Impact of freshwater fish oil-fortified feed on nutrition and growth of *Hemibagrus nemurus* (Valenciennes, 1840) larvae

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Abstract. This study was conducted to determine the effect of feed fortified with freshwater fish oil (FFO) on the composition of fatty acids, growth performance, survival, and larvae yield of Asian redtail catfish *Hemibagrus nemurus* (Valenciennes, 1840). A total of 1,125 larvae (1.5 mg, 5 mm in length) were divided into five groups, each with three replications. Rearing was carried out in 15 aquariums (15 L of water, 75 larvae per aquarium) for 40 days. The feed was fortified with 0%, 8%, 10%, 12%, and 14% FFO, and the larvae were fed to satiation. The results showed that feed fortified with FFO significantly affected the composition of fatty acids, growth performance, survival, and yield ($p < 0.05$). Proximate analysis revealed differences in crude protein and lipid content in feed, with total n-3 increasing from 0.42 to 0.72%, and n-9 increasing from 21.96 to 27.3%. Meanwhile, n-6 decreased from 8.73 to 5.17%, and unsaturated fatty acids increased from 52.88 to 64.14%. The EPA content increased from 1.33 to 1.67% in the 14% FFO diet. The final body weight and survival rate of larvae fed with 10% FFO were 875.27 mg and 82.22%, respectively. In conclusion, among the FFO-fortified diets, the 10% FFO diet resulted in the highest growth, survival, and larvae yield of *H. nemurus*. This study suggests fortifying feed with 10% FFO to enhance feed efficiency for *H. nemurus* larvae.

Key Words: Asian Redtail catfish, freshwater fish oil, fatty acid, growth performance, survival rate.

Introduction. Zero Hunger (SDG 2) is a fundamental goal of the 2030 Agenda for Sustainable Development, which was adopted by the United Nations in 2015 to eliminate hunger, ensure food security and nutrition, and promote sustainable agriculture. A promising avenue for overcoming hunger and malnutrition problems lies in the consumption of fish (Hasselberg et al 2020; Tran et al 2022), which is widely known for its rich nutritional profile, comprising protein, lipids, vitamins, and minerals (Mohanty et al 2019; Azrita et al 2024a). In Indonesia, wild-caught and farmed fish provide about 10.32 grams of protein per capita per day (Statistical Yearbook of Indonesia 2015), with the Asian redtail catfish *Hemibagrus nemurus* (Valenciennes, 1840) serving as a source of essential nutrients. In addition, this freshwater species is predominantly obtained from wild catches by small-scale fishermen in rivers and reservoirs (Aryani et al 2020). A small portion is also obtained from farming in earthen freshwater ponds and cages in river flows (Hasan et al 2019). In 2019, the production of *H. nemurus* from freshwater cage aquaculture reached 821 tons, accounting for approximately 0.18% of the total output of 467,991 tons (Marine and Fisheries Statistics 2022). In the local market of Pekanbaru City, Indonesia, the price of table-size *H. nemurus* ranges from IDR 80,000 to 90,000 per kilogram. To meet the market demand from the aquaculture sector, the production of *H. nemurus* needs to be increased. Therefore, larvae must be provided in the right amount, at the right time, and of high quality. One approach to achieve this is by adding fatty acids through fish oil to commercial feeds. Several studies have investigated the use of live and formulated feeds enriched with fatty acids to support larval growth and survival (Campoverde & Estevez 2017; Choi et al 2021).

Despite its significant potential, there is a knowledge gap in efforts to increase the fatty acid content of feeds for *H. nemurus* larvae, which aims to improve the quantity and quality, as well as growth and survival performance. Therefore, several studies have been conducted to improve the nutritional quality of formulated feeds to support the growth and survival of *H. nemurus* larvae and juveniles. The methods used include the enrichment of formulated feeds with EPA and DHA through marine fish oil for *H. nemurus* juveniles (Aryani et al 2023a), a combination of *Tubifex* sp. and artificial feed was used for *H. nemurus* fry (Aryani et al 2013), and the enrichment of *H. nemurus* larvae feed using *Tubifex* fortified with black cumin oil (Juliana et al 2016).

Over the past decade, researchers have relied on marine fish oil to enrich freshwater fish larvae feed (Al-Soutia et al 2012; Singh et al 2012). However, the relatively high market price of marine fish oil has prompted a shift in focus. Currently, efforts are directed toward utilizing waste from pangasius catfish processing as a suitable source of fish oil, supporting a green economy (Aryani et al 2023b). This alternative is considered more environmentally friendly, potentially more sustainable, and potentially better suited to the nutritional needs of certain species. Therefore, enriching the feed with varying levels of freshwater fish oil (FFO) is crucial for *H. nemurus* larvae. Fish oil is a source of essential fatty acids, such as EPA and DHA, which play a crucial role in various physiological processes in larvae (Gesto et al 2021), including cell membrane formation and growth hormone regulation (Weinrauch et al 2023), as well as tissue differentiation and development (Hossain et al 2024a). In addition, fatty acids contribute to nutrient absorption efficiency in the digestive tract and enhance larvae's resistance to environmental stress (Hossain et al 2024b). Therefore, the inclusion of freshwater fish oil (FFO) in the diet has the potential to improve nutritional quality and biologically support optimal larval growth.

This study aims to evaluate the effects of different doses of freshwater fish oil (FFO) on the proximate composition, fatty acid profile, biometric parameters, survival rate, and yield of *H. nemurus* larvae. The results are expected to serve as a basis for sustainable feed formulation and to support the increased production of catfish larvae in the freshwater aquaculture sector.

Material and Method

Animal materials. The larvae used in this study were three-day-old *H. nemurus* larvae obtained through artificial spawning. The broodstock was first maintained in fiberglass tanks for two days. Broodstock with mature gonads were injected with GnRH analogs combined with a dopamine antagonist (Ovaprim) (Manufactured for Syndel Laboratories Ltd, 2595 McCullough Rd., Nanaimo, B.C.V9S 4M9 Canada) at a dosage of 0.5 mL kg^{-1} for females and 0.3 mL kg^{-1} for males.

Preparation of FFO oil extract. The raw material used was mesenteric fat waste from *Pangasius hypophthalmus*, obtained from a traditional market. Dirt and blood adhering to the fat were removed using tissue paper. The fat was then finely chopped with a knife and heated in a Vienta-brand oven (manufactured by PT. Blue Gas Indonesia) at 60°C for one hour until the oil was extracted. From one kilogram of catfish mesenteric fat (Pangasius hypophthalmus), 1000 mL of freshwater fish oil (FFO) can be obtained, which shows high efficiency (1:1). The resulting oil was filtered, packaged in dark glass bottles, and stored in a refrigerator. Before use, the bottle containing the oil was immersed in hot water at 70°C, then weighed according to the treatment dosage and sprayed evenly into commercial feed.

Experiment with FFO in diets. The experiment used was a 0.25 mm commercial feed (Powdered Larvae Feed) with proximate composition (based on dry weight %) of 10.23% moisture, 38.61% crude protein, 7.39% crude fat, 13.17% ash, 2.88% crude fiber, and 40.81% total carbohydrates. All experimental diets were formulated to be isonitrogenous (~38.5% crude protein) and isofat (~7.4% crude fat) to ensure that observed differences were attributable to lipid sources. In addition, the sample had a 37.93% nitrogen-free extract, an energy content of 1,608 kJ $(100\ g)^{-1}$, and 328.31 µg vitamin D. Various minerals present include 17.88 mg g^{-1} Ca, 10.82 mg g^{-1} P, 2.98 mg g^{-1} Mg, 0.095 mg g^{-1} Mn, 0.055 mg g^{-1} Na, and 0.141 mg g^{-1} Zn.

Each 100 grams of feed was fortified with FF0 sourced from Pangasius catfish with dosages differing: 0%, 8%, 10%, 12%, and 14%. FFO was added to the feed using manual stirring for 4 minutes to ensure proper distribution. After being fortified with freshwater fish oil (FFO) at doses of 8%, 10%, 12%, and 14%, the powdered larvae feed was air-dried at room temperature (±28°C) for 12 hours to allow proper absorption and surface drying of the oil without exposing it to high temperatures that could degrade essential fatty acids. The samples obtained were then given to experimental animals.

Trial setting and animal sampling. The weight of the larvae was measured using an AD-600i balance (ACIS model number AD-600i, China) with a precision of 0.01 g, while the initial length was measured with a ruler using a precision of 1 mm. A total of 1,125 *H. nemurus* larvae, three days post-hatching, with an initial weight of 1.5 mg and a body length of 5 mm, were used as samples for the experiment. In the hatchery, 15 glass aquariums height 30 cm in height and with a water volume of 15 L, were set up and provided with continuous aeration. The experiment consisted of 5 treatment groups, each with three replicates. Furthermore, each glass aquarium was stocked with a total of 75 *H. nemurus* larvae.

FFO was sprayed onto the feed based on the experimental dosage level and manually stirred for five minutes to ensure uniform distribution. The fortified feed was then provided to the experimental larvae *ad libitum*. Feeding was conducted daily at 09.00, 13.00, 18.00, and 22.00 Western Indonesian Time (WIB, UTC+7) for 40 days between June and July 2024. During the 40-day experiment, larvae were sampled every 10 days to assess body weight and calculate actual feed consumption. Before sampling, the larvae were fasted for 12 hours to ensure intestinal clearance. In each glass aquarium, 15 *H. nemurus* larvae were collected. The length and weight were measured, followed by a return to the respective glass aquarium based on the assigned treatment groups.

Proximate composition analysis. The biochemical compositions of feed samples were analyzed using standard AOAC methods (AOAC 2000). The samples were dried until a constant weight was achieved at 105°C, and the crude protein content (N × 6.25) was analyzed using the Kjeldahl method. Hydrolysis was performed three times using 6N hydrochloric acid for 24 hours at 11°C. Furthermore, crude fat content in feed ingredients was analyzed using the Soxhlet method with ether extraction. Ash content was determined by incinerating the samples at 550°C for 16 hours.

Percent from total carbohydrates (%) = 100 - (% crude protein + % fat + % ash) (Onyeike et al 2000). After calculating the total percentage of carbohydrates, the gross energy value (calories) was calculated using the following equation: Gross energy value (kJ) = (4 × percentage of crude protein) + (9×percentage of fat) + (4×percentage of total carbohydrates) (Jobling 1983). Calculations were carried out per 100 g of samples. Nitrogen- Free Extract, NFE (%) was calculated with the formula 100 - (% moisture + % ash + % lipid + % protein) (NRC 1983).

Fatty acids analysis. The diets from each treatment were subjected to fatty acid analysis using the gas chromatography-mass spectrometry (GC-MS) method. Total lipid extraction was conducted following a modified version of the Folch et al (1957) method as described by Rajion et al (1985), using a chloroform:methanol 2:1 (v/v) solvent system. Furthermore, transmethylation was carried out using 14% methanolic boron trifluoride. The fatty acid composition of the diets was analyzed by Unit Laboratorium Terpadu IPB University, Bogor, Indonesia.

Growth performance, survival, and yield analysis. The following equations were used to analyze the parameters measured in this study:

$$Weight\ \ gain\ (\%) = \frac{Final\ body\ weigt\ (mg)\ -\ Initial\ body\ weight\ (mg)}{Initial\ body\ weight\ (mg)} \times 100$$

$$Average\ daily\ gain\ (ADG) = \frac{Final\ body\ weight\ (mg)\ -\ Initial\ body\ weight\ (mg)}{Duration\ of\ rearing\ period\ (days)}$$

$$Length\ gain\ (\%) = \frac{Final\ total\ length\ (mm)\ -\ Initial\ total\ length\ (mm)}{Initial\ total\ length\ (mm)} \times 100$$

$$Specific\ growth\ rate, weight\ (\%\ day^{-1}) = \frac{ln\,Wt\ -\ ln\,Wo}{Duration\ of\ rearing\ period\ (days)} \times 100$$

$$Specific\ growth\ rate, length\ (\%\ day^{-1}) = \frac{ln\,Lt\ -\ ln\,Lo}{Duration\ of\ rearing\ period\ (days)} \times 100$$

$$Survival\ rate\ (\%) = \frac{Number\ of\ surviving\ fry}{Number\ of\ fry\ stocked} \times 100$$

$$Mortality\ rate\ (\%) = \frac{Number\ of\ fry\ stocked\ -\ Number\ of\ survived\ fry}{Number\ of\ fry\ stocked} \times 100$$

$$Fulton\ condition\ factor = \frac{Total\ weight\ (TW)}{Standard\ length (SL)^3} \times 100$$

$$Yield\ (mg\ L^{-1})\ = \frac{Final\ \ weight\ of\ fry\ \times\ Number\ of\ survived\ of\ fry}{Water\ volume\ (litre)}$$

$$Coefficient\ of\ variation\ (CV)\ of\ weight\ (\%) = \frac{Standard\ deviation\ of\ weight}{Mean\ weight} \times 100$$

$$Coefficient\ of\ variation\ (CV)\ of\ length\ (\%) = \frac{Standard\ deviation\ of\ length}{Mean\ length} \times 100$$

Water quantity parameter analysis. The water quality parameters in the glass aquarium used for rearing *Hemibagrus nemurus* larvae were recorded weekly at 10.00 Western Indonesian Time (WIB, UTC + 7). Measurements were taken at a depth of 10 cm from the water surface in each aquarium. In addition, the water temperature in each aquarium is. Water temperature was measured using a Celsius-scale thermometer, and the dissolved oxygen content (O_2; mg L^{-1}) was assessed with an oxygen meter (YSI Model 52, Yellow Instrument Co., Yellow Spring, OH, USA). A digital pH meter (Mini 0–14 pH I.Q., Scientific Chemo Science Thailand) was used to record the pH value of the water. The importance of nitrate-nitrogen (NO_3-N; mg L^{-1}) was analyzed with standard APHA procedures (APHA 1995).

Statistical analysis. Data were statistically analyzed using SPSS version 16.0 (SPSS; Chicago, IL). Analysis was carried out using one-way ANOVA to determine the effect of feed enrichment tests with different levels of FF0. When there were significant differences at the 5% significance level, further analysis was carried out using Duncan's post hoc multiple range test to compare treatment mean±SD concerning proximate composition, fatty acid, and amino acid compositions in the diets. Additionally, biometric performance was determined at a 95% confidence level ($p < 0.05$) based on the method described by Duncan (1955).

Results

Proximate analysis and fatty acid profile of the experimental diets. The proximate composition of diets fortified with various doses of FFO is presented in Table 1. Based on the result, significant content was found across all five diets, with the primary differences occurring in crude protein and fat content (7% < value 12% < dry weight). The analysis results showed that all treatments contained significant nutritional values, with the main differences observed in crude protein and lipid contents. The highest protein content was recorded in the 0% FFO treatment (38.61%), while the lowest was found in the 14% FFO treatment (31.61%). In contrast, the highest lipid content was observed in the 14% FFO treatment (19.08%), whereas the lowest was found in the control (0% FFO), at 7.39%. These findings indicate that the addition of FFO in the feed positively contributes to increasing lipid content, although it is accompanied by a reduction in protein content, as shown in Table 1.

Table 1

Proximate composition of experimental diets containing 0%, 8%, 10%, 12%, and 14% FFO (dry weight basis)

Proximate composition	*Experimental diets*				
	0% FFO	*8% FFO*	*10% FFO*	*12% FFO*	*14% FFO*
Moisture (% WW)	10.23 ± 0.02^a	9.17 ± 0.01^b	8.71 ± 0.02^c	8.34 ± 0.01^d	7.91 ± 0.01^e
Crude protein (% DW)	38.61 ± 0.16^a	34.19 ± 0.02^b	33.28 ± 0.01^c	32.08 ± 0.02^d	31.61 ± 0.02^e
Crude lipid (% DW)	7.39 ± 0.02^a	13.41 ± 0.01^b	16.69 ± 0.01^c	17.53 ± 0.02^d	19.08 ± 0.01^e
Crude fiber (% DW)	2.88 ± 0.01^a	1.16 ± 0.01^b	1.67 ± 0.01^c	1.63 ± 0.01^d	1.13 ± 0.01^e
Crude ash (% DW)	13.17 ± 0.01^a	11.82 ± 0.02^b	11.45 ± 0.01^c	11.25 ± 0.01^d	10.92 ± 0.01^e
Total carbohydrates (% DW)	40.81 ± 0.15^a	40.57 ± 0.03^b	38.58 ± 0.03^c	39.31 ± 0.01^d	38.37 ± 0.03^e
Nitrogen-free extract (% DW)	37.93 ± 0.16^a	39.41 ± 0.04^b	36.91 ± 0.02^c	37.68 ± 0.01^d	37.24 ± 0.04^e
Energy value (kcal $(100\ g)^{-1}$ DM)	384.30 ± 0.09^a	419.74 ± 0.04^b	437.65 ± 0.04^c	441.76 ± 0.10^c	451.72 ± 0.03^d

Notes: 0% FFO: feed fortified with 0% freshwater fish oil; 8% FFO: feed fortified with 8% freshwater fish oil; 10% FFO: feed fortified with 10% freshwater fish oil; 12% FFO: feed fortified with 12% freshwater fish oil, and 14% FFO: feed fortified with 14% freshwater fish oil. Values are presented as mean±SD (Standard Deviation) from three independent determinations. Different superscripts (a, b, c, d) within the same row indicate statistically significant differences ($p < 0.05$).

The fatty acid composition of the experimental diets is presented in Table 2. The results show that the highest content of unsaturated fatty acids was found in the diet containing 14% FFO, reaching 64.14%, which was higher than that of the control (52.88%) and the diets fortified with 8% (59.56%), 10% (60.89%), and 12% FFO (62.74%). Statistically, the unsaturated fatty acid content differed significantly among the FFO-fortified treatments ($p < 0.05$). The three groups of fatty acids—Σ n-3, Σ n-6, and Σ n-9—play important roles in fish physiology. However, in the context of larval fish growth, n-3 fatty acids (Σ n-3), particularly eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), are the most influential components. In this study, the highest EPA content was recorded in feed fortified with 10% FFO (2.08%). Nevertheless, a high EPA level was not always accompanied by a high DHA level. The highest DHA content was observed in the control treatment (without FFO), at 2.26%. These findings indicate variations in the EPA and DHA ratio across treatments, which may be influenced by the composition of the freshwater fish oil used.

Table 2

Fatty acid composition of the experimental diets containing FFO (dry weight basis)

Fatty acids	*Experimental diets*				
	0% FFO	*8% FF0*	*10% FFO*	*12% FFO*	*14% FFO*
Butyric (4:0)	0.04 ± 0.01^{a}	0.09 ± 0.06^{b}	0.07 ± 0.01^{c}	0.09 ± 0.01^{bd}	0.07 ± 0.01^{ce}
Lauric (C12:0)	0.11 ± 0.01^{a}	0.14 ± 0.01^{b}	0.15 ± 0.01^{bc}	0.11 ± 0.01^{ad}	0.12 ± 0.01^{de}
Myristic (C14:0)	2.60 ± 0.01^{a}	3.28 ± 0.01^{b}	3.24 ± 0.01^{c}	3.19 ± 0.01^{d}	3.24 ± 0.01^{ce}
Pentadecanoic (C15:0)	0.17 ± 0.02^{a}	0.35 ± 0.01^{b}	0.38 ± 0.01^{c}	0.22 ± 0.01^{d}	0.32 ± 0.01^{e}
Palmitic (C16:0)	22.31 ± 0.05^{a}	22.95 ± 0.02^{b}	22.75 ± 0.02^{c}	23.35 ± 0.03^{d}	24.01 ± 0.04^{e}
Heptadecanoic (C17:0)	0.21 ± 0.01^{a}	0.32 ± 0.01^{b}	0.28 ± 0.01^{c}	0.26 ± 0.01^{d}	0.29 ± 0.02^{e}
Stearic (C18:0)	4.96 ± 0.01^{a}	5.54 ± 0.04^{b}	5.46 ± 0.02^{c}	5.70 ± 0.02^{d}	5.82 ± 0.02^{e}
Arachidic (C20:0)	0.34 ± 0.00^{a}	0.26 ± 0.01^{b}	0.24 ± 0.01^{c}	0.29 ± 0.01^{d}	0.29 ± 0.02^{de}
Behenic (C22:0)	0.02 ± 0.00^{a}	0.17 ± 0.02^{b}	0.13 ± 0.00^{c}	0.15 ± 0.01^{d}	0.15 ± 0.01^{de}
Palmitoleic (C16:1)	0.12 ± 0.01^{a}	0.23 ± 0.02^{b}	2.06 ± 0.00^{c}	1.89 ± 0.02^{d}	1.24 ± 0.02^{e}
Heptadecanoic (C17:1)	0.02 ± 0.02^{a}	0.09 ± 0.01^{b}	0.06 ± 0.00^{c}	0.06 ± 0.01^{cd}	0.07 ± 0.00^{e}
Elaidic (C18:1n9t)	nd	0.08 ± 0.00^{b}	0.06 ± 0.01^{c}	0.09 ± 0.01^{d}	0.06 ± 0.00^{ce}
Oleic (C18:1 n-9)	21.34 ± 0.03^{a}	25.30 ± 0.05^{b}	25.37 ± 0.20^{c}	26.58 ± 0.14^{d}	27.07 ± 0.04^{e}
Eicosenoic, (C20:1)	0.62 ± 0.01^{a}	0.72 ± 0.02^{b}	0.68 ± 0.01^{c}	0.68 ± 0.01^{cd}	0.69 ± 0.01^{ea}
Linoleic acid (C18:2 n-6)	14.08 ± 0.03^{a}	15.39 ± 0.03^{b}	14.71 ± 0.02^{c}	14.47 ± 0.02^{d}	14.34 ± 0.02^{e}
Eicosetrienoic, C20:3n6	0.24 ± 0.01^{a}	0.27 ± 0.00^{b}	0.30 ± 0.01^{c}	0.35 ± 0.01^{d}	0.32 ± 0.01^{e}
Arachidonic (C20:4 n-6)	0.35 ± 0.01^{a}	0.37 ± 0.01^{ab}	0.36 ± 0.02^{c}	0.40 ± 0.02^{d}	0.37 ± 0.01^{e}
EPA (C20:5 n -3)	1.33 ± 0.02^{a}	1.35 ± 0.02^{b}	2.08 ± 0.01^{c}	1.82 ± 0.02^{d}	1.67 ± 0.02^{e}
DHA (C22:6 n-3)	2.26 ± 0.02^{a}	1.84 ± 0.02^{b}	1.72 ± 0.01^{c}	1.54 ± 0.02^{d}	1.41 ± 0.01^{d}
∑SFA	30.77 ± 0.08^{a}	33.12 ± 0.03^{b}	32.65 ± 0.04^{c}	33.43 ± 0.04^{d}	34.32 ± 0.09^{e}
∑MUFA	22.10 ± 0.02^{a}	26.44 ± 0.08^{b}	28.24 ± 0.02^{c}	29.31 ± 0.06^{d}	29.82 ± 0.03^{e}
∑PUFA	18.3 ± 0.02^{a}	20.5 ± 0.08^{b}	19.4 ± 0.02^{c}	18.9 ± 0.06^{ad}	18.4 ± 0.03
∑PUFA/∑SFA	3.67 ± 0.02^{a}	4.45 ± 0.05^{b}	4.02 ± 0.02^{c}	3.69 ± 0.04^{ad}	3.36 ± 0.02^{e}
Unsaturated fatty acid	52.88 ± 0.04^{a}	59.56 ± 0.12^{b}	60.89 ± 0.05^{c}	62.74 ± 0.17^{d}	64.14 ± 0.05^{e}
Σ n-3 fatty acid	0.42 ± 0.00^{a}	0.63 ± 0.01^{b}	0.58 ± 0.01^{c}	0.73 ± 0.03^{d}	0.65 ± 0.01^{e}
Σ n-6 fatty acid	8.73 ± 0.04^{a}	7.06 ± 0.13^{b}	6.93 ± 0.17^{bc}	5.04 ± 0.17^{d}	5.17 ± 0.07^{e}
Σ n-9 fatty acid	21.96 ± 0.01^{a}	26.12 ± 0.07^{b}	26.12 ± 0.04^{bc}	27.36 ± 0.12^{d}	27.83 ± 0.05^{e}
Σn-6/ Σn-3	0.58 ± 0.01^{a}	1.27 ± 0.00^{b}	1.20 ± 0.00^{c}	1.17 ± 0.01^{d}	1.18 ± 0.00^{e}
EPA/DHA	0.59 ± 0.00^{a}	0.61 ± 0.00^{b}	0.59 ± 0.00^{ac}	0.56 ± 0.00^{d}	0.53 ± 0.00^{e}
DHA/EPA	1.70 ± 0.03^{a}	0.78 ± 0.01^{b}	0.82 ± 0.01^{c}	0.84 ± 0.01^{c}	0.84 ± 0.00^{de}
EPA/ARA	3.81 ± 0.15^{a}	6.35 ± 0.15^{b}	5.78 ± 0.14^{c}	4.58 ± 0.17^{d}	4.52 ± 0.10^{de}

Notes: 0% FFO: feed fortified with 0 % freshwater fish oil; 8% FFO: feed fortified with 8% freshwater fish oil; 10% FFO: feed fortified with 10% freshwater fish oil; 12% FFO: feed fortified with 12% freshwater fish oil, and 14% FFO: feed fortified with 14% freshwater fish oil. Means±SD (Standard Deviation) of three separate determinations, a, b, c, d: significant in a row, and nd: not detected.

Growth performance parameters. During the larvae rearing experiment using feed fortified with FFO at different doses, there was a significant difference ($p < 0.05$) in growth performance, like weight gain (WG), average daily weight gain (ADG), and specific growth rate of weight (% day^{-1}) are presented in Figures 1A, 1B, 1C. The highest WG was recorded in the group receiving 10% FFO fortification (873.77 mg $fish^{-1}$), followed by 12% FFO (784.63 mg $fish^{-1}$), 8% FFO (720.33 mg $fish^{-1}$), 0% FFO (606.83 mg $fish^{-1}$), and the lowest in 14% FFO (572.90 mg $fish^{-1}$). A similar trend was observed in ADG, with the highest value also at 10% FFO (21.84 mg $fish^{-1}$ day^{-1}) and the lowest at 14% FFO (14.32 mg $fish^{-1}$ day^{-1}). Likewise, the highest SGR was recorded in the 10% FFO group (15.92% day^{-1}), while the lowest was observed at 14% FFO (14.86% day^{-1}).

The highest average daily length increment, survival rate, condition factor, and overall performance were recorded in the group fed with feed fortified with 10% FFO (Table 3). Additionally, the lowest coefficient of variation in weight (1.07 %) was also observed in this treatment, indicating that the body weights of the fish in this group were the most uniform. In contrast, the highest value (2.09 %) was recorded in the 14% FFO group, suggesting the greatest variation in body weight among individuals. A low coefficient of variation in length (0.31 %) was likewise found in the 10% FFO treatment, indicating that the length growth of the fish in this group was relatively uniform (Table 3).

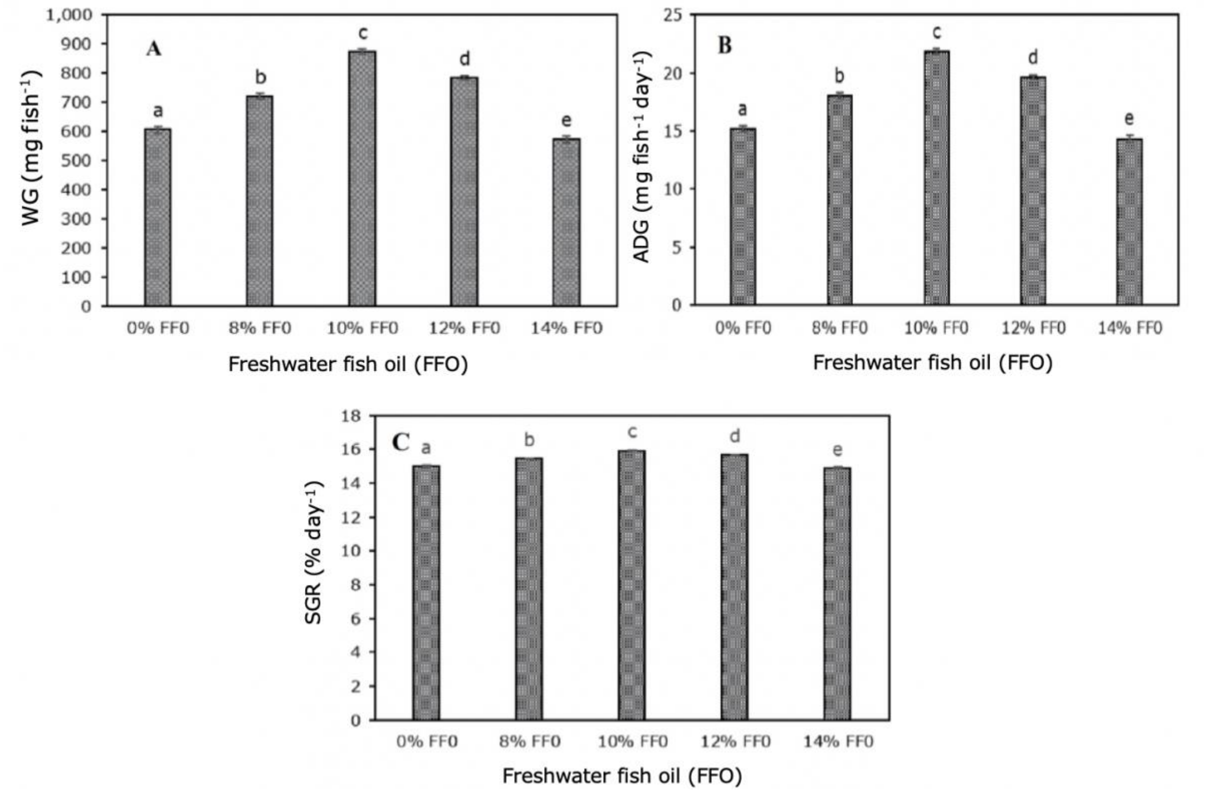

Figure 1. Growth parameters of *Hemibagrus nemurus* larvae exposed to various doses of freshwater fish oil (FF0). The graphs include weight gain (A), average daily growth (B), and specific growth rate (C). Growth parameters are presented as mean±SD (n = 3; 75 larvae per replicate glass aquarium).

Table 3

Differences in growth performance indicators of *Hemibagrus nemurus* larvae fed diets containing varying levels of FFO

Parameters	*Experimental diets*				
	0% FFO	*8% FFO*	*10% FFO*	*12% FFO*	*14% FFO*
Length gain (mm)	36.13±0.35^a	37.96±0.15^b	43.36±0.15^c	40.66±0.15^d	35.06±0.25^e
Length average daily gain (mm)	0.90±0.00^a	0.94±0.00^b	1.08±0.00^c	1.01±0.00^d	0.87±0.00^e
Survival rate (%)	68.88±0.76^a	71.55±2.03^b	82.22±2.03^c	78.66±1.33^d	65.77±2.03^e
Mortality rate (%)	31.11±0.76^a	28.44±2.03^b	17.77±2.03^c	21.33±1.33^d	34.22±2.03^e
Condition factor	0.87±0.02^a	0.77±0.00^b	0.91±0.02^c	0.82±0.01^d	0.89±0.01^e
Yield (mg L^{-1})	2,095.51±55.94^a	2,582.36±72.93^b	3,598.76±119.05^c	3,091.84±30.66^d	1,889.13±70.75^e
Coefficient of variation of weight (CV of weight (%)	1.94±0.03^a	1.47±0.02^b	1.07±0.01^c	1.98±0.17^d	2.09±0.04^e
Coefficient of variation of length (CV of length (%)	0.85±0.00^a	0.35±0.00^b	0.31±0.00^c	0.33±0.00^d	0.62±0.00^e

Notes: 0% FFO: feed fortified with 0% freshwater fish oil; 8% FFO: feed fortified with 8% freshwater fish oil; 10% FFO: feed fortified with 10% freshwater fish oil; 12% FFO: feed fortified with 12% freshwater fish oil, and 14% FFO: feed fortified with 14% freshwater fish oil. Values are presented as mean±SD (Standard Deviation) from three independent determinations. Different superscripts (a, b, c, d) within the same row indicate statistically significant differences.

Water quality during period larvae rearing. During the larval rearing period, water quality fluctuated, which is thought to affect the growth and survival of Hemibagrus nemurus larvae. The parameters observed included dissolved oxygen (DO) levels, ammonia (NH_3) concentrations, and pH. The average value of each parameter per FFO fortification treatment is presented in Table 4. In general, the decrease in dissolved oxygen (DO) and pH, along with the increase in ammonia concentration, tended to correspond with the increasing levels of FFO in the diet. The FFO 14% treatment exhibited the poorest water quality conditions, with DO levels approaching the minimum tolerance threshold for larvae (< 4.0 mg L^{-1}) and ammonia concentrations nearing toxic limits. Conversely, in the FFO 8% and 10% treatments, water quality remained within a relatively optimal range to support larval physiology.

Interestingly, although the water quality in the FFO 10% treatment was slightly lower than that in the FFO 8% group, larval growth performance and survival rates were notably higher. This suggests that the nutritional composition of the 10% FFO diet may have compensated for the slight decline in water quality, offering an optimal balance between dietary nutrition and environmental conditions.

Table 4

Water quality parameters observed during the feeding trial with experimental diets containing 0%, 8%, 10%, 12%, and 14% FFO

Treatment FFO (%)	*DO (mg L^{-1})*	*NH_3 (mg L^{-1})*	*pH*
FFO 0%	5.3-5.5	0.0620-0.0700	6.8-6.9
FFO 8%	4.9-5.2	0.0704-0.0789	6.7-6.8
FFO 10%	4.3-4.7	0.0785-0.0843	6.5-6.6
FFO 12%	3.9-4.2	0.0837-0.0885	6.4-6.5
FFO 14%	3.6-4.1	0.0873-0.0902	6.3-6.4

Discussion. Analyzing fish feed biochemical composition and fatty acids profile offers valuable insights for improving the growth rate and feed use of specific species (Qin et al 2022). This investigation showed that supplementing feed with FFO at a dosage of 14% (equivalent to 140 g FFO kg^{-1} feed) caused a significant increase of up to 11.69% in fat content. Furthermore, the total n-3 fatty acid content of the diets increased from 0.42% to 0.73%, while the total n-9 fatty acid content experienced an increment from 21.96% to 27.83%. This heightened fat content directly correlated with the quantity of FFO introduced into the diet, showing a positive correlation between feed composition and FFO levels.

Based on the results, increasing the fat content in the feed was for optimizing the growth of *H. nemurus* larvae. The fat content of commercial (Powdered Larvae Feed) fortified with different levels of FFO at 8%, 10%, 12%, and 14% was 13.41%, 16.69%, 17.53%, and 19.08%, respectively. Similarly, feed formulations fortified with EPA + DHA from marine fish oil at levels of 0.31%, 0.63%, and 0.94% resulted in lipid contents of 6.42%, 7.57%, and 7.69%, respectively (Aryani et al 2023a). These findings suggest that increasing dietary fat content through FFO supplementation contributes to improved lipid availability, which is crucial for supporting the metabolic needs of *H. nemurus* larvae.

The optimal dietary fat requirement varies among fish species, particularly between carnivorous and herbivorous species. Previous studies indicate that juvenile pompano, *Trachinotus ovatus,* range from 12.39 to 12.65% (Zhang et al 2019). The species *Hemibagrus wyckioides* required dietary fat levels between 10.60 and 13.22% (Deng et al 2021), while grouper (*Epinephelus coioides)* required 10.80 up to 11.00% (Wang et al 2017). Carnivorous fish generally require higher fat levels in the diet compared to herbivorous species (Zaretabar et al 2022). As *H. nemurus* is a carnivorous fish species (Aryani et al 2020), this study's observed dietary fat content of 16.69% aligns with the lipid requirements necessary to support its optimal growth. These results emphasize the importance of tailoring dietary fat content to meet the metabolic demands of *H. nemurus*, ensuring efficient feed utilization and improved growth performance.

Adding FFO to *H. nemurus* larvae feed could increase the levels of the PUFAs group fatty acids, particularly EPA and ARA. In this study, the addition of 10% FF0 increased EPA levels in the samples from 1.33 to 1.67%, while DHA decreased from 2.26 to 1.72% of total fatty acids. Several studies reported that increasing EPA and DHA in the diet was positively associated with whole-body lipid deposition (Zhang et al 2019; Aryani et al 2023a). According to Morshedi et al (2022), the lipid content in rotifers (*Brachionus rotundiformis*) and Artemia nauplii (*Artemia franciscana*), used as feed for yellowtail sea bream (*Acanthopagrus latus*) larvae, increased despite the enrichment with docosahexaenoic acid (DHA) being at very low levels. This response depended on factors such as the species, initial weight, feeding habits, and aquaculture conditions (Zhang et al 2019).

The Fulton condition factor (CF) for *H. nemurus* larvae remained below 1 during the early rearing period up to day 40, ranging from 0.77 to 0.91. Statistical analysis revealed significant differences in condition factor among treatments ($p < 0.05$), with the highest value observed in larvae fed with 10% FFO-fortified feed.

A CF value below 1 typically indicates that the larvae were still in a phase of active morphological development and had not yet accumulated sufficient body mass relative to their length. This is common during early larval stages, when energy is primarily allocated to organogenesis, tissue differentiation, and skeletal formation rather than fat or protein deposition (Gisbert & Williot 1997). The significant variation in CF among treatments suggests that dietary composition played an important role in influencing larval body robustness and growth uniformity (Banu et al 2020). According to Azrita et al (2024b), a condition factor < 1 indicates deteriorating habitat conditions and a possible imbalance in feed quality.

The highest CF value recorded in the 10% FFO group reflects the positive impact of moderate freshwater fish oil supplementation on larval condition, likely due to an optimal balance of essential fatty acids that support energy metabolism and membrane development. This finding aligns with previous studies reporting that adequate lipid fortification in larval diets can enhance physiological resilience and overall health status (Choi et al 2021; Ritu et al 2024). Therefore, the inclusion of 10% FFO appears to improve not only growth performance but also body condition, which is a critical indicator of the larvae's adaptive capacity and readiness for further development.

Although *H. nemurus* larvae were provided with nutritionally balanced and high-quality feed throughout the rearing period, water quality factors were suspected to be suboptimal for optimal growth. The DO level declined from 4.9-5.2 to 3.6-4.1 mg L^{-1}, while ammonia (NH_3) concentration increased from 0.0704-0.0893 to 0.0843-0.0902 mg L^{-1}. In addition, the pH (acidity) level declined from 6.7-6.8 to 6.3-6.5, which could negatively affect the physiological condition and growth performance of the larvae. Low DO concentrations can impair aerobic metabolism, reduce feed intake, and increase susceptibility to stress and disease (Qiang et al 2019). Meanwhile, elevated ammonia levels, even at sublethal concentrations, are known to interfere with gill function and osmoregulatory balance, leading to reduced energy available for growth. The decline in pH may also disrupt enzymatic activity and metabolic efficiency, further compromising larval development (Fin 2007).

In this study, the DHA/EPA ratios in diets fortified with FFO at 8%, 10%, 12%, and 14% were 0.78, 0.82, 0.84, and 0.84, respectively. Notably, feed fortified with 10% FF0 showed a significantly higher growth rate, survival rate, and yield (mg L^{-1}) than other diets (Table 3). The high survival and yields obtained in the 10% FFO treatment were likely related to the optimal balance of essential fatty acids, especially the DHA/EPA ratio, which supported the physiological functions of the larvae efficiently. The DHA/EPA ratio of 0.82 found in the 10% FFO diet appeared to be sufficient to meet the structural and energy needs of the larvae during the critical early life stages. Similarly, enrichment of *Artemia* nauplii with low DHA/high EPA could improve the survival of greater amberjack (*Seriola dumerili*) larvae (Roo et al 2023). Fatty acids, either too low or too high, can cause lipid metabolism disorders and reduce stress resistance, which ultimately affect survival and productivity (Choi et al 2021).

In line with these results, larvae growth of *Gadus macrochepalus* and *S. dumerili* was significantly inhibited by diets containing elevated DHA/EPA ratios (Choi et al 2021; Roo et al 2023). The corresponding whole-body lipid levels of juvenile black sea bream

(*Acanthopagrus schlegelii*) decreased when the dietary DHA/EPA ratio increased from 2.03 to 2.67 (Jin et al 2017). These results suggest that an optimal DHA/EPA ratio is essential not only to support lipid metabolism and growth but also to improve the overall survival and production yield of fish larvae.

Additionally, a strong relationship was observed between the DHA/EPA ratio in the diet and larval size, with larvae typically exhibiting a DHA/EPA ratio of < 2:1 (Copeman et al 2002). This indicates that excessive DHA relative to EPA may disrupt essential physiological processes, possibly by altering membrane fluidity and enzymatic activity related to lipid metabolism. Thus, the findings of this study emphasize the importance of maintaining a balanced DHA/EPA ratio in formulated feeds. The 0.82 DHA/EPA ratio in the diet supplemented with 10% FFO appears to optimize the growth and survival of *H. nemurus* larvae, highlighting the potential role of dietary lipid composition in aquaculture nutrition strategies.

H. nemurus larvae were reported to experience better growth rates when fed commercial feed fortified using 10% FFO, with a DHA/EPA ratio of 0.82. On the other hand, growth was lower when given feed fortified using 14% and 0% FF0, with a DHA/EPA ratio of 0.84 and 1.70, respectively. Similar results were also obtained for other freshwater fish, such as largemouth bass (*Micropterus salmoides*), which achieved the best weight with a DHA/EPA ratio of 0.25 (Yadav et al 2020). While marine fish, such as amberjack (*S. dumerili*), thrived well with a DHA/EPA ratio of 0.49 (Roo et al 2023), grouper, *E. coioides*, had higher growth with a ratio of approximately 1.0 (Chen et al 2017). A study on Japanese seabass, *Lateolabrax japonicus* showed that the SGR increased significantly with a DHA/EPA ratio of 2.08 (Xu et al 2016). These findings suggest that the optimal DHA/EPA ratio for fish varies depending on species and feeding ecology, with carnivorous freshwater fish generally requiring lower DHA/EPA ratios compared to marine species.

Conclusions. This study demonstrated that incorporating freshwater fish oil (FFO) into feed at levels between 8% and 14% enhanced the fat content, fatty acid composition, growth performance, and survival of *Hemibagrus nemurus* larvae. The optimal results, especially in terms of total polyunsaturated fatty acids (∑PUFA), growth rate, fry yield, and survival, were achieved with 10% FFO supplementation. These findings highlight the practical value of using FFO, particularly from local fish processing by-products, as a sustainable and cost-effective lipid source in aquaculture feed formulations. Its application can improve nutrient utilization, increase fry production, and reduce dependency on marine fish oil, thereby supporting more resilient and locally-sourced hatchery systems. Further research should investigate the long-term physiological effects of FFO, explore feed formulation strategies combining FFO with other lipid sources, and evaluate its impact on the flesh quality of cultured fish.

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

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