamin C is anticipated to enhance ATP availability, thereby promoting vitellogenin synthesis in liver cells and facilitating the growth and development of embryos into larvae.

Optimal levels of vitellogenin are essential for oocyte development. In addition, the anti-inflammatory activity of katuk leaf extract has been reported to improve ovarian health (Desnita et al., 2018). Research indicates that food supplements like turmeric flour enhance vitellogenesis, gonad development, and egg diameter in striped catfish (Dewi et al., 2017). Vitamin C has been demonstrated to influence ovarian development. Research indicates that the administration of vitamin C can enhance the total volume of ovaries and oocytes, as well as the average number of follicles (Abdollahifar et al., 2019). Furthermore, vitamin C has been shown to restore ovarian follicle reserves in aging mice and mitigate lipid peroxidation in ovaries exposed to hexavalent chromium (Nasr et al., 2014; Soleimani Mehranjani et al., 2016). Substantial quantities of ATP are required during embryonic development; therefore, the optimal supply of ATP during reproduction is essential.

Individual research on the effects of katuk extract, turmeric flour, and vitamin C has demonstrated their potential to enhance ovarian development across different animal species. However, there have been no reports regarding the combination of katuk extract, turmeric flour, and vitamin C and the potential synergistic effects of these compounds on ovarian development. The content of these three natural ingredients suggests that their combination may enhance egg and ovarian development, thereby improving the quality and quantity of oocytes and seeds produced.

MATERIALS AND METHOD

Preparation of katuk extract, turmeric flour, and vitamin C

Katuk leaves originated from Tarakan, North Kalimantan. Katuk leaves were extracted through the maceration method utilizing a 70% ethanol solvent for three days. Katuk leaves were detached from their stems, chopped into small pieces, and dried at room temperature for 14 days until dry. The dried katuk leaves were ground until smooth, then soaked in 70% alcohol for 3 x 24 hours at room temperature with a ratio of 1:3. The katuk leaf extract solution was filtered, subsequently evaporated with an evaporator, and dried in a water bath at 40°C. The turmeric flour was sourced from the Cimanggu Medicinal Plants and Aromatics Research Center (BALITRO) in Bogor. The Vitamin C utilized was Ascorbyl monophosphate sourced from Luwei Pharmaceutical Group.

Experimental Animal

This research was conducted at the Sukamandi Fish Research and Breeding Center, Subang, West Java. The research procedure has been approved by the animal ethics committee of the School of Veterinary Medicine and Biomedicine, IPB University, with Number 059/KEH/SKE/VI/2023. The study involved 24 individual female striped catfish, each averaging 150 grams in weight and aged two years, all of which were in their first reproductive cycle. This study used eight treatments with three replications. The treatment included katuk extract at 0.6 grams/kg, turmeric flour at 4.8 grams/kg (Dewi *et al.*, 2017), and vitamin C at 599 mg/kg (Sarmento *et al.*, 2018). The research design included eight treatments: CON, a control without katuk extract, turmeric flour, or vitamin C; SA, katuk extract at 0.6 grams/kg; TU, turmeric flour at 4.8 grams/kg; VC, vitamin C at 599 mg/kg; SATU, a combination of katuk extract (0.6 grams/kg) and turmeric flour (4.8 grams/kg); SAVC, katuk extract (0.6 grams/kg) and vitamin C (599 mg/kg); TUVC, turmeric flour (4.8 grams/kg) and vitamin C (599 mg/kg); and COM, a combination of katuk extract (0.6 grams/kg), turmeric flour (4.8 grams/kg), and vitamin C (599 mg/kg), with three replications for each treatment. The broodstock were kept in a 10 m x 8 m x 1 m earthen pond using 3 m x 3 m x 0.5 m nets for three individuals each. The brood striped catfish were kept for 8 weeks, during which their diet was supplemented with katuk extract, turmeric flour, and vitamin C, accounting for up to 5% of their body weight. Weekly examinations of the urogenital papilla were conducted, which was characterized by a reddish hue, signifying the presence of mature gonads. The parameters of the

maintenance water quality were temperature 28-29°C, pH 7.75-8.20, and DO 3.59-5.24 mg/kg. Gonad samples were obtained through surgical procedures in the eighth week. The fish were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 1 mL per liter of water.

Gonad Analysis

The gonads were removed and weighed to determine the gonad somatic index (GSI), calculated using Equation 1.

$$GSI(\%) = \frac{Bg}{Bt} \times 100 \quad (1)$$

The gonads of the striped catfish broodstock were observed using Hematoxylin Eosin (HE) staining, with a section thickness of 5 μm, under a light microscope at 10x magnification. The diameter of the oocytes was measured using ImageJ.

Data Analysis

Data were analyzed using SPSS. Broodstock weight, gonad weight, GSI value, and oocyte diameter were analyzed using one-way ANOVA, followed by the Tukey test to assess differences across treatments. The histological data of gonads were presented descriptively, focusing on the development of oocytes within the ovaries.

RESULTS

The virgin broodstock utilized in this study was sourced from TKG I and was maintained for eight weeks, with a diet supplemented with katuk extract, turmeric flour, and vitamin C following the treatment protocols. The findings from the observations following the administration of katuk extract, turmeric flour, and vitamin C, encompassing broodstock weight, gonad weight, and GSI value for each treatment, are detailed in Table 1. Table 1 indicates variations in gonad weight and GSI values among the treatment groups. The gonad weight exhibited a statistically significant difference ($P < 0.05$), with the highest weight recorded in the treatment group receiving a combination of katuk extract, turmeric flour, and vitamin C (COM), measuring 155.50 ± 8.48 . In contrast, the control treatment group (CON) did not receive supplementation and showed the lowest weight at 54.70 ± 10.56 . COM treatment was able to increase gonad weight by 2.8 times compared to CON treatment. The treatment group receiving a combination of katuk extract, turmeric

Table 1. Fish broodstock weight, gonad weight, and GSI

Treatment	Broodstock weight	Gonad Weight (g)	Somatic Gonad Index (%)	Diameter of oocytes in the endogenous vitellogenin phase (μm)
CON	2.21±0.52 ^a	54.70±10.56 ^c	2.50±0.13 ^a	412.60±39.76 ^c
SA	2.64±0.66 ^a	110.03±32.88 ^{abc}	4.12±0.27 ^b	490.40±92.40 ^{abc}
TU	2.72±0.92 ^a	121.90±26.85 ^{ab}	4.58±0.52 ^{bc}	493.80±25.67 ^{abc}
VC	2.29±0.37 ^a	88.53±19.86 ^{bc}	3.84±0.27 ^b	474.60±46.16 ^{bc}
SATU	2.64±0.24 ^a	136.36±17.10 ^{ab}	5.15±0.18 ^{cd}	552.40±25.85 ^{ab}
SAVC	2.58±0.70 ^a	126.50±35.70 ^{ab}	4.92±0.36 ^c	548.00±49.87 ^{ab}
TUVC	2.31±0.20 ^a	134±5012.32 ^{ab}	5.82±0.32 ^{de}	561.60±81.51 ^{ab}
COM	2.38±0.12 ^a	155.50±8.48 ^a	6.53±0.33 ^e	603.60±99.09 ^a

Description: CON: Control, SA: Katuk, TU: Turmeric, VC: Vitamin C, SATU: Katuk and Turmeric, SAVC Katuk and Vitamin C, TUVC: Turmeric and Vitamin C, COM: Katuk, Turmeric, and Vitamin C

flour, and vitamin C (COM) exhibited the highest GSI value ($P<0.05$) at 6.53 ± 0.33 . In contrast, the control treatment group (CON) recorded the lowest value at 2.50 ± 0.13 . The GSI value in the COM treatment increased by 2.6 relative to the CON treatment.

The gonadal development of broodstock striped catfish after eight weeks of supplementation with katuk extract, turmeric flour, and vitamin C exhibited distinct morphological variations, as illustrated in Figure 1, with differing sizes observed. The COM treatment resulted in larger gonads than the other treatments, particularly the CON treatment. The oocyte development varied among the treatment groups (Figure 2). The variation in oocyte development stages within the ovaries suggests that each treatment group of fish underwent distinct phases of oocyte maturation. The combination treatment involving supplementation with katuk extract, turmeric flour, and vitamin C resulted in oocyte development advancing to the exogenous vitellogenesis phase. In contrast, many oocyte development stages in the CON treatment were still in the previtellogenic phase. This suggests that striped catfish undergo partial or asynchronous spawning. Statistical analysis revealed significant differences ($P<0.05$) in the diameter of oocytes during the endogenous vitellogenesis phase. The treatment group receiving supplementation of katuk extract, turmeric flour, and vitamin C (COM) exhibited an average diameter of 603.60 ± 99.09 μm , while the control group (CON) recorded a lower average diameter of 412.60 ± 39.76 μm .

DISCUSSION

Feed supplementation with katuk extract, turmeric flour, and vitamin C for 8 weeks affects fish reproduction as evidenced by gonad weight,

gonadosomatic index (GSI), and histological development of the gonads. This study found that the supplementation of katuk extract, turmeric flour, and vitamin C in feed enhanced gonad development relative to the control group.

The treatment group that received katuk extract (SA, SAVC, and COM) exhibited superior gonad development compared to the control group (CON). The estrogenic effect of katuk leaves may influence the hormonal processes associated with gonad development. Estrogenic stimulation from katuk leaves given in feed also stimulates vitellogenin synthesis and deposition in follicle cells, apart from endogenous estrogen. The development of follicle cells initiates with the supplementation of katuk extract, which supplies phytoestrogens essential for the synthesis of vitellogenin. This vitellogenin is subsequently deposited in follicle cells to facilitate oocyte development.

Katuk leaves contain active compounds such as androstane-17-one and 3-ethyl-3-hydroxy-5-alpha, precursors in synthesizing steroid hormones, including estrogen. In addition, katuk leaf extract contains phenol and terpenoid fatty acids, which can increase fish reproduction (Awaludin *et al.*, 2020; Susilowati *et al.*, 2020). The high antioxidant content in katuk leaves may protect gonad tissue from oxidative damage, thereby supporting gonad development (Fatmawati *et al.*, 2022; Hikmawanti *et al.*, 2021). Therefore, this demonstrates that the estrogenic effect of katuk extract can facilitate ovarian development and maturation.

Supplementation with turmeric flour (TU, SATU, TUVC, and COM) accelerated gonadal development compared to the control group (CON). This is likely due to turmeric containing curcumin, a bioactive compound with pharmacological properties, including

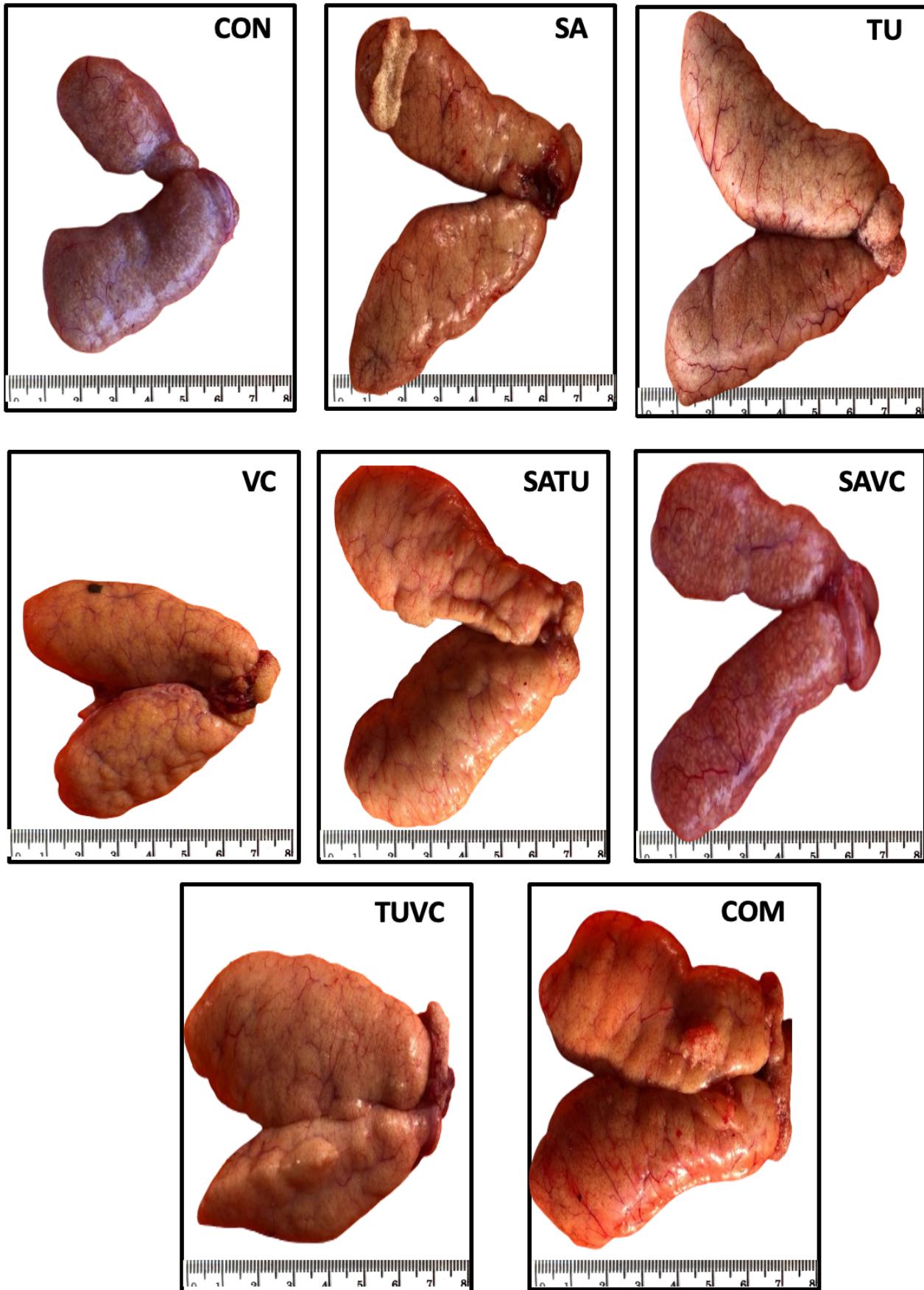


Figure 1. Gonads of treated catfish. Description CON: Control, SA: Katuk; TU: Turmeric; VC: Vitamin C; SATU: Katuk and Turmeric; SAVC Katuk and Vitamin C; TUVC: Turmeric and Vitamin C; COM: Katuk, Turmeric, and Vitamin C.

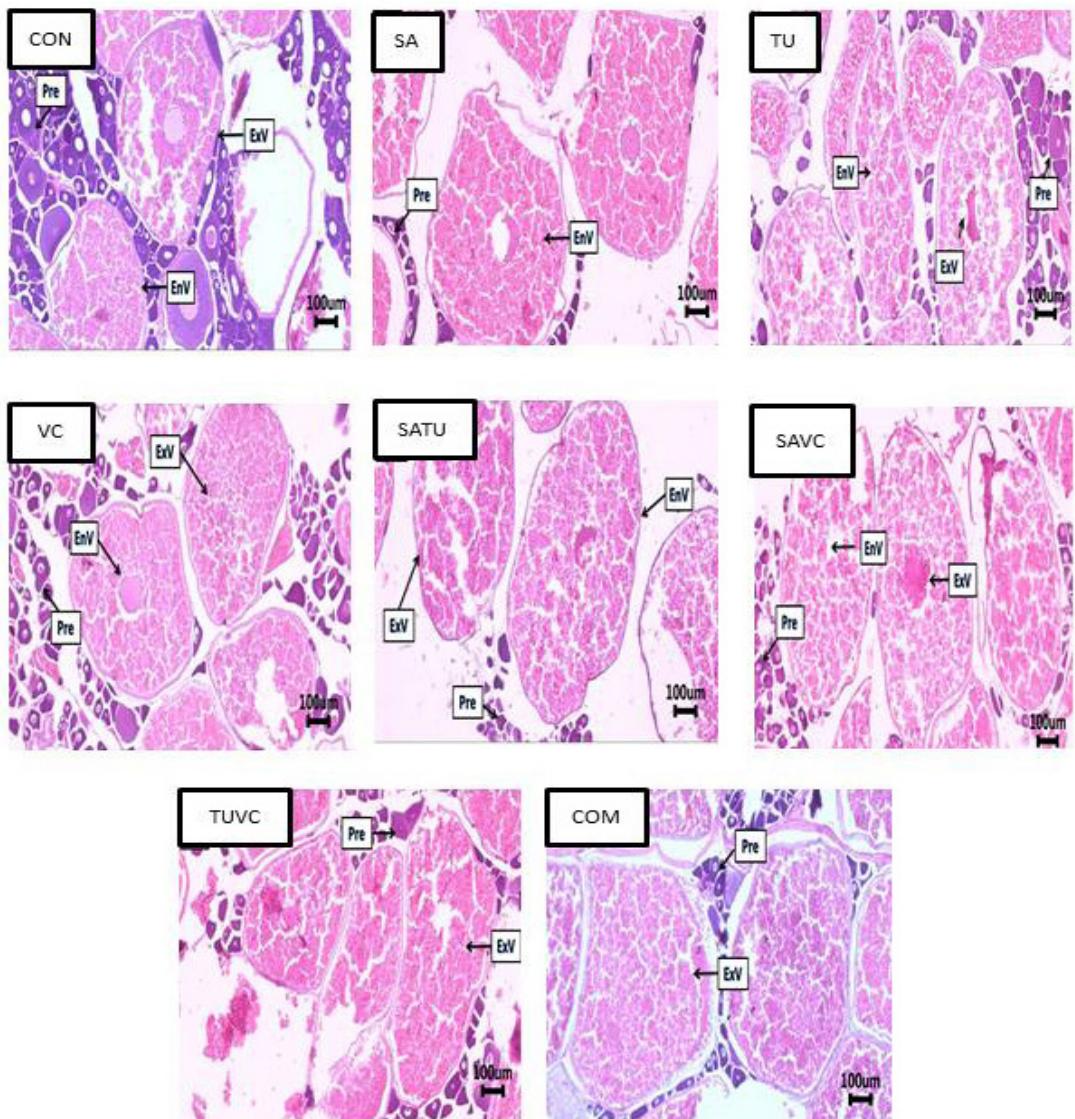


Figure 2. Histological examination of the broodstock ovary of the catfish, utilizing HE staining at 10X magnification. Description: CON: Control; SA: Catfish; TU: Turmeric; VC: Vitamin C; SATU: Catfish and Turmeric; SAVC: Catfish and Vitamin C; TUVC: Turmeric and Vitamin C; COM: Catfish, Turmeric, and Vitamin C; Pre: Previtellogenin; ExV: Exo vitellogenin; EnV: Endo vitellogenin.

antioxidant and immunomodulatory effects that can potentially impact fish reproduction (Abdelkhalek *et al.*, 2021; Dewi *et al.*, 2017; Kumari *et al.*, 2017; Li *et al.*, 2020; Manju *et al.*, 2012).

Studies indicate that the inclusion of vitamin C in striped catfish feed can enhance the GSI value. The rise in GSI value correlates with the development of oogenesis, indicating the progression and maturation of oocytes (Prayogo *et al.*, 2016). Vitamin C is essential for animal development and is involved in numerous metabolic processes. Striped catfish that receive supplemental vitamin C in their diet exhibit optimal oocyte development compared to the control group. The results presented in Figure 2 indicate that the treatment involving the addition of vitamin C has facilitated the development of oocytes, as evidenced by histological observations confirming that the

oocytes have attained the developmental stage of exogenous vitellogenesis. This stage represents the peak of oocyte development. The oocyte sizes across the treatments exhibit variation, with those receiving katuk extract, turmeric powder, and vitamin C being larger compared to the other treatment groups. Ovarian development in striped catfish involves multiple stages of oocyte development within a single ovary, indicative of asynchronous ovarian development (Kabir *et al.*, 2012). The development of these oocytes is influenced by vitellogenin levels in the parent, particularly in the treatment group supplemented with vitamin C, which rises towards the end of the rearing period. Vitellogenin is produced in the liver and subsequently transported to the ovaries during the initial phase of oogenesis (Tocher, 2003).

CONCLUSION

Supplementation of striped catfish extract, turmeric flour, and vitamin C in the feed of striped catfish broodstock can optimize gonadal development. Oocytes develop optimally towards final development, namely exogenous vitellogenesis.

ACKNOWLEDGMENT

Authors acknowledge the Ministry of Education, Culture, Research, and Technology and the Ministry of Finance for the 2021 Indonesian Education Scholarship (BPI) program with Contract Number 1054/J.5.2.3/BPI.06/10/2021. Additionally, appreciation is extended to the Ministry of Fisheries and Maritime Affairs KKP at the Fish Breeding Research Center in Sukamandi, Subang, West Java, where the research was conducted.

“The authors state no conflicts of interest with the parties involved in this study”.

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