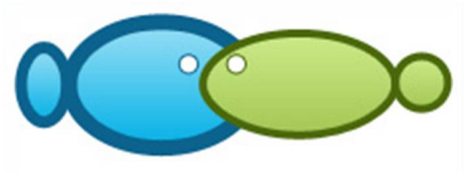

The reproduction performance of female Indonesian Javaen barb *Systomus orphoides* after dietary curcumin supplementation and PMSG hormone induction

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Abstract. One of the important phases in the reproductive cycle is the vitellogenesis process, which involves gonadotropin and steroid hormones. This study aimed to evaluate the effects of dietary curcumin and PMSG hormone induction on the reproductive performance of the Indonesian Javaen barb and was conducted at the Fisheries Field Laboratory in the College of Vocational Studies, IPB University. The experiments used a completely randomised design with 13 treatments and three replications with 10 fish in each treatment, namely KPA1-3 (curcumin 250 mg 100 g^{-1} feed with PMSG level 5, 10, and 15 IU kg^{-1} body weight), KPB1-3 (curcumin 500 mg 100 g^{-1} feed with PMSG level 5, 10, and 15 IU kg^{-1} body weight), KPC1-3 (curcumin 750 mg 100 g^{-1} feed with PMSG level 5, 10, and 15 IU kg^{-1} body weight), KPD1 (curcumin 1000 mg 100 g^{-1} feed with PMSG level 5, 10, and 15 IU kg^{-1} body weight), and the control (without curcumin supplementation and PMSG induction). The measured parameters included the hepatosomatic index (HSI), gonadosomatic index (GSI), vitellogenin and testosterone levels, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), malondialdehyde (MDA), and superoxide dismutase (SOD). All parameters were measured at weeks 0, 2nd, 4th, 6th and 8th week. The observation results showed that the KPC3 treatment (curcumin 750 mg 100 g^{-1} feed with PMSG level 15 IU kg^{-1}) can accelerate the process of gonad maturity better than the other treatments in parameter HSI and GSI. Moreover the KPC3 treatment can improve vitellogenin in blood plasma, and reduce the testosterone level. In addition, this treatment can also enhance liver performance and function, based on the increased SOD levels and decreased MDA levels, SGPT, and SGOT in female Indonesian Javaen barb fish.

Key Words: curcumin, fish reproduction, Indonesian Javaen barb, PMSG hormone, vitelogenesis.

Introduction. The Indonesian Javaen barb *Systomus orphoides* (Figure 1) culture development success is closely related to reproductive efficiency efforts to prepare mature broodstocks. This fish is highly favoured by the public as a consumption fish as well as an ornamental fish with economic value in Indonesia, especially in West Java. At present, the need for this fish is still fulfilled by natural catches. The size of the fish caught varies, and the fish are relatively young; if fishing is continuous, it can disrupt the sustainability of this fish population and eventually lead to extinction (Iskandar et al 2023). To address this, preventive measures must be taken by taming aquaculture tanks for breeding. In order to be cultured properly, Indonesian Javaen barb must be adapted to the cultivation environment because the conditions are different from the original habitat of this fish. Environmental signals that affect the reproductive physiology of fish in nature are different from those in culture containers. This is often an obstacle that disrupts the reproductive cycle, hampering the breeding process.

One of the important phases in the reproductive cycle is the vitellogenesis process involving gonadotropin and steroid hormones (Ho 1987). Farrell (2011) reported that vitellogenesis is a seasonal or cyclic process dependent on gonadotropins. Gonadotropin-releasing hormone (GnRH) production in the brain (hypothalamus) is mediated by a variety of endogenous and environmental factors including innate metabolism, nutritional status, and seasonal changes in day length and water temperature. In response to GnRH, pituitary gonadotrophs secrete follicle-stimulating hormone (FSH), which causes the theca and granulosa cells of ovarian follicles to secrete estradiol-17β (E2), directing the liver to synthesise Vtg cells and secrete them into the bloodstream. The anterior hypophysis secretes gonadotropin to accelerate theca cells and produce testosterone. Testosterone diffuses into granulosa cells and aromatized to estradiol-17β (Kagawa et al 1982). Estradiol-17β is transferred by the blood vessels to the liver for vitellogenin biosynthesis (Pelissero et al 1991; Peyon et al 1992; Mylonas & Zohar 2001; Devlin & Nagahama 2002). Vitellogenin is then transferred by blood vessels and internalized in the oocytes through micropinocytosis by a specific receptor, before being processed as a smaller yolk protein that will be used as feed reserve for embryos (Tyler et al 1991; Devlin & Nagahama 2002; Sun & Pankhurst 2004).

In female oviparous fish reproduction, vitellogenesis is an important process in oocyte development, reaching more than 95% of the initial size at the maximum size (Tyler & Sumpter 1996). Under estrogen control and regulation, liver has a role as the main organ that synthesizes and produces vitellogenin. During the vitellogenesis process, hepatocytes work continuously to fulfil the vitellogenin requirements of all developing oocytes and support the performance of most organs inside the body. This condition gradually causes oxidative stress that affects the physiological condition of the liver by producing vitellogenin, thereby decreasing the reproduction capability and broodstock productivity, followed by increasing lipid peroxidation and free radicals. Imbalanced oxidant and antioxidant levels cause cell disruption, which leads to liver damage (Zaetun et al 2017).

Figure 1. Indonesian Javaen barb *Systomus orphoides*.

To fix this condition, turmeric powder as a feed supplement can be utilized by mixing it in the feed. The supplementation of certain nutrients, such as nutrient materials (feed supplements) or non-nutrient materials (feed additives) in feed, can accelerate the maturation process and improve gonad quality (Carman et al 2023). The supplementation of turmeric powder can enhance liver tissue growth, as proven by the increased liver weight. In addition, the supplementation of turmeric powder can decrease malondialdehyde (MDA) in liver whereas it is an indicator of lipid peroxidation and secondary inhibition of enzymes in the liver. Furthermore, the turmeric powder is able to reduce serum glutamic pyruvic transaminase/serum glutamic oxaloacetic transaminase (SGPT/SGOT) in plasma, whereas these two enzymes is an indicator of decreased liver function (Gorman et al 2009; Bigoniya et al 2009; Saraswati 2015; Mizan et al 2018; Naseer 2019).

Fish reproduction is controlled by the hypothalamic-pituitary-gonad complex, which involves three factors, such as environmental signals, hormonal system, and reproductive organs. The environmental signal induces hormone secretion by the brain and pituitary, which combine various related organ activities in the reproductive system

and become an integrated biochemical-physiological response (Zohar et al 2016). In the culture media, the environmental signal becomes extinct, which causes physiological inhibition of the reproductive system. To overcome this problem, hormonal manipulation is necessary (Lam 1983). Specifically, the gonadotropin hormones in the reproductive process secreted by gonadotrophic cells of the pituitary gland are follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Balasch et al 2006).

Pregnant mare serum gonadotropin (PMSG) has been used in mammals to trigger superovulation and induce follicle production (Gosalvez et al 1994). Furthermore, PMSG application has also been performed in fish by Nagahama et al (1991) in the medaka *Oryzias latipes* at 100 IU mL^{-1} *in vitro*, which resulted in an improvement in estradiol-17β production in follicle layers. PMSG injection at 10-20 IU kg^{-1} fish dose could induce gonadal maturation of the shortfin eel *Anguilla bicolor bicolor* (Tomasoa et al 2015). Pamungkas et al (2019) induced the female striped catfish *Pangasianodon hypophthalmus* broodstock reproduction performance apart from the spawning season with 20 IU kg^{-1} PMSG. The 1.25 g kg^{-1} PMSG dose trial could increase the gonadosomatic index (GSI) and hepatosomatic index (HSI) values of female snakehead *Channa striata* broodstock by 3.35% and 1.37%, respectively (Hutagalung et al 2015), while the 1.5 mL kg^{-1} dose could accelerate gonadal maturation until the vitellogenesis phase and showed the best results compared to other treatments (Ath-thar et al 2017). The induction of PMSG has also been applied in *Tor soro* fish (Wahyuningsih 2012). Putra et al (2017b) reported that PMSG could induce the gonadal development of silver pomfret fish *Tracinotus blochii*. The current study has led to the examination of the combination of curcumin supplementation and PMSG hormone on liver function, with the goal of improving reproduction in the female of Indonesian Javaen barb *Systomus orphoides*.

Material and Method

Description of the study sites. The experiment was conducted from August to December 2021. The preparation, rearing, and treatment stages were performed in the field laboratory of fishery farms at the Study Program of Technology and Management of Applied Aquaculture, College of Vocational Studies, IPB University. The analysis of HSI, GSI, vitellogenin, and testosterone hormone concentrations, SGPT, SGOT in the plasma, MDA, and SOD in the liver tissues was conducted at the Primate Animal Study Center, IPB University.

Fish preparation and rearing. The fish samples used in this experiment were female Indonesian Javaen barb broodstock candidates originating from Garut (West Java, Indonesia). Fish were acclimatized and reared for 30 days in a 16 m^2 concrete tank before the treatment application. During acclimatization, the fish were fed a commercial diet (36-38% protein) twice daily in the morning and afternoon. Fish were selected based on their body weight (100-150 g $fish^{-1}$) and stocked in a 3 m^2 rearing tank equipped with aeration with 10 fish per tank. The water used for rearing was supplied from the well, and water exchange was performed daily by reducing the water volume by 30% in the rearing tank.

Experimental design. This study used a completely randomized experimental design with 13 treatments and three repetitions. The treatments were composed of KPA1 (curcumin 250 mg 100 g^{-1} feed + PMSG 5 IU kg^{-1} body weight), KPA2 (curcumin 250 mg 100 g^{-1} feed + PMSG 10 IU kg^{-1} body weight), KPA3 (curcumin 250 mg 100 g^{-1} feed + PMSG 15 IU kg^{-1} body weight), KPB1 (curcumin 500 mg 100 g^{-1} feed + PMSG 5 IU kg^{-1} body weight), KPB2 (curcumin 500 mg 100 g^{-1} feed + PMSG 10 IU kg^{-1} body weight), KPB3 (curcumin 500 mg 100 g^{-1} feed + PMSG 15 IU kg^{-1} body weight), KPC1 (curcumin 750 mg 100 g^{-1} feed + PMSG 5 IU kg^{-1} body weight), KPC2 (curcumin 750 mg 100 g^{-1} feed + PMSG 10 IU kg^{-1} body weight), KPC3 (curcumin 750 mg 100 g^{-1} feed + PMSG 15 IU kg^{-1} body weight), KPD1 (curcumin 1000 mg 100 g^{-1} feed + PMSG 5 IU kg^{-1} body weight), KPD2 (curcumin 750 mg 100 g^{-1} feed + PMSG 10 IU kg^{-1} body weight), KPD3

(curcumin 750 mg 100 g^{-1} feed + PMSG 15 IU kg^{-1} body weight), and control (without curcumin supplementation and PMSG induction).

Diet and feeding management. The diet provided for feeding was a special commercial feed for freshwater fish broodstock (36-38% protein) supplemented with curcumin following the applied treatments. The curcumin was obtained from a commercial product of Guangzhou Phytochem Sciences Inc., China. The treatment diet was mixed with curcumin by coating with chicken egg whites as a binder, whereas the control diet was only coated with chicken egg whites. The diet was produced following the method described by Arfah et al (2018a). Diets were produced daily, following the requirements. The diets were then fed to the fish twice a day (morning and afternoon) at 3% of body weight during the rearing period.

Hormonal injection. Before the hormonal injection, fish were anesthetized using MS222 at 0.4-1.2 g L^{-1} dose (Popovic et al 2012). The hormone was dissolved in a phosphate-buffered saline (PBS) and diluted following the treatment application. The diluted hormone was injected intramuscularly once a week or four times during the rearing period, following Putra et al (2017a). For control treatment, fish were injected with PBS solution.

HSI and GSI observation. Gonad sampling was performed at the initial period, before applying the treatment (week 0), on the 14^{th} (week 2), 28^{th} (week 4), 42^{nd} (week 6), and 56^{th} day (week 8) of rearing. Sampling was performed on three fish from each treatment. Fish were then surged to obtain liver and gonad organs for HSI and GSI calculations. The HSI was determined by comparing the liver weight to the fish's overall weight, and the GSI was calculated by comparing the gonad weight to body weight of the gonadally-mature broodstock.

Vitellogenin and testosterone concentrations. The vitellogenin concentrations on ovulated eggs were determined by an enzyme-linked immunosorbent assay (ELISA) method using the Cusabio CSB-E14116Fh-Grouper Vitellogenin VTG ELISA Kit. The testosterone concentration was analysed using the Cusabio CSB-E17554Fh Fish Testosterone (T) ELISA Kit. Both products are manufactured by Wuhan Huamei Biotech Co., LTD., China.

Liver performance. The SGPT and SGOT analyses used a spectrophotometry method (Reitman & Frankel 1957; Bigoniya et al 2009; Kasetti et al 2010). Blood and liver samples were taken from the fish at the final rearing period (3 fish $sampling^{-1}$). For MDA and SOD analyses, 3 g of liver tissue was minced under cold conditions. The liver MDA and SOD levels were measured using thiobarbituric acid reactive substances (TBARs) (Maggi et al 2002).

Statistical analysis. The HSI, GSI, vitellogenin, testosterone, plasma SGPT and SGOT, MDA, and SOD in liver tissues were tabulated in Microsoft Excel and analyzed using analysis of variance (ANOVA) in SPSS software 22.0. If there were any significant differences, the data were analyzed further using Duncan's test to determine the difference level among treatments at a 95% confidence level.

Results

Hepatosomatic index (HSI). The results showed that the HSI value tended to increase from week 0 to week 8 (Figure 2). At week 0, the highest HSI value was found in the KPD1 treatment and significantly different ($p < 0.05$) from the control, KPA1, and KPC2 treatments. At week 2, the results showed the highest HSI values on the KPC3 and KPD1 treatments, that were significantly different ($p < 0.05$) from the control and KPC1 treatments. At week 4, the highest HSI value was found in the KPC3 treatment and significantly different ($p < 0.05$) from the KPB1, control, KPC1, KPA1, KPB3, KPA2, KPB2, and KPD3 treatments. At week 6, the highest HSI value was found in the KPC3 treatment

but not significantly different from the other treatments and only significantly different ($p < 0.05$) from the KPA2 treatment. Furthermore, at week 8, the highest HSI values were found in the KPA2 and KPC3 treatments, and significantly different ($p < 0.05$) from the KPA3, KPA1, KPD1, control, and KPB2 treatments.

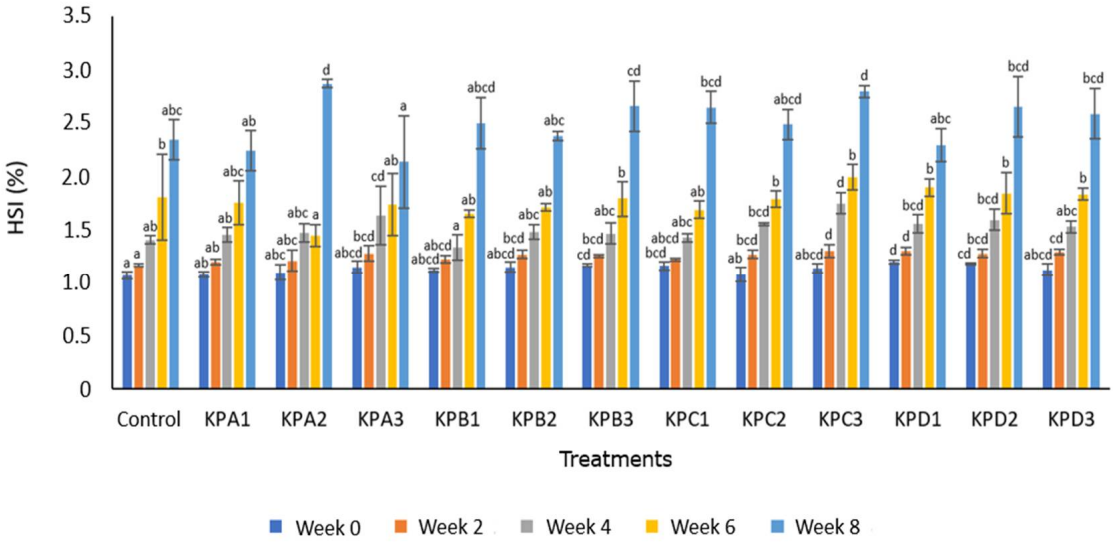

Figure 2. Hepatosomatic index (HSI) of female Indonesian Javaen barb *Systomus orphoides* values before and after treatment. Different letters above the standard deviation (SD) line in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviation.

Gonadosomatic index (GSI). The highest GSI value at week 0 was produced by the KPB2 treatment and the lowest was produced by the KPD2 treatment, but these treatments were insignificantly different ($p > 0.05$). In week 2, increased GSI value was found, as the KPA3 treatment produced the highest GSI value, and significantly different ($p < 0.05$) from the KPD2, KPB1, KPB2, KPB3, KPA1, and KPA2 treatments. In general, the KPA3 treatment was insignificantly different ($p > 0.05$), from the control treatments. However, at week 4, the highest GSI value was produced by the KPC2 treatment significantly different ($p > 0.05$) from the control treatment. At week 6, the GSI value was produced by the KPD3 treatment and was significantly different ($p < 0.05$) from the KPB1 and control treatments. At week 8, the highest GSI value was produced by the KPD2 and KPD3 treatments, and significantly different from other treatments ($p < 0.05$) (Figure 3).

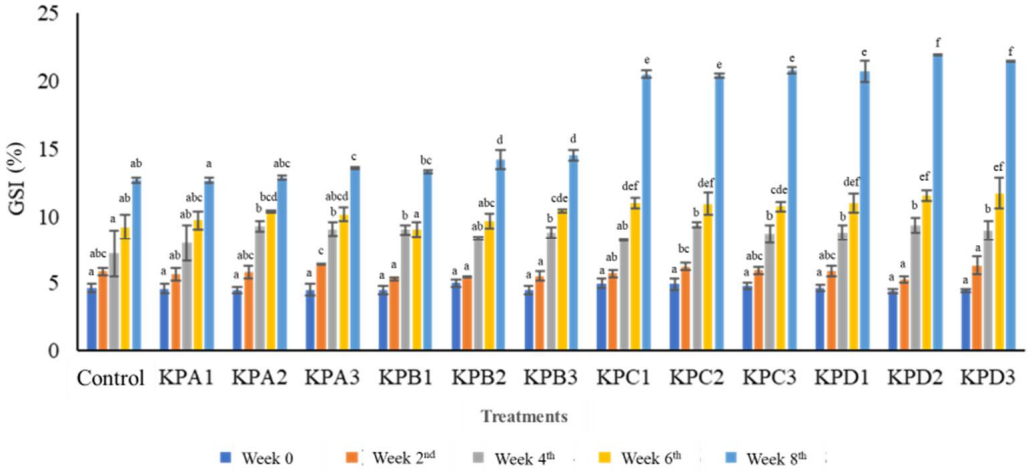

Figure 3. Gonadosomatic index (GSI) of female Indonesian Javaen barb *Systomus orphoides* values before and after treatment. Different letters above the standard deviation (SD) line in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean± standard deviation.

Vitellogenin concentration. The vitellogenin concentration at week 0 to 8 was increased (Figure 4). At week 0, the highest vitellogenin value was found in the KPD3 and KPC2 treatments and significantly different from the control treatment. At week 2, the highest vitellogenin value was found in the KPD2 treatment, followed by the KPB1, KPC3, KPB2, KPC2, KPB3, KPD3 treatments, yet no significant difference ($p > 0.05$) was found among these treatments, but only showing significant different ($p < 0.05$) with the control treatment. At week 4, the highest vitellogenin value was found in the KPD1 treatment and significantly different ($p < 0.05$) from the control treatment. At week 6, the highest vitellogenin value was found in the KPD1 treatment, followed by the KPD2, KPC3, KPC2 treatments, yet no significant different ($p > 0.05$) was obtained among these treatments, and only showing a significant different ($p < 0.05$) with the control treatment. Furthermore, the highest vitellogenin concentration was produced by the KPD3 treatment and significantly different ($p < 0.5$) from other treatments.

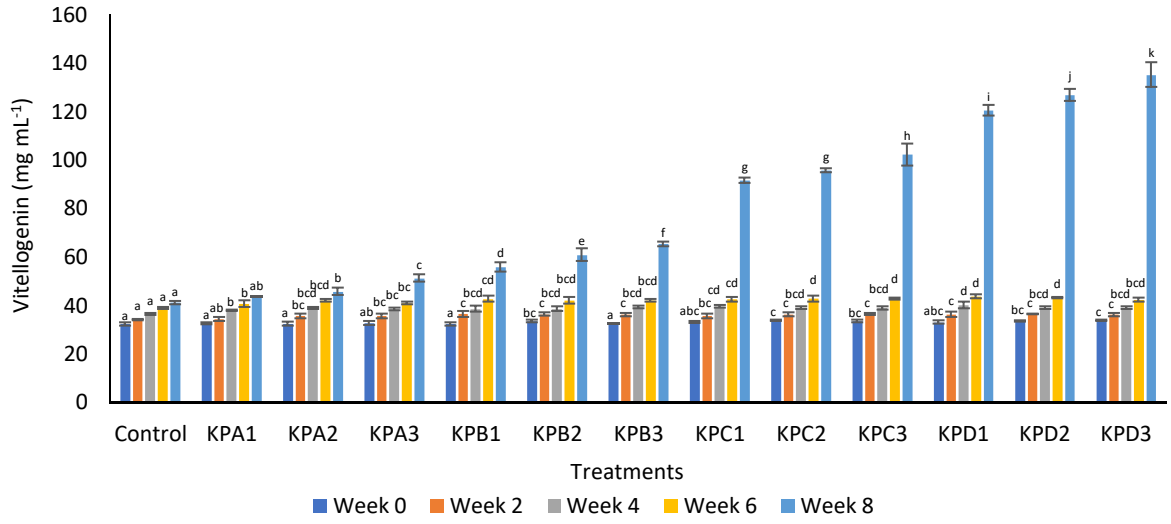

Figure 4. Vitellogenin concentration in fish blood plasma results of female Indonesian Javaen barb *Systomus orphoides* values before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean± standard deviation.

Testosterone concentration. The analysis revealed that the highest value of testosterone concentration at week 0 was found in the KPD3 treatment and significantly different ($p < 0.05$) from other treatments. In week 2, decreased testosterone concentration was occurred in each treatment. The highest testosterone value was found in the KPD3 treatment, but showing no significant difference ($p < 0.05$) with other treatments. At week 4, the highest testosterone concentration value was found in the KPB2 treatment and the lowest was found in the KPB1 treatment but showing no significant difference ($p > 0.05$) with other treatments. At week 6, the highest testosterone concentration value was found in the KPD1 treatment and significantly different ($p < 0.05$) from the KPC3 and KPB3 treatments. At week 8, decreased testosterone concentration was re-occurred in all treatments. The highest value of testosterone concentrations at week 8 was found in the KPD2 treatment, and the lowest level was found in the KPB1 treatment. At week 8, the value of testosterone concentration was in significantly different ($p > 0.05$) among the treatments (Figure 5).

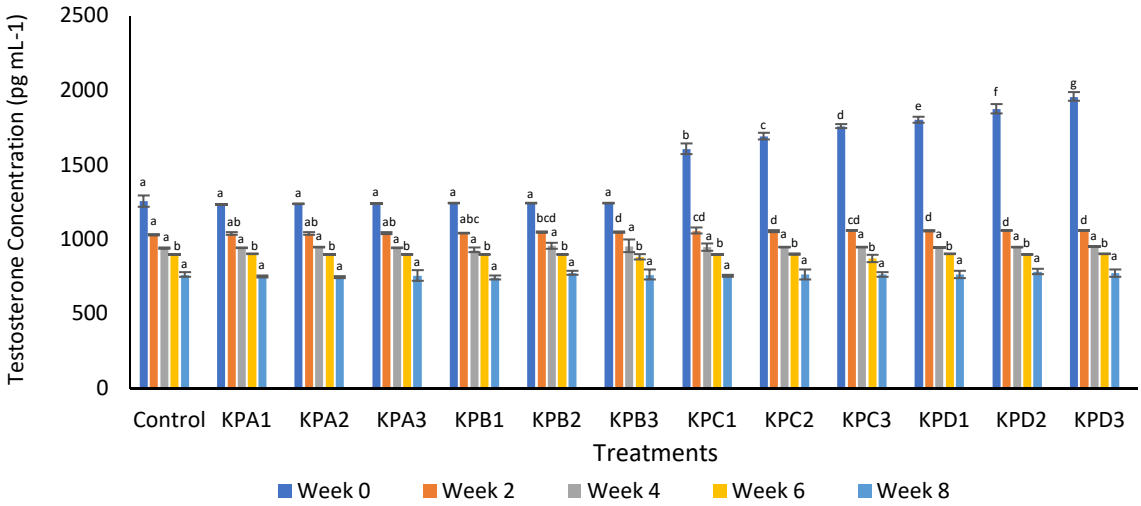

Figure 5. Testosterone changes in blood plasma of female Indonesian Javaen barb *Systomus orphoides* before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviation.

SGPT concentrations. The highest SGPT value in week 0 was produced by the control treatment and significantly distinct ($p < 0.05$) from other treatments. At week 2, the highest SGPT value was produced by the KPB2 treatment and the lowest was produced by the KPD1 treatment, whereas these treatments were significantly different ($p < 0.05$) from other treatments. At week 4, the highest SGPT value was produced by the control treatment and significantly different ($p < 0.05$) from the other treatments. The control, KPA3, and KPA2 treatments were in significantly different ($p > 0.05$). Furthermore, at week 6, the highest SGPT value was produced by KPC2 treatment and statistically significant ($p < 0.05$) from other treatments, while the SGPT value of KPC2 was insignificantly different ($p > 0.05$) with KPB3 treatment. At week 8, the highest SGPT value was produced by the control treatment and significantly different from the other treatments ($p < 0.05$) (Figure 6).

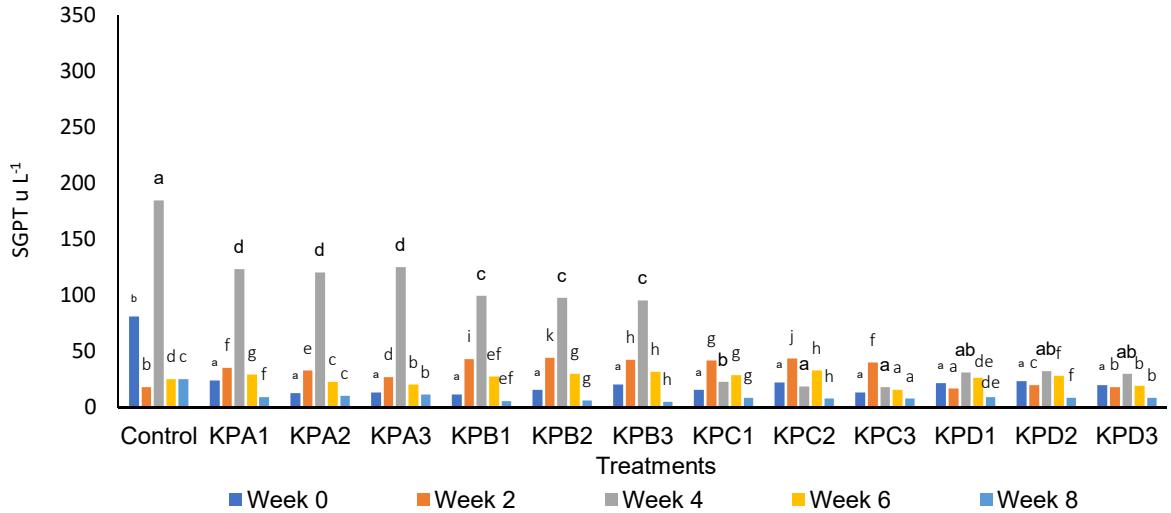

Figure 6. SGPT values of female Indonesian Javaen barb *Systomus orphoides* before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviation.

SGOT concentrations. Based on the observation result, the SGOT level in week 0 obtained the highest value in the KPB1 treatment, and the lowest SGOT value was produced by the KPC3 treatment ($p < 0.05$). At week 2, the highest SGOT value was produced by the KPA1 treatment and the lowest was found in the KPD1 treatment ($p <$

0.05). At week 4, decreased SGOT values where occurred, as the highest SGOT value was produced by the control treatment and the lowest was produced by the KPB1 treatment. These treatments were significantly different ($p < 0.05$) from other treatments. At week 6, the highest SGOT value was produced by the control treatment, and the lowest value was produced by the KPA1 treatment, whereas these treatments were significantly different ($p < 0.05$) from other treatments. Furthermore, at week 8, the highest SGOT value was produced by the control treatment, and the lowest value was produced by the KPC3 treatment. Both treatments were significantly different ($p < 0.05$). The KPA1, KPB3, KPA2, KPB1, and KPB2 treatments were insignificantly different ($p > 0.05$) (Figure 7).

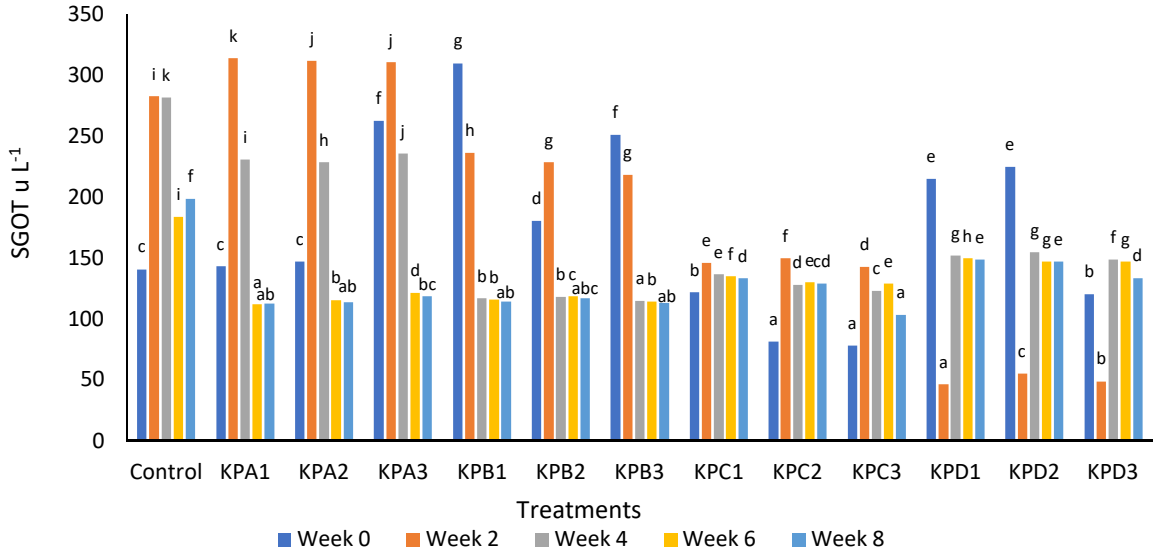

Figure 7. SGOT concentration of female Indonesian Javaen barb *Systomus orphoides* before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviation.

MDA concentrations. The MDA concentration tended to decrease from week 0 to 8. At week 0, the higher MDA concentration was produced by the KPA1 and KPD1 treatments. Both treatments were significantly different ($p < 0.05$) from KPC3 and KPD3 treatments, while the other treatments were insignificantly different ($p > 0.05$) from the following treatments. At week 2, the highest MDA concentration were produced by KPB3 and KPC1 treatments, but insignificantly different ($p > 0.05$) from KPB1, KPC2, KPD3, KPA2, KPB2, KPA1, control, KPC3, and KPD2 treatments, and significantly different ($p < 0.05$) from KPD1 KPA3 treatments. At week 4, the highest MDA concentration was produced by KPB1 treatment and significantly different ($p < 0.05$) from KPA2, KPC1, control, KPD1, KPD3, and KPA3 treatments. At week 6, the highest MDA concentration was produced by the KPB1 treatment, and lowest MDA concentration was produced by the KPB3 treatment, but not significantly different ($p > 0.05$) from other treatments. At week 8, the highest MDA concentration was produced by the KPA3 treatment, but insignificantly different from the control, KPB3, KPC1, KPA2, KPC3, KPA1, KPC2, and KPD3 treatments. These values were significantly different ($p < 0.05$) from the KPD1, KPB1, and KPB2 treatments (Figure 8).

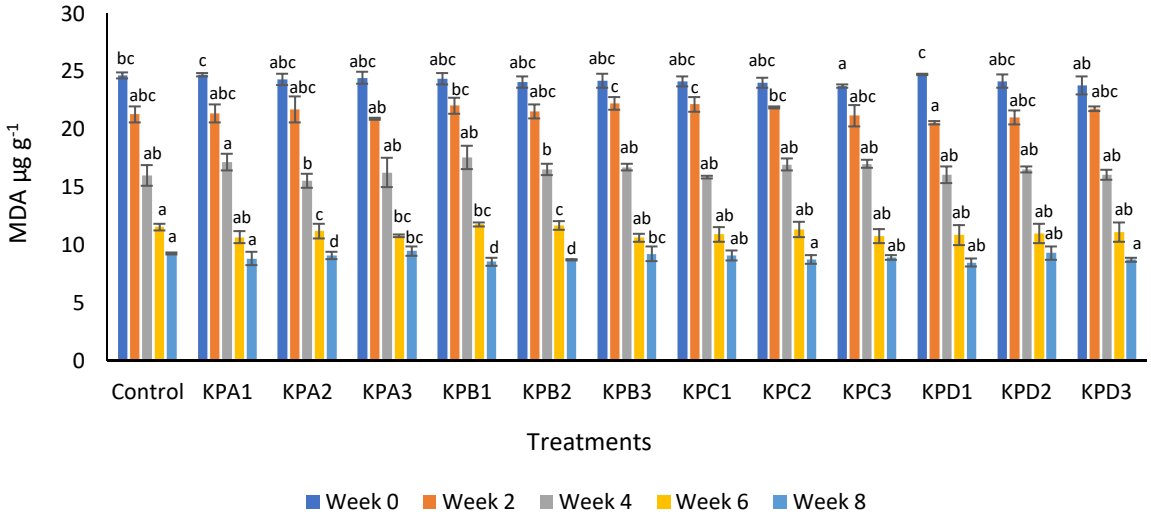

Figure 8. MDA value results of female Indonesian Javaen barb *Systomus orphoides* before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviations.

SOD concentrations. At week 0, the highest SOD value was produced by the KPB2 treatment, and the lowest value was produced by the KPA1 treatment. Both treatments were significantly different ($p < 0.05$), while KPB2 showed no significant difference ($p > 0.05$) with other treatments. At week 2, the higher SOD value was produced by the KPC1 treatment and was insignificantly different ($p > 0.05$) from the KPD3, KPC2, KPD2, KPB3, KPD1, and KPC3 treatments. These values were significantly different ($p < 0.05$) from KPA1, control, KPB1, KPA3, KPA2, and KPB2 treatments. Furthermore, at week 4, the highest SOD values were produced by KPB2 and KPA2 treatments which were significantly different ($p < 0.05$) from the KPA1 treatment, but showing no significant difference ($p > 0.05$) with other treatments. At week 6, the highest SOD values were produced by KPB2 and KPA2 treatments, but insignificantly different ($p > 0.05$) from KPB1 and KPA3 treatments. However, the KPB2 and KPA2 treatments were significantly different ($p < 0.05$) from other treatments. At week 8, the highest SOD value was produced by the KPA2 and KPB2 treatments, but insignificantly different ($p > 0.05$) from the KPB1 treatment, but showing a significant difference ($p < 0.05$) with other treatments (Figure 9).

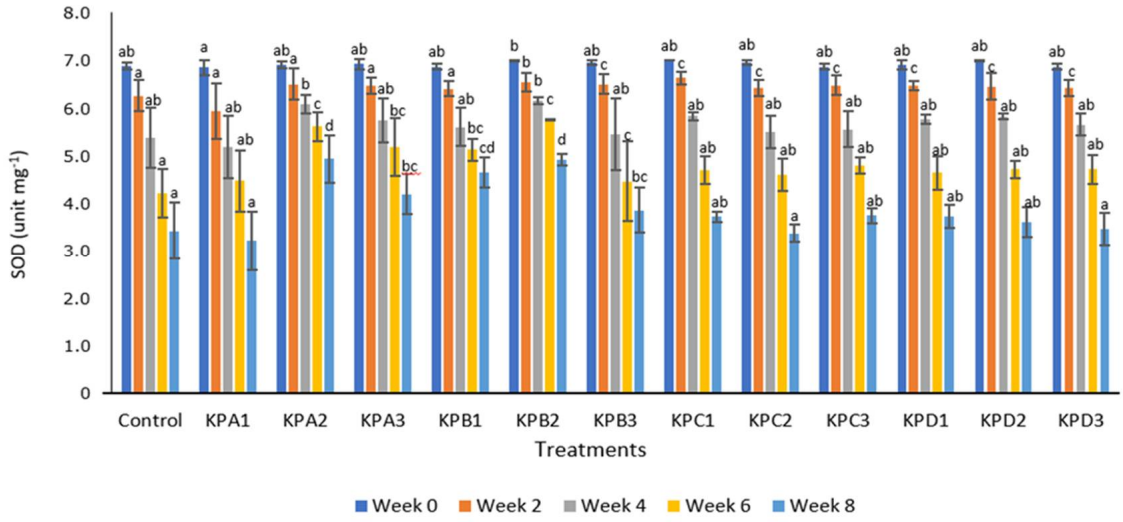

Figure 9. SOD value results of female Indonesian Javaen barb *Systomus orphoides* before and after treatment. Different superscript letters in the same observation period indicate significant differences ($p < 0.05$). The values shown are mean±standard deviation.

Discussion. The HSI tended to increase from week 0 to week 8. The highest HSI values were produced in the KPA2 and KPC3 treatments, that were significantly different ($p < 0.05$) from the KPA3, KPA1, KPD1 (2.29%), control (2.34%), and KPB2 (2.38%) treatments (Figure 2). Increased HSI value was occurred due to the vitellogenin synthesis in the liver. The HSI value shows the ratio of the total weight and liver weight of female Javaen barb fish broodstock after the treatment application that affects the vitellogenesis process. Increased HSI value was occurred to the vitellogenin synthesis and secretion (Hara et al 2016). Dietary curcumin supplementation combined with PMSG hormone induction resulted in major changes in the plasma vitellogenin level (Gunadi et al 2021). Matzkin et al (2021) mentioned that curcumin has a function as an antioxidant and plays a role in body cells repairment, including the liver and oviduct, while PMSG contains FSH hormone, as an instrumental part in the process of vitellogenesis and gonadal maturation (Gallego et al 2012; Brzuska 2021).

Increased HSI value with dietary phytoestrogen supplementation, namely curcumin, showed significantly different results compared to the control treatment in striped catfish *Pangasianodon hypophthalmus* (Arfah et al 2018a; Arfah et al 2018b; Dewi et al 2018), red tilapia *Oreochromis* sp. (Mainassy et al 2022). When the vitellogenesis process takes place in the liver, increased HIS value was occurred at week 8^{th}. Damsteegt et al (2020) mentioned that the HSI is an indicator of vitellogenesis emergence in the liver with the induction of estradiol 17β as a stimulator of yolk formation. In some cases, HSI is not significant compared to the control, as the synthesis is stimulated from outside (exogenous hormone), that takes place very quickly (Sinurat et al 2009; Lawhavinit et al 2011; Pereira et al 2020). The percentage value of HSI depends on the body weight of the fish and the weight of the liver, as liver is allegedly carrying out the estradiol 17β steroids synthesis by blood through the liver, that will be converted into vitellogenin and increase the vitellogenin in blood to fulfill the oocytes in the female gonads of Indonesian Javaen barb fish (Dewi et al 2018; Rawung & Saruan 2020; Rawung et al 2021). The distribution pattern of increasing HSI values will be directly proportional to the GSI value, so the HSI value in broodstock fish will increase toward vitellogenesis (Hutagalung et al 2015). This condition also indicates that the HSI value parameter can be used to observe the reproductive process in female Indonesian Javaen barb fish.

The GSI is a value that describes quantitative changes in the gonads during gonadal development in the reproductive process, that will reach a maximum value when spawning occurs (Parker et al 2018). Increased GSI value indicates the oocyte growth, which has an impact on increasing the body weight. In this study, the highest GSI value was produced by the KPD2 and KPD3 treatments, that were significantly different ($p < 0.05$) from the other treatments (Figure 3). Increase GSI value indicates the oocyte development process, which causes the ovaries enlargement in female Indonesian Javaen barb fish broodstock. Similar result were also obtained in Zahri et al (2015), Putra & Razai (2020), and Iskandar et al (2023).

The dietary supplementation of curcumin could stimulate the liver to produce vitellogenin, as the main product in the vitellogenesis process (Awaludin et al 2021; Syamsuryadi et al 2021; Awaludin et al 2023). According to Riley et al (2004) and Sinjal et al (2014), increased estradiol-17β hormone concentration in blood will spur the liver to carry out the vitellogenesis process and accelerate the gonadal maturation process. Increased vitellogenin synthesis activity in the liver will also increase the GSI value of fish (Haque et al 2023). This process is strongly influenced by estradiol as a stimulator in vitellogenin biosynthesis (Dahlia et al 2023). Matozzo et al (2008) and Sullivan & Yilmaz (2018), stated that vitelogenin is a yolk precursor protein, synthesized in the liver under the control of the estrogen hormone, and secreted into the bloodstream. Similar theory was also mentioned by Jones et al (2000) and Hara et al (2016). The results showed that the vitellogenin value female Indonesian Javaen barb fish broodstock at week 0 to 8 increased. At week 0, the higher vitellogenin value was found in the KPD3 treatment (33.74 mg mL^{-1}), and significantly different from the control treatment (32.26 mg mL^{-1}). The highest vitellogenin value at week 8 was produced in the KPD3 treatment (135.01 mg mL^{-1}) and significantly different from the other treatments. Based on observations,

increased vitellogenin in blood plasma was emerged along with the increase estradiol concentration (Figure 4). Pamungkas et al (2019) found the same condition in striped catfish *P. hypopthalmus* induced with PMSG hormone, which was thought due to the stimulation of estradiol hormone that caused plasma vitellogenin concentration increase. Furthermore, Amaral et al (2019) mentioned that estradiol plays a role in inducing the vitellogenin synthesis in the liver, then vitelogenin secretion is distributed through the bloodstream as compounds with Ca^{2+} (Sullivan & Yilmaz 2018). Suwarta & Suryani (2019) reported that curcumin as a phytoestrogen could affect reproductive ability and improve the performance and egg quality of Japanese quail *Coturnix coturnix japonica*. Phytoestrogens induce vitelogenin synthesis in hepatocytes, resulting in an increased vitelogenin deposition in the yolk (Levi et al 2009).

Based on the testosterone concentration result (Figure 5) the dietary curcumin supplementation and PMSG hormone increased in the early weeks of treatment, then decreased at the end of treatment. This indicates that the gonadal maturation process in the treatment occurred earlier than the control treatment. This condition is thought that PMSG hormone induction plays a role in early gonadal maturation and vitellogenesis, as this hormone contains high follicle-stimulating hormone (Gallego et al 2012; Hutagalung et al 2015; Brzuska 2021; Gustiano et al 2020). PMSG can stimulate the formation of follicles, growth of interstitial cells, and formation of luteal cells. It is generally agreed that PMSG hormones contain high FSH concentration and low LH concentration. The FSH will later help process the egg formation in fish. The use of PMSG hormones in fish has been widely reported to increase and accelerate the gonad maturity of striped catfish *P. hypopthalmus* (Tahapari et al 2014; Dewi et al 2018; Arfah et al 2018a; Pamungkas et al 2019), snakehead fish *Channa striata* (Hutagalung et al 2015), eel *A. bicolor bicolor* (Tomasoa et al 2015), silver pompano *Trachinotus blochii* (Putra & Razai 2020).

Figures 6 and 7 present the SGPT and SGOT levels of female Indonesian Javaen barb fish, after eight weeks of treatment. The results indicate that the treatments influenced the SGPT and SGOT values. SGPT and SGOT values tend to fluctuate at the beginning of the treatment (weeks 2 to 6), but at the end of the study, both values tend to decrease. Kumar et al (2021) mentioned that the concentration of SGPT and SGOT enzymes are parameters that indicate liver function decline. The SGPT value is a specific parameter in liver damage, while SGOT value is not specific as a parameter of liver damage, because it is also present in myocardial infarction, muscle necrosis, kidney, brain, and intravascular hemolysis (Vasudevan et al 2013; Rolfes et al 2014). In this study, curcumin dietary supplementation treatment could prevent the increased SGPT and SGOT significantly ($p < 0.05$), compared to the control group. Farzaei et al (2018) mentioned that curcumin is known to help improve the liver function, by accelerating the liver cell regeneration and protecting the liver from the influence of toxic substances that can damage the liver. Curcumin has antioxidant (Nisha & Anbu 2017), anti-inflammatory, antiviral, anti-protozoal, antifungal and hepatoprotector (Tajodini et al 2015) and antibacterial (Shome et al 2016) activities. This antioxidant activity is allegedly related to the hepatoprotective activity of curcumin. Reactive free radicals cause cell damage through two main mechanisms, namely covalent bonding and lipid peroxidation. The lipid peroxidation process has been shown to promote collagen synthesis and fibrosis; therefore antioxidants may play a role in inhibiting liver cell damage (Ezhilarasan 2018).

The MDA value in female Indonesian Javaen barb fish broodstock fed with curcumin supplemented diet showed a lower value than the control treatment (Figure 8). The same result was also reported by Dewi et al (2018) and Arfah et al (2018a), that the dietary supplementation of turmeric powder could produce low MDA values in striped catfish *P. hypopthalmus*, female carp *Cyprinus carpio* (Rawung & Saruan 2020) and in red tilapia (Mainassy et al 2022). Likewise, dietary turmeric supplementation in Siagian et al (2021) on catfish *Clarias gariepinus* could reduce MDA values due to antioxidant compounds that can increase immune response and antioxidant activity in the body (Geier et al 2002; Tung et al 2019). Furthermore, Rahmadani et al (2020) mentioned that decreased MDA value has a relationship with antioxidant enzymes in counteracting ROS (reactive oxygen species), namely SOD. ROS is a high reactive molecule to lipid membranes, proteins, and DNA (El-Beltagi & Mohamed 2013). The effect of curcumin

supplementation as an antioxidant on the performance of female Indonesian Javaen barb fish liver is thought to encourage the low value of MDA concentration as a result of free radical inhibition by curcumin. Harmeet et al (2011) mentioned that curcumin analogs as antioxidants will donate hydrogen atoms to superoxide anions, which causes this reaction to produce more stable compounds and inhibit the peroxidation of polyunsaturated fatty acids in cell membranes, thereby reducing the liver MDA concentrations.

SOD is an endogenous antioxidant produced by the liver to neutralize oxidant compounds in cells (Mainassy et al 2022). The results showed that dietary curcumin supplementation could increase the SOD enzyme in the liver of female Indonesian Javaen barb fish (Figure 9). This condition was in line with Sadeghi & Moghaddam (2018), who stated turmeric supplementation in broiler chickens could increase the SOD production. Likewise, Mainassy et al (2022) found that supplementation of curcumin analogs to improve the liver performance and support the reproductive performance of red tilapia could also increase the SOD concentration. According to Zhou et al (2014), curcumin is a compound to induce translocation of the NRF2 nucleus and increase the expression of several ROS detoxification and antioxidant genes in hepatocytes to produce more SOD enzymes. This statement was reaffirmed by Iqbal et al (2003), who stated that dietary curcumin supplementation could increase cellular antioxidant status, such as glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and catalase, which is also accompanied by an increase in phase II metabolic enzymes, namely glutathione s-transferase and quinone reductase in the liver and kidneys, thus reducing ROS activity (Sharma et al 2005).

Conclusions. The dietary curcumin supplementation combined with PMSG hormonal induction in the KPC3 treatment is the best treatment, that can greatly accelerate the gonad maturity process and improve the vitellogenesis process in female Indonesian javaen barb fish. Also, the dietary curcumin supplementation can improve liver performance and prevent liver damage, based on the increased of vitellogenin value, SOD and decreased liver MDA, SGPT, and SGOT levels in female Indonesian javaen barb fish.

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