Original article DOI: 10.19027/jai.19.1.74-83

Reproductive performance of catfish *Clarias* sp. with probiotics *Bacillus* sp. NP5 addition through feed

Performa reproduksi ikan lele *Clarias* sp. yang diberi probiotik *Bacillus* sp. NP5 melalui pakan

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(Received September 5, 2017; Accepted September 11, 2019)

ABSTRACT

The aim of this study was to evaluate the reproductive performance of catfish given probiotics *Bacillus* sp. NP5 with different concentrations through feed. This study used a completely randomized block design (CRBD) with the administration of probiotic *Bacillus* sp. NP5 at 2% concentration of 10^8 CFU/mL and 10^{10} CFU/mL in feed given and control (without probiotics), then repeated four times. Catfish broodstocks with an average size of 955 ± 261.27 g were kept in a net sized 1 m×1 m×1 m with seven broodstocks per net. Parameters observed included the Gonadosomatic Index (GSI), fecundity, number of matured eggs, frequency distribution of egg diameter, fertilization rate, and hatching rate. The result showed that the GSI value of female catfish broodstock was significantly different in sixth week as the highest value was obtained from 10^{10} CFU/mL probiotics with 5.19%. Moreover, the fecundity of all treatments showed significantly different results ($P<0.05$) as the highest value was on the concentration of 10^{10} CFU/mL *Bacillus* sp. NP5 with 137,123 ± 32,635 eggs/broodstock. This study concludes that the administration of probiotics *Bacillus* sp. NP5 with 10^{10} CFU/mL in feed given to catfish broodstock increases the fecundity (137,123 eggs/broodstock), GSI (5.19%), number of matured eggs percentage (64.50%), and hatching rate (82.53%).

Keywords: *Bacillus* sp. NP5, catfish, reproductive performance

ABSTRAK

Tujuan penelitian ini adalah mengevaluasi performa reproduksi ikan lele yang diberi probiotik *Bacillus* sp. NP5 dengan konsentrasi berbeda melalui pakan. Penelitian ini menggunakan rancangan acak kelompok lengkap (RAKL) dengan pemberian probiotik *Bacillus* sp. NP5 pada konsentrasi 10^8 CFU/mL dan 10^{10} CFU/mL sebanyak 2% dari pakan yang diberikan serta kontrol (tanpa pemberian probiotik), masing-masing dengan empat ulangan. Induk ikan lele dengan ukuran 955 ± 261.27 g dipelihara pada jaring hapa ukuran 1 m × 1 m × 1 m sebanyak tujuh ekor per jaring hapa. Parameter yang diamati meliputi indeks kematangan gonad (IKG), fekunditas, jumlah telur matang, sebaran frekuensi diameter telur, derajat pembuahan, dan derajat penetasan. Hasil penelitian menunjukkan bahwa nilai IKG induk betina ikan lele berbeda nyata pada minggu keenam dengan nilai tertinggi diperoleh pada perlakuan probiotik 10^{10} CFU/mL yaitu sebesar 5.19%. Selain itu, fekunditas semua perlakuan menunjukkan hasil yang berbeda nyata ($P<0.05$) dengan nilai tertinggi pada perlakuan pemberian probiotik *Bacillus* sp. NP5 dengan konsentrasi 10^{10} CFU/mL sebesar 137,123 ± 32,635 telur/induk. Kesimpulan, pemberian probiotik *Bacillus* sp. NP5 dengan konsentrasi 10^{10} CFU/mL melalui pakan kepada induk ikan lele mampu meningkatkan nilai fekunditas 137,123 telur/induk, GSI 5,19%, persentase jumlah telur matang 64,50%, dan derajat penetasan telur 82,53%.

Kata kunci: *Bacillus* sp., ikan lele, performa reproduksi

INTRODUCTION

Clarias sp. catfish is one of important aquaculture commodities that is mostly cultured in Indonesia. This catfish is obtained from male African catfish *Clarias gariepinus* and female Taiwanese catfish *Clarias fuscus* crossing (Iswanto, 2013). The average production of catfish in Indonesia increases continuously as an increased production of 19.39% was produced in 2014–2016 (KKP, 2017). An effort to improve the production can be performed through an intensive to super-intensive culture through the increased stocking density, which respectively increases the seed requirement.

One method to increase the number of seeds is by inducing the broodstock reproduction performance. Nadio (2015) reported that a microbial-based catfish broodstock rearing with biofloc technology could improve the catfish broodstock reproduction performance by accelerating the gonad maturation recovery (rematuration). A broodstock rearing with biofloc technology only required four- week period of rematuration process, while control treatment was until seven-week period. Another parameter, such as gonadosomatic index (GSI), showed a higher value in biofloc treatment than control. Moreover, biofloc technology was also proven to produce more fecundity value twice than control treatment.

Widanarni *et al*. (2012) also stated that biofloc technology in a Nile tilapia intensive culture could improve the production, water quality, and reproduction. Ekasari *et al*. (2015) reported that the biofloc technology could improve the average weight, GSI, and fecundity level of Nile tilapia broodstock until 1,243 ± 217 eggs/broodstock compared to control treatment with 890 ± 73 eggs/broodstock, while the hepatosomatic index (HIS) and egg diameter were insignificantly different. Emerenciano *et al*. (2012) reported that biofloc technology could improve spawning performance of blue shrimp *Litopenaeus stylirostris*. In this study, male and female broodstock weight with biofloc treatment was higher than control treatment. The number of eggs per gram weight on each spawning was insignificantly different between biofloc and control, however the broodstock gonad maturation level given biofloc treatment was higher (3.6 ± 0.8) than control (2.5 ± 0.5). Furthermore, the latent period of spawning (one spawning period to another) in biofloc treatment was faster (7.4 ± 1.8 days) than control (10.7 ± 4.6 days). Meanwhile, Emerenciano *et al*. (2014) stated that biofloc technology did not influence the spawning performance of pink shrimp *Farfantepenaeus duorarum*, except producing a greater egg (268 ± 1.6 µm) than control (258 ± 0.5 µm).

Another microbial-based culture that is potentially studied to improve the reproduction performance is a probiotic administration. Probiotics are living microbes that provides a beneficial influence for the culture organisms due to the capability of modifying the microbial community, repairing the nutrient value, maintaining the host response against diseases, controlling the environment quality (Cruz *et al*. 2012), and induce the immune response (Nayak, 2010). Probiotics in fish culture is often utilized as a growth promoter and pathogen inhibitor, as well as maintaining the feed digestibility, water quality, and stress tolerance (Cruz *et al*., 2012). The use of probiotics to maintain the reproduction performance and gamete quality in fish culture are still limited. Cruz *et al*. (2012) reported that probiotics *Bacillus subtilis* could maintain the reproduction performance of guppy *Poecilia reticulata* and platy *Xiphoporus maculatus*.

A previous study performed by Gioacchini *et al*. (2011) showed that probiotics *Lactobacillus rhamnosus* could improve the gonad development containing growth, gonad maturation phase, and fecundity level of zebra fish *Danio rerio*. The aim of this study was to evaluate the reproduction performance of catfish given probiotic *Bacillus* sp. NP5 through feed. This study result is expected to become an alternative method to improve the reproduction performance of catfish with the administration of probiotics through feed.

MATERIALS AND METHOD

Period and location

The study was performed on February, 2017 until January, 2018 in the experimental pond station, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor.

Experimental design

The study used a completely randomized block design (CRBD) with the administration of probiotic *Bacillus* sp. NP5 at 2% (v/w) concentration of 10^8 CFU/ml and 10^{10} CFU/ml in feed and control (without probiotic administration), then repeated four times. The

following table shows the experimental design used in this study with the administration of probiotic *Bacillus* sp. NP5 (Table 1).

Table 1. The experimental design of probiotic *Bacillus* sp. NP5 administration on catfish broodstock through feed

Treatment	Description
A (control)	Feed without *Bacillus* sp. NP5 administration
B	Feed with *Bacillus* sp. NP5 10^8 CFU/ mL administration
C	Feed with *Bacillus* sp. NP5 10^{10} CFU/ mL administration

Rearing media and fish preparation

This study used 12 nets sized 1 m ×1 m ×1 m placed on a pond with the temperature of 27–30°C. The fish used were catfish broodstocks with the average size of 955 ± 261.27 g obtained from Catfish and Striped Catfish Development and Stock Inducement Center, Cijengkol, Subang, West Java. The broodstocks used in this study were seven female broodstocks per net, therefore the total broodstocks used were 84 fish. Fish were acclimatized based on media and water quality condition for a week during the initial rearing period fed with a commercial feed as much as 2% of biomass and no *Bacillus* sp. NP5 administration.

Broodstock rearing

Before rearing, the catfish broodstocks were selected and artificially spawned to equalize the initial reproduction status. The artificial spawning was performed using a hormone mixture of gonadotropin releasing hormone analog and domperidone (*Ovaprim Syndel Lab. Ltd., Canada*). The female broodstock eggs were stripped after 12 hours of hormone injection. The female broodstocks were distributed into a recovery medium for 24 hours, then moved into the experimental media and reared until their gonads were matured. Sampling was performed every three weeks to observe the gonad maturation and other parameters.

Probiotic preparation

The probiotics used in this study were *Bacillus* sp. NP5 bacteria obtained from the laboratory of fish health, Department of Aquaculture, IPB. *Bacillus* sp. NP5 bacteria were prepared by culturing these bacteria in a tryptic soy broth (TSB) as much as 10 mL to obtain 10^{10} CFU/ mL concentration (bacteria stock). *Bacillus* sp. NP5 bacteria from the stock solution were taken as much as 1 mL and moved into a polyethylene tube, then centrifuged for five minutes at 3000 rpm. The bacterial cell pellet was added with 1 mL PBS (phosphate buffer saline) solution and diluted to obtain a concentration based on the given treatments in this study.

Feed preparation

The feed used in this study was a commercial feed for broodstocks with 38% protein contents. The feed treatments were made by administering the *Bacillus* sp. NP5 based on the given concentrations. Probiotics were mixed with the feed by adding 3% (v/w) of egg whites, then air dried to decrease the humidity. A commercial feed was mixed with PBS and egg whites without a probiotic administration on the control treatment. A daily feed was given 1–2% of the biomass weight twice a day.

Spawning preparation

An artificial spawning was performed using an *ovaprim* injected on muscle below dorsal fin. The *ovaprim* dose used for the female broodstocks were 0.3 mL/kg broodstock, while the male broodstocks were 0.2 mL/kg broodstock. The *ovaprim* hormone injection was given once at 16.00 GMT+7.

Eggs and sperms collection

The eggs were taken by removing the female broodstock from the media carefully using a wet fabric. The abdomen part was pressed to withdraw the eggs, then the eggs were kept into a dried container. Meanwhile, the sperms were taken by removing the male broodstock from the media carefully, then dissected to remove its gonads and measured. The gonads were cut on one of the lobes and pressed slowly to withdraw the sperms. Sperms were then kept in a polyethylene tube.

Fertilization

Five grams of eggs from each female broodstock were mixed with 0.2 mL sperms and incubated into an aquarium. The fertilization rate value was determined after 8 hours of eggs and sperms mixing by counting the fertilized eggs (transparent color) in different five striped glass squares placed randomly, then compared to the total number of eggs.

Hatching

The hatching media used were aquaria sized 40 × 30 × 20 cm filled with water at 25–27°C and aerated. Hatching process was performed by incubating the fertilized eggs for 24–40 hours. The hatching rate was determined by counting the hatched eggs (larvae) compared to the total number of fertilized eggs.

Water quality

Water exchange and syphonization (for an aquarium observation) performed in this study were to maintain the water quality. The water quality parameter for catfish culture used was based on Aneesh *et al*. (2013).

Gonad maturity index

Treated female broodstocks were artificially spawned and dissected to obtain their gonads. Gonads were measured to obtain the gonad weight data. The gonad maturity index value was determined by comparing the gonad weight against the fish body weight.

Fecundity

Fecundity was calculated from the measurement of broodstock weight on pre- and post-ovulated period. The egg weight produced from the ovulation process was obtained from the difference of broodstock weight on pre- and post-ovulated period. Eggs produced from the ovulation process were taken and measured as much as 0.1 g, then the egg was counted and multiplied by 10 to obtain the total number of eggs in 1 g.

Matured egg size determination

The determination of matured egg size was performed by comparing the egg size from matured broodstock on pre-ovulation process against the remaining egg size in broodstock gonads on post-ovulation process. Three matured broodstocks were injected with an *ovaprim* and the other three broodstocks were injected with a physiological solution as control. *Ovaprim* and physiological solution were injected on the muscle below dorsal fin, then the broodstocks were reared for 12 hours in the net. Broodstocks those injected with *ovaprim* underwent ovulation, while in the control did not. Broodstocks were stripped after 12 hours to withdraw the ovulated eggs and dissected to obtain their gonads. The matured eggs were determined by comparing the remaining egg size from *ovaprim* injected broodstocks against the egg size in the control broodstocks. The frequency distribution of matured eggs was determined by taking 100 eggs, then measured their diameters using a microscope with 40 times magnification. The egg diameter measurement result on each size group was calculated its percentage and created its frequency distribution. Matured egg size was the size that was absent in gonads on the post-ovulation process.

Parameters

Gonadosomatic index (GSI)

The gonadosomatic index (GSI) is the relationship of gonad and body weight. GSI was calculated using the following formula (Yalcin, 2001) at a percentage unit:

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Fecundity

Fecundity is the total number of eggs produced per broodstock. Fecundity was determined by the following formula:

$$\text{Fecundity} = \text{Total number of eggs} \times \text{Ovulated egg weight}$$

Egg diameter measurement

The egg diameter (μ) was calculated with:

$$\text{Egg diameter} = \frac{\text{Ocular micrometer scale}}{\text{Correction factor}} \times 0.01$$

Egg diameter frequency distribution

The egg diameter frequency distribution was obtained from egg diameter data measured using an ocular micrometer and made in a histogram graphic (Jusmaldi *et al*., 2018).

Fertilization rate (FR)

The fertilization rate (FR) was calculated using a formula (Tilahun *et al.,* 2016):

$$FR = \frac{\text{Total number of fertilized eggs}}{\text{Total number of counted eggs}} \times 100$$

Hatching rate (HR)

The hatching rate (HR) was calculated using the following formula at a percentage unit:

$$HR = \frac{\text{Total number of hatched eggs}}{\text{Total number of counted eggs}} \times 100$$

Water quality

The water quality parameters measured in this study were temperature, pH, DO, nitrite, and total ammonia-nitrogen (TAN). The following table presents the units and tools of measured water quality parameters. The water quality standard in this study was based on Aneesh *et al*. (2013).

Table 2. Water quality parameter units and measurement tools

Parameter	Unit	Tool
Temperature	°C	Thermometer
pH	-	pH meter
DO	mg/L	DO meter
NH_3	mg/L	Spectrophotometer

Data analysis

Data result for fecundity and GSI were analyzed using *SPSS 18.0* continued with a Duncan test with the significance level of α= 5%. The data of total matured eggs, frequency distribution, egg diameter, fertilization rate, hatching rate, and water quality were analyzed descriptively using Microsoft Excel 2010.

RESULT AND DISCUSSION

Result

Gonadosomatic index (GSI)

The observation result of female catfish broodstock gonadosomatic index (GSI) fed with probiotic *Bacillus* sp. NP5 is presented on Figure 1. The GSI value of female catfish broodstock started to become significantly different at the 6th week with the highest value was obtained from 10^{10} CFU/mL(C) probiotic concentration (5.19%). The highest GSI value at the 9th week was obtained from 10^{8} CFU/mL(B) probiotic concentration with 2/49%. The GSI value at the 12th week did not increase followed with an insignificant difference among treatments.

Fecundity

The observation result of catfish broodstock fecundity level fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Figure 2. All treatment fecundity levels at the end of the study showed a significantly different result (P<0.05). The highest fecundity level was obtained from the probiotic *Bacillus* sp. NP5 concentration of 10^{10} CFU/mL (C) with

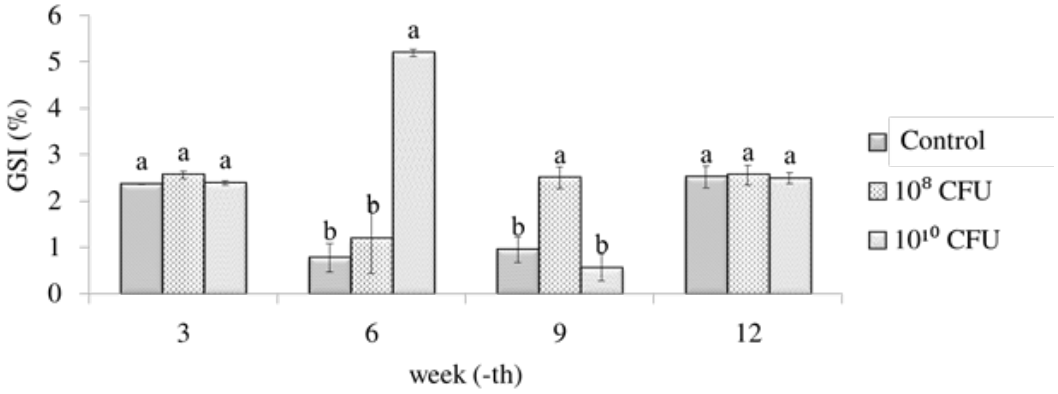

Figure 1. Gonadosomatic index (GSI) of catfish *Clarias* sp. broodstock fed with the different concentrations of probiotic *Bacillus* NP5.

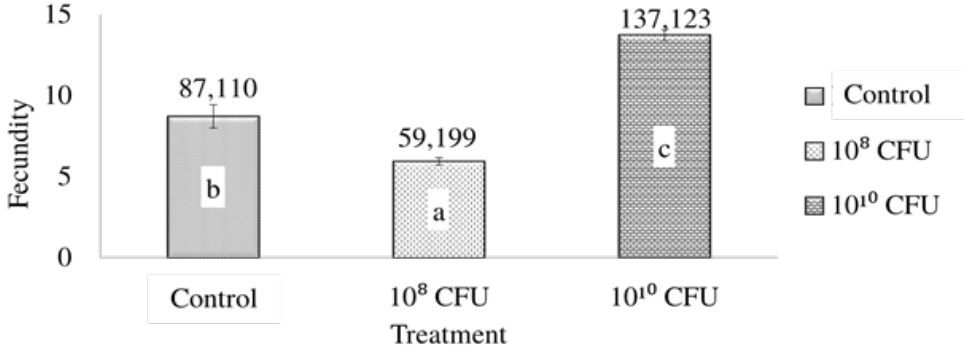

Figure 2. Fecundity level of catfish bloodstock *Clarias* sp. fed with different concentrations of probiotic *Bacillus* sp. NP5

137,123 ± 32,635 eggs/broodstock, followed with the control treatment (A) with 87,110 ± 29,260 eggs/broodstock and the lowest was on 10^8 CFU/mL (B) with 59,199 ± 38,781 eggs/broodstock.

The total number of matured eggs

Observation result of egg diameter to evaluate the total number of matured egg development is presented on Table 3. The highest matured egg percentage at the 3rd week was on the probiotic *Bacillus* sp. NP5 concentration treatment of 10^8 CFU/mL (B) with 49.75%, followed with the control treatment (37.25%) and probiotic *Bacillus* sp. NP5 concentration of 10^{10} CFU/mL (C) (33,50%). The percentage of matured eggs increased at the 6th until 12th week with a greater value of probiotic administrations than control. Catfish broodstock fed with 10^8 CFU/mL(B) probiotic *Bacillus* NP5 at the 12th week had the highest percentage (74.00%) on the total number of matured eggs, followed with 10^{10} CFU/mL (C) probiotic *Bacillus* sp. NP5 (64.50%) and control (A) (64.00%).

Frequency distribution of egg diameter

The egg diameter frequency distribution of catfish fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Figure 3. Figure 3a shows a frequency distribution of catfish at 0th week as the initial study period. The development shows two inclined peaks of egg size at the 3rd week (Figure 3b) until 9th week (Figure 3d). However, probiotic *Bacillus* sp. NP5 with 10^8 CFU/mL concentration at the 12th week (Figure 3e) shows a declined peak of immatured egg size. Nevertheless, a large egg size was occurred during this week (1.17 mm) with 6.5% occurrence on 10^{10} CFU/mL (C) probiotic *Bacillus* sp. NP5 treatment, 5% on 10^8 CFU/mL (B) probiotic *Bacillus* sp. NP5 treatment, and only 0.75% on control treatment.

Fertilization rate (FR)

The fertilization rate (FR) value of catfish broodstock eggs fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Figure 4. The fertilization rate values in this study were insignificantly different. Control treatment (A) obtained 99.14%, probiotic treatment with 10^8 CFU/g concentration (B) obtained 97.59%, and probiotic treatment with 10^{10} CFU/g concentration (C) obtained 96.82%.

Hatching rate (HR)

The hatching rate of catfish broodstock eggs fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Figure 5. Based on Figure 5, the highest hatching rate value (HR) was obtained from the administration of 10^{10} CFU/g(C) probiotic *Bacillus* sp. NP5 with 82.53%.

Water quality

Result of water quality measurement during broodstock rearing and egg hatching period of catfish fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Table 4. Data in Table 4 showed that all of water quality parameters, i.e. temperature, dissolved oxygen, pH, and NH_3 were in the normal range.

Table 3. Matured egg percentage of female catfish *Clarias* sp. fed with different concentrations of probiotic *Bacillus* sp. NP5

Treatment	Matured egg percentage (>0.85 mm)			
	3rd week	6th week	9th week	12th week
A (Control)	37.25	42.75	34.50	64.00
B (10^8 CFU/mL)	49.75	45.00	52.50	74.00
C (10^{10} CFU/mL)	33.50	43.75	35.50	64.50

Table 4. Water quality during the catfish broodstock rearing and egg hatching period

Parameter	Broodstock	Hatching	Standard (Aneesh *et al*., 2013)	
			Broodstock	Hatching
Suhu (˚C)	27.38 ± 0.36	27.90 ± 1.08	26 ± 3.2	27 ± 1.79
DO (mg/L)	4.70 ± 1.79	6.26 ± 1.33	5.0 ± 2.6	6.0 ± 2.2
pH (unit)	7.41 ± 1.41	8.06 ± 0.05	7 ± 0.85	7 ± 1.26
NH_3	0.06 ± 0.07	0.02 ± 0.02	0.06 ± 0.07	0.08 ± 0.08

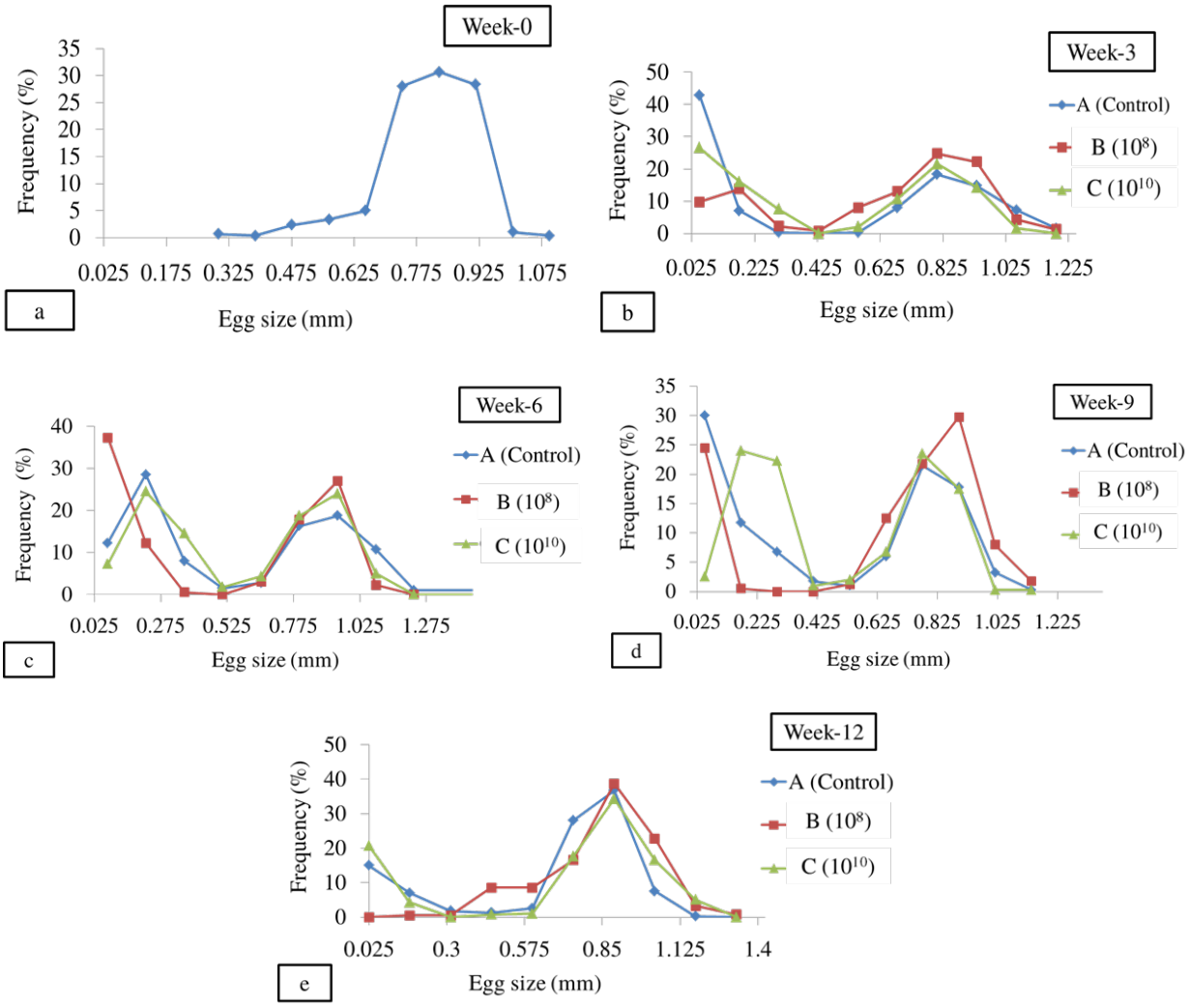

Figure 3. Frequency distribution of catfish *Clarias* sp. egg diameter fed with different concentrations of probiotic Bacillus sp. NP5 at the 0th week (a), 3rd week (b), 6th week (c), 9th week (d), and 12th week (e) of rearing period.

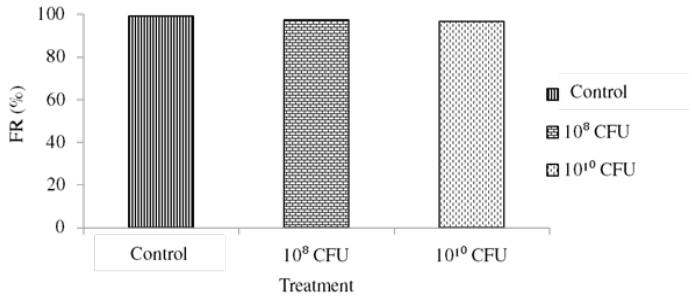

Figure 4. Egg fertilization rate of catfish *Clarias* sp. fed with different concentrations of probiotic *Bacillus* sp. NP5

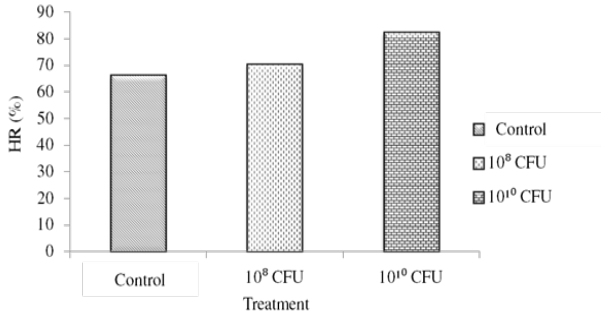

Figure 5. Hatching rate of catfish *Clarias* sp. eggs on different treatment concentrations of probiotic *Bacillus* sp. NP5

Discussion

Gonadosomatic index (GSI) reflects the relationship between gonad and body weight. Based on Figure 1, the GSI value of female catfish broodstock was significantly different at the 6th week with the highest value was on 10^{10} CFU/mL(C) probiotic treatment, namely 5.19%. Increased GSI value was similar to the study result of Gioacchini *et al.* (2011), which showed that the probiotic administration of *Lactobacillus rhamnosus* IMC 501® on zebra fish had a higher GSI value than the control treatment. Moreover, Gioacchini *et al*. (2011) explained that the increased GSI value happened due to the increased estradiol receptors (erα) and *vtg* gene expression correlated with the significant inclination of *cyp19a* gene expression. The expression of *cyp19a* gene indicate an enzyme activity controlling the androgenic to estrogenic alteration, therefore the inclined *cyp19a* genes can increase the estradiol level in blood plasma, which continuously increases vitellogenins (vtg) in vitellogenesis process. The highest GSI value at the 9th week was obtained from 10^{8} CFU/mL (B) probiotic treatment with 2.49%. Decreased GSI value at the 9th week on the 10^{10} CFU/mL (C) probiotic treatment was suspected due to the over-riped eggs. The GSI value at the 12th week did not increase again following the treatments. This was suspected because the nutrient content in probiotics after the 9th week was insufficient for the broodstock to improve the reproduction performance.

Fecundity is the broodstock capability of producing eggs that are ready to be spawned. Fecundity is closely related to the vitellogenesis process, namely the egg yolk synthesis and filling the yolk into the egg (Gioacchini *et al*., 2011). The increase in fecundity was suspected due to the probiotics indirectly inactivates the potential hormone in metabolism, such as leptins. Increased leptin levels are correlated with high expressions of *kiss1*, *kiss2*, and also *GnRH3* gene in the female brain given treatment, improving the fecundity level. Leptins regulate the reproduction in hypothalamus by modulating the mRNA transcription and GnRH release, as well as in hypophysis by modulating *LH* gene expression release (Gioacchini *et al*., 2010). The present study found that the fecundity level (Figure 2) on all treatments at the final study period showed a significantly different result ($P<0.05$). The highest fecundity level was found on 10^{10} CFU/mL (C) probiotic *Bacillus* sp. NP5 with 137,123 ± 32,635 eggs/broodstock, followed with control treatment (A) 87,110 ± 29,260 eggs/broodstock, and the lowest was on 10^{8} CFU/mL (B) probiotic *Bacillus* sp. NP5 with 59,199 ± 38,781 eggs/broodstock. Generally, this was in line with the study result of Gioacchini *et al*. (2010) which indicated that the administration of probiotic *L. rhamnosus* in zebra fish caused a higher fecundity level than the control treatment.

Female reproduction process is started from the vitellogenesis and finished on the dormant phase as fish awaits for ovulation and spawning signals. According to Sarkar *et al*. (2014), matured fish eggs have larger size and weight. Based on Table 3, catfish broodstock fed with 10^{8} CFU/mL (B) probiotic *Bacillus* sp. NP5 treatment at the 12th week had the highest number of matured egg percentage (74.00%), followed with 10^{10} CFU/mL (C) probiotic *Bacillus* sp. NP5 (64,50%) and control (A) (64.00%). This value indicated that administration of probiotic *Bacillus* sp. NP5 in feed increase egg yolk content and then increasing the total number of matured eggs. The number of matured eggs can be predicted through a frequency distribution of egg diameter. The result of catfish egg diameter frequency distribution after fed with different concentrations of probiotic *Bacillus* sp. NP5 is presented on Figure 3. Figure 3a shows a frequency distribution of catfish at 0th week (initial study period). The development shows two inclined peaks of egg size at the 3rd week (Figure 3b) until 9th week (Figure 3d). This result was similar to the study result of Tyor and Pahwa (2017) which stated that the existence of egg diameter similarity at third and fourth level was found to be capable of spawning at several times in a year.

However, probiotic *Bacillus* sp. NP5 with 10^{8} CFU/mL concentration at the 12th week (Figure 3e) shows a declined peak of immatured egg size. A large egg size was occurred during this week (1.17 mm) with 6.5% occurrence on 10^{10} CFU/mL (C) probiotic *Bacillus* sp. NP5 treatment, 5% on 10^{8} CFU/mL (B) probiotic *Bacillus* sp. NP5 treatment, and only 0.75% on control treatment. This indicates that eggs from the broodstock fed with probiotic *Bacillus* sp. NP5 have greater size than control.

The fertilization rate value in this study was around 96.82–99.14% and insignificantly different among probiotic and control treatments. This indicates that the administration of probiotic *Bacillus* sp. NP5 has no negative effect on the egg quality. Meanwhile, the highest hatching rate was

found on 10^{10} CFU/mL probiotic *Bacillus* sp. NP5 concentration with 82.53%. The hatching rate value on probiotic treatments were higher than control as suspected due to higher gonadosomatic index, resulting more perfect eggs with higher egg yolk content. Egg yolk is an energy source for embryo required for fulfilling the embryonic to hatch (Fajrin *et al*., 2012).

Some factors influencing eggs and sperms quality come from the environmental factors such as temperature, salinity, and photoperiod. However, temperature becomes the main factor which influences egg quality, especially during the reproductive and spawning season. Low temperature can have a negative impact on the egg quality (Bobe & Labbe, 2010). In the present study, water quality parameters which consisted of temperature, dissolved oxygen (DO), pH, and NH_3 were still in the normal range.

CONCLUSION

The administration of probiotic *Bacillus* sp. NP5 with 10^{10} CFU/mL concentration through feed on catfish (*Clarias* sp.) broodstock could increase the fecundity level (137,123 eggs/ broodstock), GSI (5.19%), percentage of matured egg (64.50%), and hatching rate (82.53%).

ACKNOWLEDGEMENTS

Authors would like to thank the head of Catfish and Striped Catfish Development and Stock Inducement Center, Cijengkol, Subang, West Java for providing the materials in this study and West Java Province stakeholders for funding support through *Beasiswa Pemprov Jabar* in 2016.

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