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Effect of zinc (Zn) supplementation on quality and quantity of striped catfish *Pangasianodon hypophthalmus* sperm

Suplementasi seng (Zn) terhadap kualitas dan kuantitas sperma ikan patin siam *Pangasianodon hypophthalmus*

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ABSTRACT

This study aimed to evaluate the effects of Zinc (Zn) supplementation on the quality and quantity of striped catfish sperm. Experimental design for this study was a complete randomized design with five treatments and five replications. Male broods fed with Zn supplementation for eight weeks. The Zn supplemented into the fish diet at different concentrations (0, 50, 100, 150 and 200 mg/kg of feed). The results showed that Zn supplementation could improve the quality and quantity of striped catfish sperm. The treatments also showed significant effects on semen volume, sperm motility, sperm viability, and sperm concentration (P<0.05). Zn supplementation at a dose of 200 mg/kg feed demonstrated the best result has indicated by enhancement of quality and quantity of striped catfish sperm, increasing 51% of the volume, 11.6% of motility, 5.81% of viability, 54.1% of concentrations. The results suggested that Zn played an important role in improving reproductive performances of male striped catfish reproduction.

Keywords: quality of sperm, a quantity of sperm, striped catfish, supplementation zinc

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh suplementasi zinc (Zn) terhadap kualitas dan kuantitas sperma ikan patin Pangasianodon hypophthalmus. Penelitian ini menggunakan rancangan acak lengkap dengan lima perlakuan dan lima ulangan. Induk jantan diberi pakan dengan suplementasi Zn selama 8 minggu. Zn disuplementasikan dengan dosis berbeda (0, 50, 100, 150 dan 200 mg/kg pakan). Hasil penelitian menunjukkan bahwa suplementasi Zn dapat meningkatkan kualitas dan kuantitas sperma ikan patin sehingga berpengaruh signifikan terhadap volume semen, motilitas, viabilitas dan konsentrasi sperma (P<0,05). Suplementasi Zn pada dosis pakan 200 mg/kg menunjukkan hasil terbaik yang ditunjukkan oleh peningkatan kualitas dan kuantitas sperma ikan patin 51% volume; 11,6% motilitas; 5,81% viabilitas; 54,1% konsentrasi sperma. Hasil penelitian ini menunjukkan bahwa Zn memainkan peran penting dalam meningkatkan reproduksi ikan patin.

Kata kunci: kualitas dan kuantitas sperma, Ikan patin, suplementasi Zn

INTRODUCTION

Striped catfish *Pangasianodon hypophthalmus* is one of the most rapidly developed freshwater species. According to the Ministry of Fisheries and Marine Affairs (KKP, 2013) the national production fo striped catfish in 2013 was 410,383 tons with 95.57% of growing each year. In 2014, the total production reached 418,002 tons with 1.82% of growing, but in 2015 there was a downturn in production as many 18.8% to 339,111 tons (KKP, 2016). The decline of production was possibly caused by inefficient broodstock management. A decline in total production could interfere with the aquaculture activity. Therefore, any kinds of interferences in aquaculture should be avoided in order to achieve a high-quality seed supply.

An excellent broodstock quality can be accomplished through genetic modification and sperm and eggs quality improvement by enhancing nutrition intake. Izquerdo *et al.* (2001) stated that nutrition intake improvement significantly boosted not only sperm and egg quality but also seed quality and quantity. Sperm quality and quantity is an essential parameter to determine the reproduction activity and it is measured through its concentration, motility, viability, morphology, metabolism activity, and the ability to fertilize an egg (Cabrita *et al.,* 2014). Several factors that can affect the sperm performance are temperature, season, stress, hormone stimulation, and broodstock feed induk (Alavi *et al*., 2008).

Protein, lipid, carbohydrates, and vitamin are essential for reproduction, but without mineral, the reproduction process can be stranded. One of the micro mineral to induce reproduction performance is zinc (Zn). Zinc is involved in 300 different biological processes, include DNA transcription, protein translation, cell proliferation, differentiation and apoptosis (Thomas, 2011). Zinc interacts with DNA, regulates gene activity, maintains biological integration, protects from oxidative damage and physiologically zinc is essential for growth, sexual development, and reproduction (Roy *et al*., 2013). Zinc deficiency potentially destructs DNA (Valco *et al,*. 2016). Several findings presented Zn as a beneficial in inducing both reproduction and growth performance and antioxidant response, i.e. Nile tilapia, *(Morone chrysops* × *more saxatili), (Oreochromis niloticus* × *Oreochromis aureus),* red sea bream *(Pagrus major),* elevate sperm quality *(Megaloma amblycephala),* trout (*Salmo coruhensis*), and rainbow trout (*Oncorhynchus mykiss*) (Gammanpila *et al*., 2007; Feng *et al*., 2015; Li *et al.,* 2015; Zhao *et al*., 2011; Buentello *et al*., 2009; Nguyen *et al.,* 2008; Jiang *et al.,* 2016; Kocabas *et al.,* 2017).

Jiang *et al.* (2016) showed that feed enhancement using Zn 20−320 mg/kg was able to induce motility and viability of sperm in blunt snout bream *Megaloma amblycephala.* According to Payaran *et al.* (2014), Zn administration in dosage 10−30 mg/kg to male *Mus musculus.* Khairi *et al.* (2014) described that vitamin E 1886 11 IU/kg, selenium 1350 mg/kg, and zinc 5200 mg/kg could decrease semen production, motility, and concentration of male cow sperm during rainfall and high humidity. It indicated that Zn is crucial in reproduction activity.

Zinc in reproduction system acts as hormone receptor modulator which behave to stimulates androgen hormone (testosterone) in Leydig cell so that it will increase sperm quality (Yamaguchi *et al*., 2010; Yungsang *et al*., 2011; Egwurugwu *et al*., 2013; Payaran *et al*., 2014). Quality and quantity of sperm are closely related to testosterone synthesis and it depends to Zn adequacy in the feed (Roy *et al.,* 2013). Zinc belongs to induce Leydig cell to produce testosterone (Syarifuddin *et al*., 2017). Zinc is also required in embryo development. Anggreini (2007) presented that Zn deficiency potentially interfered sperm formation and both secondary and primary sexual organ, deficiency of testicular function (testicular hypofunction) which resulted in testosterone hormone production in Leydig cell. It also inhibited DNA synthesis in the germ cell, apoptosis response induction, and reduce sperm motility (Yamaguchi *et al*., 2010). Therefore, this study was conducted to evaluate zinc supplementation effect towards the quality and quantity of male striped fish sperm.

MATERIALS AND METHODS

Feed experimental formulation

The main ingredient in making feed experimental was fish flour, soybean meal, meat bone meal, Pollard, fish oil, corn oil, vitamin premix, mineral, and binder (carboxymethyl cellulose). An inorganic Zn ($ZnSO_4.7H_2O$) was added in different dosage (0, 50, 100, 150, 200 mg/kg feed). All of the ingredients were weighed based on its composition and stirred well. After that, the feed would be formed using pellet extruder and baked for 24 hours in 40°C. The protein content of the feed was 38%, lipid 7.5%, crude fiber 3%, ash 16.6%, and moisture 10%.

Table 1. Formulation and proximate analysis of experimental feed

Ingredients (g)	Zn supplementation dosage (mg/kg)				
	A (0)	B (50)	C (100)	D (150)	E (200)
Fish flour	150	150	150	150	150
MBM *(meat bone meal)*	170	170	170	170	170
Soybean meal	290	290	290	290	290
Pollard	290	290	290	290	290
Fish oil	15	15	15	15	15
Corn oil	15	15	15	15	15
Premix*	50	50	50	50	50
$ZnSO_4.7H_2O$	0	0.05	0.1	0.15	0.2
Binder (CMC)	20	20	20	20	20
Total	1000	1000	1000	1000	1000
Experimental feed proximate					
Protein (%)	38.90	37.63	39.30	37.62	37.80
Lipid (%)	7.00	7.31	7.34	7.85	8.17
Ash (%)	16.61	15.85	17.17	16.75	16.75
Crude fiber (%)	3.03	2.56	3.06	3.19	3.39
Zn (mg Zn /kg)	66.60	151.31	224.33	256.68	274.72
BETN	34.51	36.64	33.02	34.59	33.85
GE (kcal /g)	425.131	429.666	424.458	426.158	427.263

Note : BETN = Non nitrogen free extract; GE = gross energy, 1 g protein = 5.6 kcal, 1 g lipid = 9.41 g carbohydrate/BETN = 4.1 kcal (Watanabe, 1988).

Broodstock rearing

The experimental fish were two-year-old male striped catfish *Pangasianodon hypophthalmus* as many of 30 fish. The average initial weight was 2.5 ± 0.28 kg. Each treatment has 5 replications. The selected broodstocks were adapted in rearing cage net, then all of the broodstocks were spawned to empty the gonad before treatment. During the maturation process, all of the experimental broodstocks were reared in five cage nets sized in 3×4×3 m^3 with density 5 ind/net and all of the nets were placed in a 25×20×1.5 m^3 pond. The feeding rate was 3% and feeding frequency was twice a day (07.00 and 16.00) for 2 months.

Observation of quantity and quality of sperm

The observation of gonad development was done once in 14 days started from week 2, week 4, week 6, and week 8. The observation included semen volume, sperm motility, viability, and concentration. The semen collection was conducted using stripping technique on the abdomen to urogenital that has been cleaned using NaCl 0.9% so that the semen would not be mixed with any dirt and it was stored in a 16 mL tube. The semen collection was done at 08.00 in the morning. The semen was evaluated macroscopic and microscopically. The macroscopic evaluation consisted of volume (mL), color, consistency or viscosity (viscous and dense), and pH (pH indicator paper 6–8). Microscopic evaluation consisted of motility (%), viability (%), and concentration (10^{-9} spermatozoa cell/mL). Semen volume was calculated using the printed scale on the tube. Sperm motility was determined from the amount of progressive sperm that moves forward while it was observed on the object glass using a microscope. Aquadest was put on the edge of the object glass, let it spread and activated the sperm. The observation using microscope used 400 times of magnification (Rahardianto *et al*., 2012).

Sperm viability was observed by dropping one drop of semen approximately 0.01mL, then eosin-nigrosin was added using ratio 1:2. It was mixed well and spread preparation was made. It was air-dried and then observed using a microscope with three different sites. The active sperm would absorb the eosin, while the inactive or dead sperm cell would be transparent. The viability assessment was handled by comparing active sperm and total

sperm observed (Maulana, 2014). The sperm concentration was observed in Neubauer chamber using semen dilution 200 times (199 µL formol-saline and 1 µL semen). It was homogenized and counted the density each box using a microscope with 400 times of magnification. The observation was repeated five times and stated in unit 10^9 cell /mL (Gammanpila *et al.,* 2007). The viability and concentration were calculated using software Optilab image raster version 1.3.2.

RESULTS AND DISCUSSIONS

Results

Zn supplementation in male striped catfish broodstocks feed influenced semen volume, motility, viability, and sperm concentration. The supplementation treatment of 200 mg/kg was considered as the best treatment according to semen volume, motility, viability, and sperm concentration. The semen volume analysis of striped catfish was directed for 8 weeks (Figure 1). The semen volume increased on week 4 on 150 and 200 mg/kg treatment, while 1, 50, and 100 mg/kg treatments increased on week 6. On the week 6 to week 8, semen volume in each treatment evenly increased and 200 mg/kg treatment increased 51% compared to the control.

The sperm motility tended to raise after 4 weeks of rearing. It presented that 50–200 mg/kg supplementation treatment showed a significant difference (P<0.05) compared to control (P<0.05) in boosting sperm motility. Figure 2 described that Zn supplementation in dosage 200 mg/kg on week 8 significantly affected sperm motility. The highest motility percentage (11.6%) was obtained from 200 mg/kg of Zn supplementation compared to control. Briefly, it could be described that the higher Zn got, the motility would get higher.

The sperm viability of male striped catfish broodstocks was shown below in Figure 3. A similar order was shown by the average value of sperm viability which increased along with Zn dosage as well. The Zn supplementation (50–200 mg/kg) on week 2 and week 8 significantly affected sperm viability compared to control.

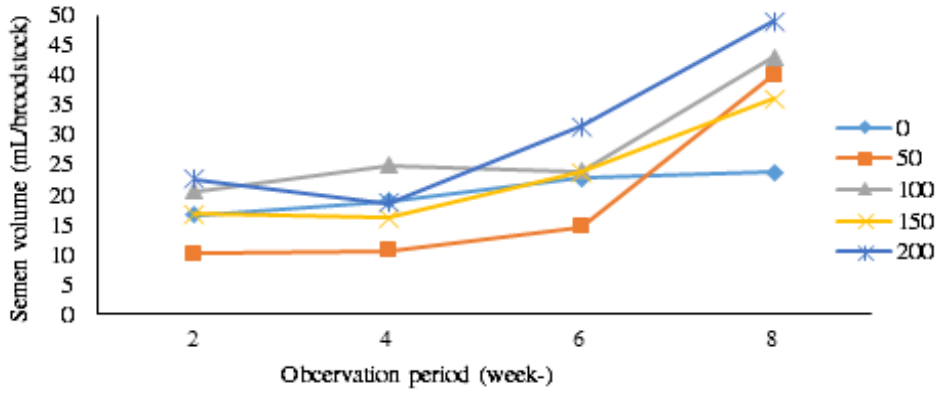

Figure 1. Striped catfish semen volume supplemented using different Zn dosage for 8 weeks.

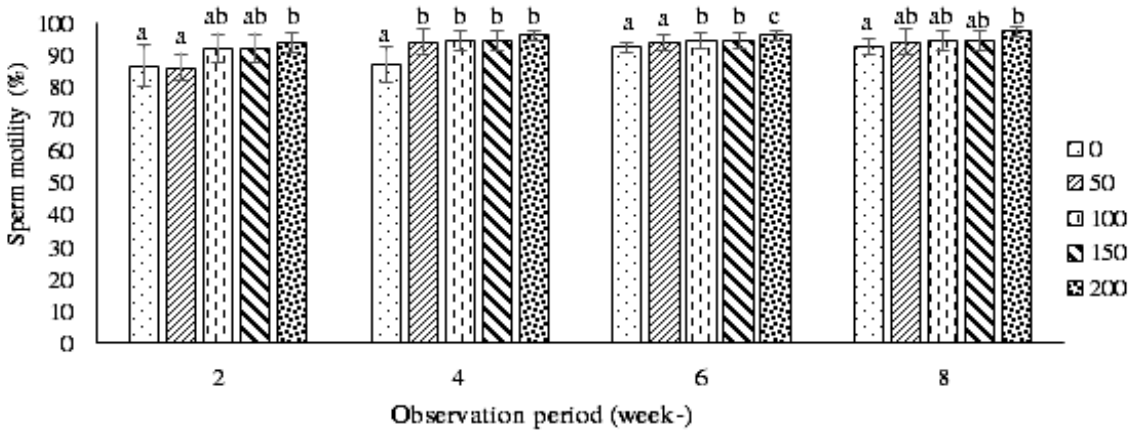

Figure 2. Sperm motility of striped catfish treated with of Zn feed supplementation for 8 weeks. The different letter above the bar indicates a significant difference (P<0.05).

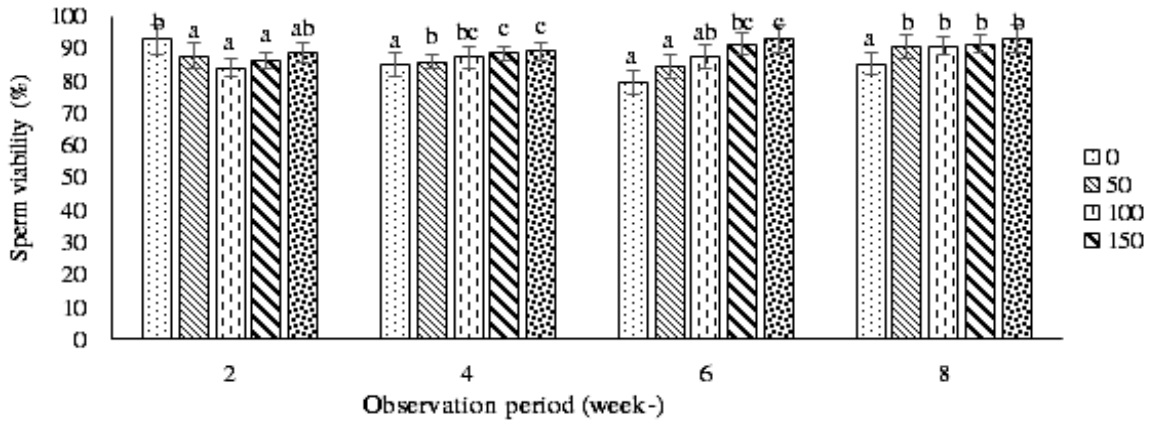

Figure 3. Sperm viability of striped catfish broodstocks treated with Zn feed supplementation for 8 weeks. The different letter above the bar indicates a significant difference (P<0.05).

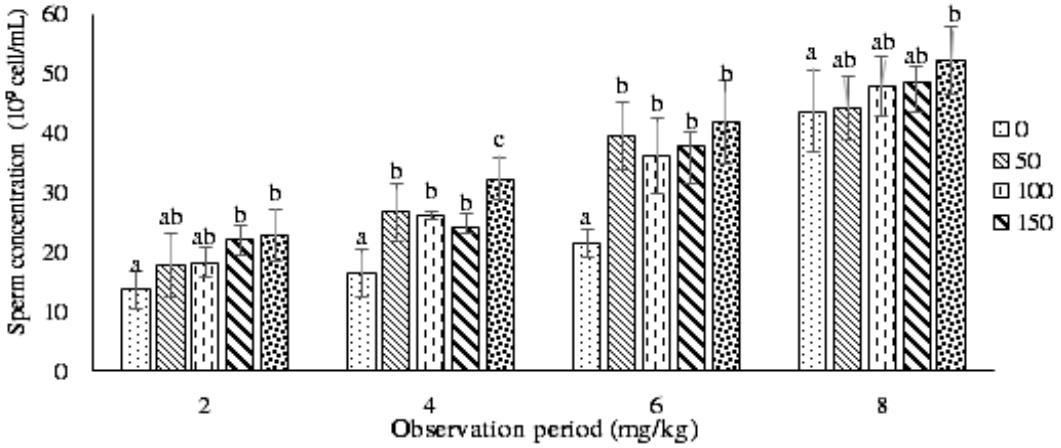

Figure 4. Sperm concentration of striped catfish broodstocks treated with Zn feed supplementation for 8 weeks. The different letter above the bar indicates a significant difference (P<0.05).

According to the result, the highest percentage (5.81%) was obtained in 200 mg/kg treatment compared to control.

Figure 4 showed that each treatment significantly raised sperm concentration. Generally, the higher Zn dosage got, sperm concentration would be higher, although dosage 50–150 mg/kg was not different significantly (P>0.05). The highest average value of sperm concentration was 200 mg/kg on week 8 or increased as many of 54.1% compared to control.

Discussions

Zn is an essential micronutrient which functioned as metalloenzyme and cofactor in the enzymatic system (NRC, 2011). Zn is involved in the various enzymatic activity in DNA synthesis and signal regulation which indirectly related to hormone regulation activity and cell cleavage (Carniola *et al,.* 2008). Different Zn supplementation to male striped fish broodstock influenced the semen volume, motility, viability, and sperm concentration. The semen volume increased almost in every treatment, but a different time. The dosage 50–100 mg/kg increased in week 6, while 150–200 mg/kg rose in week 4, and the treatment without any Zn supplementation showed relatively constant. The highest semen volume was obtained from 200 mg/kg treatment (49 mL) and it increased by 51% compared to no Zn supplementation treatment. This result was in line with Kumar *et al.* (2012) who stated that application of Zn supplementation as many of 150 mg/kg feed elevated the sperm volume in a cow. In addition, 5200 mg/kg of Zn in livestock feed was able to avoid semen production declining in rain season with high humidity (Khairi *et al.*, 2014).

Zinc maintains sperm survival and acts a lot in catalyzing, activating, synthesizing several important enzymes related to spermatogenesis, such as adenyl cyclase, alkaline phosphatase, and phosphatase acid (Jiang *et al.*, 2016). Similar reports by Yamaguchi *et al.* (2010), Yungsang *et al.* (2011), Egwurugwu *et al.* (2013), Surhayati

et al. (2013), Payaran *et al*. (2014) stated that zinc acts as a hormone receptor modulator to stimulate androgen hormone (testosterone) in Leydig cell and sperm activation and maturation. Zinc also contributes to steroidogenesis (Smith & Akinbamijo, 2000). On the contrary, zinc deficiency decreased androgen hormone (testosterone). A low level of testosterone could possibly distract spermatozoa maturation, low testosterone secretion from Leydig cell (Khoobbakht *et al*., 2018).

Sperm motility, viability, and concentration are a substantial parameter to characterize the ability of sperm to fertilize the egg. Based on the result of 200 mg/kg of Zn supplementation resulted in 96% of motility, 93% of viability, and 37.23×10^{9} cell /mL of sperm concentration or those parameters increased 11.6%, 5.81%, and 54.1%, respectively (Figure 2, 3, 4). Jiang *et al*. (2016) reported that 20 mg/kg until 320 mg/kg of Zn supplementation in feed potentially increased sperm motility and viability on blunt snout bream *Megaloma amblycephala.* The related result also stated by Kocabas (2017) that 0.5–1 mM of Zn was sufficient to induce sperm quality of rainbow trout *Oncorhynchus mykiss.* Yamaguchi *et al.* (2010) presented that Zn had such a crucial mineral towards rate regulation and motility duration in Japanese eel *Anguilla japonica.* The concentration of Zn mineral will rise and be accumulated in the germinal cell. Zn supplementation significantly increased semen volume, sperm motility, normal sperm percentage (Zhao *et al.,* 2016), viability and sperm concentration (Kumar *et al.*, 2012), activated secretion and testosterone activity, increased spermatogenic efficiency and germ cell in tubules seminiferous (Abdella *et al.*, 2011).

Astuti *et al.* (2008) reported that Zn addition as many of 27 mg/kg could increase mouse sperm motility and concentration. Payaran *et al.* (2014) also reported that Zn dosage 10–30 mg/kg was able to boost male rat sperm motility and concentration. The most significant result of this study was shown in 200 mg/kg of Zn supplementation. The sperm motility, viability, volume, and concentration were higher when supplemented by higher dosage of dosage. It indicated that Zn holds an essential role in boosting the quality and quantity of male striped catfish sperm.

The requirement of Zn increases, especially growth and reproduction phase (Widhyari *et al.*, 2015). Zn is definitely demanded in RNA catabolism, RNA and DNA polymerase, dehydrogenase, phosphatase, reverse transcriptase, AMP aminohydrolase, ATP synthesis and carbonic acid which physically needed in sperm motility (Yamaguchi *et al*, 2009). Zinc is also drawn in lipid catabolism which is the energy source for sperm to actively motile. In addition, zinc is also associated with testosterone secretion, so that it's undoubtedly required in male reproduction (Chu *et al.*, 2016). The activity of ribonuclease enzyme also demanded zinc existence to process spermatogenesis, during spermatogenesis, and at the end of spermatogenesis (Widhyari *et al*., 2015). Zinc also contributes to 5α-reductase enzyme activity which functioned to transform testosterone to active biological form becomes 5α-dihydroxy testosterone (Ali, 2007). Zinc is dependently affected reproduction hormone system which causes distractions in the spermatogenesis process (Sedigh, 2014).

CONCLUSION

Zinc supplementation in male striped catfish broodstock boosted the semen volume, sperm motility, viability, and concentration. The most significant result was presented by 200 mg/kg of zinc supplementation and elevated 51% of semen volume, 11.6% of motility, 5.81% of viability, and 54.1% of concentration compared to control.

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