The addition of fish oil to commercial feed as omega-3 fatty acid source in biofloc *Clarias* sp. culture system

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Abstract. Biofloc farming is a technology for recycling organic matter and improving the water quality and fish performance in aquaculture systems. The present study investigates the impact of biofloc culture system (BCS) on fish performance by adding fish oil to commercial feed as an omega-3 fatty acid source. The fish oil containing the omega-3 fatty acid was mixed with commercial fish feed. The growth and feeding efficiency of *Clarias* sp. were monitored in a recirculating tank system (RTS). Fat and Omega-3 levels were observed by analyzing the proximate test in *Clarias* sp. These results suggest that the combination of biofloc systems and the addition of fish oil could provide efficient systems to stimulate fish growth with high contents of omega-3 in *Clarias* sp.
Key Words: farming, lipids, RTS, growth, feeding.

Introduction. Aquaculture is an approach to elevating and maintaining the fish production at a mass scale. Fish products demand worldwide increased every year, needing an efficient method to sustain the fish stocks in the industry (Avnimelech 2012). Biofloc is a technology for maintaining the fish growth and increasing the fish production in aquaculture systems (Dediyanto et al 2017). Biofloc technology can improve the water quality and enrich the useful microbes or bacteria (*Bacillus* sp., *Nitrobacter* sp., *Nitrosococcus* sp.) that can convert NH_3 to NH_4, NO to NO_3, in order to suppress toxic compounds from the fish culture media. Leveraging biofloc technology in aquaculture system will have beneficial impacts on maintaining a high stocking density with limited water sources (Nasrudin 2010).

The supply of marine and freshwater fish reached the sustainable limits, which also impacted the production of fish oil industry. Fish oil contains approximately 25% saturated fatty acids (MUFA) and 75% polyunsaturated fatty acids (PUFA) (Panagan et al 2011). n-3 fatty acid is one of polyunsaturated fatty acids found in fish, that are beneficial in human health (Pratiwy & Pratiwi 2020). Omega-3 fatty acids comprise low triglyceride levels and prevent the inflammation. Omega-3 fatty acids are a type of fat that contain two or more double bonds (Gammone et al 2018). n-3 fatty acids belong to the group of essential fatty acids needed by our body and supplied by food intake. The omega-3 are characterized the by their LNA (alpha-linolenic acid) double bounds and their longer chain formed EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Da Boit et al 2017). Fatty acids are found in high amounts in fish and vegetable oil (Holub & Holub 2004; Turchini et al 2011; Pasaribu et al 2014). However, fish cannot synthesize de novo fatty acids, which are obtained from algae and plankton (Bell & Tocher 2009). It was reported that the vegetable and marine fish oils can be used as alternative sources of lipids for freshwater fish feeds (Montero et al 2005; Wassef et al 2009; Yildiz et al 2018). The content of omega-3 fatty acids in freshwater fish is lower than in marine fish (Ugoala et al 2008). In addition, the metabolites of 18C PUFA from n-3 and n-6 series can be converted to highly unsaturated fatty acids (HUFAs) via the desaturation and elongation pathways in the freshwater fish (Tocher & Sargent 1984; Glencross 2009). Catfish is a freshwater fish relatively richer in omega-3 fatty acids than other freshwater fish species (Suryaningrum et al 2010). The addition of fish oil in catfish can increase the fat content from 7 to 14% (Rostika et al 2020). The high fat level content in fish treated with additional fish oils causes the decreasing of lipogenic enzymes activity that inhibit the fatty acid synthesis (Mukti et al 2014). The utilization of biofloc technology with additional fish oil could attain the increasing production efficiency for *Clarias* sp. production.

In present study, we developed a biofloc culture system in *Clarias* sp. Biofloc technology has been applied in improving the fish production with limited water sources. However, the most efficient technology is a combination of the biofloc technology and fish oil with commercial feed. This study demonstrates that such a culturing technology, applied to the *Clarias* sp., stimulates the growth of specimens treated with fish oil. Subsequently, the omega-3 levels were observed in *Clarias* sp. treated with fish oil, providing information on alternative systems for efficient farming based on the administration of high omega-3 fatty acids to *Clarias* sp.

Material and Method

Experiment period. The experiment was carried out over a period of 30 days, from September to October 2021, and it was located in the Green House complex of Faculty of Fisheries, Ciparanje, Padjadjaran University.

Fish feeds analysis. Four feeding rates with additional fish oil (0, 2, 4 and 6%) food weight were tested. Commercial fish feeds were based on Hi-Provite 781. Fish oil were extracted from commercial swordfish. In the experiment, 3% of fish oil of the total live fish weight were added into the commercial fish feed with the addition of progol as feed adhesive.

Fish culture. *Clarias* sp. were obtained from a private fish farm, Cileunyi. 240 fish, with an average initial weight of 20.04 g and a length of 14 cm were randomly allocated in 16 fiber tanks of 68x68x68 cm^3 (15 fish per tank). All tanks were designed with a blower, as aeration system, a heater, to adjust the temperature, and an aeration tube. Each group/treatment was fed twice per day, at 08:00 h and 16:00 h. The body weight of *Clarias* sp. was measured every seven days, for all treatments, with addition of molasse and Paraqua bioflok probiotic for one or two weeks.

Biofloc set-up. The procedure of biofloc construction followed several steps: (1) Arranging material and tools used such as fiber tank and sterile aeration; (2) Approximately 1 g of Paraqua probiotic was dissolved into the tank; (3) The fiber tank was filled with 143 L of water and connected to the aeration system. 3 gr of fish feeding and 90 gr molasse were distributed; (4) The biofloc culturing medium was incubated for 1-5 days. The floc was observed as a coagulation in the culturing media.

Parameter analysis.
Specific Growth Rate (SGR). The experimental fish specimens were measured using the formula described by Steffens (1989):

$$SGR = \frac{ln\ ln\ Wt - ln\ ln\ Wo}{t} \text{ X } 100$$

Where:
Wt - final fish average weight (g);
Wo - initial fish average weight (g);
T - culturing period (days).

Feed efficiency. The feed efficiency calculation was based on the formula (Effendi 1997):

$$EP = \{[(Wt+D)-Wo]/F\}\ X\ 100$$

Where:
EP - feedr efficiency (%);
W_t - final fish average weight (g);
W_o - initial fish average weight (g);
D - weight of death fish;
F - total feed consumed (g).

Hepatosomatic index (HIS). The hepatosomatic index was calculated as follows (Effendi 1997):

$$HSI = \frac{B_{ht}}{B_t} \times 100$$

Where:
HSI - hepatosomatic index (%);
B_{ht} - liver weight (g);
B_t - fish total weight (g).

The fish meat lipids level was determined by proximate analysis. Lipids level was measured as follows:

$$\text{Lipid level} = [(C - B) /A] \times 100$$

Where:
A - sample weight (g);
B - empty dry flask weight (g);
C - flask content extraction oil (g).

Omega-3 analysis. The content of omega-3 level was measured by using the chromatogram (peak) fractionation residue of fatty acid methyl ester through chromatography capillary. Methyl ester was obtained from methyl lipids based on Modified Standard British method and methyl lipids then extracted by using modified Bligh-Dyer method. Fatty acids were identified by reference to known standards (Andhikawati et al 2020).

Water quality. Water quality analysis such as temperature, pH, DO, nitrite, nitrate, and ammonia were measured.

Results and Discussion

Specific growth rate analysis of Clarias sp. It is reported that addition of fish oil results in diverse average individual weight gain rates in catfish. The result showed that the average individual weight in catfish increases when the breeding period is longer. Average individual weight in the whole treatment was of 20.04 g t the beginning of the breeding period, while at the end it was of 30.41-31.72 g. The best weight growth rate results during the breeding period were obtained in treatment C (4%), at 32.73 g, while the lowest were observed in treatment D (6%), at 30.60 g. According to Mukti (2014), the feed given during the experiment has met primary need in fish, for its breeding and growth. This is supported by previous studies, stating that the growth began to occur when the availability of the required energy for breeding was fully reached (Halver & Hardy 2002).

The growth occurs because of the excess of energy input and protein from the feed. According to (Puteri et al 2015), not all feed in water can be used for growth, while most of the energy from food is used for breeding, reproduction and activities. The specific growth rate serves to measure the growth rate or increase in the percentage of fish growth per certain breeding period (Steffan 1989). This research shows that the administration of fish oil in feed resulted in a specific growth rate in catfish of approximately 1.53-1.73%. The use of fish oil at 4% in feed resulted in the highest growth rate, of 1.73%. Meanwhile, without the use of fish oil or control, it resulted in the lowest growth rate, of 1.53% (Table 1, Figure 1). The variance analysis shows that the use of fish oil had a significantly different effect to the growth of catfish. In order to analyze the difference among treatments, a Duncan test was conducted.

Table 1

Average specific growth rate of *Clarias* sp.

Treatment	*Growth rate (%)*
A	1.57 ± 0.14^{ab}
B	1.66 ± 0.18^{ab}
C	1.73 ± 0.05^{b}
D	1.53 ± 0.05^{a}

The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Treatment C (4%) resulted in the highest specific growth rate, compared with treatment D (6%). Growth in the cultivated fish depends on the chemical quality in feed, namely macro and micro nutrients (Lall & Kaushik 2021). Macro nutrients that can affect the fish growth are protein, carbohydrate, fat, and crude fiber. Protein is the source of essential amino acids required by fish to support an optimum growth, as well as the main energy source for fish (Schulz et al 2008). The optimum use of fat in feed can accelerate the fish growth (Beamish & Medland 1986). Fish oil administration in feed is the source of fatty acid and it will result into triglycerides as the source of energy (Bhouri et al 2010). In line with the result of a research by Haetami (2018), fat addition is quite effective for the growth of juvenile tilapia fish, at the stage of fingerling. However, high fat as the consequence of fish oil addition will disturb the activities of enzyme in the cell membranes, thus leading to the decrease of growth rate (Takeuci & Watanabe 1979).

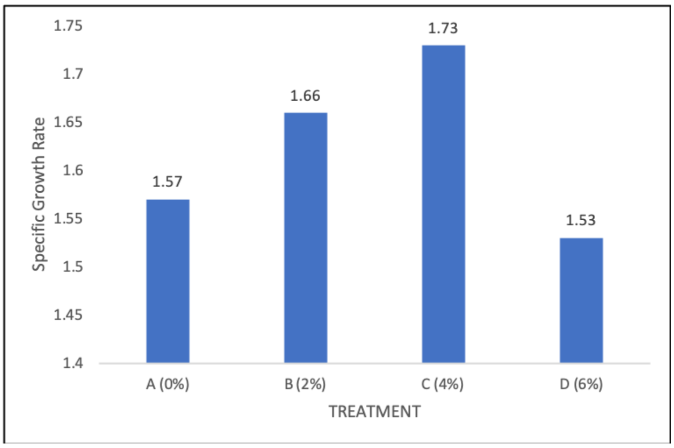

Figure 1. Specific growth rate of *Clarias* sp. cultured with three different treatments. *Clarias* sp. fed with 2, 4, and 6% fish oil, respectively.

Feeding efficiency. Feed efficiency is a comparison between the feed consumed and the weight gain, expressed in percentage (Mudjiman 2004). The weight gain in fish is directly proportional to the feed efficiency (Hariyadi et al 2005). Feed is considered efficient when its efficiency value is larger than 50% or almost 100% (Puteri et al 2012). The use of fish oil at different concentrations resulted in various efficiency values in feeding. This value is obtained from the difference between the final and the initial fish biomass, compared to the amount of feed and expressed as percentage. In the current research, the feeding efficiency is approximately between 71.5-83.39%. The highest feeding efficiency, 83.39%, was observed in treatment C (4%). Meanwhile, the lowest feeding efficiency, 71.5%, was observed in treatment D (6%) (Table 2, Figure 2).

Table 2

Total growth rate with the addition of fish oil to the commercial feed in *Clarias* sp.

Treatment	*Growth rate (%)*
A	76.5±5.07[ab]
B	76.5±10.21[ab]
C	83.39±3.40[b]
D	71.5±3.15[a]

The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Result of variance analysis shows that the use of fish oil in feed had a significantly different effect on the feeding efficiency. It was continued with Duncan's Multiple Range Test at a confidence level of 95% and it shows that the treatment C (4%) was significantly different from treatments A (0%), B (2%), and E (6%). The feeding efficiency resulted from the use of fish oil at 6% of the body weight was of 83.39% (the highest value). The reason is the oil digestibility, which improves the growth. Meanwhile, the treatment D (6%) resulted in a low feeding efficiency, at 71.5%, because fish cannot use fat excess for growth. Fish oil is considered a main source of fat in cultivation, which stimulates growth in the cultivated species, by providing polyunsaturated fatty acids and high unsaturated fatty acids (Sargent et al 2002). Feeding efficiency is higher when fish oil concentration is in accordance with the need for nutrients in catfish.

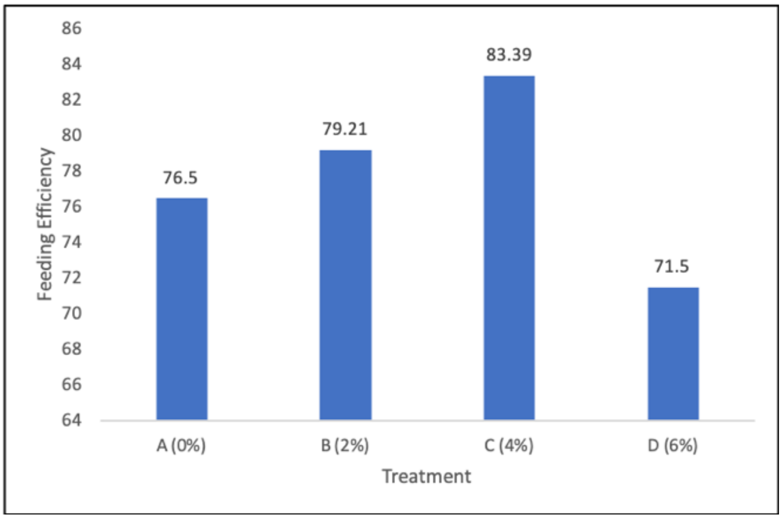

Figure 2. Feed efficiency of *Clarias* sp. fed with three different treatments. *Clarias* sp. fed the fish oil with 2, 4 and 6% of body weight, respectively.

Hepatosomatic index (HSI). The Hepatosomatic Index (HSI) is one of the indicators used in identifying growth. Centre of the metabolism in body and liver has been generally used as growth indicator (Ighwella et al 2014). HSI is a quantitative value that can describe the weight gain in liver along with the gonad development and the GSI increase (Effendie 1997). The HSI value relates to vitellogenesis, which occurs in liver (Syarif et al 2020). The increase of vitellogenin synthesis activity will result in the weight gain in liver, which increases the HSI (Putra 2017).

Table 3

Hepatosomatic index of *Clarias* sp. treated at three different concentrations of fish oil

Treatment	*Hepatosomatic index (HSI) (%)*
A (0%)	1.61±0.14[a]
B (2%)	1.70±0.23[ab]
C (4%)	1.91±0.08[b]
D (6%)	1.85±0.65[ab]

The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Based on the result of research, HSI value of catfish was 1.61% in treatment A (0%); 1.70% treatment B (2%); 1.92% in treatment C (4%); and 1.85% in treatment D (6%). The largest HSI was observed in the treatment C (4%), while in the treatment with the lowest HSI value was observed in the control treatment (Figure 3). HSI value from treatments A, B and C increased, along with the increase of the fat level. According to Yandes et al (2003), the increase of nutrient absorption causes accumulation in the liver, as a result of the increase in HSI value. However, in treatments C and D, the increase of HSI value is a result of the increase in feed protein. In accordance with the research conducted by Tibbets et al (2005) and Arnason et al (2010), the HSI decreased along with the increase of feed protein. The result of variance analysis shows that fish oil administration gave a significantly different effect on the hepatosomatic index (HSI). Based on the result of an advanced test, Duncan's Multiple Range Test at confidence level of 5%, the treatment C (4%) was significantly different, compared with treatments A (0%), B (2%) and D (6%) (Table 3). According to Mukti et al (2014), an increase of the liver's volume will directly support the increase of fish growth. Liver will grow faster to support the growth of other organs. According to Hidayaturrahmah et al (2017), when the hepatosomatic index (HSI) increases as a result of the glycogen level in liver, it is supposedly due to several substances in catfish oil, EPA/DHA in particular.

Water quality analysis. Water quality is one of the aspects that must be considered in cultivation because it is capable of affecting growth and development of fish. Water quality should meet criteria of water quality standards. During the research, measurements were conducted on some water quality parameters, namely temperature, pH, dissolved oxygen (DO), nitrite, nitrate, and ammonia. Average water quality during the research was in an appropriate range (Table 4). Temperature is one of the factors affecting the feeding response in fish. An optimal range of water temperatures determine an optimal fish response to the feed. However, when temperature is out of the range of optimal temperatures, fish response to feed will decrease (Gusrina 2008). Based on the results of measurement in the breeding tub, temperature was between 26-30^{o}C, still in the good quality category, based on the Indonesian National Standard for catfish, at 25-30^{o}C. When water temperature in the breeding tub gets higher, oxygen solubility in water will get lower, and vice versa (Ghufran 2011). Therefore, temperature closely relates to the value of dissolved oxygen (DO). The results of measurement in the dissolved oxygen during the research were approximately 6.0-6.64 mg L^{-1}, while the dissolved oxygen during the breeding period were supplied by an aerator. According to the Indonesian National Standard, an appropriate dissolved oxygen value is at least 3 mg L^{-1}, while according to Gusrina (2008), the ideal dissolved oxygen level for fish cultivation is of 4-9 mg L^{-1}. It means that the fish needs for dissolved oxygen were met during the research. The acidity degree (pH) during the research was of 6.4-6.59, while the water pH in the breeding tub has been suitable for catfish cultivation, with the quality standard limit at 6.5-8, according to Indonesian National Standard.

Table 4

Water quality analysis.

Parameter	*Unit*	*Range value*	*Quality standard*
Temperature	^{o}C	26-30	25-30^{a}
pH	-	6.4-6.59	6.5-8^{a}
Dissolved oxygen	mg L^{-1}	6.0-6.64	Minimum 3^{a}
Nitrite	mg L^{-1}	0-1	<1.0^{b}
Nitrate	mg L^{-1}	0-25	< 100^{c}
Ammonia	mg L^{-1}	0-0.25	Maximum 0.1^{a}

The mean values (±SD) at the same column with similar superscripts are not significantly different (P<0.05).

Water quality in the breeding period can be maintained by controlling some parameters, and by using biofloc technology during the breeding process. Biofloc technology is one of the techniques for alternative cultivation, solving the issue of the organic waste in the water, related to the fish feces and feed residue (Adharani et al 2016). During the research, water in the breeding tub was not replaced, but probiotics and molasses were added twice a week. Thus, nitrite, nitrate, and ammonia had an appropriate value. The ammonia value was between 0 and 0.25 mg L^{-1}, according to Pillay (2004), toxic ammonia value on the short term was between 0.6 and 2.0 mg L^{-1}. When ammonia concentration in water is high, it will cause death in fish. Ammonia concentration is affected by temperature, pH, and dissolved oxygen (Rahayu 2019). Furthermore, nitrate value during the research was between 0-25 mg L^{-1}. High nitrite concentrations in water can endanger cultivated organisms, while nitrate in water will be quickly converted into nitrite in fish digestion, which is harmful for fish (Rahayu 2019). Nitrate level increase indicates an ammonia nitrification process due to Nitrobacter bacterium (Prayogo et al 2016). Nitrate levels for a feasible fish cultivation are >3 mg L^{-1}. Meanwhile, the nitrite value during the research was approximately 0-1 mg L^{-1}. Nitrite in water is a result of ammonia oxidation process through the nitrification process occurring through an aerobic pathway. Feasible nitrite for fish cultivation is <1 mg L^{-1} (Wedmeyer et al 1977). Nitrite concentration passing the limit can be fatal for fish and cause death.

Fat in fish meat identified by proximate test. Fat is one of the nutrients required by body to provide energy (Angelia 2016). In the body, fat levels are regulated by the liver, as this is the center of fat metabolism (Siregar & Makmur 2020). During the research, the highest hepatosomatic index (HSI) and specific growth rate (SGR) values were observed in the treatment C (4%), while the lowest were observed in the treatment A (0%). Proximate tests were conducted in treatment C (4%) and A (0%) to identify the fat levels. Proximate tests were conducted in the Laboratory of Ruminant Livestock Nutrition and Feed Chemicals, Padjadjaran University. In the result of proximate test, the fat, protein, carbohydrate, and energy increased, compared with their values before the treatment and treatments with 0% and 4% fish oil concentrations. Fat levels obtained from fish before treatment were of 5.03%; during the research, fat level was 9.14% in treatment C (4%) and 5.35% in treatment A (0%) (Table 5). Addition of fish oil in feed modified the fat content un feed. Diana (2016) stated that fish oil addition at 2% in feed can already improve fish by changing the nutrient composition and absorption rate, thus optimizing the energy for growth.

Table 5

Proximate analysis of *Clarias* sp.

Composition	*Initial catfish*	*Catfish A (0%)*	*Catfish C (4%)*
Protein (%)	15.46	16.25	18.66
Fat (%)	5.03	5.35	9.14
Carbohydrate (%)	62.61	68.82	69.71
Gross energy (Kcal kg^{-1})	3500	3711	4136

Addition of fat level in treatment C with fish oil at 4% was of 4.11%, while in treatment A without fish oil administration was only 0.32%. When fat level is too high, as a result of excessive fish oil administration, the lipogenic enzyme activity decreases, thus hampering the fatty acid synthesis (Ismayanti et al 2019) and decreasing the capability of fish to digest the feed (Sargent et al 2002). In treatment D (6%), HSI value decreased, after obtaining optimum value in treatment C (4%), from 1.92 to 1.85%. Based on the result of a research by Mukti (2014), a low HSI value and a high fat level in fish will decrease the growth rate, feed efficiency, energy retention, and protein retention. The high HSI value in treatment C (4%) shows that catfish is capable of absorbing and metabolizing protein, fat, and carbohydrate more optimally.

Omega-3 content. Omega-3 is a fatty acid with many double bonds, commonly found in fish oil, containing EPA and DHA (Vilka 2008). Fish oil addition to feed can improve Omega-3 in catfish's body, EPA and DHA in particular. Fish oil had 1,698.5 mg 100 g^{-1} of EPA and 11,175.8 mg 100 g^{-1} of DHA. A proximate test was conducted in fish with treatments C (4%) and A (0%), in order to determine EPA and DHA. Diverse compositions of fatty acids in fish's body can be obtained by fish oil addition at different levels in feed (Ismayanti et al 2019). Treatment C (4%) had 36 mg 100 g^{-1} of EPA and 148.5 mg 100 g^{-1} of DHA. EPA and DHA values in the catfish (*Clarias gariepinus*) with fish oil addition at 4% were almost the same as in the torpedo scad (*Magalapris cordyla*): 34.56 g and 145.92 g, respectively (Wan Rosli et al 2012). In the current study, treatment A (0%) generated 16.7 mg 100 g^{-1} of EPA and 69.9 mg 100 g^{-1} of DHA, while the values of EPA and DHA observed in other studies in catfish (*Claris gariepinus*) without fish oil addition were similar to those of the mackerel fish (*Rastrelliger kanagura*), with 17.74 mg g^{-1} of EPA and 67.46 mg g^{-1} of DHA (Rosli et al 2012). The values obtained in treatment C (4%) were twice the levels generated by treatment A (0%) (Table 6). Thus, fish oil administration in feed result in various levels of EPA and DHA.

Table 6

Omega-3 fatty acid in *Clarias* sp.

No.	*Parameter*	*Unit*	*Fish C*	*Fish A*	*Fish oil*
1	DHA	mg 100 g^{-1}	148.5	69.9	11175.8
2	EPA	mg 100 g^{-1}	36	16.7	1698.5

The fatty acid levels increase through fish oil addition in feed are similar in freshwater fish and saltwater fish. According to Hadipranoto (2005), long-chain omega-3 fatty acids, EPA and DHA, can be naturally obtained from saltwater fish fat, in particular, but it is also possible to get it from freshwater fish fat. The difference is due to feeding habits: the seawater fish eat plankton, which can synthesize these two fatty acids (Nettleton 1995). Saltwater fish need more essential fatty acid, due to the water salinity and environmental temperatures (Cowey & Sargent 1979). Meanwhile, freshwater fish only requires C18 n-3 fatty acid (linolenic acid) in concentrations between 0.5 and 1.5% in feed (Craig & Helfrich 2002).

Omega-3 EPA and DHA fatty acid in fish are highly required in carrying out the cell membrane function, while DHA is very important for the neural network cells' membrane; its derived eicosanoate is a precursor in the synthesis of several hormones (Tocher 2003). Meanwhile, the omega-3 fatty acid in humans has a role as antihypertensive anti-thrombotic, and anti-cancer, and is involved also in the vision and cognitive development, fat metabolism, inflammation control and immune system activation (Ardi 2019). Consuming food with omega-3 fatty acid can support medication and healing of patients infected by SARS-CoV-2 (Rogero et al 2020).

Conclusions. This study has successfully determined that the optimum dose of fish oil addition is 4%, with the highest specific growth rate at 1.73%, the highest feeding efficiency at 83.39%, the highest hepatosomatic index at 1.92%, a fat level at 9.14%. This treatment generated 36 mg 100 g^{-1} of EPA and 148.5 mg 100 g^{-1} of DHA, by increasing the fat level, without reducing the protein level in fish.

Acknowledgements. The authors would like to thank to the undergraduate alumni of FPIK UNPAD (Alpriyanto Ginting, S.Pi; Elvida Usman, S.Pi; Dzikri Ahmad Fauzi, S.Pi; Fajar Nurul Arifah, S.Pi) that have helped with data collection in the laboratory.

Conflict of interest. The authors declare no conflict interest.

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Received: 11 October 2022. Accepted: 21 December 2022. Published online: 05 January 2023.
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How to cite this article:
Rostika R., Pratiwy F. M., Andhikawati A., Pasaribu B., Dewanti L. P., 2023 The addition of fish oil to commercial feed as omega-3 fatty acid source in biofloc *Clarias* sp. culture system. AACL Bioflux 16(1):55-65.