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Biochemical study of striped catfish *Pangasianodon hypophthalmus* broodstock induced by PMSG hormone + antidopamine and turmeric addition

Kajian biokimia induk ikan patin yang diberi hormon PMSG dan antidopamin ditambah kunyit

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ABSTRACT

This study aimed to evaluate biochemical changes (cholesterol, triglyceride, HDL, LDL, glucose, and plasma protein) on striped catfish *Pangasianodon hypophthalmus* broodstock induced with PMSG hormone and turmeric addition. An observation was also done to blood glycogen content. The striped catfish broodstock was fed on commercial feed without any addition (control) and with turmeric addition (HKu). In control treatment, there was a decreasing on cholesterol, meanwhile, the triglyceride (TG) value was increased. The HDL concentration was decreased in 2nd sampling and increased in 4th sampling. In 1st until 4th sampling, glucose was quite stable, while LDL was on extremely low concentration. In HKu treatment, the cholesterol value was higher than the control treatment. The TG concentration also higher than control in 3rd sampling and decreased in 4th sampling. The HDL concentration was increased and higher than the control treatment, while LDL concentration was lower. The liver glycogen content on the control and HKu treatment were 0.015 (mg/100 mL) and 0.181 (mg/100 mL) respectively; while in the flesh of the control and HKu treatment were 0.76 (mg/100 mL) and 1.19 (mg/100 mL) respectively; and in the gonad of control and HKu treatment were 0.10 (mg/100 mL) and 0.70 (mg/100 mL) respectively. It was shown that the glycogen content in the liver, flesh, and gonad on experimental fish was higher than control treatment.

Keywords : biochemistry, hormone, turmeric, channel catfish, reproduction

ABSTRAK

Penelitian ini bertujuan untuk mengaji perubahan biokimia (kolesterol, trigliserida, HDL, LDL, glukosa dan protein plasma) pada induk ikan patin Pangasianodon hypophthalmus yang diberi perlakuan hormon PMSG dan kunyit. Pengamatan juga dilakukan terhadap glikogen dalam darah induk patin. Induk ikan patin diberi pakan tanpa penambahan kunyit (kontrol) dan pakan yang diberi kunyit (HKu). Hasil penelitian pada kontrol menunjukkan adanya penurunan kolesterol, sedangkan pada trigliserida (TG) mengalami peningkatan. HDL menurun pada sampling ke−2 dan meningkat pada sampling ke−4. Sementara itu, pada LDL rendah sekali dan pada glukosa terlihat stabil dari sampling ke−1 sampai ke−4. Pada perlakuan HKu terlihat bahwa pada kolesterol menghasilkan nilai lebih tinggi dibanding kontrol. Pada TG terlihat juga nilai lebih tinggi dibanding kontrol pada sampling ke−3 dan menurun pada sampling ke−4. Konsentrasi HDL meningkat dan lebih tinggi dibanding kontrol, sedangkan nilai LDL lebih rendah. Data yang diperoleh pada kadar glikogen hati perlakuan kontrol adalah 0,015 (mg/100 mL) dan HKu 0,181 (mg/100 mL); sedangkan pada daging kontrol sebesar 0,76 (mg/100 mL) dan HKu 1,19 (mg/100 mL);); serta gonad kontrol 0,10 (mg/100 mL) dan HKu 0,70 (mg/100 mL). Hal ini menunjukkan kadar glikogen pada hati, daging, dan gonad ikan yang diberi perlakuan bernilai lebih tinggi dibanding kontrol.

Kata kunci: biokimia, hormon, kunyit, ikan patin, reproduksi

INTRODUCTION

Striped catfish *Pangasianodon hypophthalmus* is one of the farmed freshwater species in Indonesia. The rapid development of striped catfish aquaculture tends to increase the production target. This issue has to be followed by an adequate seeds supply. According to histology analysis, the development of oocyte in striped catfish takes approximately 18 weeks in a culture tank with sufficient feed. Kabir *et al.* (2012) described a beneficial information about the oocyte development and biological reproduction in striped catfish.

According to Forlano (2007) and Phodorec *et al.* (2016), dopamine play an essential role in gonadal maturation. An inhibitor compound, dopamine, causes signal inhibition in secretion of pituitary gonadotropin (Sudrajat *et al*., 2014). PMSG (pregnant mare serum gonadotropin) hormone utilization together with anti-dopamine (domperidone and pimozide) is more effective to induce gonadal maturation in various fish species, such as common carp and catfish (Sharaf, 2011), Redfin (Islami *et al*., 2017), and eel (Ahlina *et al*., 2017). Nugraha (2014) stated that supplmentation of PMSG (0.25 mg/kg) plus anti-dopamine (0.01 mg/kg) through feed was able to increase gonadal maturation in striped catfish.

Turmeric has a high content of phytoestrogen and it is acknowledged to support liver function by accelerating liver cell regeneration and protect it from any toxic (Hwa-Young *et al*., 2016). Turmeric is also able to improve the performance of digestion organ (Mahmoud *et al*., 2014; Thongon *et al*., 2017). Polipoch *et al.* (2014) stated that curcumin is a hepatoprotector compound so that it will protect liver cell structure. Turmeric flour is a curcumin compound which also acts as an antioxidant and plays a role to repair body tissues, such as liver and oviduct (Alinezhad, 2017; Kyung *et al*., 2018). Feed enrichment with turmeric flour is effective to induce gonadal maturation, particularly outside the spawning season (Tahapari & Dewi, 2015).

Cholesterol is a compound that directly related to reproduction and gonadal maturation (Hiramatsu *et al.* 2015; Shankar & Kulkarni, 2007). Cholesterol is a precursor for various types of steroid hormone (Pattisiana *et al*., 2010). Basically, cholesterol comes from lipid absorption inside the digestive tract and fatty acid metabolism in the fish liver (Di Marco *et al*., 2008). Cholesterol is divided into low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL is considered as unhealthy cholesterol because it will cling on a blood vessel, while HDL is considered as harmless cholesterol because it takes back the cholesterol to the liver to be processed.

Triglycerides are one of blood lipid in various body organ and it is used during the reproduction phase (Wahyuningsih *et al*., 2012). According to Mukhopadhyay and Ghosh (2007), the triglyceride is a major energy source in the fish body, both in liver and yolk-sac. Various concentration of triglycerides can be used as an indicator of the reproduction phase (Kocaman *et al*., 2005). The concentration of triglycerides shows lipid flow from the liver to fulfill a massive energy requirement during vitellogenesis process (Hachero−Cruzado *et al*., 2007).

Blood glucose is one of the essential carbohydrates as the main energy source to assist cell metabolism (Putri *et al.,* 2013). The blood glucose concentration depend on several factors, such as age, size, nutrition requirement, and reproduction (Yousefian *et al.,* 2010). Frequently, glucose is required continuously and has to be available in adequate concentrationin protein plasma. In the reproduction process, the protein plasma content depend on nutrition supply, so that both hormone and turmeric was used in this study to maintain the protein plasma.

The protein plasma concentration is related to vitellogenesis process. When spawning occurred, the protein plasma concentration will decrease (Saxena, 2002). Glycogen is a glucose deposit in most of the fish organ, especially hepatopancreas, flesh, or muscle (Akbar *et al*., 2011). Glycogen is stored as glucose inventory and act as high-energy phosphate manufacturer (Effendi, 2016).

Information on biochemistry during fish maturation is highly required in fish reproduction study, notably that diversity affected by species, age, maturation, and broodstock condition. Therefore, in this study, various biochemistry parameters were examined, such as glucose concentration, cholesterol, triglyceride, HDL, LDL, and protein plasma. This study addressed to find biochemistry changes in striped catfish that fed using enhanced feed (PMSG, anti-dopamine, and turmeric).

MATERIALS AND METHODS

Experimental design

This study was conducted during low rainfall and dry season. The temperature was ranged from 31.4−34.9°C and pH 6.47. This study was

designed using two treatments, i.e. control (K) and HKu treatmens (PMSG + anti-dopamine treatments and added with turmeric). The PMSG, anti-dopamine, and turmeric were added through the commercial feed with 30% of protein content. The experimental hormone premix was PMSG and anti-dopamine in dosage

0.25 mL and 0.1 mg per kg, while turmeric flour was in dosage of 480 mg/100 g. The commercial feed was weighed based on 3% of feeding rate. As many of 700 mL of egg-white was mixed with 200 mL of water. The turmeric flour was blended with 0.25 mL/kg of PMSG and 0.1 mL of anti-dopamine. The mixture was then mixed together with egg-white. Furthermore, the mixture was added to the experimental feed and mixed up well. Lastly, the experimental feed was dried in temperature of 40°C for 24 hours. Finally, when the experimental feed was ready, those were put in a plastic container to keep it dry.

The experimental fish were fed twice a day using a feeding rate of 3%, and sampling was conducted every two weeks. As many of 30 broodstocks were treated using the treatment with stocking density 1 fish/m^2 for three months. The experimental broodstock in this study has spawned before and has no eggs. The broodstocks were reared in the earthen pond sized in 10×20×0.7 m^3 and each treatment was separated using a net.

Biochemistry observation

Blood chemistry analysis

Glucose analysis was conducted using glucose liquicolor GOD-PAP kit. Cholesterol and lipoprotein (LDL and HDL) analysis were performed using the CHOD-PAP method. Triglyceride was analyzed using enzymatic colorimetric method through CHPA-PAP commercial kit.

Data analysis

Blood biochemistry data was analyzed using least significant difference (LST test) if significant difference was detected. Chemical analysis was done descriptively. Data administration was conducted using Ms. Word 2007 and Minitab 16.

RESULTS AND DISCUSSION

Result

Biochemistry analysis

The blood chemistry parameters were cholesterol, triglyceride (TG), HDL, LDL, glucose, and total protein (Figure 1, 2 and 3). The cholesterol in control K treatment decreased straightly until 4th sampling (284.14–180.73 mg/dL), while in TG increased until 3rd sampling (473.77 mg/dL) and decreased on 4th sampling. The HDL concentration decreased from 1st sampling (147.16 mg/dL) until 4th sampling (94.42 mg/dL). Unlike HDL, the LDL kept increasing from 1st until 4th sampling. The blood glucose content decreased from 1st sampling to 4th sampling.

Figure 2 described blood chemistry in striped catfish broodstock in HKu treatment. The cholesterol was quite stable from 1st sampling to 4th sampling (248.48–282.48 mg/dL). The triglyceride was relatively high on the 1st sampling (523.55 mg/dL), then decreased on the 2nd sampling and increased again on the 3rd sampling until 4th sampling (307.77 mg/dL). The

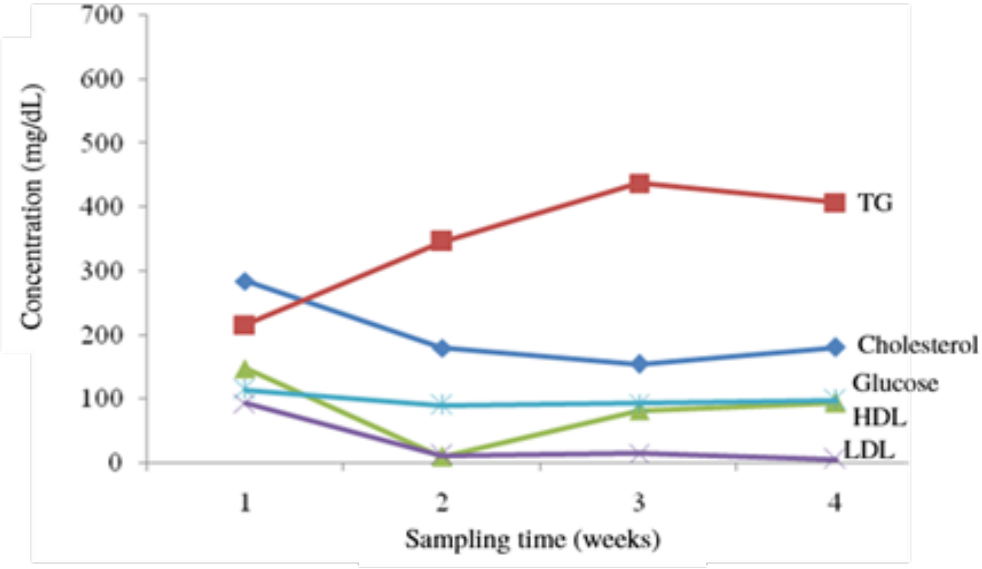

Figure 1. Blood chemistry of striped catfish broodstock in K treatment

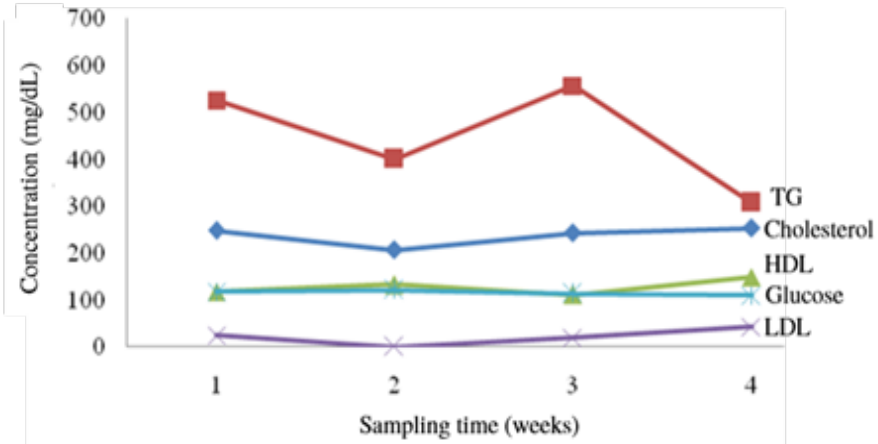

Figure 2. Blood chemistry of striped catfish broodstock treated with PMSG + anti-dopamine and added with turmeric through the diet.

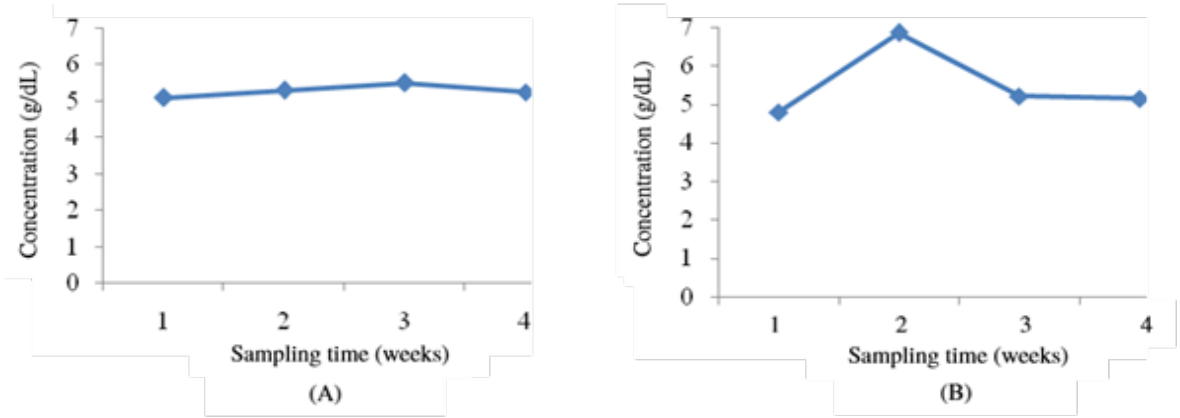

Figure 3. Total blood protein of striped catfish broodstock in control K (A), and HKu (B) treatments. HKu: fish treated with PMSG + anti-dopamine and added with turmeric through the diet.

Table 1. Glycogen content in striped catfish broodstock liver

Sample code	Water content (%)	Glycogen (mg/100 mL)
Liver		
Control (K)	79.918	0.015
Treatment (HKu)	78.078	0.181
Flesh		
Control (K)	72.39	0.76
Treatment (HKu)	74.12	1.19
Gonad		
Control (K)	80.24	0.10
Treatment (HKu)	75.79	0.70

HDL seemed to be stable on the 1st (117.707 mg/dL), but it increased on the 4th sampling (148.83 mg/dL). The LDL tended to decrease until 2nd sampling, then it started to increase on the 3rd and 4th sampling (42.55 mg/dL). On the contrary, the blood glucose showed stable result through the experiment.

In the control treatment, the total blood protein in striped catfish broodstock remained stable on the 1st sampling until 4th sampling. In the HKu treatment, the total blood protein increased on the 2nd sampling, but it went back to stable on the 3rd sampling and 4th sampling.

Liver glycogen, flesh, and gonad

The liver glycogen content is presented in Table 1. In the K treatment, the liver glycogen (0.015 mg/100 mL) was lower than the HKu treatment (0.181 mg/100 mL). Both flesh and gonad of striped catfish broodstock in HKu treatment showed higher glycogen content.

Discussion

The experimental broodstock were reared in low stocking density (15 ind/m^2), thus stress condition could be avoided. It is related to physiological and biochemistry of the broodstock (Gaber *et al*., 2012). Cholesterol is a lipid and triglyceride at the same time that plays essential role both in energy source and reproduction. The blood plasma showed various result each treatment. The cholesterol increased on the 3rd and 4th week in HKu treatment. It triggered estradiol (PMSG) increased from the beginning of the study until the 4th week. Increasing blood plasma estradiol indiceted that the vitellogenesis process was occurring and the value of cholesterol was affected by curcumin compound in turmeric.

Curcumin acts as cholesterol–7α-hydroxylase enzyme stimulation (El-Wakf *et al*., 2011). The enzyme is located in the liver cell and it potentially increases blood cholesterol. Cholesterol in blood will be modified to bile salt and several parts of it will be utilized in steroid hormone secretion (Fitriyah *et al.*, 2008). Arifin (2015) reported that turmeric extract addition boosted cholesterol in *Osphronemus goramy.* Cholesterol is a precursor for steroid synthesis and it will affect gonadal maturation (Hiramatsu *et al.,* 2015) so that the escalation of cholesterol was followed by estradiol. In this study, the greatest increase of cholesterol occurred before spawning and it returned to be low right after spawning.

The diversity of cholesterol and triglyceride is profoundly affected by the reproduction phase. In this study, HKu treatment apparently affected cholesterol and triglyceride. According to Cheng *et al.* (2006), cholesterol and triglyceride content of feed also showed a positive result in grouper *Epinephelus coioides.* Broodstock feeding with a high amount of lipid and a similar amount of protein will cause cholesterol and triglyceride higher than using a high amount of protein, but a similar amount of lipid.

The increase of cholesterol and triglyceride was affected by HKu treatment. This indicated that the broodstocks were undergoing vitellogenesis and cholesterol decreased after oocyte maturation phase. This process was important for female broodstock. The enlarged liver implied that the liver also had a role to synthesis vitellogenin. Estradiol-17β will spread in the liver and diffused into body tissues and induced the liver to produce vitellogenin (Hana *et al*., 2016).

At the end of sampling, the HDL increased in HKu treatment. A similar result was also obtained by Javed and Usman (2015) in *Channa punctatus.* The concentration of protein plasma always related to the fluctuation of vitellogenin in catfish broodstock. The decreasing of protein plasma during spawning also happened in *Channa* spp. (Saxena, 2002). A similar result also occurred in this study, but on the other hand, there was no protein plasma changes in K treatment.

The glycogen content was also measured in the experimental broodstock. Glycogen is another deposit form of glucose, located in almost most body tissues, such as liver, flesh, and gonad. The experiment result showed that glycogen content in HKu treatment was higher than in the K treatment. The stored glycogen in a fish body was able to be transformed into glucose as an energy source. The result described that the treated broodstock underwent gonadal maturation faster than no treated broodstock. It is explained that HKu treatment was able to increase liver performance and nutrient metabolism. The outcome would be utilized as yolk material, so that follicle development would be higher compared to control treatment.

CONCLUSION

The changes in blood plasma and glycogen presented significant result between treatments. The spawning pattern of broodstock between treatments was described clearly in treated broodstock. Glycogen in liver tissues, flesh, and gonad in treated broodstock showed greater result in fish treated with PMSG + anti-dopamine and turmeric through the diet than non treated broodstocks.

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