







## RESEARCH ARTICLE

**REVISED** Fatty acid composition on diet and carcasses, growth, body indices and profile serum of Asian redbtail catfish (*Hemibagrus nemurus*) fed a diet containing different levels of EPA and DHA [version 2; peer review: 2 approved]

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**Abstract**

**Background:** The Asian redbtail catfish *Hemibagrus nemurus* is a promising commercial aquaculture freshwater big-sized Bagridae catfish across Asian countries such as the Mekong, Malay Peninsula, and Indonesia. This study analysed the effect of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) supplementation in diets on changes in fatty acid compositions in feed and fish meat, lipid quality (atherogenic index and thrombogenic index), growth rate, body indicators, and serum metabolites of *Hemibagrus nemurus* juveniles.

**Methods:** A total of 180 Asian redbtail catfish (initial weight  $54.80 \pm 2.72$  g) were fed four levels (0, 3,150, 6,300, and 9,450 mg of EPA+DHA/kg feed) sourced from fish oil. Diets were fed in triplicate in freshwater tarpaulin ponds, with 15 fish per tarpaulin pond. During the experiment, fish were fed 3% per day of the biomass weight.

**Results:** Categorically, there were significant differences in the composition of fatty acids in the feed and fish meat. The atherogenic index was between 1.76 and 1.84, and the thrombogenic index was between 0.81 and 0.89 in all fish meat. Growth performance was significantly different between diets, while body indices did not make a significant difference between diets. The fish meat EPA and DHA showed positive linear relationships with diet EPA ( $p < 0.001$ ,  $r^2 = 90\%$ ) and DHA diet ( $p < 0.001$ ,  $r^2 = 85\%$ ). Serum metabolites among treatments D2 and D3 diet-fed feed for 60 days did not significantly differ. Glucose (GLU) levels had moderate relationships with triglycerides (TAG) ( $r^2 = 65\%$ ), and GLU levels strongly correlated with low-density lipoprotein cholesterol (LDL-C) ( $r^2 = 81\%$ ).

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**Conclusions:** Based on diets and whole-body carcass compositions, growth performance, and serum metabolites, Asian redbtail catfish fed a diet containing 6,300 mg of EPA+DHA/kg feed are best for food safety.

### Keywords

Aquaculture, Asian redbtail catfish, essential fatty acids, growth rates, serum metabolites



This article is included in the [Agriculture, Food and Nutrition gateway](#).

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**REVISED Amendments from Version 1**

1. The abstract cannot be revised because it is limited to a maximum of 300 words.
2. There are changes and additions in the method according to the Reviewer Reports.
3. In the discussion, there is an additional discussion about enzyme activity that cannot be developed yet.
4. At the conclusion, there is an additional statement about "AI and TI values in the meat of Asian redtail catfish at 6,300 mg of EPA+DHA were 0.81 and 1.85. Foods with low AI and TI promote excellent human health, for example, by preventing human health coronary diseases."

**Any further responses from the reviewers can be found at the end of the article**

## Introduction

Global capture fisheries production from marine sources has stagnated in the last few decades.<sup>1,2</sup> Therefore, food sources from freshwater fish farming are increasingly being recognized for their role in an environmentally sustainable and nutritionally sustainable food system.<sup>3,4</sup> However, freshwater fish contain fewer omega ( $\omega$ )-3 fatty acids than omega ( $\omega$ )-6 polyunsaturated fatty acids (PUFAs), making them a healthy food.<sup>5,6</sup> Therefore, fish feed freshwater must be provided with the addition of omega ( $\omega$ )-3 FUFAs carried out sustainably. Several researchers have reported feed enrichment for freshwater fish, such as the addition of linolenic acid, fish oil, and soybean oil to Nile tilapia fish feed.<sup>6-8</sup> Omega ( $\omega$ )-3, as well as EPA and DHA for the feed of Atlantic salmon.<sup>9,10</sup> Dietary lipid levels for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*) and silver barb (*Puntius gonionotus*) fingerling.<sup>11,12</sup>

Aquafeed is rich in numerous significant nutrients, such as amino acids, fatty acids, vitamins, and minerals. Of these, feed is rich in nutrients and can be used by fish to increase body weight and survival,<sup>13</sup> disease resistance, and changes in the aquaculture environment.<sup>14,15</sup> This factor could also increase feed efficiency, which is usually used as a success indicator of fish farming.<sup>16</sup> However, better feed quality is related to the nutrients of the whole-body carcass and is beneficial for human health.<sup>1,4,5,8,14,17</sup>

*Hemibagrus nemurus*, commonly known as "Asian redtail catfish," is a promising commercial aquaculture freshwater big-sized Bagridae catfish across Asian countries such as Mekong, Malay Peninsula, and Indonesia. Because of its dominant consumer preference, it is most abundant in ordo Bagridae.<sup>18</sup> This species has a high growth rate, disease resistance, year-round reproduction,<sup>19</sup> and wide adaptability to the cultivation environment.<sup>20,21</sup> In recent decades, research on feed nutrition for the growth performance of Asian redtail catfish has garnered the interest of researchers, such as experiments on dietary protein levels to increase growth performance, feed efficiency, and survival rate of juveniles,<sup>22,23</sup> and the utilization of salted trash fish meal in the diet as a substitute for fish meal.<sup>21</sup> Substitution of fish meal with a mixture of by-catch and fish viscera meal mixtures.<sup>24</sup> Additionally, the addition of turmeric (*Curcuma longa*) in artificial feed,<sup>25</sup> and addition of *Saccharomyces cerevisiae* in commercial feed,<sup>26</sup> as well as different lipid level on daily growth coefficient and feed conversion ratio of Asian redtail catfish, *Hemibagrus wyckioides*.<sup>11</sup> Until now, there has been no information on the utilities of optimum EPA and DHA levels on the sensible diet for the growth rate and fish meat value of Asian redtail catfish. We hypothesized that supplemented levels of EPA + DHA *via* fish oil in the diet could improve the nutritional quality of dietary fatty acid and fish meat, feed efficiency, growth rate, body indices, and lipid profiles in the serum of Asian redtail catfish. Therefore, the first aim of this study was to investigate the effect of the EPA and DHA levels in diets on the fatty acid composition. The second aim was to analyse fish meat fatty acids, growth rate, feed efficiency, and nutritional quality of lipids.

## Methods

### Ethical statement

This study was conducted under the project entitled 'Optimization of the use of EPA- and DHA-type fatty acids in the feed of Asian redtail catfish to strengthen food security after coronavirus disease (COVID-19)'. This research was funded by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, which has been approved by the Institute for Research and Community Service, Universitas Riau (grant letter: 1633/UN19.5.1.3/PT.01.03/2022). The Ethics Community Research and Community Service Universitas Riau approved collecting and rearing of *Hemibagrus nemurus* juveniles with the ARRIVE guidelines, which have been stated in the letter of grant No. 1633/UN19.5.1.3/PT.01.03/2022, May 11, 2022. Additionally, according to Indonesian Legislation, the Asian redtail catfish were not categorized as a protected species. The research was carried out in the Hatchery Laboratory, Faculty Fisheries and Marine, Riau University for 60 days from May to June 2022. This study is reported in line with the ARRIVE guidelines.<sup>27</sup> We have made efforts to alleviate the suffering of experimental animals in this study, except for some euthanized animal experiments that were carried out by piercing part of the fish brain. The total number of fish in the study was 180 fish consisting of 45 fish for each treatment. Each treatment consisted of 15 fish per replicate (replicate = 3). A total of three

fish were anesthetized in each replicate for the analysis of whole-body flesh, and body indices. Before the fish was euthanized, it was soaked in fresh water at a temperature of 10°C for five minutes. The goal is for the fish to be calmer and pierce their brain easier. After that, the fish were put to sleep on a board with a cork bottom, and their brain was pierced with a large animal syringe (9G × 1 inch). Then the fish were dissected to measure their body indices. In addition, the flesh was collected to measure its fatty acid composition.

### Preparation experiment diet

Asian redtail catfish adapted for 30 days to standard, 2 mm pellet feed. The dietary composition (% dry weight) consisted of an 8.76% moisture level, 34.52% crude protein, 5.94% crude fat, 41.13% carbohydrates, 9.65% ash content and 3.85% crude fibre (Table 1). The total calories were 356.06 Kcal/100 g vitamin D (328.31 mcg/100 g). Minerals consisted of calcium (1,788.11 mg/100 g), phosphorus (1,081.764 mg/100 g), magnesium (298.42 mg/100 g), manganese (9.50 mg/100 g), sodium (549.30 mg/100 g), and zinc (14.10 mg/100 g).

The vitamin mix contains vitamins A, 2,750 IU; Vit D, 550,000 IU; Vit E, 25,000 IU; Ca D-pantothenate, 25,000 mg, Mg, 25,000 mg; P, 50,000 mg.

After adaptation, the fish were fed supplemented with fish oil (EPA + DHA) at a dosage of 0 g/kg feed (labelled D1). A total of 5 g/kg feed, consisting of 1,950 mg EPA and 1,200 mg DHA (labelled D2). The 10 g/kg feed consisted of 3,900 mg EPA and 2,400 mg DHA (labelled D3). The 15 g/kg feed consisted of 5,850 mg EPA and 3,600 mg DHA (labelled D4). EPA and DHA were mixed manually to the aquafeed to produce quality feed, which was then given to the animal experiment.

### Experimental procedure and sampling

Fish samples were weighed using the AD-600i, with an accuracy of 0.01 g (ACIS model number AD-600i, China). The Indonesian Directorate of Metrology has approved the use of ACIS model AD-600i. Additionally, body length was measured with a measuring board with an accuracy of 1 mm. A total of 180 juvenile *Hemibagrus nemurus* were counted (initial body weight was  $54.80 \pm 2.72$  g, and initial body length was  $15.77 \pm 2.5$  cm). The age of the fish was 120-day post-hatching (120 DPH), whose sex has not been determined. Juveniles were obtained from the Hatchery Laboratory Faculty of Fisheries and Marine Science Universitas Riau. This species' health status was good, not a hybridization

**Table 1. List of ingredients and analysis of experimental diets containing 0, 0.5, 1 and 1.5% fish oil.**

Ingredients (%)	D1	D2	D3	D4
Fish meal	25	25	25	25
Soybean meal	27	27	27	27
Rice bran	41	40.5	40	39.5
Fish oil	0	0.5	1	1.5
Corn oil	3	3	3	3
CMC (carboxy methyl cellulose)	2	2	2	2
Vitamin-Mineral Mix	1	1	1	1
Cellulose	1	1	1	1
	100	100	100	100
Proximate composition (dry weight)				
Moisture	8.64	9.04	8.67	9.17
Ash	9.58	9.42	9.61	9.59
Crude Protein	34.54	34.53	33.72	33.75
Crude Fat	5.90	6.42	7.57	7.69
Crude fibre	3.82	3.96	3.88	3.69
NFE	46.22	45.57	45.37	45.22
Energy (Kcal/g)	356.54	358.18	364.71	363.01

Note: nitrogen-free extract (NFE) = 100 - (% crude protein + % crude fat + % ash + % crude fibre).

genetic modification. A total of 12 round tarpaulin ponds (diameter = 80 cm, height = 60 cm, water depth is 50 cm with a water volume of 400 litres) were placed in Hatchery Laboratory and equipped with continuous aeration. This experiment consisted of four treatments and three replicates, and each round tarpaulin pond was stocked with 15 juveniles randomly stocked. The Asian redbtail catfish were fed pellets 2 mm in size supplemented with EPA and DHA, namely, D1, D2, D3, and D4 diets. Daily feeding was performed at 07:00, 13:00, 18:00, and 22:00 at a body weight rate of 3% per day for 60 experimental days. A feeding rate of 3% has been optimal for the growth of *Hemibagrus nemurus* in this study, although some researchers reported a range of 3 - 8%. Every 20 days, five fish samples were taken from each tarpaulin pond. Before sampling, the fish fasted for 12 hours to vacate their visceral contents. Fish samples were anesthetized orally using clove oil, and body weight was measured to determine the amount of fed feed. Then, their weights were measured. After that, the fish were returned to their respective tarpaulin ponds according to treatment and replication.

### Fatty acid analysis

The feed and whole-body meat of the fish of each treatment were examined utilizing a fatty acid composition by gas chromatography-mass spectrometry (GC-MS). The extraction of the total lipid was carried out according to the method described by Folch *et al.* (1957), as explained by Rajion<sup>28</sup> utilizing a chloroform: methanol (2.1. v/v) solvent system. Transmethylation was carried out using 14% methanolic boron trifluoride. Transmethylation was carried out using 14% methanolic boron trifluoride. The derivatized fatty acid methyl esters (FAMES) were separated on a Quadrex 007 series bonded phase fused silica capillary column (Quadrex Corporation, New Haven, CT, USA) (30 m × 0.25 mm ID, 0.20 mm film thickness, 007 Carwax/BTR) in a 5890 Hewlett-Packard Gas-Liquid Chromatograph (Hewlett-Packard Co., Avondale, PA). Individual fatty acids were recognized and measured by proportion with retention time and peak areas of FAMES standards (Supelco 37 Component FAME mix and Nu-Check Prep Inc., GLC-569). The fatty acid composition of feed and whole-body meat of Asian redbtail catfish were examined by Saraswanti Indo Genetech Laboratory, Bogor-Indonesia (SIG Laboratory, Accredited Testing Laboratory- LP -184-IDN).

### Tarpaulin pond water quality

The water quality values of the round tarpaulin ponds that were used to rear the Asian redbtail catfish juveniles were recorded weekly. Water quality was measured at 10:00 AM at a distance inward of 10 cm from the water surface of each tarpaulin pond to detect the water temperature, dissolved oxygen, and pH. A thermometer (Celsius scale) was used to measure water temperature. Water oxygen (O<sub>2</sub>; mg/L) was measured by an oxygen metre (YSI Model 52, Yellow Instrument Co, Yellow Spring, OH, USA). The pH value of the water was calculated on a digital pH metre (Mini 0–14 pH IQ, Scientific Chemo Science Thailand). Additionally, we also measured the level of nitrate-nitrogen (NO<sub>3</sub>-N; mg/L), total alkalinity (mg/L), and hardness (mg/L) calculated according to standard American Public Health Association (APHA) procedures.<sup>29</sup>

### Calculations

All fish from each treatment and replicate (n=15 fish per replicate) were measured in length and weighed body weight separately for the final experiment. Final length (FL), final weight (FW), weight gain (WG, %), and specific growth rate (SGR, %/day). The feed conversion ratio (FCR) and survival rate (SR) were analysed using the support formula as follows:

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

$$\text{Specific growth rate (\%/day)} = \frac{\ln \text{Final body weight (g)} - \ln \text{Initial body weight (g)}}{\text{Culture day}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed supply in kg}}{\text{Total harvest weight in kg}}$$

$$\text{Survival rate} = \frac{\text{The final number of fish}}{\text{The initial number of fish}} \times 100$$

For the analysis of the body indices of Asian redbtail catfish (*Hemibagrus nemurus*), three fish were sacrificed each in the tarpaulin pond, and their weight and length were measured. After that, it was immediately dissected to determine the Condition Factor (CF), Hepatosomatic Index (HSI), Viscerosomatic Index (VSI), and Liposomatic Index (LSI) as given below:

$$\text{Condition Factor} = \frac{\text{Body weight of the fish (g)}}{\text{The body length of the fish (cm)}^3} \times 100$$

$$\text{Hepatosomatic Index} = \frac{\text{Liver weight of the fish (g)}}{\text{Body weight of the fish (g)}} \times 100$$

$$\text{Viscerosomatic Index} = \frac{\text{Viscera weight of the fish (g)}}{\text{Body weight of the fish (g)}} \times 100$$

$$\text{Liposomatic Index} = \frac{\text{Visceral fat weight of the fish (g)}}{\text{Body weight of the fish (g)}} \times 100$$

The nutritional quality of lipid AI and TI was calculated based on the equations<sup>30</sup>

$$\text{Atherogenic Index (AI)} = \frac{[(\text{C12:0} + 4 + \text{C14:0} + \text{C16:0})]}{[(\sum \text{MUFA} + \sum n-6 + \sum n-3)]}$$

$$\text{Thrombogenic Index (TI)} = \frac{[(\text{C14:0} + \text{C16:0} + \text{C18:0})]}{[(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum n-6) + (3 \times \sum n-3) + (\sum n-3 / \sum n-6)]}$$

Where:

C12:0 = Lauric acid

C14:0 = Myristic acid

C16:0 = Palmitic acid

C18:0 = Stearic acid

$\sum$ MUFA = Sum concentrations of all unsaturated fatty acid

$\sum$ n-6 = Sum concentrations of n-6 polyunsaturated fatty acid

$\sum$ n-3 = Sum concentrations of n-3 polyunsaturated fatty acid

### Lipid profiles in serum

In the present study, to minimize stress on the experimental animals, the animals within the containers were lightly anesthetized with 1 ml/10 L clove oil for 2–3 min until the loss of coordination was visible. Afterward, the blood was collected by puncturing the caudal vertebrae tail vessels using a hypodermic needle of 1 ml (made in Indonesia). Briefly, blood samples from each specimen were placed in an Eppendorf tube, and then centrifuged at 3,000 rpm for 15 minutes (5804R, Eppendorf), and the supernatant serum rather than plasma was stored at -21 °C. Then it was analysed at the Centre for Primate Animal Studies at IPB University, Bogor, Indonesia. Serum glucose (GLU) was analysed using the glucose oxidase method using commercial kit (Pathology Laboratory, the Centre for Primate Animal Studies, IPB University, Bogor, Indonesia), Primate Animal Study Center, IPB University-Bogor, has standardized commercial kits to measure blood fish parameters. Triglycerides (TAG) were measured using the GPO-PAP method, total cholesterol (TC) by the CHOP-PAP method, high-density lipoprotein (HDL) with direct method-select inhibition method, low-density lipoprotein (LDL) with direct method-surfactant removal were also measured using commercial investigation kit (Pathology Laboratory, the Centre for Primate Animal Studies, IPB University, Bogor, Indonesia), and the ratio of LDL-C and HDL-C were approximated as outlined previously.<sup>31</sup>

## Data analysis

To determine the trial effect of supplemented EPA and DHA in diets, body meat, growth performance, body indices, and serum metabolite variables were measured using one-way ANOVA. The data from the experiment were analysed using SPSS (RRID:SCR\_002865) 16.0 software package (SPSS, Chicago, IL). The data homogeneity was analysed with Levin's test and followed up with the post hoc Duncan's multiple range test.<sup>32</sup> Relationship between dietary EPA and DHA with whole-body meat; glucose levels, TAG levels, and LDL-C levels were analysed using Regression with curve estimation. For the figures presented, Microsoft Office Professional Plus 2019 was used.

## Results

### Fatty acid concentrations in the experimental diet

In this study, the  $\sum$ MUFA in the four diets showed higher values than  $\sum$ SAFA and  $\sum$ PUFA. Regarding the  $\sum$ MUFA, the D1 diet had the highest value, followed by the D2, D3, and D4 diets with C-oleic acid (C18:1, n-9) abundance in all diets. The SAFA palmitic acid (C16:0) and stearic acid (C18:0) were abundant in all diets. However, in the PUFA, the higher composition was linoleic acid (C18:2 n-6) (Table 2).<sup>27</sup>

**Table 2. Fatty acid profile (% of total FA) and total lipids in the diet supplemented with the EPA and DHA.**

Fatty acids	Fish oil	Experiment diets				P-Value	SE <sup>1</sup>
		D1	D2	D3	D4		
C12:0, Lauric	nd	0.220 <sup>a</sup>	0.233 <sup>b</sup>	0.200 <sup>c</sup>	0.220 <sup>ad</sup>	0.619	0.024
C14:0, Myristic	1.31	1.590 <sup>a</sup>	1.593 <sup>b</sup>	1.633 <sup>c</sup>	1.590 <sup>d</sup>	0.063	0.015
C15:0, Pentadecanoic	0.17	0.173 <sup>a</sup>	0.177 <sup>b</sup>	0.170 <sup>c</sup>	0.167 <sup>d</sup>	0.400	0.005
C16:0, Palmitic	10.84	25.363 <sup>a</sup>	27.540 <sup>b</sup>	27.550 <sup>c</sup>	26.713 <sup>d</sup>	0.000	0.197
C17:0, Margaric	1.23	0.350 <sup>a</sup>	0.388 <sup>b</sup>	0.417 <sup>c</sup>	0.427 <sup>d</sup>	0.000	0.007
C18:0, Stearic	3.95	4.973 <sup>a</sup>	4.993 <sup>b</sup>	4.903 <sup>c</sup>	3.490 <sup>d</sup>	0.000	0.023
C20:0, Arachidic	0.40	0.350 <sup>a</sup>	0.033 <sup>b</sup>	0.337 <sup>c</sup>	0.257 <sup>d</sup>	0.035	0.027
C21:0, Heneicosylic	0.10	0.053 <sup>a</sup>	0.050 <sup>b</sup>	0.057 <sup>c</sup>	0.070 <sup>d</sup>	0.132	0.007
C23:0, Tricosylic	nd	0.050 <sup>a</sup>	0.033 <sup>b</sup>	0.020 <sup>c</sup>	0.021 <sup>d</sup>	0.001	0.004
C24:0, Lignoceric	nd	0.117 <sup>a</sup>	0.087 <sup>b</sup>	0.107 <sup>c</sup>	0.103 <sup>d</sup>	0.023	0.007
C14:1, Myristoleic	0.15	0.043 <sup>a</sup>	0.070 <sup>b</sup>	0.110 <sup>c</sup>	0.150 <sup>d</sup>	0.000	0.007
C16:1, Palmitoleic	3.62	1.913 <sup>a</sup>	1.900 <sup>b</sup>	1.950 <sup>c</sup>	1.420 <sup>d</sup>	0.074	0.192
C17:1, cis-10 Heptadecanoic	0.79	0.177 <sup>a</sup>	0.187 <sup>b</sup>	0.200 <sup>c</sup>	0.223 <sup>d</sup>	0.000	0.005
C18:1, Oleic	15.47	34.130 <sup>a</sup>	33.477 <sup>b</sup>	32.850 <sup>c</sup>	32.167 <sup>d</sup>	0.000	0.066
C20:1, Paullinic	5.29	0.610 <sup>a</sup>	0.680 <sup>b</sup>	0.760 <sup>c</sup>	0.833 <sup>d</sup>	0.000	0.024
C20:2, Dihomo-linoleic	0.23	0.283 <sup>a</sup>	0.263 <sup>b</sup>	0.250 <sup>c</sup>	0.233 <sup>d</sup>	0.002	0.008
C22:1 n-9, Erucic	7.74	0.053 <sup>a</sup>	0.583 <sup>b</sup>	1.023 <sup>c</sup>	0.140 <sup>d</sup>	0.000	0.008
C22:2, cis-13,16 Docosadienoic	0.11	0.300 <sup>a</sup>	0.183 <sup>b</sup>	0.180 <sup>c</sup>	0.140 <sup>d</sup>	0.000	0.007
C18:2 n-6, Linoleic	2.74	19.487 <sup>a</sup>	19.843 <sup>b</sup>	18.770 <sup>c</sup>	18.033 <sup>d</sup>	0.000	0.102
C18:3 n-6, Gamma-Linolenic	0.28	0.140 <sup>a</sup>	0.113 <sup>b</sup>	0.147 <sup>c</sup>	0.137 <sup>d</sup>	0.007	0.007
C20:3 n-6, Dihomo-gamma-linolenic	0.14	0.147 <sup>a</sup>	0.147 <sup>b</sup>	0.137 <sup>c</sup>	0.137 <sup>d</sup>	0.370	0.007
C20:4 n-6, Arachidonic	1.53	0.463 <sup>a</sup>	0.530 <sup>b</sup>	0.547 <sup>c</sup>	0.550 <sup>d</sup>	0.000	0.007
C18:3 n-3, $\alpha$ -Linolenic	1.48	1.890 <sup>a</sup>	1.970 <sup>b</sup>	1.923 <sup>c</sup>	1.863 <sup>d</sup>	0.000	0.012
C20:3 n-3, Eicosatrienoic	0.10	0.067 <sup>a</sup>	0.067 <sup>b</sup>	0.060 <sup>c</sup>	0.050 <sup>d</sup>	0.109	0.006
C20:5 n-3, EPA	23.82	1.320 <sup>a</sup>	2.023 <sup>b</sup>	2.850 <sup>c</sup>	3.387 <sup>d</sup>	0.000	0.014
C22:6 n-3, DHA	18.28	2.270 <sup>a</sup>	2.503 <sup>b</sup>	3.048 <sup>c</sup>	3.123 <sup>d</sup>	0.000	0.029
$\Sigma$ n-3	43.89	6.147 <sup>a</sup>	6.563 <sup>b</sup>	7.882 <sup>c</sup>	8.823 <sup>d</sup>	0.000	0.050
$\Sigma$ n-6	4.69	20.237 <sup>a</sup>	20.633 <sup>b</sup>	19.600 <sup>c</sup>	18.857 <sup>d</sup>	0.000	0.111

**Table 2.** Continued

Fatty acids	Fish oil	Experiment diets				P-Value	SE <sup>1</sup>
		D1	D2	D3	D4		
Σn-6/Σn-3	0.10	3.292 <sup>a</sup>	3.144 <sup>b</sup>	2.487 <sup>c</sup>	2.137 <sup>d</sup>	0.000	0.019
DHA/EPA	1.30	2.174 <sup>a</sup>	1.237 <sup>b</sup>	1.070 <sup>c</sup>	1.040 <sup>d</sup>	0.000	0.009
EPA+DHA	42.1	4.190 <sup>a</sup>	4.527 <sup>b</sup>	5.898 <sup>c</sup>	6.910 <sup>d</sup>	0.000	0.041
ΣSFA	18.08	33.090 <sup>a</sup>	35.440 <sup>b</sup>	35.483 <sup>c</sup>	33.140 <sup>d</sup>	0.109	1.143
ΣMUFA	33.57	38.777 <sup>a</sup>	37.360 <sup>b</sup>	37.315 <sup>c</sup>	36.817 <sup>d</sup>	0.000	0.245
ΣPUFA	48.68	26.38383 <sup>a</sup>	27.197 <sup>b</sup>	27.489 <sup>c</sup>	27.680 <sup>d</sup>	0.000	0.139
ΣFA	100	100 <sup>a</sup>	100 <sup>b</sup>	100 <sup>c</sup>	100 <sup>d</sup>	0.000	0.038
Lipid content (%)		5.937 <sup>a</sup>	6.49 <sup>b</sup>	7.64 <sup>c</sup>	7.75 <sup>d</sup>	0.265	1.448

Notes: 0 g fish oil/kg feed (labelled D1); 5 g fish oil/kg feed, consisted of 1,950 mg EPA and 1,200 mg DHA (labelled D2); 10 g fish oil/kg feed consisted of 3,900 mg EPA and 2,400 mg DHA (labelled D3); and 15 g fish oil/kg feed consisted of 5,850 mg EPA and 3,600 mg DHA (labelled D4). Means ± SE (standard error) of three separate determinations, a b c d = significant in a row, nd = not detected FA, fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

### Fatty acid concentrations in body meat

The Asian redbtail catfish fed feed D1, D2, D3, and D4 showed higher levels of ΣMUFA in the body meat than in ΣSAFA and ΣPUFA. Regarding MUFA, oleic acid (C18:1) is the most abundant. Palmitic acid (C16:0) and stearic acid (C18) were significant concentrations of SAFAs. Conversely, linoleic acid (C18:2, n-6) abounds in PUFA. We noted that EPA and AA concentrations were lacking in all body meat, while DHA was higher (Table 3). EPA and DHA levels in feed have strong relationships with EPA and DHA levels in body meat ( $r^2 = 0.897$  for EPA; Figure 1 and  $r^2 = 0.812$  for DHA; Figure 2). AI and TI were significantly higher on the D3 diet than on the other treatments. Duncan's post hoc test showed that the AI and TI for fish fed D1 were not significantly different ( $p > 0.05$ ) from those provided for D2 and D4 (Figures 3 and 4).

**Table 3.** Fatty acid concentrations (% of total FA) of the Asian redbtail catfish (*Hemibagrus nemurus*) fed with different diets after the 60 days reared.

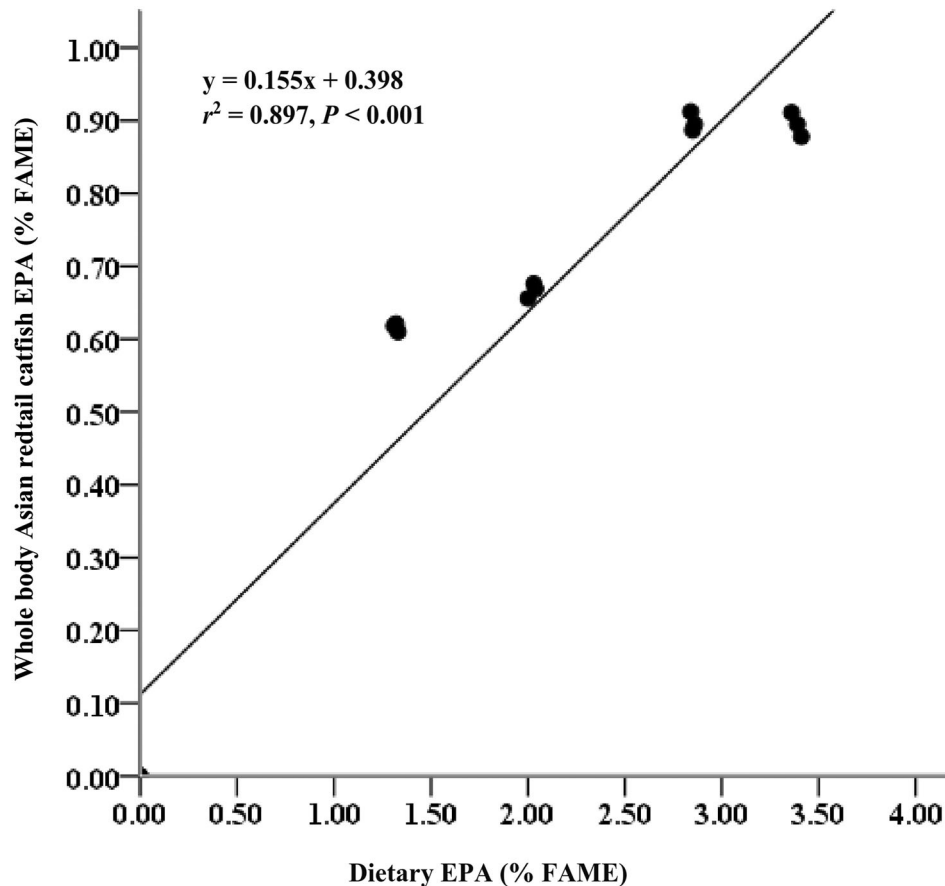
Fatty acids	Experiment diets				P-value	SE <sup>1</sup>
	D1	D2	D3	D4		
C12:0, Lauric	0.760 <sup>a</sup>	0.614 <sup>b</sup>	0.773 <sup>c</sup>	0.730 <sup>d</sup>	0.000	.007
C14:0, Myristic	2.277 <sup>a</sup>	1.881 <sup>b</sup>	2.367 <sup>c</sup>	2.372 <sup>cd</sup>	0.000	.027
C15:0, Pentadecanoic	0.169 <sup>a</sup>	0.155 <sup>b</sup>	0.181 <sup>c</sup>	0.181 <sup>cd</sup>	0.002	.004
C16:0, Palmitic	27.266 <sup>a</sup>	25.480 <sup>b</sup>	25.824 <sup>c</sup>	26.783 <sup>d</sup>	0.000	.093
C17:0, Margaric	0.248 <sup>a</sup>	0.245 <sup>ab</sup>	0.285 <sup>c</sup>	0.245 <sup>ad</sup>	0.000	.026
C18:0, Stearic	8.156 <sup>a</sup>	8.566 <sup>b</sup>	9.226 <sup>c</sup>	9.271 <sup>cd</sup>	0.000	.133
C20:0, Arachidic	0.244 <sup>a</sup>	0.252 <sup>b</sup>	0.306 <sup>c</sup>	0.253 <sup>bd</sup>	0.000	.029
C24:0, Lignoseric	0.109 <sup>a</sup>	0.078 <sup>b</sup>	0.112 <sup>b<sup>c</sup></sup>	0.053 <sup>d</sup>	0.000	.001
C16:1, Palmitoleic	2.177 <sup>a</sup>	1.938 <sup>b</sup>	2.348 <sup>c</sup>	2.413 <sup>cd</sup>	0.000	.036
C17:1, cis 10 Heptadecanoic	0.162 <sup>a</sup>	0.154 <sup>b</sup>	0.182 <sup>c</sup>	0.183 <sup>d</sup>	0.000	.002
C18:1, Oleic	37.565 <sup>a</sup>	35.097 <sup>b</sup>	35.342 <sup>c</sup>	36.439 <sup>d</sup>	0.000	.303
C20:1, Paullinic	0.839 <sup>a</sup>	0.750 <sup>b</sup>	1.025 <sup>c</sup>	0.935 <sup>d</sup>	0.000	.006
C20:2, Dihomo-linoleic	0.677 <sup>a</sup>	0.614 <sup>b</sup>	0.748 <sup>c</sup>	0.656 <sup>d</sup>	0.000	.009
C22:2, cis 13,16 Docosadienoic	1.093 <sup>a</sup>	1.500 <sup>b</sup>	nd	nd	0.000	.011
C18:2 n-6, Linoleic	12.874 <sup>a</sup>	11.668 <sup>b</sup>	14.603 <sup>c</sup>	13.726 <sup>d</sup>	0.000	.172
C18:3 n-6, Gamma-Linolenic	1.271 <sup>a</sup>	0.345 <sup>b</sup>	0.369 <sup>c</sup>	0.404 <sup>d</sup>	0.000	.004
C20:3 n-6, Dihomo-Gamma-linolenic	0.913 <sup>a</sup>	0.816 <sup>b</sup>	1.011 <sup>c</sup>	0.930 <sup>d</sup>	0.000	.012
C20:4 n-6, Arachidonic	0.465 <sup>a</sup>	0.425 <sup>b</sup>	0.503 <sup>c</sup>	0.496 <sup>d</sup>	0.000	.005



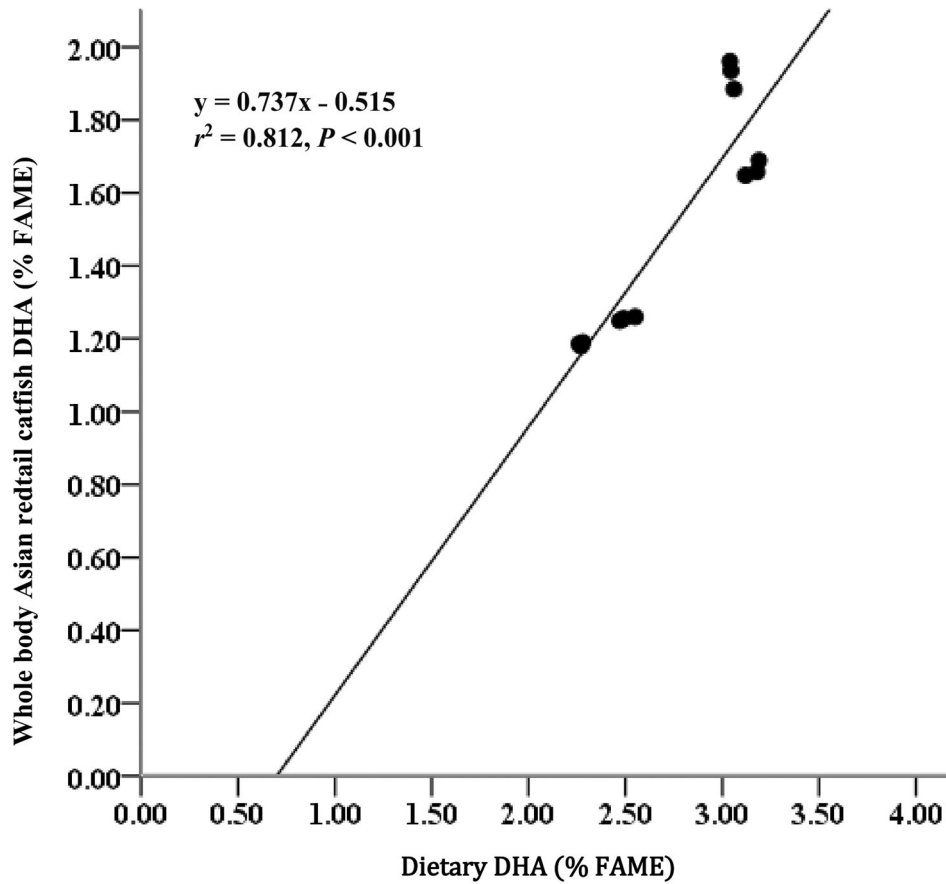
**Table 3.** *Continued*

Fatty acids	Experiment diets				P-value	SE <sup>1</sup>
	D1	D2	D3	D4		
C18:3 n-3, $\alpha$ -linolenic	0.853 <sup>a</sup>	0.768 <sup>b</sup>	1.006 <sup>c</sup>	0.895 <sup>d</sup>	0.000	.126
C20:3 n-3, Eicosatrienoic	nd	0.813 <sup>b</sup>	0.907 <sup>c</sup>	nd	0.000	.011
C20:5 n -3, EPA	0.616 <sup>a</sup>	0.667 <sup>b</sup>	0.898 <sup>c</sup>	0.895 <sup>cd</sup>	0.000	.009
C22:6 n-3, DHA	1.186 <sup>a</sup>	1.255 <sup>b</sup>	1.927 <sup>c</sup>	1.665 <sup>d</sup>	0.000	.020
$\Sigma$ n-3	2.655 <sup>a</sup>	3.503 <sup>b</sup>	4.738 <sup>c</sup>	3.455 <sup>d</sup>	0.000	.038
$\Sigma$ n-6	15.524 <sup>a</sup>	15.255 <sup>b</sup>	15.487 <sup>c</sup>	15.557 <sup>d</sup>	0.420	.188
$\Sigma$ n-3: $\Sigma$ n-6	0.171 <sup>a</sup>	0.230 <sup>b</sup>	0.306 <sup>c</sup>	0.222 <sup>d</sup>	0.000	.029
DHA/EPA	1.924 <sup>a</sup>	1.881 <sup>b</sup>	2.146 <sup>c</sup>	1.862 <sup>d</sup>	0.000	.030
EPA+DHA	1.802 <sup>a</sup>	1.922 <sup>b</sup>	2.825 <sup>c</sup>	2.560 <sup>d</sup>	0.000	.023
$\Sigma$ SAFA	39.231 <sup>a</sup>	37.272 <sup>b</sup>	39.074 <sup>ac</sup>	39.890 <sup>d</sup>	0.000	.166
$\Sigma$ MUFA	42.514 <sup>a</sup>	40.053 <sup>b</sup>	39.645 <sup>bc</sup>	40.626 <sup>d</sup>	0.000	.325
$\Sigma$ PUFA	18.179 <sup>a</sup>	16.758 <sup>b</sup>	21.225 <sup>c</sup>	19.012 <sup>d</sup>	0.000	.222
$\Sigma$ FA	99.925 <sup>a</sup>	94.083 <sup>b</sup>	99.943 <sup>c</sup>	99.527 <sup>d</sup>	0.000	.040
Lipid content	9.200 <sup>a</sup>	9.380 <sup>b</sup>	11.567 <sup>c</sup>	10.400 <sup>d</sup>	0.000	.461

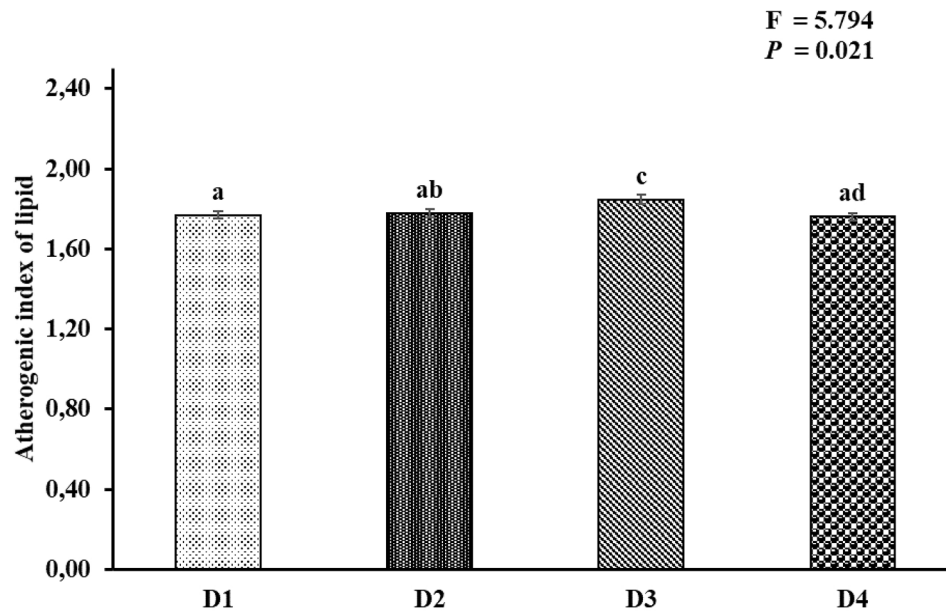
Notes: 0 g fish oil/kg feed (labelled D1); 5 g fish oil /kg feed, consisted of 1,950 mg EPA and 1,200 mg DHA (labelled D2); 10 g fish oil/kg feed consisted of 3,900 mg EPA and 2,400 mg DHA (labelled D3); and 15 g fish oil/kg feed consisted of 5,850 mg EPA and 3,600 mg DHA (labelled D4).FA, fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. <sup>1</sup> Means  $\pm$  SE (standard error) of three separate determinations, a b c d = significant in a row, nd= not detected



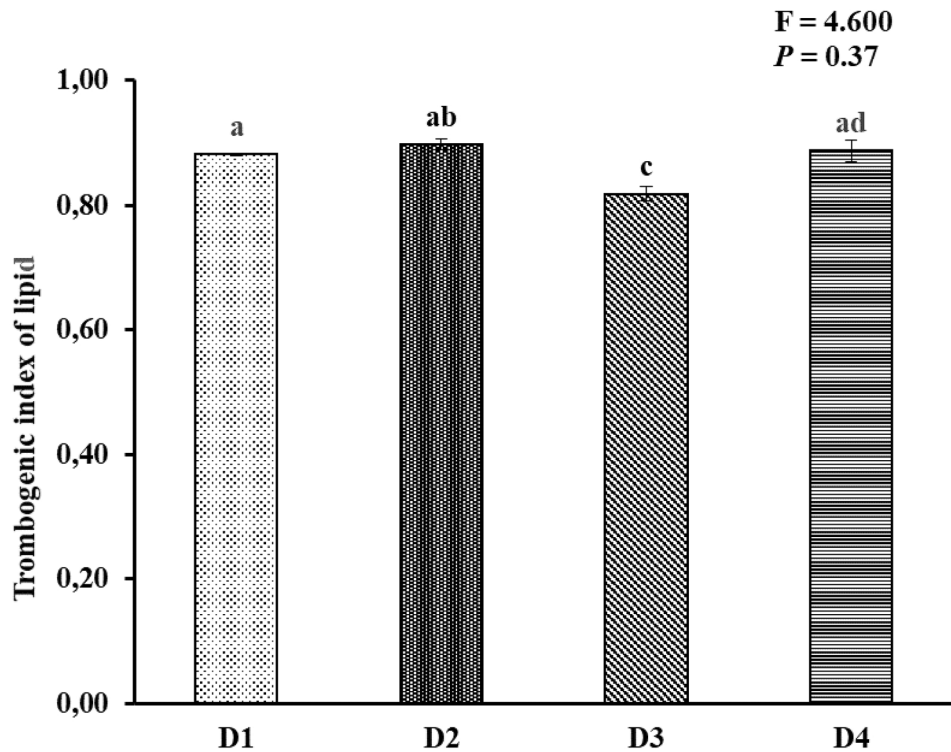
**Figure 1.** Relationship between dietary EPA content (% FAME) and whole body EPA content of Asian redbtail catfish, feed diets containing increasing EPA levels over 60 days. EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester.



**Figure 2.** Relationship between dietary DHA content (% FAME) and whole body DHA content of Asian redtail catfish feed diet containing increasing DHA levels over 60 days. DHA, docosahexaenoic acid; FAME, fatty acid methyl ester.



**Figure 3.** Nutritional quality of lipid atherogenic index of Asian redtail catfish whole body containing EPA and DHA levels over 60 days. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Note: A different superscript letter on each bar chart indicates a significant difference ( $P < 0.05$ ). The same superscript letter on each bar chart does not show a significant difference ( $P > 0.05$ ).



**Figure 4. Nutritional quality of lipid thrombogenic index of Asian redbtail catfish whole body containing EPA and DHA levels over 60 days.** EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Note: A different superscript letter on each bar chart indicates a significant difference ( $P < 0.05$ ). The same superscript letter on each bar chart does not show a significant difference ( $P > 0.05$ ).

#### Growth performance and body indices

All experimental diets given to Asian redbtail catfish presented a significant ( $p < 0.05$ ) effect on final body weight, body weight gain (%), and specific growth rate (%/day). The higher growth was contributed by the D3 diet, followed by a better feed conversion ratio (FCR). The mean values of CF, HSI, VIS, and LSI did not differ significantly ( $p > 0.05$ ) among the D1, D2, D3, and D4 diets (Table 4). The survival rate of various diet experiments was insignificant ( $p > 0.05$ ).

**Table 4. Growth performance and body indices of Asian redbtail catfish (*Hemibagrus nemurus*) during the 60-day experimental period.**

Parameters	D1	D2	D3	D4	P-value	SE <sup>1</sup>
FL (cm)	19.43	19.64	19.97	20.03	0.411	0.463
FW (g)	92.412 <sup>a</sup>	103.242 <sup>b</sup>	110.097 <sup>c</sup>	101.499 <sup>d</sup>	0.002	2.770
WG (%)	63.983 <sup>a</sup>	81.269 <sup>b</sup>	91.952 <sup>c</sup>	75.663 <sup>d</sup>	0.003	4.860
SGR (%/day)	0.824 <sup>a</sup>	0.991 <sup>b</sup>	1.086 <sup>c</sup>	0.937 <sup>c</sup>	0.002	0.039
FCR	1.988 <sup>a</sup>	1.633 <sup>b</sup>	1.469 <sup>b</sup>	1.737 <sup>d</sup>	0.002	0.087
SR (%)	85.925	86.666	88.888	86.666	0.902	4.190
CF (%)	0.988	0.981	1.030	0.922	0.839	0.119
HSI (%)	0.950	0.942	1.150	1.110	0.690	0.214
VSI (%)	0.901	1.071	0.969	0.947	0.816	0.181
LSI (%)	0.191	0.510	0.240	0.190	0.175	0.148

<sup>1</sup> Values are presented as mean  $\pm$  SE, n = 3. Note: Numbers followed by different superscript of letters in the same row indicate a significant differences ( $P < 0.05$ ). Numbers followed by superscript of the same letter in the same row showed no significant difference ( $P > 0.05$ ). FL, final length; FW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate; CF, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index; LSI, liposomatic index.

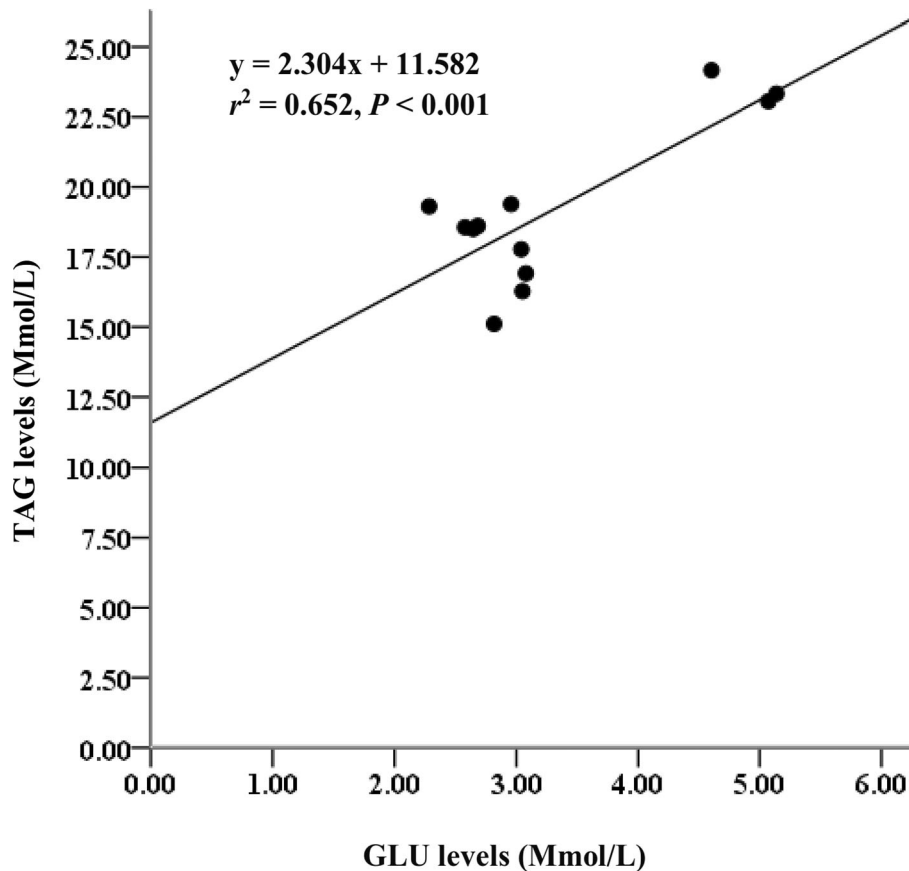
**Table 5.** Serum metabolites (Mmol/L) of Asian redbtail catfish (*Hemibagrus nemurus*) juveniles fed an experimental diet for 60 days.

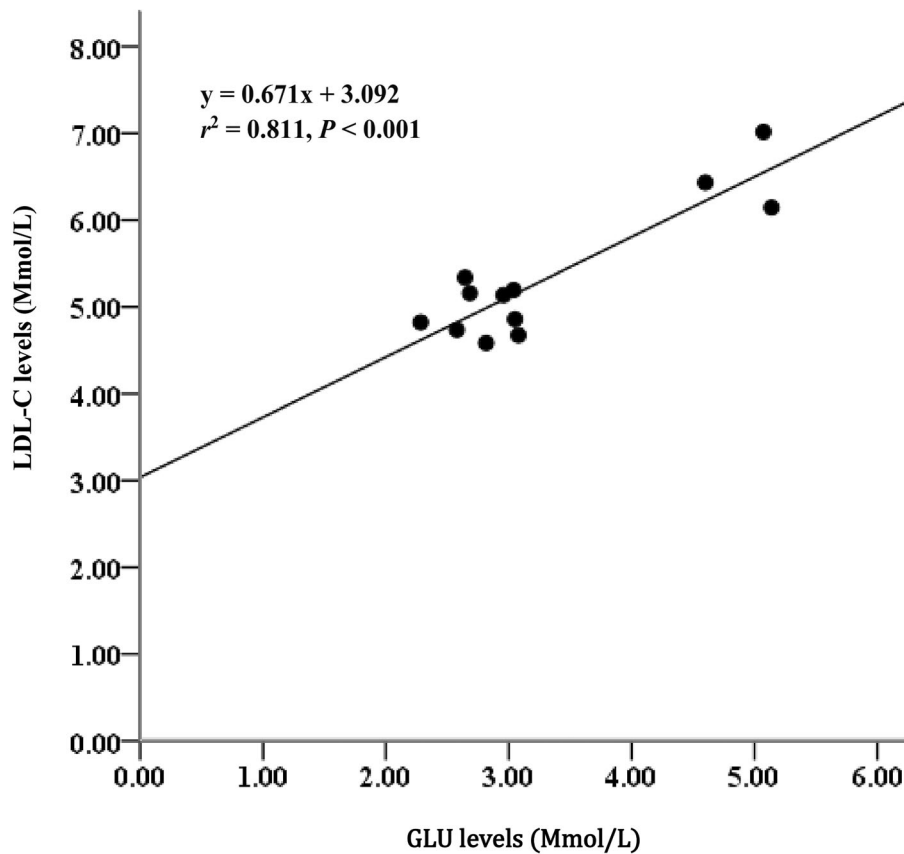
Serum metabolites	D1	D2	D3	D4	P-value	SE <sup>1</sup>
GLU	4.94 <sup>a</sup>	2.50 <sup>b</sup>	2.89 <sup>bc</sup>	2.98 <sup>cd</sup>	0.000	3.103
TAG	23.52 <sup>a</sup>	18.79 <sup>b</sup>	18.59 <sup>bc</sup>	16.10 <sup>d</sup>	0.000	10.454
TC	9.92 <sup>a</sup>	8.50 <sup>b</sup>	8.44 <sup>bc</sup>	7.00 <sup>d</sup>	0.000	5.675
HDL-C	4.03 <sup>a</sup>	3.20 <sup>b</sup>	3.35 <sup>bc</sup>	2.85 <sup>d</sup>	0.001	3.129
LDL-C	6.53 <sup>a</sup>	4.97 <sup>b</sup>	5.60 <sup>bc</sup>	4.71 <sup>d</sup>	0.001	5.003
LDL-C/HDL-C ratio	1.62 <sup>a</sup>	1.55 <sup>b</sup>	1.67 <sup>c</sup>	1.65 <sup>d</sup>	0.000	0.002

<sup>1</sup>Values are presented as means  $\pm$  SE, n = 3. Note: Numbers followed by different superscript of letters in the same row indicate a significant differences ( $P < 0.05$ ). Numbers followed by superscript of the same letter in the same row showed no significant difference ( $P > 0.05$ ). GLU, glucose; TAG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol.

### Lipid profile in serum

The serum GLU, TAG, TC, HDL-C, and LDL-C levels in fish fed a diet with no supplemental fish oil (control, D1) were significantly higher than those in fish-fed diets D2, D3, and D4. Glucose levels in experimental animals fed D2 and D3 and D3 and D4 showed no significant difference ( $p > 0.05$ ), except for D2 and D3 diets. Additionally, LDL-C and HDL-C ratios indicated a significant effect ( $p < 0.05$ ) among the experimental diets (Table 5). The GLU level parameter also had moderate relationships with TAG ( $r^2 = 0.652$ , Figure 5). Additionally, the GLU level parameter strongly correlated with LDL-C ( $r^2 = 0.811$ , Figure 6).

**Figure 5.** Relationship between GLU and TAG levels and Asian redbtail catfish fed diet containing EPA + DHA over 60 days. GLU, glucose; TAG, triglycerides; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.



**Figure 6. Relationship between GLU and LDL-C levels and Asian redtail catfish fed diet containing EPA + DHA over 60 days.** GLU, glucose; LDL-C, low-density lipoprotein cholesterol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

### Pond water quality

The physicochemical water parameters in the tarpaulin ponds for rearing juvenile Asian redtail catfish were as follows: water temperatures ranged from 28 to 30°C, oxygen between 6.4 and 6.7 mg/L, and pH between 6.6 and 6.8. The water alkalinity increased from 60.5 to 67.5 mg/L HCO<sub>3</sub>, and the hardness varied between 67.5 and 69.5 mg/L HCO<sub>3</sub>.

### Discussion

For food safety and to increase the efficiency of feed use in fish farming, it is necessary to use nutrient-rich feed, such as fatty acids, amino acids, minerals, and vitamins.<sup>7,33,34</sup> According to previous research, commercial feeds do not contain complete nutrients such as EPA and DHA.<sup>4</sup> Therefore, improving the nutrition of fish feed can be done by supplementing fish oil containing EFA and DHA. In this study, adding fish oil to feed directly correlated with the EPA and DHA composition for the D1, D2, D3, and D4 diets. The higher the level of addition of fish oil (EFA and DHA) to the feed, the higher the levels of EPA and DHA in the experimental diets.

Additionally, in all experimental diets, the MUFA level was higher than the SAFA and PUFA levels. By contrast, Nile tilapia feed supplemented with fish oil at 5, 10, and 15% showed higher SAFA levels than MUFA and PUFA levels.<sup>7</sup> However, EPA and DHA supplementation in Atlantic salmon feed were 0.25, 0.50, 0.75, and 2%, and SAFA levels were lower than MUFA and PUFA levels. By contrast, EPA + DHA in feed was detected in 1.35, 3.33, 5.48, and 15.6% of the total fatty acid methyl ester (FAME).<sup>10</sup> In this study, EPA and DHA addition to D1, D2, D3, and D4 diets were 0 g fish oil/kg feed, 5 g fish oil (consisted of 1,950 mg EPA and 1,200 mg DHA/kg feed), 10 g fish oil (consisted of 3,900 mg EPA and 2,400 mg DHA/kg feed), and 15 g fish oil (consisted of 5,850 mg EPA and 3,600 mg DHA/kg feed). The EFA + DHA values detected in the experimental feed D1, D2, D3, and D4 were 4.19, 4.53, 5.90, and 6.91%, respectively. Seemingly, EPA and DHA were detected in the D1 diet, even though it's not added with fish oil, but levels were lower than D2, D3, and D4. However, it has been reported that EPA and DHA were not detected in commercial pellet feed.<sup>4</sup> Conversely, EFA and DHA were found in commercial fish feed, which enriches coconut water and palm sap sugar, fermented with various fungi.

The ratios of DHA and EPA are determined by the levels of DHA and EPA in the feed. In this study, DHA/EPA ratios between 1.05 and 2.17 were lower in the D4 and D3 diets than in D1 and D2. In this context, a ratio of 1.05 in the D4 diet indicated that DHA and EPA were at their highest levels in the experimental diet. In this study, the DHA/EPA ratio differences within the experimental diet were due to the addition of EPA and DHA at different levels. Zhang *et al.*<sup>35</sup> state that the diet's proportion of DHA/EPA must be precise to develop better feed formulations. For this case, the DHA/EPA ratio of the diet for maximum growth of Nile tilapia was 1.42,<sup>7</sup> including 1.70 for giant gourami<sup>4</sup> and 0.53 for Atlantic salmon.<sup>10</sup> Supplementation with fish oil in aquafeed directly reflects the chemical composition of aquafeed and can influence DHA/EPA ratios.<sup>10,36</sup> Arachidonic acid (ARA), EPA, and DHA are the main essential HUFAs in fatty acid compositions, and these elements may be preventively supplemented in the diet.<sup>37</sup> In general, certain types of fatty acids are principal energy sources and crucial constructional portions for the diet.<sup>38</sup>

In this study, the fish feed supplemented with 10 g of fish oil (consisting of 3,900 mg EPA and 2,400 mg DHA/kg of feed) had the highest fat content in the whole-body carcass, 11.567%, with 39.074% SAFAs, 39.645% MUFAs, and 21.225% PUFAs. By contrast, the fat level in whole body carcasses fed the D1 diet with the lowest fat content was 9.20% with 39.231% SAFA, 42.514% MUFA, and 18.179% PUFA, respectively. This study found that MUFA levels were higher than SAFA levels in the all-body carcass of Asian redbtail catfish fed fish D1, D2, D3, and D4. In Nile tilapia-fed pellets with fish oil added at 0, 5, 10, and 15% for 60 days, MUFA levels were also higher than SAFAs and PUFAs.<sup>7</sup> However, Atlantic salmon in freshwater-fed feed were enriched at levels of 0.25, 0.5, 0.75, and 2.0% of EPA + DHA, and their whole-body carcasses also contained higher MUFA levels than SAFAs and PUFAs.<sup>10</sup> By contrast, in wild specimens, sea bass (*Dicentrarchus labrax*) showed the highest level of PUFA compared with SAFAs and MUFA.<sup>39</sup> In common carp (*Cyprinus carpio*), Rohu (*Labeo rohita*), and tilapia (*Oreochromis niloticus*) specimens captured in the Indus River, Pakistan, the levels of SAFA were higher than those of MUFA and PUFA.<sup>40</sup> The total SAFA, MUFA, and PUFA in body meat depends on the supplemented fish oil, levels of DHA+EPA in the diet, and fish species. Additionally, it also depends on whether the body meat is from farmed fish or wild catch. Whether the SAFA, MUFA, and PUFA levels of Asian redbtail catfish from cultured and wild captured sources are different is poorly understood.

Palmitic acid (C16:0) was the major metabolite in the body meat, followed by stearic acid (C18:0). Palmitic and stearic acid levels were higher in fed fish D4. Oleic acid (C18:1) was identified as the essential MUFA in the fish carcass fed D1, D2, D3, and D4. The higher oleic acid (C18:1) in all carcasses could be due to its dominance in the pellet feed. Several authors reported that commercial fish feeds contain more oleic acid.<sup>4,7,39</sup>

The current study showed that Asian redbtail catfish (*Hemibagrus nemurus*) fed feed supplemented with different levels of EPA and DHA obtained from fish oil contained higher EPA and DHA than control feed (D1). These results match those for other fish species.<sup>4,6,7</sup> The EPA and DHA contents in the diet had strong relationships with the EPA and DHA contents in whole-body carcasses ( $r^2 = 0.897$  for EPA and  $r^2 = 0.812$  for DHA). This fact showed that adding EPA and DHA to the feed positively contributed to the EPA and DHA in the carcass of Asian redbtail catfish. Regarding PUFAs, Asian redbtail catfish freshwater can be considered a good source of n-3 series fatty acids, especially EPA and DHA, which have the highest content in fish fed a D3 diet. However, the levels were low, in line with the contents of EPA and DHA for Atlantic salmon freshwater fish,<sup>10</sup> Nile tilapia,<sup>7</sup> and common carp, Rohu, and tilapia.<sup>40</sup> However, this level of EPA and DHA in freshwater finfish is lower than that in seawater finfish, such as sea bass and other fish species.<sup>5,39</sup>

EPA and DHA are essential nutrients in a portion of healthy food, so finding an affordable source of PUFA from fish is crucial for consumer guidance because fish is one of the significant sources of EPA and DHA. The ratio of  $\Sigma n-3$ :  $\Sigma n-6$  fatty acids was higher in fish fed D3 than in fish fed D1, D2, and D4, which showed that EPA and DHA supplements in the diet can improve carcass quality. Fish oil is one of the low-cost supplies of EPA and DHA. It is essential to aquafeed, especially in freshwater fish farming, such as Asian redbtail catfish (*Hemibagrus nemurus*).

In the current study, the atherogenic index (AI) ranged from 1.76 to 1.84, and the thrombogenic index ranged from 0.81 to 0.89 in all whole-body carcasses fed feed D1, D2, D3, and D4. This appeared to be connected to a discrepancy in SAFA compositions among experimental diets. The AI levels in the whole-body carcasses of Asian redbtail catfish fed feed D1 and D2 and D4 were insignificant, while fish fed feed D3 differed in D1, D2, and D4. The AI and thrombogenic index (TI) indices were significantly associated with the content of myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), all of which are thrombogenic promoters.<sup>7</sup> The Food Agricultural Organization and the Health World Organization recommend AI and TI values between 0.4 and 0.5. Even though the AI and TI scores of the Asian redbtail catfish were higher than 0.5, we stated that consuming Asian redbtail catfish flesh is a healthy food. In fish farming, AI and TI indices correlated with feed supplemented with fish oil, including EPA and DHA,<sup>7,10</sup> feed quality used,<sup>4</sup> fish species, and environmental factors.<sup>5,41</sup>

Fish feeds with different levels of EPA and DHA directly impact the growth rate of Asian redbtail catfish juveniles. In this study, the final body weight, body weight gain (%), specific growth rate (%/days), and low feed conversion ratio were shown in fed fish D3. In this trial, enrichment pellet feed with EPA and DHA has a positive effect on the fatty acid composition of the feed. This factor causes the better growth rate of Asian redbtail catfish. The SAA, MUFA, and PUFA contents were higher in feed supplemented with EPA and DHA. Higher fat levels in the D3 diet also increased the growth rate of *Hemibagrus nemurus* and have been observed in other species.<sup>4,10</sup>

In this study, feed supplemented with EPA and DHA at different levels sourced from fish oil did not affect the condition factor (CF) or body indices (HSI, VSI, LSI) of Asian redbtail catfish during the 60-day experimental period. The results were the same as those for *Micropterus salmoides* and other species.<sup>42-45</sup>

The nutritional status and metabolism of reared fish indirectly reflect the blood biochemistry of fish. The protein, carbohydrate, and fat contents in food can change the levels of GLU, TAG, HDL, and LDL in blood serum and are closely linked to the activity of digestive enzymes. In this study, we did not analyze digestive enzyme activity, so the discussion related to the levels of GLU, TAG, HDL, and LDL in blood serum cannot get discussed yet; maybe in the future can be developed. A consistent GLU level is one of the crucial indicators of human health.<sup>34</sup> In the current study, the amount of GLU in the blood serum of fish was lower in fish fed the D2, D3, and D4 diets than in fish fed the D1 diet, with a constant level of GLU between 2.50 and 2.98 Mmol/L. However, TAG is the usual variety of fat that can be utilized behind time by the body for energy.<sup>46</sup> The present study showed a difference in the serum TC, HDL-C, LDL-C levels, and LDL-C/HDL-C ratios, except between the D2 and D3 diets. These results indicated that EPA and DHA levels up to 9,400 mg/kg feed were conducive to energy storage for fish health and can be recommended for consumer food safety. This finding can minimize feed costs overall, whether it is fish oil or future sources of EPA + DHA, because the aquaculture sector needed 836 thousand tonnes of fish oil.<sup>47</sup>

## Conclusions

Asian redbtail catfish (*Hemibagrus nemurus*) diet containing 6,300 mg of EPA + DHA *via* fish oil (diet D3) showed fatty acid compositions in the diets better than those other feed diets. The D3-fed feed to *Hemibagrus nemurus* also had a better effect on the fatty acid composition of body meat, nutritional quality of lipid AI and TI, growth rate, body indices, and serum metabolites. AI and TI values in the meat of Asian redbtail catfish at 6,300 mg of EPA+DHA were 0.81 and 1.85. Foods with low AI and TI promote excellent human health, for example, by preventing human health coronary diseases. According to our research, the current inclusion of EPA and DHA *via* fish oil in fish feed is approximately 9,450 mg EPA + DHA/kg diet, which could be reduced as much as 3,150 mg EPA + DHA/kg diet. This finding could minimize the overall cost of aquafeed, whether it is fish oil or future sources of EPA + DHA.

## Data availability

### Underlying data

Figshare: Fatty acid composition on diet and whole body, growth performance, body indices, and profile blood serum of Asian redbtail catfish (*Hemibagrus nemurus*) fed a diet containing different levels of EPA and DHA. <https://doi.org/10.6084/m9.figshare.21164425>.<sup>27</sup>

This project contains the following underlying data:

- Table 1. Raw data list ingrediennts and proximate of feed.pdf
- Table 2. Raw data fatty acid of experiment diet.pdf
- Table 3. Raw data fatty acid of whole body of Asian redbtail catfish over 60 daysdocx.pdf
- Table 4. Raw data initial body weight of Asian redbtail catfish.pdf
- Table 5. Raw data initial body length of Asian redbtail catfish.pdf
- Table 6. Raw data final body weight of Asian redbtail catfish each experimental diet.pdf
- Table 7. Raw data final body length of Asian redbtail catfish each experimental diet.pdf

- Table 8. Raw Data of growth performance and body indices of Asian redbtail catfish.pdf
- Table 9. Raw data serum metabolites of Asian redbtail catfishdocx.pdf
- Authors ceklist Manuscript No. 126487.pdf (completed ARRIVE checklist)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

## Acknowledgements

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## Version 2

Reviewer Report 31 May 2023

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**Christopher Marlowe Caipang** 

Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, Iloilo City, Philippines

The authors have addressed the comments/suggestions that were raised in the earlier version.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Aquaculture, fish health management, aquatic biotechnology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 24 May 2023

<https://doi.org/10.5256/f1000research.147971.r173675>

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**Rudy Agung Nugroho** 

Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia

Present revised article is better than previous version. All suggestion and comment from reviewer have been applied in this version.

This result of this study can be developed based on the molecular study in the next research.

Based on my expertise, this article can be indexed.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Animal Physiology, Fish Nutrition

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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Version 1

Reviewer Report 31 January 2023

<https://doi.org/10.5256/f1000research.138904.r157014>

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**Rudy Agung Nugroho**

Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia

#### Title

- Good title.

#### Abstract

- Please add the results: Condition Factor (CF), Hepatosomatic Index (HSI), Viscerosomatic Index (VSI), and Liposomatic Index (LSI).

#### Introduction

- Brief background and aims.

#### Materials and Methods

- Table 1, at proximate composition NEE should be NFE (Nitrogen Free Extract).
- Table 1: Please describe the content of the vitamin and mineral mix.
- This species' health status was good. Please be specific on what categorize fish indicated as good in health status.
- Does the feeding rate of 3% was optimum for this fish? Or any previous experiment mentioned that 3% is optimum for this fish?
- It is stated in the methods that "Every 20 days, five fish samples were taken from each tarpaulin pond". However, the results did not appear in the results section.

#### Results and Discussion

- The discussion of The link between digestive enzymes activity and the content of protein, carbohydrate, and fat contents which is related to the levels of GLU, TAG, HDL, and LDL in blood serum can be developed.

**Conclusion**

- Sufficient.

Current article is good and has a contribution in aquaculture field. However, the discussion need to be developed, based on the finding/results.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Animal Physiology, Fish Nutrition

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 02 Feb 2023

**Netti Aryani**

**Abstract**

1. We state that whole-body indices such as Condition Factor (CF), Hepatosomatic Index (HSI), Viscerosomatic Index (VSI), and Liposomatic Index (LSI) were not significant between diets experimental, so the result we do not write in quantity in the Abstract. On the other hand, the number of words in the abstract is limited to 300 words.

**Methods**

1. We agreed formula NEE should be changed to NFE (Nitrogen Free Extract) as written on the note under Table 1.
2. The vitamin mix contains vitamins A, 2,750 IU; Vit D, 550,000 IU; Vit E, 25,000 IU; Ca D-pantothenate, 25,000 mg, Mg, 25.000 mg; P, 50.000 mg.

3. A feeding rate of 3% has been optimal for the growth of *Hemibagrus nemurus* in this study, although some researchers reported a range of 3 - 8%.
4. Five fish samples were measured every 20 days, and their data were used for estimating the amount of feed given based on biomass weight (15 fish/pond) every 20 days. Hence, the results did not appear in the results section.

#### Result and Discussion

1. In this study, we did not analyze digestive enzyme activity, so the discussion related to the levels of GLU, TAG, HDL, and LDL in blood serum cannot get discussed yet; maybe in the future can be developed.

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 26 January 2023

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**Christopher Marlowe Caipang** 

Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, Iloilo City, Philippines

This manuscript evaluated the effects of various levels of EPA and DHA on selected growth and body composition indices of Asian redbtail catfish. Results showed that the incorporation of 6,300 mg of EPA+DHA/kg in the diets gave the best effects on whole-body carcass compositions, growth performance, and serum metabolites of the fish.

Comments: This data obtained from this study is important in developing the aquaculture industry for this species of fish particularly in the establishment of commercial feeds. Comments and suggestions for the improvement of the manuscript are indicated below.

1. In the Conclusion (Abstract), why did the authors conclude that the level of 6,300 mg of EP+DHA in the feeds is the best for food safety? Please justify and support this claim with data from the work.
2. Please indicate the area or volume of the tarpaulin ponds so the stocking density could be determined.
3. Please justify the stocking of 15 fish per pond.
4. The use of commercial kits to measure blood parameters should be justified and authors should state whether these kits have been standardized for fish.

5. Is there a need to write the formula of the different growth parameters in the text? Please explain.

6. From Tables 2 - 5, the values of the SE were only placed in one column. Authors mentioned that they have triplicate in each treatment and 3 fish were sampled per replicate. Therefore, the number of fish per treatment should be 9. I suggest that each column (Control and D1-D4) should have its own SE. Kindly revise the tables accordingly.

I hope that the authors will take these suggestions/comments when they revise the manuscript.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Aquaculture, fish health management, aquatic biotechnology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 31 Jan 2023

**Netti Aryani**

1. Although some studies recommended fat meat values between 0.5 and 1.0, no such guidelines exist for AI and TI. AI and TI values in the meat of Asian redtail catfish at 6,300 mg of EPA+DHA were 0.81 and 1.85. Foods with low AI and TI promote excellent human health, for example, by preventing human health coronary diseases.
2. In the present study, the water volume of the tarpaulin ponds is 300 liters, with a stocking density of 15 fish per tarpaulin pond.

3. Primate Animal Study Center, IPB University-Bogor, has standardized commercial kits to measure blood fish parameters.
4. In the methods and calculations part, we revised the initial body weight formula, changed it to the final length (cm), and the final body weight (FBW) changed to the final weight (FW); we have presented in Table 4.
5. Standard Errors (SE) In Table 2-5, we wrote down the results of data analysis from multiple comparisons of the Post Hoc Tests for each analyzed parameter.

**Competing Interests:** No competing interests were disclosed.

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