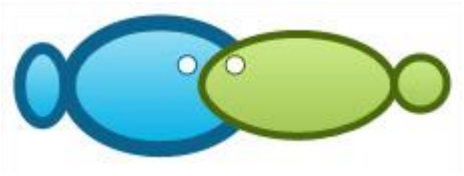

The effect of different diets on the quality of sperm in striped catfish (*Pangasianodon hypophthalmus*)

Wahyu Pamungkas, Jadmiko Darmawan, Ikhsan Khasani

Research Center for Fishery, National Research and Innovation Agency, Cibinong, Bogor, West Java, Indonesia. Corresponding author: W. Pamungkas, yhoe_pamungkas@yahoo.co.id

Abstract. Sperm quality is crucial to the success of fish breeding in captivity. The present study evaluated the effects of different diets on sperm quality in the striped catfish (*Pangasianodon hypophthalmus*) during a 3-month feeding trial. 30 striped catfish were divided evenly between the two groups (A and B). Group A was fed a commercial diet, while group B was fed a formulated diet containing essential fatty acids. 15 males with a body weight between 2.59-3.09 kg for each group were used as experimental fish. The motility and velocity of the catfish sperm, fertilization, and hatching rate were examined to evaluate sperm quality and its association with fertilization and hatching rate. The analysis of sperm motility and velocity has been performed using computer-assisted sperm analysis (CASA). The research data were analyzed by an independent sample T-test. Group A significantly differed from group B in terms of motility, progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), and hatching rate (HR). These findings imply that essential fatty acid rich diets improve the sperm quality of striped catfish.
Key Words: CASA, essential fatty acid, male, motility, velocity.

Introduction. The striped catfish (*Pangasianodon hypophthalmus*) is one of the most rapidly developing freshwater species in aquaculture. According to the Ministry of Marine Affairs and Fisheries, Republic of Indonesia (MMAF 2013), the national production of striped catfish in 2013 was 410383 tons, and the increase in striped catfish production from 2010 to 2013 reached 95.57%. Furthermore, according to the Ministry (MMAF 2016), total production reached 418002 tons in 2014, but declined by 18.8% to 339111 tons in 2015. In 2017, there was another decline in production by 18.67% of the total production in 2016 (392918 tons). Ineffective brood stock management may have contributed to the drop in fish production. Problems in aquaculture activities contributed to the decline in total production. Therefore, anticipating these issues is necessary to ensure the continued availability of high-quality juveniles.

The availability of good quality juveniles is related to the availability of superior brood stock quality. Improving the quality of broodstock can be done through a genetic approach and nutrition. The nutrients contained in broodstock diets will affect the quality of sperm and eggs produced, and in the end, they will also affect the quality of the juveniles produced. According to Izquierdo et al (2001), good nutrition improves not only the quality of sperm and egg cells, but also the quality and quantity of juveniles. Few studies have been able to directly link the nutrition of broodstock to spermatozoa kinetics, even though numerous studies have linked diet to reproductive success. Sperm concentration, motility, viability, morphology, metabolic activity, and ability to fertilize an egg are important factors that must be considered in determining reproductive performance (Cabrita et al 2014). Meanwhile, temperature, season, stress, hormone stimulation, and parent nutrition are factors that affect sperm quality (Alavi et al 2008).

In the case of broodstock nutrition, the fatty acid composition of the broodstock diet has been discovered to be a primary dietary component that affects fish sperm quality, owing to carnivorous fish being unable to manufacture specific fatty acids, hence needing

them in their feed. In general, both freshwater and marine species require PUFA or HUFA in their diets (Izquierdo et al 2001). Adding these fatty acids to broodstock diets can result in significant improvements in sperm motility indices. Beirao et al (2015) discovered that a DHA-enriched diet improved sperm quality in Senegalese sole (*Solea senegalensis*), specifically the sperm velocity (VCL) and percentage of progressive sperm. In European eels (*Anguilla anguilla*), diets high in arachidonic acid (ARA) generated medium milt volumes and high sperm motility (Baeza et al 2015; Butts et al 2015), whereas diets high in eicosapentaenoic acid (EPA) induced noteworthy milt volumes and high sperm motility. In freshwater fish, such as rainbow trout (*Oncorhynchus mykiss*), some breeders fed a diet deficient in essential fatty acids (n-3) had lower sperm motility than breeders fed a control diet (Vassallo-Agius et al 2001), while fish fed a proper HUFA/PUFA ratio had the highest sperm motility percentage and duration than other treatments in another rainbow trout trial (Hajiahmadian et al 2016).

The quality of sperm is critical to the effectiveness of farmed fish spawning. In practice, any quantitative indicator that is directly connected to fertilization capacity can be used to estimate sperm quality. Spermatozoa motility is the most often used parameter in determining sperm quality (Kime et al 2001). The motility and velocity of spermatozoa, as evaluated by computer-assisted sperm analysis (CASA), are frequently used to link male gamete quality to fertilization potential. Spermatozoa with higher velocity and motility have a shorter window of time to reach the micropyle, which is important for fish that spawn in highly competitive situations. Evaluation of sperm motility as well as other kinetic characteristics such as curvilinear, straight-line, and average path velocities, as well as morphology, are important aspects of determining sperm quality in many fish species (Gallego et al 2013). This experiment aimed to investigate the impact of different diets on the sperm quality of striped catfish.

Material and Method. The research was carried out at the Research Institute for Fish Breeding (RIFB), Ministry of Marine Affairs and Fisheries, West Java, Indonesia. The striped catfish were acquired from the institute's broodstock population.

Experimental design. The fish were separated into two groups of 15 fish each (groups A and B) and subjected to the following experimental protocol. A formulated diet containing essential fatty acids was provided to group A, while a commercial feed was given to group B. The n-6 fatty acid source in the formulated feed was 2% corn oil, while the n-3 fatty acid source was 1.5% fish oil (Table 1) (Pamungkas et al 2020). Proximate analysis of the diet was carried out according to AOAC (2005). Total lipid was determined using the method of Folch et al (1957). Fatty acid analysis of the diet was carried out using Gas-Liquid Chromatography (Mazurek et al 2017). The proximate composition of the experimental diets is presented in Table 2. The fatty acid composition of the diets is presented in Table 3.

Table 1

Feed formulation of the striped catfish diet during the experiment

Ingredient	*%*
Fish meal	50.76
Soybean meal	18.60
Fish oil	1.50
Corn oil	2.00
Coconut oil	6.96
Wheat flour	11.68
Tapioca	5.70
Premix	2.00
Choline chloride	0.50
CMC[1]	0.30

Note: CMC - carboxymethyl cellulose.

Striped catfish rearing. Thirty males (bodyweight 2.84±0.25 kg) and thirty females (bodyweight 2.5–4.0 kg) of striped catfish were used as experimental models. The experimental fish were from the Research Institute for Fish Breeding (RIFB), Subang, West Java, Indonesia. Fish were acclimated to experimental conditions for 2 weeks before treatment after being selected based on their gonad maturity (no eggs and no sperm). Fish were kept in six net cages measuring 3x5x1.5 m, located in a 6000 m^2 earthen pond. Each net cage was stocked with 10 fish, consisting of 5 females and 5 males, and the fish were cultured for 3 months. During the experiment, fish were fed commercial or formulated feeds according to the treatment, at 3% of their body weight (BW) twice a day. The experimental catfish were tagged using a microchip. The gonadal maturity stage of the experimental catfish was observed every two weeks. Fish with mature gonads, both males and females, were spawned to measure the rate of fertilization, hatching, and larval production.

Table 2

Proximate composition of the experimental diet (%)

Proximate (%)	*Experimental diet*	
	A (Formulated)	*B (Commercial)*
Protein	36.80±0.60[a]	35.80±0.01[b]
Fat	12.70±0.01[a]	4.90±0.16[b]
Fiber	2.99±0.02[a]	2.50±0.48[a]
Ash	16.40±0.02[a]	7.80±0.05[b]
NFE	31.12±0.61[a]	49.10±0.70[b]
Energy (Kcal kg^{-1} feed)	455.620	449.780
Energy/protein	12.370	12.580

Note: values (means ± SD, n=3) in the same row with different superscripts show significant differences ($p<0.05$); NFE - nitrogen-free extract.

Table 3

The fatty acid composition of the experimental diets (%)

Fatty acid	*Commercial diet*	*Formulated diet*
Σn−3 fatty acid (%)	10.10±0.02[a]	2.60±0.05[b]
Σn−6 fatty acid (%)	4.50±0.04[a]	13.80±0.08[b]
Σn−9 fatty acid (%)	47.80±0.04[a]	22.20±0.01[b]
ΣSaturated fatty acid (%)	53.40±0.03[a]	59.30±0.02[b]
ΣUnsaturated fatty acid (%)	46.80±0.04[a]	42.50±0.06[b]
n−6/n−3 ratio	0.45±0.00[a]	5.40±0.12[b]

Note: values (means ± SD, n=3) in the same row with different superscript letters show significant differences ($p<0.05$).

Sperm collection. Milt from fish was collected by abdominal massage into scaled-marked glass beakers whenever it was present. The milt was stripped in such a way that it did not become contaminated with blood, feces, or urine. During stripping, no anesthetic was utilized. Until further investigation, sperm samples were stored in insulated boxes (4°C) without direct contact with ice.

Measurement of sperm motility. Motility parameters were evaluated using CASA (CEROS II Hamilton-Thorne) connected to a CX41 microscope (Olympus) after 10 s of activation with a digital camera (U-TV1X- 2). The motility variables measured included the percentage of motility and progressive motility, non-progressive static motility, VCL, straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), and straightness (STR). Measurements were performed in triplicate, and the average result was used in the data analyses. Sperm with a velocity of ≥20 μm s^{-1} was defined as motile.

CASA assessment. Semen was diluted and placed in a Leja slide that had been conditioned to 37°C. The CASA used is a Sperm Class Analyzer (SCA) 5.2 Micro-optics (Spain). The CASA settings were as follows: pH 1 contrast phase, 10x10 magnification,

and a green filter on the reflector mirror. The light intensity was adjusted to meet the standards and printed on the monitor screen. The pictures were taken in five different fields of view, and the results were displayed in Microsoft Excel.

Fertilization and hatching rates. Fertilization and hatching rate values were measured to assess the sperm's ability to fertilize eggs and produce offspring. Fertilization and hatching rates were calculated using the following equations:

Fertilization rate (%) = (Number of fertilized eggs /Total number of eggs) × 100 (Tilahun et al 2016)

Hatching rate (%) = (Number of hatched eggs /Total number of fertilized eggs) × 100 (Hanjavanit et al 2008)

Data analysis. The data on motility, velocity, fertilization rate, and hatching rate were statistically analyzed using Microsoft Excel 2016 and the SPSS program (ver. 25). The independent sample t-test was used to examine the data.

Results

Sperm motility. The results of this study revealed that the percentages of motility and progressive motility in group A were significantly different from those in group B, but the percentages of non-progressive motility were not (Table 4). Group A had a higher percentage of motility (96±1.67%) and progressive motility (32.1±5.9%) than group B (77.32±5.54% motility; 12.35±2.63% progressive motility).

Table 4

Sperm motility of striped catfish (*Pangasianodon hypophthalmus*) fed experimental diets

Parameter	*Experimental diet*	
	A (Formulated)	*B (Commercial)*
Motility (%)	96±1.67^{a}	77.32±5.54^{b}
Progressive motility (%)	32.1±5.9^{a}	12.35±2.63^{b}
Non-progressive motility (%)	63.9±4.41^{a}	64.97±4.99^{a}
Static (%)	4±4.11^{a}	22.68±13.58^{b}

Note: mean values in the same row with different superscripts show significant differences between the groups ($p<0.05$).

Sperm velocity. The sperm velocity including VCL, VSL, and VAP in group B was significantly different from group A (Table 5). The VCL (58.87±4.33 μm s^{-1}), VSL (34.30±2.80 μm s^{-1}), and VAP (45.17±2.93 μm s^{-1}) values in group B were higher than in group A (36.85±4.47 μm s^{-1} VCL, 23.28±1.69 μm s^{-1} VSL, and 29.70±2.84 μm s^{-1} VAP). The percentage of LIN and STR in group A were not significantly different from group B.

Table 5

Sperm velocity, linearity, and straightness of striped catfish (*Pangasianodon hypophthalmus*) fed experimental diets

Parameter	*Experimental diet*	
	A (Formulated)	*B (Commercial)*
VCL (μm s^{-1})	58.87±4.33^{a}	36.85±4.47^{b}
VSL (μm s^{-1})	34.30±2.80^{a}	23.28±1.69^{b}
VAP (μm s^{-1})	45.17±2.93^{a}	29.70±2.84^{b}
LIN (%)	58.10±1.33^{a}	65.00±3.36^{a}
STR (%)	75.42±1.68^{a}	79.30±2.30^{a}

Note: VCL - curvilinear velocity; VSL - straight line velocity; VAP - ; average path velocity; LIN - linearity; STR - straightness; mean values in the same row with different superscripts show significant differences between the groups ($p<0.05$).

Fertilization and hatching rate. Observation data for fertilization and hatching rate are presented in Table 6. The hatching rate in group A was significantly different from that of group B, but the percentage of fertilization was not significantly different between the groups. The value of the hatching rate (87.47±4.29%) in group B was higher than in group A (57.58±7.36%).

Table 6

Fertilization (FR) and hatching rate (HR)

Parameter	*Experimental diet*	
	A (Formulated)	*B (Commercial)*
FR (%)	86.66±4.77[a]	76.11±4.61[a]
HR (%)	87.47±4.29[a]	57.58±7.36[b]

Note: mean values in the same row with different superscripts show significant differences between the groups ($p<0.05$).

Discussion. Previous research on broodstock nutrition has primarily focused on female fish. However, few studies using male brood fish show the importance of optimizing the dietary needs of both male and female brood fish (Butts et al 2015; Hajiahmadian et al 2016). As a result, the current study investigated how different diets high in essential fatty acids affected sperm quality and reproductive performance in striped catfish.

For viable offspring, the quality of fish sperm is just as important as the quality of female eggs. Sperm morphology, density, volume, motility, and fertilizing capacity, as well as seminal plasma composition and osmolality, are common parameters used to assess sperm quality in fish (Billard & Cosson 1992; Lahnsteiner et al 1998; Rurangwa et al 2004; Alavi & Cosson 2005; Hanjavanit et al 2008; Bobe & Labbe 2010). Among the various semen quality biomarkers in fish, motility is now the most commonly used parameter (Gallego et al 2019), and it is highly correlated with fertilization success in several fish species (Gallego et al 2018).

Sperm velocities are also reliable indicators of sperm quality (Fauvel et al 2010) and are important in determining male fertility (Gage et al 2004; Lahnsteiner et al 1998; Rurangwa et al 2004). These have been linked to hatching rates and sperm motility in different fish species, such as African catfish (*Clarias gariepinus*) (Rurangwa et al 2001), turbot (*Psetta maxima*) (Dreanno et al 1999), carp (*Cyprinus carpio*) (Kaspar et al 2008a, b; Linhart et al 2000), and rainbow trout (Linhart et al 2000).

In this study, striped catfish sperm motility and velocity were affected by formulated diets containing essential fatty acids. Higher sperm motility and velocity values in catfish-fed formulated diets indicated a better effect (group B). According to Izquierdo et al (2001), the lipid content and fatty acid profile in brood fish diets are critical in determining successful reproduction and early-stage development. The dietary profile of sperm influences its FA profile (Bell et al 1997; Koprucu et al 2015; Hajiahmadian et al 2016). Lipids are the most essential substance of teleost spermatozoa (Lahnsteiner et al 1993a; Lahnsteiner et al 1994; Bell et al 1997; Dziewulska et al 2008). The functionality of spermatozoa is closely related to its fatty acid profile (Koprucu et al 2015). Fatty acid oxidation in the mitochondria of sperm cells produces energy, which is crucial for their survival and movement (Koprucu et al 2015). Thus, fatty acids are the primary energy sources in fish spermatozoa and are critical to sperm viability (Lahnsteiner et al 1993b; Lahnsteiner et al 2009).

Apart from the above, reproductive performance is the most reliable way of determining gamete quality (Izquierdo et al 2001; Rurangwa et al 2004). Fertilization is one method for determining the quality of sperm (Bobe & Labbe 2010). Fatty acids content in sperm, which is affected by broodstock dietary lipids, has been shown to affect sperm quality, regulate its ability to fertilize eggs successfully, and increase the hatching rate (Lahnsteiner et al 2009; Henrotte et al 2010; Beirao et al 2012). In this study, different diets influenced the value of fertilization and hatching rate in striped catfish. Fertilization and hatching rate values describe the ability of the sperm to fertilize eggs and produce

offspring. According to the findings of the study, dietary fatty acids in brood fish nutrition are also very important to be considered for determining male reproductive performance.

Good nutrition in the broodstock diet will improve the broodstock's reproductive performance, allowing it to produce large amounts of high-quality juveniles. The availability of high-quality brood stock and juveniles will achieve sustainable aquaculture.

Conclusions. The different diets affected the sperm quality of the striped catfish. Formulated diets containing essential fatty acids improved sperm motility and velocity in striped catfish. The high motility and velocity of fish sperm increased fertilization and hatching rates.

Acknowledgements. The Research Institute for Fish Breeding in West Java, Indonesia, provided funding for this study. The Agency for Marine and Fisheries Research and Human Resources, Ministry of Marine Affairs and Fisheries, Republic of Indonesia, also contributed to the study. We also appreciate the valuable technical assistance provided by the Research Institute for Fish Breeding's catfish commodity team.

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

References

Alavi S. M. H., Cosson J., 2005 Sperm motility in fishes. I. Effects of temperature and pH: a review. Cell Biology International 29(2):101-110.

Alavi S. M. H., Psenicka M., Policar T., Rodina M., Kozak P., Linhart O., 2008 Sperm characteristics in *Barbus barbus* as a function of nutrition throughout the reproductive season. Cybium 32(2):200-201

Baeza R., Mazzeo I., Vilchez M. C., Gallego V., Penaranda D. S., Perez L., Asturiano J. F., 2015 Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver, and testis of European Eel. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 181:79-86.

Beirao J., Soares F., Pousao-Ferreira P., Diogo P., Dias J., Dinis M. T., Herraez M. P., Cabrita E., 2015 The effect of enriched diets on *Solea senegalensis* sperm quality. Aquaculture 435:187-194.

Beirao J., Zilli L., Vilella S., Cabrita E., Fernandez-Díez C., Schiavone R., Herraez M. P., 2012 Fatty acid composition of the head membrane and flagella affects *Sparus aurata* sperm quality. Journal of Applied Ichthyology 28(6):1017-1019.

Bell M. V., Dick J. R., Buda C., 1997 Molecular speciation of fish sperm phospholipids: Large amounts of dipolyunsaturated phosphatidylserine. Lipids 32(10):1085-1091.

Billard R., Cosson J., 1992 Some problems related to the assessment of sperm motility in freshwater fish. Journal of Experimental Zoology 261(1):122-131.

Bobe J., Labbe C., 2010 Egg and sperm quality in fish. General and Comparative Endocrinology 165(3):535-548.

Butts I. A. E., Baeza R., Strotrup J. G., Kruger-Johnsen M., Jacobsen C., Perez L., Asturiano J. F., Tomkiewicz J., 2015 Impact of dietary fatty acids on muscle composition, liver lipids, milt composition and sperm performance in European eel. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 183:87-96.

Cabrita E., Martinez-Paramo S., Gavaia P. J., Riesco M. F., Valcarce D. G., Sarasquete C., Herraez M. P., Robles V., 2014 Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture 432:389-401.

Dreanno C., Cosson J., Suquet M., Seguin F., Dorange G., Billard R., 1999 Nucleotide content, oxidative phosphorylation, morphology, and fertilizing capacity of turbot (*Psetta maxima*) spermatozoa during the motility period. Molecular Reproduction and Development 53(2):230-243.

Dziewulska K., Rzemieniecki A., Domagała J., 2008 Basic physico-chemical parameters of milt from sea trout (*Salmo trutta* m. *trutta*), brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). Journal of Applied Ichthyology 24(4):497-502.

Fauvel C., Suquet M., Cosson J., 2010 Evaluation of fish sperm quality. Journal of Applied Ichthyology 26(5):636-643.
Folch J., Lees M., Stanley G. H. S., 1957 A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry 226:497-509.
Gage M. J. G., Macfarlane C. P., Yeates S., Ward R. G., Searle J. B., Parker G. A., 2004 Spermatozoal traits and sperm competition in Atlantic salmon: Relative sperm velocity is the primary determinant of fertilization success. Current Biology 14(1):44-47.
Gallego V., Asturiano J. F., 2019 Fish sperm motility assessment as a tool for aquaculture research: a historical approach. Reviews in Aquaculture 11(3):697-724.
Gallego V., Herranz-Jusdado J. G., Rozenfeld C., Pérez L., Asturiano J. F., 2018 Subjective and objective assessment of fish sperm motility: when the technique and technicians matter. Fish Physiology and Biochemistry 44(6):1457-1467.
Gallego V., Pérez L., Asturiano J. F., Yoshida M., 2013 Study of pufferfish (*Takifugu niphobles*) sperm: Development of methods for short-term storage, effects of different activation media and role of intracellular changes in Ca^{2+} and K^{+} in the initiation of sperm motility. Aquaculture 414-415:82-91.
Hajiahmadian M., Moghanlou K. S., Agh N., Ardabili F. F., 2016 Semen characteristics of rainbow trout (*Oncorhynchus mykiss*) following diets containing different vegetable fatty acid levels. Reproduction in Domestic Animals 51(6):979-984.
Hanjavanit C., Kitancharoen N., Rakmanee C., 2008 Experimental infection of aquatic fungi on eggs of African catfish (*Clarias gariepinus Burch*). Khon Kaen University Science 36:36-43.
Henrotte E., Kaspar V., Rodina M., Psenicka M., Linhart O., Kestemont P., 2010 Dietary n-3/n-6 ratio affects the biochemical composition of Eurasian perch (*Perca fluviatilis*) semen but not indicators of semen quality. Aquaculture Research 41(9):e31-e38.
Izquierdo M. S., Fernandez-Palacios H., Tacon A. G. J., 2001 Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197(1-4):25-42.
Kaspar V., Hulak M., Kohlmann K., Vandeputte M., Rodina M., Gela D., Linhart O., 2008a *In vitro* study on sperm competition in common carp (*Cyprinus carpio L.*). Cybium 32(2):303-306.
Kaspar V., Vandeputte M., Kohlmann K., Hulak M., Rodina M., Gela D., Kocour M., Linhart O., 2008b A proposal and case study towards a conceptual approach of validating sperm competition in common carp (*Cyprinus carpio L*.) with practical implications for hatchery procedures. Journal of Applied Ichthyology 24(4):406-409.
Kime D. E., Van Look K. J. W., McAllister B. G., Huyskens G., Rurangwa E., Ollevier F., 2001 Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 130(4):425-433.
Koprucu K., Yonar M. E., Ozcan S., 2015 Effect of dietary n-3 polyunsaturated fatty acids on antioxidant defense and sperm quality in rainbow trout (*Oncorhynchus mykiss*) under regular stripping conditions. Animal Reproduction Science 163:135-143.
Lahnsteiner F., Berger B., Weismann T., Patzner R. A., 1998 Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 163(1-2):163-181.
Lahnsteiner F., Mansour N., McNiven M. A., Richardson G. F., 2009 Fatty acids of rainbow trout (*Oncorhynchus mykiss*) semen: Composition and effects on sperm functionality. Aquaculture 298(1-2):118-124.
Lahnsteiner F., Patzner R. A., Weismann T., 1993a The spermatic ducts of salmonid fishes (Salmonidae, Teleostei). Morphology, histochemistry, and composition of the secretion. Journal of Fish Biology 42(1):79-93.
Lahnsteiner F., Patzner R. A., Weismann T., 1993b Energy resources of spermatozoa of the rainbow trout *Oncorhynchus mykiss* (Pisces, Teleostei). Reproduction, Nutrition, Development 33(4):349-360.
Lahnsteiner F., Patzner R. A., Weismann T., 1994 The testicular main ducts and the spermatic ducts in some cyprinid fishes-II. Composition of the seminal fluid. Journal of Fish Biology 44(3):459-467.

Linhart O., Rodina M., Cosson J., 2000 Cryopreservation of sperm in common carp *Cyprinus carpio*: Sperm motility and hatching success of embryos. Cryobiology 41(3):241-250.
Mazurek B., Chmiel M., Gorecka B., 2017 Fatty acids analysis using gas chromatography-mass spectrometer detector (GC/MSD) - Method validation based on berry seed extract samples. Food Analytical Methods 10:2868-2880.
Pamungkas W., Jusadi D., Zairin M. Jr., Setiawati M., Supriyono E., Imron I., 2020 Effect of dietary essential fatty acids on level of oestradiol-17β and vitellogenin, reproductive performance and larval quality of striped catfish (*Pangasianodon hypophthalmus*) in out-of-spawning season. Aquaculture Research 51(9):3900-3909.
Rurangwa E., Kime D. E., Ollevier F., Nash J. P., 2004 The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture 234(1-4):1-28.
Rurangwa E., Volckaert F. A. M., Huyskens G., Kime D. E., Ollevier F., 2001 Quality control of refrigerated and cryopreserved semen using computer-assisted sperm analysis (CASA), viable staining and standardized fertilization in African catfish (*Clarias gariepinus*). Theriogenology 55(3):751-769.
Tilahun G., Dube K., Chtruvedi C. S., Kumar B., 2016 Assessment of reproductive performance, growth and survival of hybrids of African catfish (*Clarias gariepinus*) and Indian catfish (*Clarias batrachus*) compared to their parental lines crosses. Turkish Journal Fisheries and Aquatic Science 16:123-133.
Vassallo-Agius R., Watanabe T., Yoshizaki G., Satoh S., Takeuchi Y., 2001 Quality of eggs and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and its effects on the lipid and fatty acid components of eggs, semen, and livers. Fisheries Science 67(5):818-827.
*** AOAC, 2005 Official methods of analysis. 19th Edition. The Association of Official Analytical Chemists, Washington DC, 1786 p.
*** MMAF (Ministry of Maritime Affairs and Fisheries), 2013 [Annual report of the production directorate in 2013 DJPB]. 45 p. [In Indonesian].
*** MMAF (Ministry of Maritime Affairs and Fisheries), 2016 [Annual report of the production directorate in 2016]. [In Indonesian].

Received: 09 March 2023. Accepted: 05 May 2023. Published online: 16 August 2023.
Authors:
Wahyu Pamungkas, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor No. 32 Cibinong, 16915 West Java, Indonesia, e-mail: yhoe_pamungkas@yahoo.co.id
Jadmiko Darmawan, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor No. 32 Cibinong, 16915 West Java, Indonesia, e-mail: micho_jad@yahoo.co.id
Ikhsan Khasani, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor No. 32 Cibinong, 16915 West Java, Indonesia, e-mail: ikhsankhasani@gmail.com

How to cite this article:
Pamungkas W., Darmawan J., Khasani I., 2023 The effect of different diets on the quality of sperm in striped catfish (*Pangasianodon hypophthalmus*). AACL Bioflux 16(4):2166-2173.