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Evaluation of dietary α-lipoic acid supplementation on the growth performance and physiological status of striped catfish *Pangasianodon hypophthalmus*

Evaluasi suplementasi asam α-lipoat pada pakan terhadap kinerja pertumbuhan dan status fisiologis ikan patin siam *Pangasianodon hypophthalmus*

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ABSTRACT

This study aimed to evaluate the dietary supplementation of α-lipoic acid (ALA) on the growth performance, antioxidative capacity, and protein-sparring effect of striped catfish (*Pangasianodon hypophthalmus*) fingerling. The striped catfish (individual weight of 1.4 ± 0.0 g) were stocked in 25 cages at 2×1×1.5 m size in a 200 m pond at 50 fish. Fish were fed formulated diets with ALA supplementation, namely: diet A (27% protein: 390 kcal), diet B (27% protein: 390 kcal +ALA 8 g/kg), diet C (27% protein: 400 kcal), diet D (27% protein: 400 kcal +ALA 8 g/kg), and diet E (30% protein: 390 kcal). Feeding was performed until apparent satiation for 60 days. The ALA supplementation significantly produced higher final individual weight, specific growth rate, and protein efficiency ratio, followed by a lower feed conversion ratio on the 27:390+ALA diet treatment (P<0.05) than other diet treatments. The highest liver performance based on superoxide dismutase enzyme activity and glycogen contents was obtained from the 27:390+ALA diet treatment. The malondialdehyde, triglycerides, and lipid accumulation in the liver on the 27:390+ALA diet treatment were lower than other diet treatments (P<0.05). Therefore, the best growth performance and physiological status in striped catfish was obtained from 27:390+ALA diet treatment.

Keywords: α-lipoic acid, antioxidative capacity, growth, striped catfish

ABSTRAK

Penelitian ini bertujuan mengevaluasi suplementasi asam α-lipoat (ALA) dalam pakan terhadap kinerja pertumbuhan, kapasitas antioksidan dan protein-sparring effect ikan patin siam (*Pangasianodon hypophthalmus*). Ikan patin siam (bobot individu 1,4 ± 0,0 g ditebar dalam 25 hapa ukuran PLT 2×1×1,5 m yang ditempatkan dalam kolam seluas 200 m. Setiap hapa diisi 50 ekor ikan. Ikan diberi pakan formulasi dengan dan tanpa suplementasi ALA yaitu: pakan A (27%: 390 kkal) B (27%: 390 +ALA 8 g/kg), C (27%: 400 kkal), D (27%: 400 kkal +ALA 8 g/kg) dan pakan E (30%: 390 kkal). Pemberian pakan dilakukan secara at satiation selama 60 hari masa budidaya. Penambahan ALA secara signifikan menghasilkan bobot individu ikan akhir, laju pertumbuhan harian, rasio efisiensi protein lebih baik serta rasio konversi pakan yang lebih rendah pada perlakuan 27:390+ALA (P<0,05) dibandingkan keempat perlakuan lainnya. Kapasitas kinerja hati (aktivitas enzim superoksida dismutase dan glikogen hati) tertinggi diperoleh pada perlakuan 27:390+ALA. Nilai malondialdehyde dan trigliserida lebih rendah dibandingkan keempat perlakuan lainnya (P<0,05). Kinerja pertumbuhan dan status physiologi terbaik diperoleh pada perlakuan pakan 27: 390 + ALA.

Kata kunci: asam α-lipoat, kapasitas antioksidan, pertumbuhan, ikan patin.

INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) is one of the most cultured fish species in Indonesia (Slembrouck *et al*., 2009). A problem occurred in striped catfish production is a qualified feed availability with competitive price. Currently, the feed ingredient has been increasing by 40-70% (Suprayudi *et al*., 2012), which may not be followed by the increased fish price. Therefore, it will surely increase the feed cost to produce the fish. Efforts to reduce the production cost through feed efficiency can be performed by applying feed supplements to shorten the metabolism process by improving the antioxidant properties and energy utilization. One of the feed supplements that can be used is α-lipoic acid (ALA) (Samuki *et al.*, 2020; Xiong *et al*., 2012; Liu *et al*., 2018).

ALA or 1,2-dithiolane-3-pentanoic acid contains disulfide bonds originated from the octanoic acid synthesized in mitochondria and found in the plant or animal microorganisms (Navari-Izzo *et al*., 2002). ALA is a water- and lipid-soluble organic compound with various important roles in metabolism, mainly related to antioxidative properties and non-protein energy utilization. ALA can also be utilized as an antioxidative agent in the free-radical reaction as a reactive oxygen species (ROS) by producing glutathione and superoxide dismutase activity (SOD) (Samuki *et al*., 2020; Shi *et al*., 2017). Moreover, ALA is a cofactor enzyme in mitochondria, which has a role in carbohydrate metabolism and energy production (Trattner *et al*., 2007; Butler *et al*., 2009). Increased lipoic acid content in the body will finally induce the glycolysis and β-oxidation processes that will lead to the increased total energy productions from carbohydrate and lipid (Kuo *et al*., 2012; Akbari *et al*., 2018). The energy produced from the non-protein source will improve the protein-sparing effect capacity level, which supplies protein as structural properties and biological function more than the energy source. The utilization of non-protein energy aside from protein will promote the fish growth. In addition to its role in the antioxidative activity, ALA is also involved in the insulin metabolism, glucose regulation, and glycogen synthesis (Islam, 2009).

Evaluation of dietary ALA supplementation has many been performed in several fish species, such as giant gourami *Osphronemus goramy* fingerling at 0.55 g/kg diet (Samuki *et al*., 2020) and African catfish (*Clarias gariepinus*) 1.15 g/kg diet (Siagian *et al*., 2021). Both studies also reported that the dietary ALA supplementation at 0.3 and 0.5 g/kg diet could increase the superoxide dismutase activity in giant gourami and African catfish (Samuki *et al*., 2020; Siagian *et al*., 2021). Moreover, the dietary ALA supplementation at 70 mg/kg diet dose could decrease the ROS concentration in the *Corydoras paleatus* fish brain (Monserrat *et al*., 2008). Meanwhile, the dietary ALA supplementation in *Trachinotus marginatus* fish at 316-524 mg/kg diet dose could promote growth rate and antioxidant status, and decrease the lipid peroxidation in the muscle (Kütter *et al*., 2012). In GIFT tilapia, the 300 mg/kg diet dose of dietary ALA supplementation showed the highest growth performance and superoxide dismutase enzyme activity in blood serum and liver (Xu *et al*., 2018b). In grass carp, the 600 mg/kg diet dose of dietary ALA supplementation could prevent the n-3 highly unsaturated fatty acid (n-3 HUFA) oxidation and induce the antioxidative enzyme production in serum, muscle, and liver (Shi *et al*., 2017). Our previous study showed that the optimum dose of ALA for striped catfish with 30% protein diet was 0.8 g/kg (Rifai *et al*., 2022).

For protein utilization, the dietary ALA supplementation in giant gourami were reported to increase the protein preservation in the body (protein retention) (Samuki *et al*., 2020), as similar as in the African catfish (Siagian *et al*., 2021). This condition indicates the high non-protein availability from lipid and carbohydrate, resulting in an increased protein retention level. A similar condition was also reported by Xu *et al*. (2019) in Chinese mitten crab (*Eriocheir sinensis*), (Shi *et al*., 2018; Huang *et al*., 2019) in grass carp (*Ctenopharyngodon idellus*), and (Xu *et al*., 2018) in GIFT tilapia (*Oreochromis* sp.), and (Hung *et al*., 2002) in Asian catfish (*Pangasius bocourti*).

From the description above, the dietary supplementation of ALA has roles in triggering the lipid and carbohydrate metabolisms to increase the non-protein energy availability level and decrease the protein breakdown as an energy source. Moreover, the supplementation of ALA also has roles in the antioxidative capacity improvement for various fish species. Therefore, this study was conducted to evaluate the influence of dietary ALA supplementation on the growth performance and physiological status of striped catfish.

MATERIALS AND METHODS

This study experiment has been ethically approved by the Commission of Animal ethics LPPM-IPB of 1999-2021 IPB.

Experimental diets

The experimental diets in this study applied five diet types, namely: Diet A contained 27.0% protein and 390.0 GE/100 g diet energy (abbreviated: 27:390), diet B contained 27.0% protein, 390.0 GE/100 g diet energy, and 0.8% ALA supplementation (27:390+ALA), Diet C contained 27.0% protein and 400 GE/100 g diet energy (27:400), Diet D contained 27.0% protein, 400 GE/100 g diet energy, and 0.8% ALA supplementation (27:400+ALA), Diet E contained 30.0% protein and 390.0 GE/100 g diet energy (30:390). The proximate analysis of experimental diet formulation after following the Association of Official Analytical Chemists (AOAC 2012) tests are presented in Table 1.

Table 1. Experimental diet formulation.

Ingredients (g/kg)	Treatments				
	A (27:390)	B (27:390 + ALA)	C (27:400)	D (27:400+ALA)	E (30:390)
Fish meal	50.0	50.0	50.0	50.0	70.0
Soybean meal	226.0	230.0	235.0	235.0	320.0
Meat bone meal	100.0	100.0	100.0	100.0	100.0
Bran pollard	360.0	360.0	353.0	365.0	230.0
Rice bran type-A	80.0	80.0	80.0	80.0	100.0
Wheat flour	147.0	135.0	128.0	108.0	150.0
Fish oil	2.0	2.0	4.0	4.0	-
Palm oil	5.0	5.0	20.0	20.0	-
α-Lipoic acid	-	8.0	-	8.0	-
Calcium carbonate	10.0	10.0	10.0	10.0	10.0
Phytase enzyme	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.5	0.5	0.5	0.5	0.5
Choline chloride	1.0	1.0	1.0	1.0	1.0
Premix	10.0	10.0	10.0	10.0	10.0
Polymethylolcarbamide	3.0	3.0	3.0	3.0	3.0
NaCl	5.0	5.0	5.0	5.0	5.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Diet proximate contents (%) Protein	27.10	27.21	27.14	27.16	30.56
Lipid	5.70	5.46	7.33	7.31	5.12
Crude fiber	4.06	4.02	4.01	4.08	3.82
NFE*	49.91	48.92	47.53	47.26	47.53
Moisture	7.51	8.07	7.80	7.86	7.82
Ash	6.95	6.85	6.65	6.88	7.67
GE(kcal/100 g diet)**	390	390	400	400	390
Energy : Protein	15.0	14.9	15.3	15.3	13.6

Note: *NFE = nitrogen-free extract = 100 - (protein + lipid + moisture + ash + crude fiber); **GE = gross energy = (% protein × 5.6 kcal) + (% lipid × 9.4 kcal) + (%NFE × 4.1 kcal) (Watanabe, 1988).

Fish maintenance

The striped catfish were obtained from the hatchery station in Parung, Kemang, Bogor, West Java, Indonesia. Fish were initially adapted to the experimental condition for 7 days. After the adaptation period, fish were fasted for 12-to 18 hours before measuring their initial weight. Fish with an average weight of 1.4 ± 0.0 g were stocked in a 2×1×1.5 m net at 50 fish net. Fish were reared for 60 days and fed three times a day until apparent satiation. The total diet weight was recorded for the calculation of feed intake and conversion ratio. The 25 nets were used in this study on a 200 m pond (20 × 10 × 1.5 m) with 0.5 m spacing for sustaining the good water circulation.

Growth performance and feed utilization analyses

At the final maintenance period, the fish biomass was measured along with the fish survival rate. Before the final sampling, fish were fasted for 24 hours and anesthetized with a natural anaesthetic, i.e. clover oil at 0.1 ml/4L water dose. The specific growth rate and survival rate of the

$$SGR = [(Wt/Wo) / (1/t)-1] \times 100$$

fish were calculated using the following formula:
Note:
SGR = specific growth rate (%/day)
Wt = average fish weight at the final maintenance period (g)
Wo = average fish weight at the initial maintenance period (g)

The total consumed diets were recorded everyday during the maintance period. The feed intake (FI) was determined by measuring the total diets fed to the fish and the total remaining diet. Feed conversion ratio was calculated based on Tacon (1987), namely:

$$FCR = \left(\frac{F}{Wt+Wd-Wo} \right)$$

Note:
FCR = feed conversion ratio
F = total consumed diets during maintenance period (g)
Wt = total fish biomass on the final maintenance period (g)
Wd = total dead fish weight (min. 2 weeks after stocking)
Wo = total fish biomass on the initial maintenance period (g)

Liver performance and physiological status

Liver performance was evaluated based on the liver glycogen level and meat glycogen level, while liver physiological status was determined based on the liver superoxide dismutase (SOD), malondialdehyde (MDA), total protein and triglyceride levels. Before sampling, fish were fasted for 24 hours and anaesthesized with a purified clover oil (Sari Daun Store, Indonesia) at 0.1 ml/4 L water dose. Fish weight was measured, before taking the blood and liver sample from its body. The liver was then moved to a gas tube filled with a liquid nitrogen and preserved in a freezer at -80°C for SOD, MDA, glycogen analyses. The liver and muscle glycogen levels were measured following Watanabe (1988) method, and the glucose level standard was determined based on Wedemeyer and Yasutake (1977) method with spectrophotometer at an absorbance value of 635 nm wavelength. The blood chemistry test was performed using the fish blood plasma by measuring the plasma triglyceride and total protein concentrations, following the reagent kit manual. The triglyceride concentrations were analyzed using the Triglycerides Liquiform (Labtest) Diagnostca SA with the absorbance value for standard and sample concentrations was 505 nm wavelength in a spectrophotometer. The total protein concentrations were analyzed using the Proteinas Totals colorimetric test content Portugels Ref. kit with an absorbance value of 545 nm wavelength.

Water quality

The water quality parameter was remained at temperature of 25-28°C, pH of 6.5-8.5, DO of 5.56 mg/L, ammonia of 0.02 mg/L, and TAN of 0.422 mg/L.

Statistical analysis

Data from the study results were processed using a Microsoft Excel 2010. Then, these data were tested with a multivariate analysis using SPSS ver.25.0 at 95% confidence level. If a significant difference condition was occurred among the treatments, a continuous test was performed using the Duncan's multiple range test (DMRT).

Results

Striped catfish growth performance

The striped catfish growth performance maintained for 60 days is presented in Table 2. Fish fed with the 27:390+ALA diet indicates the highest specific growth rate, final biomass

weight, feed intake, and protein efficiency ratio, while feed conversion ratio lower compared to other diets ($p<0.05$). Moreover, the fish survival rate obtained a similar value to all diet treatments ($P>0.05$).

Liver and muscle glycogen levels and plasma triglycerides/ protein.

The highest glycogen level in fish liver was obtained from the diet B treatment ($P<0.05$). Meanwhile, fish fed with ALA-supplemented diets (diet B and D treatments) obtained a higher muscle glycogen level than other treatments ($P<0.05$). The muscle glycogen and MDA levels in fish fed with the diet A and C were lower than other treatments ($P<0.05$). Furthermore, the SOD value of the fish fed with the diet A and C was higher than other treatments ($P<0.05$).

The highest triglyceride level was obtained from fish fed with the diet C treatment, while the diet B treatment obtained the lowest triglyceride level. Fish fed with the diet B treatment had a similar plasma protein level to the diet C, D, and E ($P>0.05$).

Discussions

The results showed that the dietary ALA supplementation improved the striped catfish growth. This condition was thought as ALA had important roles in metabolism regulation and energy transformation (Trattner *et al*., 2007; Ramamurty & Ronnet 2012; Serhiyenko *et al*., 2018). The utilization of non-protein energy such as mainly carbohydrate, is inseparable from the ALA function by i.e. inducing the insulin sensitivity, triggering the glucose transport

Table 2. Growth performance of striped catfish after feeding with dietary α-lipoic acid supplementation.

Parameters	Diet treatment (g/kg diet)				
	A (27:390)	B (27:390 + ALA)	C (27:400)	D (27:400+ALA)	E (30:390)
TIBW (g)	70.9 ± 0.3^{a}	70.9 ± 0.4^{a}	70.9 ± 0.2^{a}	70.9 ± 0.7^{a}	70.9 ± 0.5^{a}
TFBW (g)	2385.3 ± 349.3^{a}	3737.4 ± 557.6^{b}	2314.2 ± 305.9^{a}	2637.2 ± 480.5^{a}	2464.3 ± 329.8^{a}
FI (g)	4882.5 ± 68.4^{b}	5006.4 ± 67.8^{a}	4683.7 ± 93.2^{c}	4954.0 ± 84.3^{ab}	4513.8 ± 67.5^{d}
FCR	2.1 ± 0.3^{a}	1.4 ± 0.2^{b}	2.1 ± 0.3^{a}	2.0 ± 0.3^{a}	1.9 ± 0.3^{a}
SGR (%/day)	6.22 ± 0.4^{a}	6.86 ± 0.2^{b}	6.09 ± 0.2^{a}	6.35 ± 0.3^{a}	6.18 ± 0.3^{a}
PER	1.7 ± 0.3^{a}	2.7 ± 0.4^{b}	1.8 ± 0.2^{a}	1.9 ± 0.4^{a}	1.7 ± 0.2^{a}
SR (%)	91.2 ± 7.6^{a}	98.0 ± 2.8^{a}	94.0 ± 5.7^{a}	92.4 ± 7.3^{a}	95.2 ± 4.1^{a}

Noted: Values are presented in average ± standard deviation (n=5). Different superscript letters on the same line show a significant different value (DMRT $P<0.05$). TIBW = Total Initial Bimass Weight. TFBW = Total Finally Biomass Weight. FI = Feed Intake. FCR = Feed Conversion Ratio. SGR = Specific Growth Rate. PER = Protein Efficiency Ratio. SR= Survival Rate.

Table 3. Liver performance and plasma capacity of striped catfish fed with and without dietary α-lipoic acid supplementation.

Parameters	Diet treatments				
	A (27:390)	B (27:390+ALA)	C (27:400)	D (27:400+ALA)	E (30:390)
LG (mg/g)	0.28 ± 0.02^{b}	0.41 ± 0.03^{a}	0.28 ± 0.02^{b}	0.29 ± 0.02^{b}	0.23 ± 0.04^{c}
MG (mg/g)	0.46 ± 0.03^{b}	0.56 ± 0.06^{a}	0.46 ± 0.06^{b}	0.53 ± 0.07^{a}	0.52 ± 0.03^{a}
SOD (unit.mL enzyme)	79.65 ± 15.45^{b}	110.28 ± 14.54^{a}	83.38 ± 12.06^{b}	105.63 ± 6.92^{a}	105.63 ± 13.61^{a}
MDA (nmol/L)	0.059 ± 0.021^{b}	0.038 ± 0.02^{a}	0.050 ± 0.01^{b}	0.044 ± 0.04^{a}	0.044 ± 0.02^{a}
PT (mg/dL)	282.00 ± 18.88^{b}	218.03 ± 17.27^{a}	470.82 ± 51.97^{d}	275.55 ± 19.36^{b}	326.48 ± 15.71^{c}
PP (g/dL)	4.79 ± 0.29^{b}	6.44 ± 1.54^{a}	4.59 ± 0.39^{bc}	5.39 ± 0.31^{b}	5.65 ± 0.26^{ab}

Note: Values are presented in average ± standard deviation (n=5). Different superscript letters on the same line show a significant different value (DMRT $P<0.05$). LG = Liver Glycogen. MG = Meat Glycogen. SOD = Superoxide dismutase. MDA = Malondialdehyde. PT = Plasma Triglycerides. PP = Plasma Protein.

(GLUT) translocation (Yaworsky *et al.*, 2000), and activating the Akt1 gene (Wang *et al.*, 1999). Moreover, glucose will experience glycolysis and phosphorylation processes in mitochondria. This condition causes the metabolized energy production can undergo well to decline the protein breakdown into energy (Suprayudi *et al*., 2014; Samuki *et al*., 2020). From the description above, there was an increased protein efficiency ratio (PER) value in fish fed with the diet B and D. This study also showed that the declined protein level in diets from 30% (diet E) to 27% (diet B) with similar energy content and ALA supplementation could produce a higher PER value. These results followed the previous studies reported by Samuki *et al*. (2020) in giant gourami, Siagian *et al*. (2021) in African catfish, and Shi *et al*. (2018) in grass carp. Wang and Proud (2007) stated that ALA had important roles in protein synthesis through mTOR pathway and cellular nutrient-signalling. Shi *et al*. (2018) also revealed that ALA supplementation could activate the phosphorylation process through AKT/mTOR/4EBP pathway in muscle, that could finally increase the PER value and non-protein energy utilization.

Increasing non-protein energy utilization will decrease the preserved energy breakdown such as glycogen both in liver and muscle, resulting in an increased glycogen level in liver and muscle. High glycogen level in fish fed with the ALA-supplemented diets (Diet B and D) was supported by the low plasma triglyceride level (Table 3), which indicates a lipolysis occurrence. Decreased triglyceride level in blood was caused by the lipolysis along with the β-oxidation process, producing more non-protein energy and improving the protein-sparing effect performance, based on the increased PER value. A similar condition was also described by Xu *et al*. (2019) in Chinese mitten crab juvenile, Samuki *et al*. (2020) in giant gourami, and Siagian *et al*. (2021) in African catfish. Other mechanisms of ALA in suppressing the blood triglyceride level include inhibiting the liver lipogenic gene expression, reducing the liver triglyceride secretion, and inducing the protein-rich triglyceride (Butler *et al*., 2009). These mechanisms are often occurred in mice by inducing the lipolysis and reducing the lipogenesis (Butler *et al.,* 2009; Kuo *et al*., 2012).

Available non-protein energy from carbohydrate or lipid due to ALA supplementation can reduce the oxidative deamination as a stress oxidative cue in fish (Suprayudi *et al*., 2014). The oxidative deamination activity dynamics can be identified by the fish plasma protein dynamics and PER or protein retention value, which can be shown in fish fed with the ALA-supplemented diet (Diet B, Table 3). Furthermore, the increased diet energy level at 100 kcal/kg diet (Diet C) produced a similar plasma protein level to the diet supplemented with ALA (Diet D), which increased its protein plasma level (Table 3). These results were also supported by Samuki *et al*. (2020) and Siagian *et al*. (2021). Oxidative stress in the organisms occurs when the reactive oxygen species (ROS) production exceeds the antioxidative capacity of the organisms (Matés *et al*., 2008). Malondialdehyde (MDA) is a natural product from the lipid peroxidation process, commonly used to control the oxidative damage in cells and tissues due to oxidative stress (Dotan *et al*., 2004). MDA is considered as an oxidative stress marker due to free radical exposures (Kushayadi *et al*., 2020; Purnamasari *et al.,* 2020). The MDA level in fish fed without the ALA-supplemented diet (Diet A) was higher than fish fed with the ALAsupplemented diet (Diet B) (Table 3). Nevertheless, fish fed with the ALA-supplemented diet (Diet B) obtained a higher SOD value than fish fed with the diet A treatment (Table 3). SOD is functioned as a part of important antioxidative properties in defending the body from free readicals and protecting several critical targets from the superoxide anions (Fattman *et al*., 2003). A similar condition was occurred in GIFT tilapia (Xu *et al*., 2018), *Haliotis discus hannai* (Huang *et al*., 2019), *Trachinotus marginatus* (Kütter *et al.*, 2012), giant gourami (Samuki *et al*., 2020), and African catfish (Siagian *et al*., 2021). The present study occupied the energy and protein increase without the ALA supplementation (diet C and E), which triggered the oxidative stress as explained previously.

Increased fish appetite was occurred in the ALA-supplemented diet treatments, as shown in the increased the total feed intake (Table 2). Increased appetite along with the increased feed intake was caused by the ALA roles in adenosine monophosphate-activated protein kinase enzyme (AMPK) activation to provide a signal to the cells for energy consumption and hunger-full regulation (Kim *et al*., 2004; Wang *et al*., 2010). Increased feed intake, followed by the increased nutrient availability and utilization caused the diet became more efficient to consume that finally reflected on a higher growth. Fish fed with the ALA-supplemented diet (diet B; 27:390) had a

lower feed conversion ratio and a higher growth rate than other treatments. Therefore, the dietary ALA supplementation (diet B) can highly reduce the diet energy and protein level, compared to the diet C and E treatments. A similar condition was also occupied in giant gourami (Samuki *et al*., 2020) and African catfish (Siagian *et al*., 2021).

CONCLUSION

The dietary ALA supplementation at 8 g/kg could reduce the use of energy and protein contents in feed, and improve the growth performance of striped catfish (*Pangasianodon hypophthalmus*).

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Author confirmation

All authors have agreed to publish the article in Jurnal Akuakultur Indonesia (JAI).

Conflict of interests

Authors have no conflict of interests to declare.

Authors' Contribution

RR performed the experiment, analyze the data, and wrote the article; DJ designed the experiment and wrote the article; MAS analyzed the data and wrote the article; AA analyzed the data and wrote the article; SN analyzed the data and wrote the article.

Data availability statement

Data supporting the study results are available in the corresponding author upon a reasonable request.

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