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RESEARCH ARTICLE

REVISED The proximate composition, amino acid profile, fatty acid content, and mineral content of scale flour from three fish species as potential feeds for fish fry [version 2; peer review: 2 approved with reservations]

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Abstract

Background

Fish scale waste is highly valued both as a functional food ingredient and a potential feed source for farmed fish. This study aimed to analyse the chemical composition, fatty acid profile, and mineral content in fish scale flour of *Osphronemus (O) goramy*, *Cyprinus (C) carpio*, and *Oreochromis (O) niloticus* as potential feed for fish fry.

Methods

Fish scales were cleaned with 10% w/v NaCl solution at a ratio of 1:10 (w/w) for 24 hours at 4 °C. Agitation was used every eight hours to remove excess protein. Fish scales were evenly arranged in a cooker and cooked at 121 °C for 10 minutes with 15 *psi* pressure. After cooking, 100 grams of wet fish scales was dried at 50 °C for four hours. Dried fish scales were transformed into flour for proximate composition analysed via standard AOAC method, amino acid and fatty acid assessment employing HPLC and GC-MS, while mineral content was determined using AAS.

Results

The examined fish scale flour from three species displayed significant

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variations in chemical components, amino acids, and minerals ($p<0.01$). Crude protein content spanned 49.52% to 72.94%, and fat content ranged from 0.11% to 0.23%. Magnesium levels varied between 767.82 mg/kg and 816.50 mg/kg, calcium content ranged from 3.54 mg/kg to 12.16 mg/kg, iron content was within 40.46 mg/kg to 44.10 mg/kg, and zinc content ranged from 45.80 mg/kg to 139.19 mg/kg. Predominantly, glycine emerged as the main free amino acid (FAA), varying from 13.70% to 16.08%, while histidine had the lowest content, at 0.39% to 0.71%. Conversely, fatty acid content was low in all species examined ranging from 6.73% to 9.48%.

Conclusions

Flour from three farmed fish types has potential for fish fry feed due to its chemical composition, amino acid, and mineral content. Further validation is needed for amino acid comparison to fish meal.

Keywords
Fish scale flour, chemical composition, amino acids, mineral content, fatty acid profile

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REVISED **Amendments from Version 1**

The author has made a number of significant changes to this paper. These changes include, among other things, improving the background and conclusions in the abstract. In the Introduction, we add to the important role of fish scales as a culinary ingredient and explain the technological gaps in the use of fish scale flour as fish fry feed. In the Methods section, the author has included references used to analyze biometric aspects of fish samples and analyze mineral content. In the Results section, information about protein levels is explained in relative terms, and additional discussion about the importance of amino acids, fatty acids, and minerals in feed for fish survival and growth. Apart from that, the author provides other explanations in the Conclusion section. With these changes, the paper becomes more comprehensive and provides more straightforward and detailed information.

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

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), global fish production in 2018 reached approximately 179 million tonnes, with aquaculture contributing 46% of the total production.[1] In Indonesia, the total aquaculture production was recorded at 16,032,122 metric tonnes (mt). Of this, 3,374,924 mt (21.05%) originated freshwater aquaculture production; 9,884,670 mt (61.65%) from marine water aquaculture production, including seaweeds in general; and 2,772,568 mt (17.29%) from brackish water aquaculture production (CDSI; Central Data System Information).[2] Approximately 10% of global fish production is currently discarded, while byproducts from fisheries constitute 70% of the total weight of fish production. Among these byproducts, fish bones and scales constitute 14 to 20% of the total weight, and these materials are also discarded.[3]

This significant quantity of byproducts is occasionally utilized as animal feed, fishmeal, oil, or plant fertilizer, but in most instances, it is discarded.[4,5] In recent years, there has been a growing recognition of environmental sustainability and a heightened emphasis on harnessing the value of resources within green and blue economies.[6,7] As a result, fish byproducts, including fish scale waste,[3,8] have gradually been applied as raw materials for human consumption.[9]

Fish scales contain approximately 41-45% organic components, such as collagen, fat, lecithin, sclerotin, and vitamins, and 38-46% inorganic components and mineral elements, including magnesium, iron, zinc, calcium, and vitamins.[10] Furthermore, fish scales possess antioxidant and antihypertensive properties.[11] Fish scales have been used as a culinary ingredient in baked bread,[3] because they are a source of food that is rich in nutrients.[6,7] These components are also crucial for the growth and survival of fish fry. In recent years, fish fry feed has primarily been live feed, and expensive artificial feed is a bottleneck in aquaculture[12,13] Therefore, there is a technological gap in exploring alternative ingredients for fish fry feed, such as fish scale flour, which is a rich source of nutrients, has economic value, and provides an element of novelty in this study.

A significant quantity of fish scale waste is readily accessible in the Indonesian market, encompassing fish scale waste derived from the death of farmed fish in Lake Maninjau.[14] Regrettably, in the past decade, this waste has remained untapped as both a food source and an ingredient for fish fry feed. This research aims to assess the proximate, amino acid, fatty acids and mineral contents in freshwater fish scale flour, specifically flour from the scales of giant gourami (*Osphronemus (O) goramy*), carp (*Cyprinus (C) carpio*), and tilapia (*Oreochromis (O) niloticus*), with the potential to be utilized as feed ingredients for fish fry.

Methods

Ethical considerations

The research was approved by the Research and Community Service Ethics Committee at Universitas Bung Hatta with an approval letter No.057a/LPPM/Hatta/VI-2023 dated June 23, 2023. Experiments were carried out in accordance with the guidelines outlined in the Standard Operating Procedure of Laboratory Aquaculture at Universitas Bung Hatta.

Biometric measurement of fish sample

Ten *O. goramy*, *C. carpio*, and *O. niloticus* fish were obtained from a local fish market in Lake Maninjau, Indonesia. The fresh fish were carefully stored on ice and promptly transported to the laboratory.

Upon reaching the laboratory, the fish were individually weighed (TW) using AD-600i scales with a precision of 0.001 grams and measured to their standard length (SL) and maximum height (H), with distance measured from the mouth to the end of the upper lobe of the caudal fin and height measured vertically, excluding the fins.[15] Standard length and height were assessed using a meter ruler with an accuracy of 1 millimeter. The condition factor (CF) was calculated using the formula $CF = (TW/SL^3) \times 100$.[16]

Figure 1. Scales of Giant gourami (a), Common carp (b), and Tilapia (c) used in this study.

Preparation of fish scales

Upon arrival at the laboratory, the fish scales from *O. goramy*, *C. carpio*, and *O. niloticus* were collected from each fish for further processing. Fish scales were collected with a stainless steel fish scaler cleaner of 17.5 cm × 3.5 cm × 1.5 cm.

Every 1,000 grams of wet scales of *O. goramy*, *C. carpio*, and *O. niloticus* were washed thoroughly to obtain 200 grams of dry scales. The fish scales were washed in a three-litres plastic jar with 10% w/v NaCl solution with a solution ratio of 1:10 (w/w). The washing procedure was conducted for a duration of 24 hours at a temperature of 4 °C. The washing process was repeated every eight hours to further improve the effectiveness of protein removal. This frequent repetition helped ensure that any remaining unnecessary proteins on the fish scales were thoroughly removed.[3,10] By repeating the washing process at regular intervals, the purity of the fish scales was enhanced, preparing them for further processing (Figure 1).

Furthermore, scales of *O. goramy*, *C. carpio*, and *O. niloticus* were washed three times in low mineral-content water (with a TDS of around 100 mg/L) at room temperature for 10 minutes and then drained. Subsequently, the scales were evenly arranged in a cooker that was equipped with a pressure control button and cooking time settings (Model: Classic Pressure Cooker, with a ø 20 cm, 5.5-litre capacity, named Culinart, Made in China). The heating process was carried out using a cooker until the temperature reached 121 °C with a pressure of 15 *psi*, as indicated by the temperature and pressure panel. At this point, the timer was set for 10 minutes. The cooking time was calculated as the time between when the pressure in the cooker reached 15 *psi* and that when the heat source for cooking was turned off.

A total of 200 grams of scales from each species (*O. goramy*, *C. carpio*, and *O. niloticus* scales; wet weight) was dried using a 28 L stainless steel black digimatic oven tester at 50 °C for four hours until the moisture content reached 10%. The dried fish scales were then processed into flour using a Miller Powder Grinder with a 100-gram capacity. The resulting flour was then sieved using a mesh size of 60 μm to analyse the proximate composition and amino acid, fatty acid, and mineral contents (Figure 2).

Proximate and amino acid composition

The proximate composition of the fish scale samples was analysed using standard AOAC methods.[17] The samples were dried at 105 °C until a constant weight was achieved. The crude protein content was analysed using the standard Kjeldahl method, calculated as N × 6.25. Crude lipids were analysed using the Soxhlet method with ether extraction. The ash content was determined by incinerating the samples at 550 °C for 16 hours. Gross energy was measured using a bomb calorimeter.

Total carbohydrates were determined by subtracting the sum of % crude protein (CP), % fat (F) and % ash contents (A) from 100[18] by using the following equation: % Total carbohydrates = 100 – (CP + F + A). The gross energy value of

Figure 2. Fish scale flour from Giant gourami (a), Common carp (b), and Tilapia (c) was examined in this study.

each sample was determined by augmenting the percentage of crude protein (CP), fat (F), and total carbohydrate (C) contents with their respective energy values of 4, 9, and 4 kcal per 100 g of scale flour, respectively, to obtain the caloric values of the samples by using the following equation= (4CP + 9F + 4C)kcal/100 g weight.

The methods described by Ref. 19 were employed for amino acid analysis. The amino acid composition was determined using a high-performance liquid chromatography (HPLC) system, which consisted of a Waters 1525 binary HPLC pump, 717 autosamplers (Waters®), and Waters 2475 multi λ fluorescence detector optics (with wavelengths set at 250 nm for excitation and 395 nm for emission). The samples were hydrolysed in triplicate using 6 N hydrochloric acid for 24 hours at 11 °C.

Mineral content analysis

For the analysis of mineral content (Na, Mg, Ca, K, P, Fe, and Zn), the ashed GCTS sample was dissolved in 1 ml of hydrochloric acid (35% v/v Suprapur® Merck). Subsequently, the sample was filtered using cellulose filter paper (Watchman No 1, International Ltd; Maidstone, UK) and appropriately diluted for each elemental mineral and finally analyse with a Perkin-Elmer AA mod 3110 spectrophotometer (Norwalk, CT, USA).[20] Phosphorus (P) levels were analysed using a Perkin-Elmer AA spectrophotometer mod 3110 (Norwalk, CT, USA).[20]

Fatty acid analysis

The fatty acid composition of fish scale flours was examined through gas chromatography-mass spectrometry (GC-MS) analysis. The total lipid extraction followed a modification of the method by Folch *et al*. (1957), as detailed by Rajion,[21] employing a chloroform: methanol (2.1, v/v) solvent system. Transmethylation was carried out with 14% methanolic boron trifluoride.

Data analysis

Data analysis was conducted using Statistical Package for the Social Sciences (SPSS) 16.0 software (SPSS; Chicago, IL). The homogeneity of the data was assessed using the Levine test. One-way ANOVA was performed to determine the proximate and amino acid composition parameters and the mineral content for each fish scale flour of the three species. *Post hoc* analysis was carried out using Duncan's multiple-range test.[22] The results are reported as the mean values ± standard errors for each parameter.

Results and discussion

Biometric measurement

Table 1 displays the average standard length, wet weight, height, condition factor, and chemical composition of three fish species found in Lake Maninjau. Statistically significant variations were noted in the standard length, wet weight, height, and condition factor of the three examined fish species ($p < 0.05$; Table 1).[52]

Table 1. Biometric, proximate composition and mineral content of scale flour of three fish species.

	Species			
	O.goramy	***C. carpio***	***O. niloticus***	α
Biometric measurements				
Wet weight (g)	595.40 ± 32.31[a]	633.30 ± 87[b]	210.5 ± 12.12[c]	***
Standard length (cm)	23.32 ± 0.62[a]	26.01 ± 2.03[b]	18.0 ± 0.53[c]	***
Height (cm)	12.60 ± 0.36[a]	9.90 ± 0.44[b]	7.15 ± 0.21[c]	***
Condition factor	3.91 ± 0.22[a]	3.99 ± 0.65[b]	3.61 ± 0.21[c]	***
Proximate composition				
Moisture % of dry weight	6.74 ± 0.01[a]	7.41 ± 0.03[b]	5.74 ± 0.02[c]	***
Crude protein (%)	56.44 ± 0.02[a]	72.94 ± 0.10[b]	49.52 ± 0.01[c]	***
Fat (%)	0.13 ± 0.00[a]	0.23 ± 0.02[b]	0.11 ± 0.00[c]	***
Ash (%)	32.71 ± 0.58[a]	15.45 ± 0.15[b]	40.31 ± 0.03[c]	***
Fibre (%)	1.22 ± 0.03[a]	1.38 ± 0.01[b]	1.32 ± 0.02[c]	***
Total carbohydrates (%)	11.01 ± 0.55	11.56 ± 0.18	10.90 ± 0.06	ns
Energy value (kkal/100 g DM)	271.05 ± 2.36[a]	339.35 ± 0.50[b]	239.13 ± 0.16[c]	***

Table 1. *Continued*

	Species			
	O.goramy	***C. carpio***	***O. niloticus***	α
Mineral composition				
Macro mineral (mg/kg)				
Sodium (Na)	2,828.13 ± 0.87[a]	6,196.65 ± 0.30[b]	10,748.66 ± 0.13[c]	***
Magnesium (Mg)	816.50 ± 0.21[a]	767.82 ± 0.10[b]	794.79 ± 0.20[c]	***
Calcium (Ca)	10.81 ± 0.03[a]	3.54 ± 0.06[b]	12.16 ± 0.03[c]	***
Potassium (K)	2,111.29 ± 0.11[a]	133.88 ± 0.10[b]	252.84 ± 0.10[c]	***
Phosphorous (P)	6.15 ± 0.02[a]	2.74 ± 0.02[b]	7.33 ± 0.05[c]	***
Microminerals (mg/kg)				***
Iron (Fe)	44.10 ± 0.05[a]	40.46 ± 0.09[b]	41.52 ± 0.15[c]	***
Zinc (Zn)	45.80 ± 0.21[a]	139.19 ± 0.05[b]	55.43 ± 0.04[c]	***

Mean ± SD (n = 3) with different letters in the same row are significantly different.
Level of significance (α): ***p < 0.001; ns: non-significant.

Proximate composition and mineral content

In general, there was a significant difference ($p < 0.05$; Table 1) in the proximate content of fish scale flour between the three farmed fish species in Lake Maninjau. The water content, crude protein content, fat content, and energy values were higher in *C. carpio* scale flour than in *O. goramy* and *O. niloticus* scale flours (Table 1).

The highest protein content was relatively recorded in the fish scale flour of *C. carpio*, and the content did not differ by more than 23% between the three fish groups. Huang *et al.*[23] reported that tilapia fish scale flour contained 49.42% protein, 0.02% lipid, 45.18% ash, and 5.38% carbohydrates on a dry weight basis. Similarly, the protein content of spotted golden goatfish (*Parupeneus heptacanthus*) was 45.2%.[24] On the other hand, protein valuation from demineralized fish scale gelatine and nondemineralized gelatine displayed protein purities of 57.19 g/100 g and 43.37 g/100 g, respectively.[3]

Higher fat levels of *C. carpio* scale flour (0.23%) than in *O. goramy* (0.13%) and *O. niloticus* (0.11%) scale flour have also been observed for *Labeo rohita*[25] and other species.[26,27] This result could be due to various factors, including availability and dietary protein intake, fish size and age, and fish scale type.[23,25]

Furthermore, there were significant differences in the mineral content of fish scale flour between species ($p < 0.05$; Table 1). The magnesium, potassium, and iron levels were higher in the *O. goramy* fish scale flour than in the other scale flour samples. At the same time, the sodium, calcium, and phosphorous contents were higher in the *O. niloticus* fish scale flour. Moreover, zinc levels were higher in *C. carpio* fish scale flour (Table 1) than in the other scale flour samples. In general, the levels of the minerals analysed in this study are in alignment with the results of previous studies.[28,29]

The flour derived from fish scales of three farmed fish species exhibited a high mineral content, making it a potentially suitable choice for utilization as feed for fish fry. The inclusion of these minerals in fish feed is crucial because they serve as essential nutrients for the nourishment of fingerlings. As stated by Nagappan *et al.*,[30] fibre, minerals, and vitamins are essential, albeit minimal, requirements for optimal fish growth performance. In the context of fish scale flour, it was observed that all three species contained elevated mineral levels, implying that the scale flour offers enhanced support for the growth of fish fry. Nevertheless, it is important to highlight that despite the relatively high mineral content in the feed, there was no significant impact observed on the development of experimental animals, as noted by Dominquez *et al.*[31] and Wang *et al.*[32]

Free amino acids (FAAs)

The FAA profiles for the scale flour of the three fish species are presented in Table 2. Statistically significant differences were recorded in the FAA for the scales of the three fish species studied ($p < 0.05$; Table 2). The three species of farmed fish showed higher levels of aspartic acid, glycine, and alanine and lower levels of serine, histidine, methionine, and isoleucine (Table 2). *C. carpio* scale flour of showed the highest total FAA content (62.74%) compared to that of farmed fish (*O. goramy*; 48.31% and *O. niloticus*; 41.58%). The differences in the FAA profile could be related to different aspects, such as fish species, wild or farmed fish origin, diet composition, feeding habits, animal size, and age.[33–36]

Table 2. Profile of free amino acids in scale flours from three fish species (% ww/ww).

	Scales flour			
	O. goramy	*C. carpio*	*O. niloticus*	α
Aspartic acid	2.93 ± 0.04[a]	4.21 ± 0.03[b]	2.59 ± 0.01[c]	***
Glutamic acid	6.19 ± 0.01[a]	8.03 ± 0.01[b]	5.28 ± 0.01[c]	***
Serine	1.91 ± 0.01[a]	2.85 ± 0.01[a]	1.76 ± 0.01	***
Glycine	16.08 ± 0.01[a]	19.08 ± 0.01[b]	13.70 ± 0.01[c]	***
Histidine	0.39 ± 0.01[a]	0.71 ± 0.01[b]	0.42 ± 0.01[c]	***
Arginine	3.67 ± 0.01[a]	5.15 ± 0.01[b]	3.19 ± 0.01[c]	***
Threonine	1.50 ± 0.03[a]	2.04 ± 0.01[b]	1.34 ± 0.01[c]	***
Alanine	6.06 ± 0.01[a]	6.97 ± 0.00[b]	5.01 ± 0.01[c]	***
Tyrosine	1.79 ± 0.01[a]	2.29 ± 0.01[b]	1.51 ± 0.01[c]	***
Valine	1.53 ± 0.00[a]	1.89 ± 0.01[b]	1.24 ± 0.01[c]	***
Methionine	0.37 ± 0.01[a]	1.45 ± 0.01[b]	0.50 ± 0.02[c]	***
Isoleucine	0.91 ± 0.01[a]	1.46 ± 0.00[b]	0.76 ± 0.01[c]	***
Leucine	0.90 ± 0.01[a]	2.34 ± 0.01[b]	1.46 ± 0.01[c]	***
Phenylalanine	1.34 ± 0.01[a]	1.99 ± 0.01[b]	1.23 ± 0.01[c]	***
Lysine	2.03 ± 0.01[a]	2.30 ± 0.01[b]	1.57 ± 0.01[c]	***
Total	48.31	62.74	41.58	

Mean ± SD (n = 3) with different letters in the same row are significantly different.
Level of significance (α): ***p < 0.001.

In all samples, glycine was the most abundant FAA, which is in accordance with other studies on fish scale collagen in tilapia, *Oreochromis* sp.,[23] and the whole-body carcass of *Hemibagrus nemurus*[37] and *O. goramy.*[38] Glycine has been reported to be one of the essential components in the collagen molecule, helping maintain tissue strength and elasticity.[39,40]

Following glycine, the most abundant FAAs were glutamic acid, alanine, and arginine. Furthermore, histidine, methionine, and isoleucine were present in all samples but in smaller quantities; these particular FAA are commonly found in greater proportions within aquatic organisms.[41,42]

Fatty acid profile

The fatty acid composition in the fish scale flour of the three cultivated fish species is displayed in Table 3. This discovery is in line with data previously reported for Atlantic salmon (*salmo salar*) and Catla catla (*Labeo catla*),[43,44] where the fatty acid content of both species is relatively low. Variations in the fatty acid composition were evident among the three cultured fish species, with statistically significant differences ($p < 0.05$) observed among the species. Nevertheless, only two out of the fourteen saturated fatty acids (SAFAs) were identified. In monounsaturated fatty acids (MUFAs), three out of eight were observed, whereas three of the ten polyunsaturated fatty acids (PUFAs) were found. This result is in contrast to the type of fatty acids detected in the carcasses of several species of fish.[45–48]

Table 3. Fatty acids concentrations of scale flours from three fish species (%w/w).

	Fish scales flour			
	O. goramy	*C. carpio*	*O. niloticus*	α
Saturated fatty acids (SAFAs)				
Butyric Acid, C4:0	0.00	0.00	0.00	
Caproic acid, C6:0	0.00	0.00	0.00	
Caprilic acid, C8:0	0.00	0.00	0.00	
Capric acid, C10:0	0.00	0.00	0.00	
Undecanoic acid, C11:0	0.85 ± 0.01[a]	0.58 ± 0.01[b]	0.72 ± 0.01[c]	***

Table 3. *Continued*

	Fish scales flour			
	O. goramy	***C. carpio***	***O. niloticus***	**α**
Lauric Acid, C12:0	0.00	0.00	0.00	
Tridecanoic Acid, C13:0	0.40 ± 0.01^{a}	0.36 ± 0.01^{b}	0.17 ± 0.01^{c}	***
Myristic Acid, C14:0	0.00	0.00	0.00	
Pentadecanoic Acid, C15:0	0.00	0.00	0.00	
Palmitic Acid, C16:0	0.00	0.00	0.00	
Heptadecanoic Acid, C17:0	0.00	0.00	0.00	
Stearic Acid, C18:0	0.00	0.00	0.00	
Arachidic Acid, C20:0	0.00	0.00	0.00	
Behenic Acid, C22:0	0.00	0.00	0.00	
Total SAFAs	1.25	0.94	0.89	
Monounsaturated fatty acids (MUFAs)				
Myristoleic Acid, C14:1	0.00	0.00	0.00	
Cis-10-Pentadecanoic Acid, C15:1	2.02 ± 0.01^{a}	2.01 ± 0.01^{b}	$2.26 + 0.01^{c}$	***
Palmitoleic Acid, C16:1	0.00	0.00	0.00	
Cis-10-Heptadecanoic Acid, C17:1	0.73 ± 0.01^{a}	1.21 ± 0.01^{b}	1.19 ± 0.01^{c}	***
Elaidic Acid, C18:1n9t	2.09 ± 0.01^{a}	2.28 ± 0.01^{b}	3.71 ± 0.02^{c}	***
Oleic Acid, C18:1n9c	0.00	0.00	0.00	
Erucic Acid Methyl Ester, C22:1n9	0.00	0.00	0.00	
Nervonic Acid, C24:1	0.00	0.00	0.00	
Total MUFAs	4.84	5.50	7.16	
Polyunsaturated fatty acids (PUFAs)				
Linolelaidic Acid, C18:2n9	0.00	0.00	0.00	
Linoleic Acid, C18:2n6c	0.15 ± 0.01^{a}	0.12 ± 0.01^{b}	0.17 ± 0.01^{c}	***
v-Linolenic Acid, C18:3n6	0.22 ± 0.00^{a}	0.28 ± 0.01^{b}	0.40 ± 0.01^{c}	***
Linolenic Acid, C18:3n3	0.00	0.00	0.00	
Cis-8,11,14-Eicosetrienoic Acid, C20:3n6	0.00	0.00	0.00	
cis-11, 14, 17-Eicosatrienoic Acid Methyl Ester, (C20:3n3)	0.00	0.00	0.00	
Arachidonic Acid, C20:4n6	0.00	0.00	0.00	
Cis-13,16-Docosadienoic Acid, C22:2	0.00	0.00	0.00	
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	0.27 ± 0.01^{a}	0.48 ± 0.01^{b}	0.86 ± 0.01^{c}	***
Cis-4,7,10,13,16,19-Docosahexaenoic Acid, C22:6n3	0.00	0.00	0.00	
Total PUFAs	0.64	0.88	1.43	
∑ Fatty acid	6.73	7.32	9.48	

Mean ± SD (n = 3) with different letters in the same row are significantly different.
Level of significance (α): ***$p < 0.001$.

Fish scale flour from *O. niloticus* exhibited the highest cumulative fatty acid content (9.48%) in comparison to farmed fish scale flour (*C. carpio*; 7.32% and *O. goramy*; 6.73%). These findings are very similar in structure to the results obtained from analysis of the composition of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in the whole-body carcass of giant gourami and other fish species.[33,35,46]

Based on the analysis of the composition of the fatty acids contained in the scale flour of three species of farmed fish, if it is intended as feed for fish fry, it is necessary to enrich this fish scale flour with compounds containing fatty acids. Some enrichment options to consider are fish oil, chia seed oil, flaxseed oil, and walnut oil. These four sources are rich in omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as alpha-linolenic acid (ALA). Fish scale flour from three types of farmed fish can be validated for amino acids, fatty acids, and minerals for fish fry feed or as a substitute for fish meal and soybean meal. Rodrigues et al[49] showed that amino acids such as lysine increased the growth of juvenile pacu, *Piaractus mesopotamicus*. In contrast, fatty acids, such as linolenic acid, EPA, and DHA, are required in commercial feeds to meet the physiological, growth, and health needs of largemouth bass, *Micropterus salmoides*.[50] In addition, minerals such as Na, Mg, K, Cu, Fe, and Zn are very important for optimizing the growth of Largemouth bass, *Micropterus salmoides*.[51] These examples show the influence of various amino acids, fatty acids and minerals in feed when cultivating various fish species. They also highlighted the need for unique feed formulations to improve fish survival and growth performance.

Conclusion

Fish scale flour derived from *O. goramy*, *C. carpio*, and *O. niloticus* in the study region was identified as a valuable protein, amino acid, and mineral source. All fish scale flour samples across the three species contained amino acids and minerals but low fatty acids. Consequently, this needs to be validated in the context of amino acid requirements with fish meal or other ideal protein sources that meet the requirements for the cultured species. There is a lack of reliable data regarding the chemical composition, mineral, and fatty acid profiles of fish scale flours from the three local fish species in the study area. Therefore, the chemical, mineral, and fatty acid composition data presented in this study will be the groundwork for future research in fish scale flour chemistry, contributing to fish fry nutrition optimization.

Data availability

Underlying data

Figshare: The Proximate Composition, Amino Acid Profile, Fatty Acid Content, and Mineral Content of Scale Flour from Three Fish Species as Potential Feeds for Fish Fry, https://doi.org/10.6084/m9.figshare.23954799.[52]

This project contains the following underlying data:

- Table 1. Raw biometric data of three species of farmed fish samples.
- Table 2. Raw proximate composition data of fish scale flour from three Species (%W/W).
- Table 3. Raw mineral content data of fish scale flour from three Species (mg/kg).
- Table 4. Raw Amino Acid Composition Data of Fish Scale Flour from Three Species (%W/W).
- Table 5. Raw fatty Acid Composition Data of Fish Scale Flour from Three Species (%W/W).

Acknowledgements

We extend our gratitude to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for supporting this research.

References

1. FAO: *The state of world fisheries and Aquaculture.* Rome, Italy: Food and Agriculture Organization of the United Nations; 2020.
2. CDSI: **(Central Data System Information): Ministry of Marine and Fisheries Republic of Indonesia. (In Indonesian), 2018.**
3. Boronat O, Sintes P, Celis F, *et al.*: **Development of added-value culinary ingredients from fish waste: Fish bones and fish scales.** *Int. J. Gastron. Food Sci.* 2023; **31**: 100657. Publisher Full Text
4. Gehring CK, Gigliotti JC, Moritz JS, *et al.*: **Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review.** *Food Chem.* 2011; **124**(2): 422–431. Publisher Full Text
5. Gilman E, Roda AP, Huntington T, *et al.*: **Benchmarking global fisheries discards.** *Sci. Rep.* 2020; **10**: 14017. PubMed Abstract | Publisher Full Text | Free Full Text
6. Kratky L, Zamazal P: **Economic feasibility and sensitivity analysis of fish waste processing biorefinery.** *J. Clean. Prod.* 2020; **243**: 118677. Publisher Full Text
7. Venugopal V: **Green processing of seafood waste biomass towards blue economy.** *CRSUST.* 2022; **4**: 100164. Publisher Full Text
8. Silva AVS, Torquato LDM, Cruz G: **Potential application of fish scales as feedstock in thermochemical processes for the clean energy generation.** *Waste Manag.* 2019; **100**: 91–100. PubMed Abstract | Publisher Full Text
9. Islam J, Yap EES, Krongpong L, *et al.*: *Fish Waste Management – an Assessment of the Potential Production and Utilization of Fish Silage in Bangladesh, Philippines and Thailand.* FAO Fisheries and Aquaculture Circular; 2021; vol. 1216: 2021.

10. Lou L, Zhou Z, You X, *et al.*: **Mineral-chelating peptides derived from fish collagen: Preparation, bioactivity and bioavailability.** *LWT.* 2020; **134**: 110209.
Publisher Full Text

11. El-Rashidy AA, Gad AY, El-Hay A, *et al.*: **Chemical and biological evaluation of Egyptian Nile Tilapia (*Oreochromis niloticus*) fish scale collagen.** *Int. J. Biol. Macromol.* 2015; **79**: 618–626.
PubMed Abstract | Publisher Full Text

12. Lahnsteiner F, Lahnsteiner E, Duenser A: **Suitability of Different Live Feed for First Feeding of Freshwater Fish Larvae.** *Aquac. J.* 2023; **3**(2): 107–120.
Publisher Full Text

13. Syandri H, Azrita A, Thamrin R, *et al.*: **Broodstock development, induced spawning and larval rearing of the bilih, *Mystacoleucus padangensis* (Bleeker, 1852), a vulnerable species, and its potential as a new aquaculture candidate.** *F1000Res.* 2023; **12**: 420.
Publisher Full Text

14. Syandri H, Azrita, Junaidi, *et al.*: **Levels of available nitrogen-phosphorus before and after fish mass mortality in Maninjau Lake of Indonesia.** *J. Fish. Aquat. Sci.* 2017; **12**(4): 191–196.
Publisher Full Text

15. Famoofo OO, Abdul WO: **Biometry, condition factors and length-weight relationships of sixteen fish species in Iwopin fresh-water ecotype of Lekki Lagoon, Ogun State, Southwest Nigeria.** *Heliyon.* 2020; **6**(1): e02957.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Froese R: **Cube law, condition factor and weight–length relationships: History, meta-analysis and recommendations.** *J. Appl. Ichthyol.* 2006; **22**(4): 241–253.
Publisher Full Text

17. AOAC: *Official Methods of Analysis.* 15th ed. Washington, DC., USA: Association of Official Analytical Chemists (AOAC); 1990.

18. Onyeike EN, Ayoologu EO, Ibegbulam CO: **Evaluation of the nutritional value of some crude oil in polluted freshwater fish.** *Global J. Pure Appl. Sci.* 2000; **6**: 227–233.
Publisher Full Text

19. Cohen SA: *Amino acid analysis using pre-column derivatization with6-aminoquinolyl-N-hydroxysuccnimidyl carbomate. Protein Sequencing Protocols.* Smith BJ, editor. 2nd Edn. Totowa, NJ: Humana Press Inc.; 2003; vol. **211**: pp. 143–154. 9781592593422.

20. Perkin-Elmer: *Analytical methods for atomic absorption spectrophotometry.* Waltham: Perkin-Elmer Inc.; 1982.

21. Rajion MA: **Essential fatty acid metabolism in the fetal and neonatal lamb. PhD. Thesis. The University of Melbourne Australia.** 1985.

22. Duncan DB: **Multiple ranges and multiple F tests.** *Biometrics.* 1955; **11**: 1–42.
Publisher Full Text

23. Huang CY, Kuo JM, Wu SJ, *et al.*: **Isolation and characterization of fish scale collagen from tilapia (*Oreochromis* sp.) by a novel extrusion–hydro-extraction process.** *Food Chem.* 2016; **190**: 997–1006.
PubMed Abstract | Publisher Full Text

24. Matmaroh K, Benjakul S, Prodoran T, *et al.*: **Characteristics of acid soluble collagen and pepsin soluble collagen from scale of spotted golden goatfish (*Parupeneus heptacanthus*).** *Food Chem.* 2011; **129**(3): 1179–1186.
PubMed Abstract | Publisher Full Text

25. Begum M, Mun MZUAM, Mas M: **Nutritional profiling of selected fish's scales: An approach to determine its prospective use as a biomaterial.** *Int. J. Fish. Aquat. Sci.* 2021; **9**(3): 26–31.

26. Chinh NT, Manh VQ, Trung VQ, *et al.*: **Characterization of collagen derived from tropical freshwater Carp fish scale wastes and its amino acid sequence.** *Nat. Prod. Commun.* 2019; **14**: 1934578X1986628–1934578X1986612.
Publisher Full Text

27. Gil-Duran S, Arola D, Ossa EA, *et al.*: **Effect of chemical composition and icrostructure on the mechanical behavior of fish scales from Megalops Atlanticus.** *ScienceDirect.* 2016; **56**: 134–145.
Publisher Full Text

28. Maktoof A, Elherarlla RJ, Ethaib S: **Identifying the nutritional composition of fish waste, bones,scales, and fins.** *IOP Conf. Ser.: Mater. Sci.* 2020; **871**: 012013.
Publisher Full Text

29. Sukma, Andi M, Pamungkas BF, *et al.*: **Proximate composition and mineral profile of bone and scale of Papuyu Fish (*Anabas testudineus*).** *Media Teknologi Hasil Perikanan.* 2022; **10**(3): 185–191.
Abstract |

30. Nagappan S, Das P, Quadir MA, *et al.*: **Potential of microalgae as a sustainable feed ingredient for aquaculture.** *J. Biotechnol.* 2021; **341**: 1–20.
PubMed Abstract | Publisher Full Text

31. Dominquez D, Sehnine CP, *et al.*: **Dietary manganese levels for gilthead sea bream (*Sparus aurata*) fingerlings fed diets high in plant ingredients.** *Aquaculture.* 2020; **529**: 735614.
Publisher Full Text

32. Wang L, Sagada G, Wang R, *et al.*: **Different forms of selenium supplementation in fish feed: The bioavailability, nutritional functions, and potential toxicity.** *Aquaculture.* 2022; **549**: 737819.
Publisher Full Text

33. Jabeen F, Chaudhry AS: **Chemical compositions and fatty acid profiles of three freshwater fish species.** *Food Chem.* 2011; **125**(3): 991–996.
Publisher Full Text

34. Fuentes A, Fernández-Segovia I, Serra JA, *et al.* **Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality.** *Food Chem.* 2010; **119**: 1514–1518.
Publisher Full Text

35. Azrita A, Syandri H, Aryani N, *et al.*: **The utilization of new products formulated from water coconut, palm sap sugar, and fungus to increase nutritional feed quality, feed efficiency, growth, and carcass of gurami sago (*Osphronemus goramy* Lacepède, 1801) juvenile.** *F1000Res.* 2021; **10**: 1121.
Publisher Full Text

36. Kasozi N, Iwe G, Sadik K, *et al.*: **Dietary amino acid requirements of pebbly fish, Alestes baremoze (Joannis,1835) based on whole body amino acid composition.** *Aquac. Rep.* 2019; **14**: 100197.
Publisher Full Text

37. Hasan B, Putra I, Suharman I, *et al.*: **Growth performance and carcass quality of river catfish, *Hemibagrus nemurus* fed salted trash fish meal.** *Egypt. J. Aquat. Res.* 2019; **45**(3): 259–264.
Publisher Full Text

38. Azrita A, Syandri H, Aryani N, *et al.*: **Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles.** *F1000Res.* 2023; **12**: 140.
Publisher Full Text

39. Johnson AA, Cuellar TL: **Glycine and aging: Evidence and mechanisms.** *Ageing Res. Rev.* 2023; **87**: 101922.
PubMed Abstract | Publisher Full Text

40. Li C, Dai H, Wang Q, *et al.*: **Recent progress in preventive effect of collagen peptides on photoaging skin and action mechanism.** *Food Sci. Human Wellness.* 2022; **11**(2): 218–229.
Publisher Full Text

41. McLean E, Alfrey KB, Gatlin DM III, *et al.*: **Muscle amino acid profiles of eleven species of aquacultured animals and their potential value in feed formulation.** *Aquac. Fish.* 2022.
Publisher Full Text

42. Vijayaram S, Ringø E, Zuorro A, *et al.*: **Beneficial roles of nutrients as immunostimulants in aquaculture: A review.** *Aquac. Fish.* 2023.
Publisher Full Text

43. Grahl-Nielsen O, Clover KA: **Fatty acids in fish scales.** *Mar. Biol.* 2010; **157**: 1567–1576.
Publisher Full Text

44. Prabu K, Shankarlal S, Natarajan E: **Fatty acid profile of fish scale of Catla catla.** *AJB.* 2015; **14**(21): 1828–1831.
Publisher Full Text

45. Chen C, Guan W, Xie Q, *et al.*: **n-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: Bioconverting LNA to DHA is relatively efficient and the LC-PUFA biosynthetic pathway is substrate limited in juvenile fish.** *Aquaculture.* 2018; **495**: 513–522.
Publisher Full Text

46. Aryani N, Suharman I, Hasibuan S, *et al.*: **Fatty acid composition on diet and carcasses, growth, body indices and profile serum of Asian redtail catfish (*Hemibagrus nemurus*) fed a diet containing different levels of EPA and DHA.** *F1000Res.* 2023; **11**: 1409
PubMed Abstract | Publisher Full Text | Free Full Text

47. Qian C, Hart B, Colombo SM: **Re-evaluating the dietary requirement of EPA and DHA for Atlantic salmon in freshwater.** *Aquaculture.* 2020; **518**: 734870.
Publisher Full Text

48. Mohanty BP, Mahanty A, Ganguly, *et al.*: **Nutritional composition of food fishes and their importance and providing food and nutritional security.** *Food Chem.* 2019; **293**: 561–570.
PubMed Abstract | Publisher Full Text

49. Rodrigues AT, Mansano CFM, Khan K , *et al.*: **Estimation of the ideal dietary essential amino acid pattern for pacu**

(*Piaractus mesopotamicus*) in the later-juvenile growth phase. *Anim. Feed Sci. Technol.* 2022; **284**: 115146.
Publisher Full Text

50. Yadav AK, Rossi W, Habte-Tsion HM, *et al.*: **Impacts of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) level and ratio on the growth, fatty acids composition and hepatic-antioxidant status of largemouth bass (*Micropterus salmoides*).** *Aquaculture.* 2020; **529**: 735683.
Publisher Full Text

51. Zong YF, Shi CM, Zhou YL , *et al.*: **Optimum dietary fiber level could improve growth, plasma biochemical indexes and liver function of largemouth bass, *Micropterus salmoides*.** *Aquaculture.* 2020; **518**: 734661.
Publisher Full Text

52. Syandri H: The Proximate Composition, Amino Acid Profile, Fatty Acid Content, and Mineral Content of Scale Flour from Three Fish Species as Potential Feeds for Fish Fry. [Dataset]. *figshare.* 2023.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 30 October 2023

https://doi.org/10.5256/f1000research.154735.r212279

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Peter Vilhelm Skov
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[2] DTU Aqua, Section for Aquaculture, The North Sea Research Centre, Technical University of Denmark, Hirtshals, Denmark

Abstract:

"Conversely, fatty acid content was lowest among the species, ranging from 6.73% to 9.48%." should be rephrased to "Fatt acid content was low in all species examined, ranging from 6.73% to 9.48%."

"**Conclusions:** Scale flour from three farmed fish types showed potential for fish fry feed due to its chemical composition and amino acid and mineral contents. To enhance the essential fatty acid content, enriching the flour with oils containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid (ALA) is essential"

I don't agree with this statement. An ideal ingredient for feed formulation need not contain FA (e.g. fishmeal is also low in FA. The conclusion must be that it is a suitable protein source fir fry feed. This should however be validated in a context of AA requirement. Is the AA content comparable to fish meal or another ideal protein source, and would it satisfy the AA requirements of farmed species.

Introduction:

Do you means seagrass specifically, or seaweeds in general?

Methods:

Can you be more specific concerning "low mineral content water?"

"Mineral content analysis
For the analysis of mineral content (Na, Mg, Ca, K, P, Fe, and Zn), the ashed GCTS sample was dissolved in 1 ml of hydrochloric acid (35% v/v Suprapur® Merck). Subsequently, the sample was filtered using cellulose filter paper (Watchman No 1, International Ltd; Maidstone, UK) and appropriately diluted for each elemental mineral. Phosphorus (P) levels were analysed using a Perkin-Elmer AA spectrophotometer mod 3110 (Norwalk, CT, USA)."

Phosphorous was analysed using the spectrophotometer, is there a reference for the method? How were the other minerals analysed following acid treatment? This information is missing.

Proximate composition and mineral content:

"The highest protein content was recorded in the fish scale flour of *C. carpio*, and the content did not differ by more than 23%" - specify whether it is 23% in relative or absolute terms - insert "absolute" before content.

Conclusion:

As hinted at in the abstract, I would appreciate that as part of the assessment of the value of the protein, that you consider the AA composition relative to other protein sources used for fish feeds (e.g. fish meal, soy) or against the nutritional requirements of selected farmed species (e.g. tilapia, carp)

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?
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: Fish physiology, energetics and 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 31 Oct 2023

Hafrijal Syandri

Abstract:

1. The authors agreed to change the sentence: "Conversely, fatty acid content was lowest among the species, ranging from 6.73% to 9.48%, to Fatty acid content was low in all species examined, ranging from 6.73% to 9.48%.
2. The author will add in the new version that scale flour from three types of cultivated fish has potential as fish seed feed because of its close composition, amino acid, and mineral content. However, this needs to be validated in the context of amino acid requirements. Is it comparable to fishmeal or other ideal protein sources that meet the amino acid requirements for cultured species.

Introduction:

1. Do you means seagrass specifically, or seaweeds in general? : The author will change seagrass to seaweed in general in new versions.

Methods:

1. Can you be more specific concerning "low mineral content water?". The author will added "low mineral-content water (with a TDS of around 100 mg/L)" in new versions.
2. Phosphorous was analysed using the spectrophotometer, is there a reference for the method?: Phosphorus (P) levels were analysed using a Perkin-Elmer AA spectrophotometer mod 3110 (Norwalk, CT, USA) (Perkin-Elmer (1982)
3. How were the other minerals analysed following acid treatment? This information is missing: The author will add new versions after the sentence dilute appropriately for each mineral element and finally analyze with a Perkin–Elmer AA mod 3110 spectrophotometer (Norwalk, CT, USA).
4. Specify whether it is 23% in relative or absolute terms - insert "absolute" before content: The author state that the highest protein content was relatively recorded in *C. carpio* fish scale flour, and the content did not differ by more than 23%.

Conclusion:

In new revisions, in the conclusion part, the author will change the sentence "Consequently, enriching fish scale flour with animal and plant oils rich in omega-3 fatty acids is vital" to "However, this needs to be validated in the context of amino acid requirements with fish meal or other ideal protein sources that meet the requirements for the cultured species."

Competing Interests: No competing interests were disclosed.

Reviewer Report 16 October 2023

https://doi.org/10.5256/f1000research.154735.r208002

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Adhita Sri Prabakusuma

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The manuscript, entitled “The proximate composition, amino acid profile, fatty acid content, and mineral content of scale flour from three fish species as potential feeds for fish fry,” focuses on analyzing the chemical composition, fatty acid profile, and mineral content in fish scale flour of *Osphronemus* (O) *goramy*, *Cyprinus* (C) *carpio*, and *Oreochromis* (O) *niloticus* as potential feed for fish fry, provides insights into chemical properties influencing nutritional aspects in feed formulation for fish fry, and describes the enrichment of the fish scale flour with oils containing essential fatty acids. After careful review of the manuscript, I feel that it is an interesting study supported by a significant amount of data and contains an important topic within the scope of the Agriculture, Food, and Nutrition Gateway of F1000Research. However, there are some critical concerns that require attention to improve the quality of the manuscript before it is accepted for publication. The specific comments are as follows:

- Please include a statement in the background section of the abstract about the potential of fish scale flour by-products as a potential source of farmed fish feed, not only as a food additive and a functional food ingredient, aligning it with the title and overall context of the paper.

- Please include the analysis approaches for measuring tested parameters mentioned in the methods section of the abstract, as the authors outlined: proximate was analyzed using standard AOAC methods; amino acid composition was determined using high-performance liquid chromatography (HPLC); and fatty acid composition was examined through gas chromatography-mass spectrometry (GC-MS).

- Please include the comparative study results of tested parameters (the proximate composition, amino acid profile, fatty acid content, and mineral content) of three fish species in the results section of the abstract.

- In the introduction section, it is better to elaborate on the recent research on utilizing fish scales both for human consumption and fish feed formulation and to make a gap analysis by evaluating the effectiveness or performance of previous methods or results.

- In the materials and methods section, put the relevant references to support the procedures for measuring the biometric properties of fish samples and preparing fish scales.

- Support this statement “This frequent repetition helped ensure that any remaining unnecessary proteins on the fish scales were thoroughly removed,” with the relevant references.

- Why did the authors select a temperature of 121 °C with a pressure of 15 *psi* to perform the heating process? Why not choose a higher temperature and more pressure for less than or within ten minutes?

- In the results and discussion section, it is also better to elaborate on the recent research studies and compare those with the findings of this current study.
- In all sub-sections, mention the function of chemical nutrition, amino acids, and fatty acids to support the growth of farmed fish, their effect on increasing the quality of fish carcasses, and their role in maintaining fish health.
- Please double-check the use of English grammar in 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?
Yes

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: Food safety and quality, food science and technology, food waste management, and agri-food system

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 25 Oct 2023

Hafrijal Syandri

Response to comments from Adhita Sri Prabakusuma
Abstract: The author could only revise the introduction and method sections in the abstract due to the maximum abstract length of 300 words. We will change in the revised version.
Background: Fish scale waste is highly valued as a functional food ingredient and a potential feed source for farmed fish. This study aimed to analyse the chemical composition, amino acids, fatty acids, and mineral content in fish scale flour of

Osphronemus (O) goramy, Cyprinus (C) carpio, and Oreochromis (O) niloticus as potential feed for a fish fry.
Methods: Fish scales were cleaned with 10% w/v NaCl solution at a ratio of 1:10 (w/w) for 24 hours at 4 °C. Agitation was used every eight hours to remove excess protein. Fish scales were evenly arranged in a cooker and cooked at 121 °C for 10 minutes with 15 psi pressure. After cooking, 100 grams of wet fish scales was dried at 50 °C for four hours. Dried fish scales were transformed into flour for proximate composition analysis via standard AOAC methods, amino acid and fatty acid assessment employing HPLC and GC-MS, while mineral content was determined using AAS.
Results: Examined fish scale flour from three species showed significant variations in chemical components, amino acids, and minerals ($p<0.01$). Crude protein content ranged from 49.52% to 72.94%, while fat content ranged from 0.11% to 0.23%. Magnesium levels varied from 767.82 to 816.50 mg/kg, calcium content from 3.54 to 12.16 mg/kg, iron from 40.46 to 44.10 mg/kg, and zinc from 45.80 to 139.19 mg/kg. Predominantly, glycine was the main free amino acid (FAA), varying from 13.70% to 16.08%, while histidine had the lowest content, 0.39% to 0.71%. Conversely, fatty acid content was lowest among the species, ranging from 6.73% to 9.48%.
Conclusion: Scale flour from three farmed fish types exhibits potential as fish fry feed, considering its chemical composition, amino acid, and mineral content. Enriching the flour with EPA, DHA, and ALA-containing oils is crucial to boost essential fatty acids.

In the introductory part
The author will add to the new revised version a sentence highlighted in yellow in paragraph 3 of the "introduction" section to explain recent research and gaps in evaluating the effectiveness or performance of previous methods or results.
Fish scales contain approximately 41-45% organic components, such as collagen, fat, lecithin, sclerotin, and vitamins, and 38-46% inorganic components and mineral elements, including magnesium, iron, zinc, calcium, and vitamins[10]. Furthermore, fish scales possess antioxidant and antihypertensive properties[11]. Fish scales have been used as a culinary ingredient in baked bread [3], because they are a source of food that is rich in nutrients [5,7] .These components are also crucial for the growth and survival of fish fry. In recent years, fish fry feed has primarily been live feed, and expensive artificial feed is a bottleneck in aquaculture[12,13]. Therefore, there is a technological gap in exploring alternative ingredients for fish fry feed, such as fish scale flour, which is a rich source of nutrients, has economic value, and provides an element of novelty in this study.
Material and Methods Part
In the revised version, in the materials and methods section, the author will include relevant references to support procedures for measuring biometry properties of fish samples and preparing fish scales.
Upon reaching the laboratory, the fish were individually weighed (TW) using AD-600i scales with a precision of 0.001 grams and measured to their standard length (SL) and maximum height (H), with distance measured from the mouth to the end of the upper lobe of the caudal fin and width measured vertically, excluding the fins (Famoofo and Abdul, 2020). Standard length and width were assessed using a meter ruler with an accuracy of 1 millimeter. The condition factor (CF) was calculated using the formula $CF = (TW/SL^3) \times 100$ (Froese, 2006)
This frequent repetition helped ensure that any remaining unnecessary proteins on the fish

scales were thoroughly removed (Lou et al., 2020; Boronat et al., 2023).
Why did the authors select a temperature of 121 °C with a pressure of 15 psi to perform the heating process? Why not choose a higher temperature and more pressure for less than or within ten minutes?
The author ensures that at a temperature of 121°C with a pressure of 15 psi for 10 minutes, it is hoped that no damage will occur to the chemical composition of the fish scale flour to be analyzed.

Discussion part

In the new revised version, we agreed to add comparisons with current research findings in the discussion section.
The author will add to the newly revised version information regarding the function of amino acids, fatty acids, and minerals in the context of increasing fish seed growth.

English grammar in the manuscript

Please double-check the use of English grammar in the manuscript: The language contained in this manuscript has been checked by the American Journal Experts (AJE) with the English version (United Kingdom English) and is accompanied by a certificate.

Competing Interests: No competing interests were disclosed.