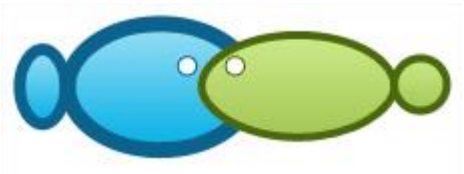


Spermatogenesis and sperm quality of male African catfish fed with *Bacillus* sp. NP5 probiotic supplemented diet

Valensia Enzeline, Hasan Nasrullah, Agus O. Sudrajat, Muhammad Zairin Jr., Alimuddin Alimuddin, Widanarni Widanarni

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Dramaga, Bogor, West Java, Indonesia. Corresponding author: W. Widanarni, widanarni@apps.ipb.ac.id

Abstract. The dietary supplementation of probiotics *Bacillus* sp. NP5 in female African catfish *Clarias gariepinus* (Burchell 1822) resulted in the improvement of the reproduction performance of the fish in one of our previous studies, but its effects on the male fish have not been evaluated yet. Thus, this study aimed to evaluate the effect of dietary *Bacillus* sp. NP5 dietary probiotic supplementation in the spermatogenesis and sperm quality of male African catfish. The immature male catfish were fed with 0 (control), 10^6, and 10^8 CFU g^{-1} probiotic supplemented in the diet. Fish were fed twice a day for 60 days. The gonadosomatic index, testis development, sperm quality (volume, density, and motility), testosterone level, and spermatogenesis-related genes were analyzed. The results showed that the dietary probiotic supplementation of *Bacillus* sp. NP5 could accelerate the spermatogenesis processes in the male African catfish and improved its sperm quality compared to the control. The transcription of the related mRNA gene was also higher in the probiotics treatments. Faster testis development and sperm quality were observed to be better in the 10^8 CFU g^{-1} probiotic treatment compared to its lower dose of 10^6 CFU g^{-1} probiotic. The dietary supplementation of 10^8 CFU g^{-1} probiotic *Bacillus* sp. NP5 could be recommended as the starting point for the improvement procedure of the reproductive performance in the male African catfish.
Key Words: beneficial bacteria, feed quality, gene expression, testis quality.

Introduction. African catfish is one of the important aquaculture species in African and Southeast Asian countries (FAO 2016). Particularly in Indonesia, catfish is one of the main cultured species that have high demand, 1.39 million tons in 2020, and are prospected to reach 1.65 million tons in 2024 (KKP 2020). African catfish are widely cultured due to their good meat quality, fast growth performance, ability to be cultured in a high stocking density that is suitable for the intensive culture system (Adamek et al 2011), it is tolerant to extreme conditions, and has a high market demand (Widyastuti et al 2019). This high demand pushes the farmers to continuously produce fry in the hatchery and send the to grow-out systems. Fry production in the hatchery depends on the spawning process, which needs a mature broodstock with good reproduction performances. To ensure the availability of broodstocks, the maturation protocols for females and males African catfish should be optimized. Male African catfish has serrated vesicular seminalis, which is unable to release sperm when stripped in the artificial spawning method (Bertha et al 2016). This condition often leads to artificial spawning by surgery, and abundant male broodstocks are needed. The disadvantage of surgery is the low sperm milt produced, resulting in a less efficient progeny production (Bertha et al 2016).

Probiotics are beneficial bacteria that give the host a health advantage when administered in appropriate quantities (Fuller 1989). Probiotics applications are becoming a promising alternative approach for the prevention of infectious diseases (Chauhan & Singh 2018). Other benefits of probiotics in aquaculture include fish growth enhancement (Giannenas et al 2015), enhanced digestion (Ramzani et al 2014), immunostimulant (Liu

et al 2017), and water quality recovery (Padmavathi et al 2012). In recent years, a few studies have begun to focus on the effects of probiotics application in accelerating the reproductive performance of fish. Gioacchini et al (2010) reported a positive influence of the probiotic *Lactobacillus rhamnosus* in the induction of oocyte maturation and fecundity in female zebrafish *Danio rerio*. The dietary supplementation of probiotic *Pediococcus acidilactici* and nucleotide in the goldfish *Carassius auratus* resulted in better reproductive performance of the fish including an increase in the motility and density of sperm, and an increase in the egg diameter and fecundity of the female fish (Mehdinejad et al 2018). Our previous study on the dietary administration of probiotics *Bacillus* sp. NP5 in the female goldfish and African catfish also showed promising results.
The administration of *Bacillus* sp. NP5 through feed was able to enhance reproductive parameters of female goldfish (unpublished) and African catfish (Ayuningtyas et al 2020). The gonadosomatic index (GSI) value, fecundity, and the number of matured eggs of female African catfish were significantly increased and higher than the control after six weeks of administration of *Bacillus* sp. NP5 (Ayuningtyas et al 2020). However, the study related to the effects of the administration of probiotic *Bacillus* sp. NP5 to the male African catfish for reproduction has not been evaluated yet. Thus, this study aimed to evaluate the effect of dietary *Bacillus* sp. NP5 probiotic supplementation in the spermatogenesis and sperm quality of male African catfish. The effects of probiotic feeding on the spermatogenesis development, sperm quality, and the molecular transcription of the spermatogenesis-related genes were assessed. The results of this study could be beneficial to the reproductive performance improvement strategy in African catfish culture.

Material and Method

Preparation of probiotic feed. The probiotic strain used in this study was *Bacillus* sp. NP5 bacteria that were previously isolated from Nile tilapia (*Oreochromis niloticus*) digestive tract (Putra & Widanarni 2015). The isolates were maintained in our laboratory and stored at −80°C in 10% v/v of glycerol. Before the experiment, probiotic isolates were re-characterized using biochemical tests. The bacteria were recovered from freezing and cultured in 10 mL tryptic soy broth at 140 rpm for 18 hours at 29°C. The cells were harvested by centrifugation at 5000 rpm for 5 min, at 4°C. The bacteria cell pellet was suspended in sterile phosphate-buffered saline (PBS) and used immediately. The diluted bacteria were supplemented in the commercial feed containing 30% protein content as described previously by Ayuningtyas et al (2020). The probiotic was mixed with the feed with a dose of 1% (v/w) of the total diet. The probiotics were sprayed to the pellets with egg whites as the binder 3% (v/w). The supplemented diets were dried and moved in a dry and closed container, and then stored at 4°C. The preparation of fresh probiotic feed was conducted every morning before the feeding. The bacterial cell was measured using the total plate count method to obtain the desired concentration.

Fish rearing. The immature adult males of African catfish (*Sangkuriang* strain; avg. body weight 305.15±32.40 g) were obtained from the National Center for Freshwater Aquaculture, Sukabumi, Indonesia. Fish came from the same batch of mass spawning, thus having the same age. Fish were acclimatized for 1 week before the experiment in the fiber tanks. During acclimatization, fish were fed with commercial feed (30% protein), twice a day. After acclimatization, fish were randomly distributed into nine 1×1x1 m circular fiber tanks. Tanks were filled with a density of 12 fish per tank. For the experiment, fish were fed until satiation twice a day at 06.00 and 18.00 with the different dose of probiotic diet: 0 (control; using PBS), 10^6 CFU g^{-1}, and 10^8 CFU g^{-1}, as presented in Table 1. Fish were reared and fed with the probiotic diet for 60 days. The fish survival was determined at the end of the rearing. Water was changed once in four weeks, 30% of the total volume. During the rearing period, the water temperature ranged from 27.7 to 27.8°C, dissolved oxygen was between 4.53–5.05 mg L^{-1}, pH between 6.6–6.8, and ammonia between 0.02–0.03 mg L^{-1}. All water parameters were not significantly different among treatments during the rearing.

Table 1
Dietary supplementation treatment of probiotic *Bacillus* NP5 with different doses in male African catfish

Treatment code	*Probiotic dose*
C	0 CFU g^{-1} *Bacillus* sp. NP5
A	10^6 CFU g^{-1} *Bacillus* sp. NP5
B	10^8 CFU g^{-1} *Bacillus* sp. NP5

Note: each treatment was replicated three times.

Gonadosomatic index and gonad histology. The gonadosomatic index (GSI) was observed at 0 (D0), 30 (D30), and 60 (D60) days of rearing. The total fish weight was measured using a digital scale with 0.01 g accuracy. The fish were euthanized, and gonads were dissected and weighed (n=3 for each treatment replication). The GSI value was calculated by comparing the percentage of gonad weight with total body weight (Sulistyo et al 2000). The gonad histology was conducted as described by Utoh et al (2005). After preparation, the gonad tissue was stained with hematoxylin-eosin. The testis developmental stage scoring was assessed from the gonad histological samples following Çek & Yilmaz (2007).

Sperm quality. The evaluated sperm quality parameters were the milt volume, spermatocrit, sperm density, pH, and motility. The observation was performed at the end of the rearing period by sacrificing 3 fish from each treatment group. Since the male African catfish cannot be stripped, the milt was collected by dissecting the gonad tissues and mincing it carefully. The milt was carefully collected using a syringe to avoid the sheared tissues being taken. The fluid was transferred into a scaled microtube to measure its volume. The pH was determined using pH indicator papers with a range of 1-14. The spermatocrit level was calculated by distributing the milt into the micro-hematocrit tube until reaching the 4/5 part of the tube. The end of the tube was clogged and the hematocrit tube was centrifuged for 5 min at 8000 rpm. The spermatozoa density was determined using a hemocytometer. Milt was first diluted 10× using a non-activating solution (0.9% w/v NaCl) and observed in five observational fields under the microscope for each replication. Sperm motility level was determined under the microscope at 20×10 magnification. Two microliters of milt were placed on an object-glass, and then the activation was achieved by adding 5 µL of water onto the milt. The sperm motility was evaluated as the time elapsed from the activation until the sperm was nonmotile. Sperm motility evaluation was done at room temperature (25-26°C) immediately after milt collection.

Testosterone level. The blood testosterone concentration was measured at D0, D30, and D60 by the enzyme-linked immunosorbent assay (ELISA) method using the commercial testosterone ELISA kit (DRG EIA-1559, Germany). Briefly, fish were anesthetized and blood was collected from the fish caudal peduncle with a syringe rinsed with 3.8% sodium citrate. Blood was placed in a sterile microtube and centrifuged at 5000 rpm for 10 min at 4°C to separate the blood cells from the blood plasma. The blood plasma was placed into the new microtube and kept at −20°C before analysis. Blood testosterone level was measured using the ELx808™ absorbance reader (BioTek Instruments Inc, USA) at 450 nm.

Gene expression level. The fish gonad and pituitary were collected at 30 and 60 days of rearing from each group. The tissues were kept in Genezol™ (Genaid, Taiwan) reagent at −80°C before analysis. The total RNA was extracted from the tissues using the Genezol reagent following the product instruction. Total RNA concentration was measured at 260 and 280 nm using GeneQuant DNA calculator (Pharamecia Biotech, USA). The cDNA synthesis was performed from 50 ng μL^{-1} of total RNA using ReverTra Ace® qPCR-RT master mix (Toyobo, Japan). The mRNA expression analysis was performed using the quantitative real-time polymerase chain reaction (qPCR) method. The qPCR reaction was

conducted in the Rotor-Gene 6000 (Qiagen, USA) using the SensiFast SYBR kit (Bioline, UK) following the recommended procedure. The qPCR reaction consisted of 10 µL SYBR-mix, 0.8 µL of sense and anti-sense primer (10 mM), 4.4 µL sterile nuclease-free water, and 4 µL of 20 ng μL^{-1} cDNA. All preparation and molecular analyses were conducted using sterile-filtered micro-tips and microtubes (ExtraGene, Taiwan) to prevent contamination. The qPCR program was performed on the following cycles: 2 minutes at 95°C; 40 cycles of 95°C for 10 seconds, 60°C for 10 seconds, 72°C for 10 seconds and ended with melting curve analysis to evaluate the reaction specificity. The mRNA expression ratio was determined using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001) after being normalized by β-actin gene (*actb*). The genes examined were the genes related to spermatogenesis, namely, follicle-stimulating hormone subunit beta (*fshb*) and its receptor (*fshbr*), and luteinizing hormone subunit beta (*lhb*) and its receptor (*lhbr*). The qPCR primers were designed based on their mRNA sequence available at the gene bank using the Primer-Quest tool (www.idtdna.com/PrimerQuest/Home). The primers sequence is presented in Table 2.

Table 2

The qPCR primers used in the study

Primer name	*Sequence (5′ – 3′)*	*Application*	*Gene bank accession no. /Reference*
qCgFshb F	GTGAGTGCAGCCAGTGTAA	qPCR analysis of *fshb*	AF324541.1
qCgFshb R	CTGATGTGACTCGGTTCAGTAG		
qCgFshr F	GGAGCACTGTAAGGATGTGATAG	qPCR analysis of *fshr*	AJ012647.2
qCgFshr R	ATGAGGACACGCAGGAAAG		
qCgLhb F	CAGAGACGTCCGCTATGAAAC	qPCR analysis of *lhb*	KM258876.1
qCgLhb R	CAGACTGCACTCACAGCTTAG		
qCgLhbr F	CGTCATCTTCAGCACAGAGAA	qPCR analysis of *lhbr*	AF324540.1
qCgLhbr R	TGATCCAGCAGGTCAGAATAAG		
qCgActb F	ACCGGAGTCCATCACAATACCAGT	qPCR analysis of *actb*	Nasrullah et al (2021)
qCgActb R	GAGCTGCGTGTTGCCCCTGAG		

Statistical analysis. The gene expression levels were normalized against the *actb* gene and their levels were relative to the expression level of the control group. The gene expression data are presented as the log2 fold change to the expression at control (control expression=0). The GSI, sperm quality, testosterone level, and gene expression level data were tabulated and analyzed statistically using the SPSS 16.0 software (IBM, USA). The significant difference among treatment groups was identified using the one-way ANOVA test, followed by the Duncan posthoc test at α=5%.

Results and Discussion. The fish survival rate, body weight, and GSI value of catfish are presented in Table 3. At the end of rearing, B treatment showed a higher survival compared to control, while A treatment was not different. Fish from the B treatment also had the highest body weight after 30 and 60 days compared to the other treatments ($p<0.05$). In line with the bodyweight, the GSI of B treatment also had a higher values compared to the A treatment and control at 30 days of rearing. However, the GSI value from all treatments was not different at 60 days of rearing ($p>0.05$).

Table 3

The survival rate, body weight, and GSI value of male African catfish after fed with different dietary supplementation doses of probiotic *Bacillus* sp. NP5

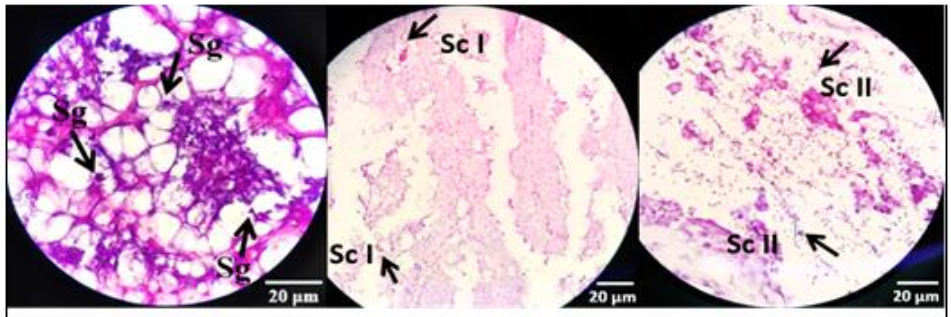
Code	Survival (%)	Bodyweight (g)			Gonad weight (g)			Gonadosomatic Index		
		D0	D30	D60	D0	D30	D60	D0	D30	D60
C	81±4.17[a]	234±21.21[a]	468±60.1[ab]	466.67±62.92[a]	1.5±0.93[a]	4.39±0.89[b]	2.65±0.75[a]	0.66±0.46[a]	0.95±0.22[b]	0.57±0.13[a]
A	92±11.79[ab]	279±2.83[a]	366.67±51.54[a]	525±100[a]	1.73±1.19[a]	1.66±0.43[a]	1.89±0.42[a]	0.62±0.43[a]	0.46±0.11[a]	0.37±0.1[a]
B	96±4.81[b]	228.5±43.13[a]	538.67±111.33[b]	733.33±194.19[a]	1.61±1.02[a]	4.46±1.74[b]	3.47±1.1[a]	0.67±0.32[a]	0.81±0.18[b]	0.48±0.09[a]

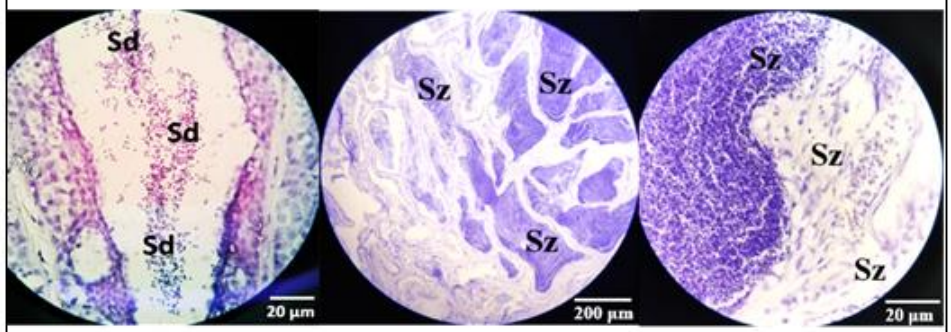
Note: values are presented as mean±SD (n=3). Different superscripts in the same column show significant differences ($p<0.05$).

Gonad development and histology. The gonad histology of male African catfish after feeding with dietary probiotics is presented in Figure 1. All the fish started with the same stage of spermatogenesis, with spermatogonia observed and absent spermatocyte. After 30 days of feeding, primary spermatocytes were detected in control fish, while in A-treatment secondary spermatocytes were already formed. The spermatocytes had already developed into spermatids in B-treatment after 30 days of feeding (Figure 1A). At D60, spermatozoa was already formed within all treatments, but at a different rate. B treatment had the fastest development, with 75-100% spermatozoa already formed, while only 25-49.9% formed in A treatment, and only 0.1-24.9% in control (Figure 1B). Scoring notes in Figure 1B followed Çek & Yilmaz (2007): 0 - spermatogonia stage, absent spermatogenesis; 1 - initial spermatogenesis, primary spermatocyte was formed; 2 - secondary spermatocyte was formed; 3 - cell was developed into spermatid; 4 - spermatozoa was formed, ranging from (a) 0.1-24.9%; (b) 25.0-49.9%; (c) 50.0-74.5%; (d) 75.0-100%.

Sperm quality. The sperm quality of male African catfish broodstock was measured after 60 days of feeding. The parameters observed are presented in Table 4. The results showed that B treatment had the highest sperm volume compared to other treatments ($p<0.05$). This result was also in line with the spermatocrit and sperm density. Fish fed with the higher dose of probiotic in B treatment had the highest spermatocrit value and sperm density compared to other treatments ($p<0.05$). The control treatment had relatively lower values compared to the probiotic treatments. The pH of the sperm ranged from 8 to 8.5 in all treatments. Sperm motility in control and A treatment was not significantly different ($p<0.05$). Sperm activity was the longest in B treatment compared to others ($p<0.05$).

A





B

Treatment group	Days after feeding		
	0	30	60
Control	0	1	4a*
A	0	2	4b*
B	0	3	4c*

Figure 1. The gonad histology (A) and gamet development scores (B) of male African catfish after treatment with different doses of probiotic *Bacillus* sp. NP5. Sg - spermatogonia; Sc I - primary spermatocyte; Sc II - secondary spermatocyte; Sd - spermatid; Sz - spermatozoa.

Table 4

The sperm quality of male African catfish after fed with different doses of probiotic *Bacillus* sp. NP5

Treatment	*Volume (mL)*	*Spermatocrit (%)*	*Density (× 10^9 cells mL $^{-1)}$*	*pH*	*Motility period (second)*
Control	0.19±0.07^a	57.84±4.31^a	2.28±0.51^a	8.0-8.5	226.00±58.00^a
A	0.26±0.01^a	68.33±1.67^b	4.73±0.77^b	8.0	393.00±279.00^a
B	1.62±0.13^b	79.05±5.38^c	6.42±1.11^c	8.0-8.5	778.67±167.26^b

Note: values are presented as mean ±SD (n=3). Different superscripts in the same column indicate significant differences ($p<0.05$).

Testosterone level. The blood testosterone level was measured at D0, D30, and D60 after the feeding treatment (Figure 2). The blood testosterone levels were increased after 30 days of rearing. However, all treatments had no significant difference in blood testosterone concentration at D0 and D30 ($p>0.05$). At D60, the blood testosterone level of the control was not significantly different from that of both the probiotics treatments. However, A treatment had lower testosterone compared to B treatment ($p<0.05$).

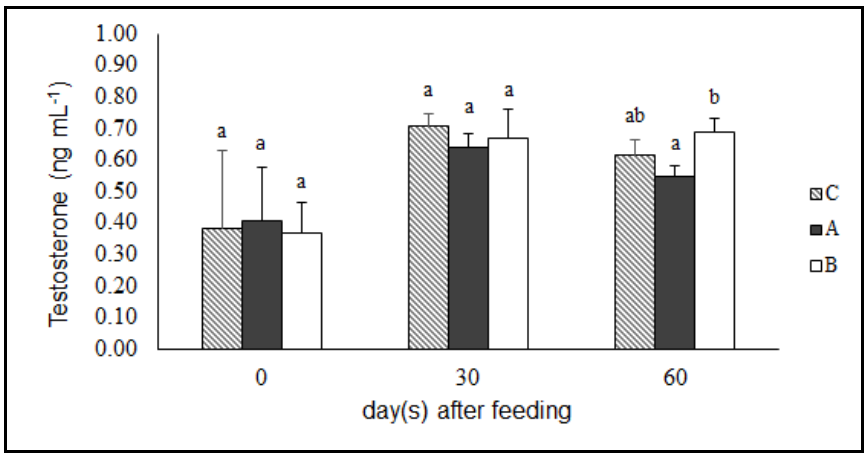


Figure 2. The blood testosterone concentration in male African catfish after fed with different doses of probiotic *Bacillus* sp. NP5.

Gene expression level. On day 30, the *lhb* mRNA expression in the fish gonad was significantly increased among all treatments compared to the D0. The probiotic treatments had higher *lhb* and *lhbr* expression compared to control at D30 ($p<0.05$). In contrast, the *fshb* expression of probiotic treatments was downregulated at D30, but its receptor expression in all treatments was upregulated in the fish gonad at D30. At D60 *lhb* and *lhbr* expression were decreased compared to the D30 in all treatments. However, the probiotic treatment still had the higher expression of *lhb* and *lhbr* compared to the control. Contrary to *lhb* and *lhbr*, significant *fshb* and *fshbr* up-regulation was observed at D60 compared to D30. At D60, probiotics treatments had significantly higher *fshb* and *fshbr* expression compared to the control treatment ($p<0.05$), and no difference in expression was found between the probiotic treatments ($p>0.05$).

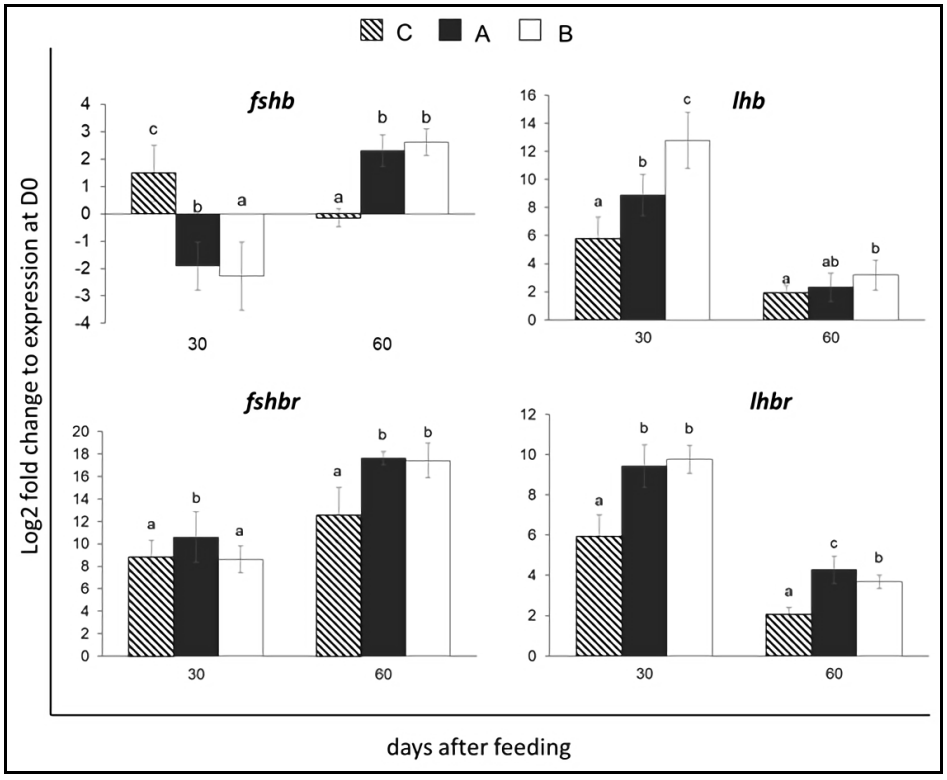


Figure 3. The mRNA expressions of *fshb*, *lhb,* and their receptor genes in the male African catfish after fed with different doses of probiotic *Bacillus* sp. NP5. Data are presented as mean log 2 fold change ±SD to the expression at D0 (n=3).

Aside from the immune responses and growth of several fish species, our previous study showed that the administration of *Bacillus* sp. NP5 through feed was able to enhance reproductive parameters of female goldfish (unpublished) and African catfish (Ayuningtyas et al 2020). The GSI value, fecundity, and the number of matured eggs of female African catfish were significantly increased and higher than in the control after six weeks of administration of 10^8 and 10^{10} CFU g^{-1} *Bacillus* sp. NP5 (Ayuningtyas et al 2020). The effects of the probiotic in the male African catfish reproduction have not yet been reported, and were evaluated in this study. This study used lower doses, 10^6 CFU g^{-1}, and 10^8 CFU g^{-1}, compared to the previous study in female African catfish.

The results showed that after 30 and 60 days, fish fed with the dose of 10^8 CFU g^{-1} *Bacillus* sp. NP5 had the significantly highest bodyweight compared to the 10^6 CFU g^{-1} dose and control (Table 3). This result was not unexpected since the previous study also reported that the administration of high doses of probiotic *Bacillus* sp. NP5 promoted growth in African catfish (Putra et al 2020, 2021) and Nile tilapia (Utami et al 2015). The gonad weight did also not increase significantly in probiotics treatment, not being higher than in the control (Table 3). However, the GSI of all treatments was not significantly different because the bodyweight had a significantly higher growth rate compared to the gonad at the corresponding treatments (Table 3).

Vertebrate spermatogenesis can be divided into four phases: mitotic stem cell renewal, mitotic spermatogonia and supporting cell proliferation, meiotic germ cell division, and germ cell maturation (spermiogenesis) into fully functional spermatozoa (Campbell et al 2003; Yoshida 2016). Spermatogenesis in fish is mainly stimulated by gonadotropin hormones such as LH and FSH and followed by sex steroid hormone production such as testosterone and 11-ketotestosterone (11-KT) (Otha et al 2007; Schulz et al 2008). The FSH regulates Sertoli cell activities and promotes germ cell survival, development, and proliferation, while LH (and also FSH) mainly induce the androgen hormone production in the gonad by activating Leydig cells (Schulz et al 2008; Ramaswamy & Weinbauer 2015). In this study, the administration of the dietary probiotic resulted in the faster meiotic division of germ cells in the male African fish. After 30 days of feeding, fish fed with 10^6 CFU g^{-1} probiotics *Bacillus* sp. NP5 had reached secondary spermatocytes, while control was still in the primary spermatocytes stage (Figure 1A). In the higher probiotics dose of 10^8 CFU g^{-1}, the spermatocytes had already begun to develop into spermatid after 30 days of feeding (Figure 1A). Spermiogenesis observed at 60 days after feeding in the probiotics treatment was also faster than control (Figure 1B). The acceleration of the spermatogenesis process might be due to the ability of probiotics to enhance the transcription of *fsh* and *lh* gene in the pituitary.

The level of *lhb* mRNA transcripts was significantly higher in the pituitary at D30 and D60 compared to control, and the *fshb was* also significantly expressed in the probiotics treatment at the spermiogenesis stage at D60 (Figure 3). High expression of *lhb* and *fshb* also leads to the modulation of their receptors in the fish gonad (Figure 3). Interestingly, instead of the high transcript expression of *fshb* and *lhb,* the blood testosterone level between treatments was not different at all time points (Figure 2). These results are previously described by Cavaco et al (2001). In African catfish, 11-ketotestosterone, but not testosterone, stimulated the male spermatogenesis process, and the 11-KT modulation was triggered by the levels of FSH and LH hormones. Different from African catfish, in male European eel, probiotics treatment enhanced the 20β-dihydroxy-4-pregnen-3-one (DHP) levels and not 11-KT, thus leading to accelerated spermatogenesis and better sperm quality (Vílchez et al 2015). However, the concentration of DHP and 11-KT was not measured in this study. Further research is also needed to assess the correlation of *fshb* and *lhb* mRNA transcripts with the circulated FSH and LH hormone.

The higher level of gonadotropin expression and androgen hormones induction by dietary probiotics feeding also resulted in the enhancement of the sperm quality in African catfish (Table 4). Spermatocrit, volume, density, and motility was significantly increased in the probiotic treatments, with the highest levels achieved by the 10^8 CFU g^{-1} probiotics *Bacillus* sp. NP5 treatment (Table 4). The ability of probiotics in accelerating fish spermatogenesis and sperm quality has been previously reported for several fish

species. The dietary feeding of probiotics *B. subtillis* and *B. cereus* in silver catfish *Rhamdia quelen* for 90 days affected the seminal volume, sperm viability, gonadosomatic and hepatosomatic indexes (Rodrigues et al 2020). In the male European eel *Anguilla anguilla*, dietary probiotic treatment with *Lactobacillus rhamnosus* IMC 501 for two weeks increased the mRNA transcription of *fshr* and androgen receptors α and β, thus increasing sperm volume, density, progressive motility, and accelerating the fish spermatogenesis (Vílchez et al 2015).

Conclusions. This study showed that the dietary probiotic supplementation of *Bacillus* sp. NP5 could accelerate the spermatogenesis processes in the male African catfish and improve its sperm quality. Thus, the dietary supplementation of 10^8 CFU g^{-1} probiotic *Bacillus* sp. NP5 could be recommended as the starting point for the improvement procedure of the reproductive performance in the male African catfish.

Acknowledgements. This work was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia under grant No. 261/SP2H/LT/DRPM/2019 for VE and MZ. The authors also thank Mrs. Lina Mulyani, Ms. Dian Novita Sari, Ms. Rani Ria Rizki, Ms. Siska Aliyas Sandra, Mr. Aldi Mulyadin, Mr. Eka Kusuma, Mr. Agil Setya Utomo, Mr. Khairul Samuki, and Mr. Dedi Supriadi for their excellent help for this study.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

Adamek J., Kamler E., Epler P., 2011 Uniform maternal age/size and light restrictions mitigate cannibalism in *Clarias gariepinus* larvae and juveniles reared under production-like controlled conditions. Aquacultural Engineering 45(1):13-19.

Ayuningtyas S. Q., Zairin M. Jr., Widanarni, 2020 Reproductive performance of catfish *Clarias* sp. with probiotics *Bacillus* sp. NP5 addition through feed. Jurnal Akuakultur Indonesia 19(1):74-83.

Bertha P. D., Zairin M. Jr., Soelistyowati D. T., 2016 The spermatogenesis process of male catfish *Clarias* sp. fed with dietary purwoceng extract. Jurnal Akuakultur Indonesia 15(1):49-55.

Campbell B., Dickey J. T, Swanson P., 2003 Endocrine changes during onset of puberty in male spring Chinook salmon, *Oncorhynchus tshawytscha*. Biology of Reproduction 69(6):2109-2117.

Cavaco J. E. B., Bogerd J., Goos H., Schulz R. W., 2001 Testosterone inhibits 11-ketotestosterone-induced spermatogenesis in African catfish (*Clarias gariepinus*). Biology of Reproduction 65(6):1807-1812.

Çek Ş., Yilmaz E., 2007 Gonad development and sex ratio of sharptooth catfish *Clarias gariepinus* cultured under laboratory conditions. Turkish Journal of Zoology 31(1):35-46.

Chauhan A., Singh R., 2018 Probiotics in aquaculture: a promising emerging alternative approach. Symbiosis 77:99-113.

Fuller R., 1989 Probiotics in man and animals. Journal of Applied Bacteriology 66(5):365-378.

Giannenas I., Karamaligas I., Margaroni M., Pappas I., Mayer E., Encarnacao P., Karagouni E., 2015 Effect of dietary incorporation of a multi-strain probiotic on growth performance and health status in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology & Biochemistry 41(1):119-128.

Gioacchini G., Maradonna F., Lombardo F., Bizzaro D., Olivotto I., Carnevali O., 2010 Increase of fecundity by probiotic administration in zebrafish (*Danio rerio*). Reproduction 140(6):953-959.

Liu H., Wang S., Cai Y., Guo X., Cao Z., Zhang Y., Xie Z., 2017 Dietary administration of *Bacillus subtilis* HAINUP40 enhances growth, digestive enzyme activities, innate

immune responses and disease resistance of tilapia, *Oreochromis niloticus*. Fish & Shellfish Immunology 60:326-333.
Livak K. J., Schmittgen T. D., 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25(4):402-408.
Mehdinejad N., Imanpour M. R., Jafari V., 2018 Combined or individual effects of dietary probiotic, *Pediococcus acidilactici* and nucleotide on reproductive performance in goldfish (*Carassius auratus*). Probiotics and Antimicrobial Proteins 11(1):233-238.
Nasrullah H., Yanti D. H., Faridah N., Hardiantho D., Nababan Y. I., Sukenda, Alimuddin, 2021 Early immune gene development and expression in African catfish *Clarias gariepinus* after challenged with *Aeromonas hydrophila*. Aquaculture International 29(2):595-607.
Ohta T., Miyake H., Miura C., Kamei H., Katsumi A., Takeshi M., 2007 Follicle stimulating hormone induces spermatogenesis mediated by androgen production in Japanese eel, *Anguilla japonica*. Biology of Reproduction 77(6):970-977.
Padmavathi P., Sunitha K., Veeraiah K., 2012 Efficacy of probiotics in improving water quality and bacterial flora in fish ponds. African Journal of Microbiology Research 6(49):7471-7478.
Putra A. N., Mustahal, Syamsunarno M. B., 2020 Effects of dietary probiotic *Bacillus* NP5 on the growth performances of catfish (*Clarias* sp.). BIOTROPIA 27(1):51-59.
Putra A. N., Mustahal, Syamsunarno M. B., Hermawan D., Fatimah D. G., Putri P. B, Sevia, Isnaini R., Herjayanto M., 2021 Dietary *Bacillus* NP5 supplement impacts on growth, nutrient digestibility, immune response, and resistance to *Aeromonas hydrophila* infection of African Catfish, *Clarias gariepinus*. Biodiversitas 22(1):253-261.
Putra A. N., Widanarni, 2015 Screening of amylolytic bacteria as candidates of probiotics in tilapia (*Oreochromis* sp.). Research Journal of Microbiology 10(1):1-13.
Ramaswamy S., Weinbauer G. F., 2015 Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. Spermatogenesis 4(2):e996025, 15 p.
Ramzani S. R., Ismail M. M., Daud H. M., Abdurofi I., 2014 Probiotic application in freshwater prawns; some implication on farm profitability. Annals of Biological Research 5(5):64-76.
Rodrigues M. L., Damasceno D. Z., Gomes R. L M., Sosa B. D. S., Moro E. B, Sanches E. A.,Bittencourt F., Signor A., 2020 Reproductive physiology of *Rhamdia quelen* is improved by dietary inclusion of probiotics. Aquaculture Research 52(4):1677-1687.
Schulz R. W., Liemburg M., García-López Á., van Dijk W., Bogerd J., 2008 Androgens modulate testicular androgen production in African catfish *Clarias gariepinus* depending on the stage of maturity and type of androgen. General and Comparative Endocrinology 156:154-163.
Sulistyo I., Fontaine P., Rincarh J., Gardeur J. N., Migaud H., Capdeville B., Kestemont P., 2000 Reproductive cycle and plasma level of steroid in male Eurasian perch (*Perca fluviatilis*). Journal of Aquatic Resource 13(2):99-106.
Utami D. A., Widanarni, Suprayudi M. A., 2015 Quality of dried *Bacillus* NP5 and its effect on growth performance of tilapia (*Oreochromis niloticus*). Pakistan Journal of Biological Sciences 18(2):88-93.
Utoh T., Horie N., Mikawa N., Okamura A., Yamada Y., Akazawa A., Tanaka S., Oka H. P., 2005 Annual change in ovarian development and plasma estradiol-17β level in reared female common Japanese conger, *Conger myriaster*. Fisheries Science 71(1):38-47.
Vílchez M. C., Santangeli S., Maradonna F., Gioacchini G., Verdenelli C., Gallego V., Peñaranda D. S., Tveiten H., Pérez L., Carnevali O., Asturiano J. F., 2015 Effect of the probiotic *Lactobacillus rhamnosus* on the expression of genes involved in European eel spermatogenesis. Theriogenology 84(8):1321-1331.
Widyastuti Y. R., Setiadi E., Taufik I., Setijaningsih L., 2019 Water quality dynamic, production and profitability of catfish, *Clarias* sp. cultured at different design

construction of aquaponic. IOP Conference Series: Earth and Environmental Science 236:012028, 11 p.
Yoshida S., 2016 From cyst to tubule: innovations in vertebrate spermatogenesis. Wiley Interdisciplinary Reviews: Developmental Biology 5(1):119-131.
*** FAO (Food and Agricultural Organization of the United Nations), 2016 Cultured aquatic species information programme: *Clarias gariepinus*. Fisheries and Aquaculture Department, Food and Agricultural Organization of the United Nations, Rome.
*** Indonesian Ministry of Marine and Fisheries/Kementrian Kelautan dan Perikanan (KKP), 2020 [Strategical plans in 2020-2024]. General Directorate of Aquaculture Fisheries, Jakarta, Indonesia. [In Indonesian].
*** Integrated DNA Technologies (IDT). Available at: www.idtdna.com/PrimerQuest/Home.

Received: 05 March 2021. Accepted: 25 July 2021. Published online: 11 February 2022.
Authors:
Valensia Enzeline, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: venzeline@gmail.com
Hasan Nasrullah, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: nasrullah.ipb@gmail.com
Agus Oman Sudrajat, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: agusom@apps.ipb.ac.id
Muhammad Zairin Junior, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: zairinmz@live.com
Alimuddin Alimuddin, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: alimuddin@apps.ipb.ac.id
Widanarni Widanarni, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University), Raya Agatis St., Dramaga, Bogor, 16680 West Java, Indonesia, e-mail: widanarni@apps.ipb.ac.id

How to cite this article:
Enzeline V., Nasrullah H., Sudrajat A. O., Zairin M. Jr., Alimuddin A., Widanarni W., 2022 Spermatogenesis and sperm quality of male African catfish fed with *Bacillus* sp. NP5 probiotic supplemented diet. AACL Bioflux 15(1):339-349.