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Comparison of Growth Performance and Muscle Nutrition Levels of Juvenile *Siniperca scherzeri* Fed on an Iced Trash Fish Diet and a Formulated Diet

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Abstract: To assess the possibility of using a formulated diet instead of an iced trash fish diet for feeding spotted mandarin fish (*Siniperca scherzeri*), a 20-week feeding trial was conducted. The objective of the study was to examine the effects of the formulated diet (FG) and the iced trash fish diet (XG) on the growth performance and muscle nutrient composition of the fish. The results showed that the spotted mandarin fish fed with an XG had slightly higher survival rates, weight gain rate, fullness, hepatic index, and viscera index compared to those fed with an FG, although the differences were not significant ($p > 0.05$). Additionally, in terms of muscle composition, the FG group had higher levels of crude protein and ash content in the fish muscle compared to the XG group ($p < 0.05$). On the other hand, the crude fat content showed the opposite trend. Among the seventeen amino acids analyzed, only lysine and proline levels differed significantly between the FG and XG groups ($p < 0.05$). In terms of muscle-hydrolyzed fatty acids, fifteen fatty acids were detected in both groups, with arachidonic acid being exclusive to the FG group. Furthermore, significant differences in the levels of thirteen fatty acids were observed between the two groups ($p < 0.05$). The FG group had lower levels of saturated fatty acids compared to the XG group ($p < 0.05$), while monounsaturated fatty acids, polyunsaturated fatty acids, and EPA + DHA contents were higher. This study demonstrates the potential of using a formulated diet as a substitute for an iced trash fish diet in the rearing of spotted mandarin fish. However, further optimization of the formulated diet is necessary to improve the growth of spotted mandarin fish in future research.

Keywords: *Siniperca scherzeri*; formulated diet; muscle composition; amino acids; fatty acids

Key Contribution: Replacing the iced trash fish diet with a formulated feed had no negative impact on the survival rate and growth performance of spotted mandarin fish. Dietary ingredient composition affects the muscle nutrient composition of mandarin fish.

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1. Introduction

The spotted mandarin fish, known as *Siniperca scherzeri*, belongs to *Perciformes* and *Siniperca*, and is a typical carnivorous and aggressive fish in China [1–3]. Because of its delicious and nutritious flesh, spotted mandarin fish has become a vital fish that is cultured in China. The annual aquaculture production has increased to 374 thousand tons [4]. However, the current culture of spotted mandarin fish is mainly based on live bait or an iced trash fish diet, bringing three unfavorable factors to the cultivation. Firstly, the transport of an iced trash fish diet and live bait requires relatively high transport conditions, raising the costs of spotted mandarin fish farming. Secondly, the iced trash fish diet and live bait may introduce some pathogenic microorganisms during the culture process, posing a disease risk to the fish [5,6]. Moreover, the low utilization of the iced trash fish diet and live bait by the fish may cause pollution to the water environment [7]. Therefore, replacing the iced trash fish diet and live bait with a formulated diet is necessary for the sustainable aquaculture of spotted mandarin fish. However, related research is still lacking, especially a comparison in the growth performance and flesh quality of spotted mandarin fish fed on iced trash fish and formulated diets.

Diet has a significant correlation with the growth, nutritional content, and taste of farmed aquatic animals [8]. Therefore, understanding the nutritional requirements of fish is very important for the development of effective aquatic feed industries [9]. Moreover, amino acids and fatty acids provide a great contribution to fish muscle, which is vital for human health [10]. The nutrient composition of fish muscle, such as amino acids and fatty acids, varies considerably by aquaculture parameters such as feeding habits and culture environments [11,12]. Therefore, it is necessary to investigate the growth and muscle nutrient composition variations in spotted mandarin fish with two experimental diets to help improve the nutritional value of farmed spotted mandarin fish. Thus, in this study, we investigated the growth and muscle nutrient composition of mandarin fish fed on two different diets. The present study provided detailed data and a theoretical basis for improving the quality of special compound diets for spotted mandarin fish under artificial culture conditions.

2. Materials and Methods

2.1. Ethical Statement

The Animal Welfare and Ethical Committee of the Freshwater Fisheries Research Institute of Fujian approved all of the experimental protocols and guidelines. Fish were maintained in effectively aerated water, and anesthetized with MS-222 before sampling, and their viscera were extracted according to the Guidelines for the Care and Use of Laboratory Animals in China.

2.2. Fish and Feeding Trial

The spotted mandarin fish used in this experiment were from the Breeding Center of the Freshwater Fisheries Research Institute of Fujian and bred from the wild population of the Minjiang River in Fujian Province. Fish were fed with a live fry of grass fish for 2 weeks, allowing them to acclimate to the cultivation conditions. The experiment used six circular tanks with a diameter of 2 m and a height of 1.2 m. The water source was streaming water, which was aerated by micropores, and 1/3 of the water was changed every 3 days. Dissolved oxygen was maintained above 6.0 mg/L, pH was maintained from approximately 7.1 to 7.8, and the water temperature ranged from 18.0 to 28.0 °C as the seasons changed. Ammonia and nitrogen were kept below 1.0 mg/L. Then, the fresh bait was reduced to cause the juveniles to be semi-starved, and this was gradually replaced by an iced trash fish diet or a formulated diet. Sixty fish were fed with an iced trash fish diet in the XG group and sixty fish were fed with a formulated diet in the FG group. Each group of three tanks was assigned to represent triplicate results for the experiment, so 20 fish were assigned to a tank. Fish were hand-fed the respective feed twice daily, at 8:00 and 17:00, until apparent satiation was achieved, based on visual observation. The trial lasted for 20 weeks.

The formulated diet was designed to contain fishmeal 65%, fermented soybean meal 5%, extruded-soybean 8%, wheat gluten 2%, seaweed gel 2%, corn starch 12%, soybean oil 2%, and carboxymethyl cellulose 2%. In contrast, the iced trash fish diet comprised captured grass carp. The proximate analysis of the two experimental diets is shown in Table 1.

Table 1. The proximate analysis of experimental diets.

Experimental Diets	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Carbohydrate (%)	Gross Energy Values (MJ/kg)
Iced trash fish diet (XG)	72.04	14.60	8.43	2.94	1.99	6.03
Formulated diet (FG)	12.05	48.51	7.50	6.34	25.6	15.54

Note: Data refer to the analyzed moisture, crude protein, crude lipid, and ash (n = 3 for each diet). Carbohydrate (%) = 100 − [Moisture + Crude protein + Crude lipid + Ash]. Gross energy values of diets were calculated based on physiological fuel values 18.83, 14.64, and 35.56 MJ/kg for protein, carbohydrate, and fat, respectively.

2.3. Growth Performance

At the end of the trial, the spotted mandarin fish were starved for 24 h before sampling. Ten fish in each tank were randomly selected and their individual body length, body weight, and total visceral weight were measured. The weight gain rate (*WG*), fullness (*K*), viscera index (*VSI*), hepatic index (*HPI*), and survival rate (*SR*) were calculated as follows:

$$WG = \frac{(W_i - W_0)}{W_0} \times 100\%$$
$$K = \frac{W_i}{L}$$
$$VSI = \frac{W_n}{W_i} \times 100\%$$
$$HPI = \frac{H_\mathrm{n}}{W_i} \times 100\%$$
$$SR = \frac{M_\mathrm{i}}{M_0} \times 100\%$$

where W_i is the weight of fish (g) at the end of the experiment; W_0 is the weight of fish (g) at the beginning of the experiment; L is the length (cm) of the fish at the end of the experiment; W_n was the total visceral weight (g) at the end of the experiment; H_n is the liver weight (g) at the end of the experiment; M_i is the number of survivors at the end of the experiment; and M_0 is the number of survivors at the beginning of the experiment.

2.4. Proximate Amino Acid and Fatty Acid Compositions

At the end of the feeding trial, the surviving fish in each tank were counted and individually weighed; 5 fish (i.e., 15 fish per treatment) were sampled and stored at −20 °C for proximate composition analysis. The fish were not fed for 24 h before sampling. Standard procedures were applied to determine the moisture, crude protein, crude lipid, and ash content in both the diets and fish samples. Moisture was measured by oven-drying at 105 °C until a constant weight was achieved. Crude protein was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyzer; Tecator, Hoganos, Sweden) after acid digestion. Crude lipid was extracted and measured by the Soxhlet method (Tecator Soxtec System HT6; Hoganos, Sweden). Ash content was measured after placing the samples in a muffle furnace at 550 °C for 24 h. A professional laboratory determined amino acid and fatty acid concentrations in the muscles (School of Food Science and Technology, Jiangnan University, China).

2.5. Amino Acids and Lipid Quality Indices

According to the standard grading model of amino acids per gram of nitrogen suggested by the FAO/WHO in 1973 and the grading model of whole egg protein proposed by the Institute of Nutrition and Food Hygiene of the Chinese Academy of Preventive Medicine in 1991, the amino acid score (*AAS*) and chemical score (*CS*) of the essential amino acids were evaluated as follows [13,14]:

$$AAS = \frac{A_\mathrm{m}}{A_s}$$
$$CS = \frac{A_\mathrm{m}}{E_s}$$

where A_m is the amino acid content of protein in the muscle of the fish (mg/g Pro); A_s is the content of the same amino acid content recommended by the FAO/WHO (mg/g Pro); and E_s is the content of the same amino acid in egg protein (mg/g Pro).

Based on the fatty acid composition in the muscle of the XG and FG groups, health parameters including atherogenicity index (*AI*), thrombogenicity index (*TI*), flesh lipid quality (*FLQ*), and fatty acid hypocholesterolemic/hypercholesterolemic ratio (*HH*) were investigated to evaluate the lipid quality of fish as follows [15,16]:

$$AI = \frac{\mathrm{C12{:}0} + 4 \times \mathrm{C14{:}0} + \mathrm{C16{:}0}}{\Sigma\mathrm{MUFAs} + \Sigma\mathrm{n3PUFAs} + \Sigma\mathrm{n6PUFAs}}$$

$$TI = \frac{\mathrm{C14{:}0} + \mathrm{C16{:}0} + \mathrm{C18{:}0}}{0.5 \times \Sigma\mathrm{MUFAs} + 3 \times \Sigma\mathrm{n3PUFAs} + 0.5 \times \Sigma\mathrm{n6PUFAs} + \frac{\Sigma\mathrm{n3PUFAs}}{\Sigma\mathrm{n6PUFAs}}}$$

$$FLQ = \frac{\mathrm{EPA} + \mathrm{DHA}}{\Sigma\mathrm{SFAs} + \Sigma\mathrm{MUFAs} + \Sigma\mathrm{PUFAs}}$$

$$HH = \frac{\mathrm{C18{:}1\ cis9} + \mathrm{C18{:}2\ n6} + \mathrm{C20{:}4\ n6} + \mathrm{C18{:}3\ n3} + \mathrm{C20{:}5\ n3} + \mathrm{C22{:}5\ n3} + \mathrm{C22{:}6\ n3}}{\mathrm{C14{:}0} + \mathrm{C16{:}0}}$$

2.6. Statistical Analysis

All data were transformed, if necessary, after evaluating assumptions of normality and the homogeneity of variances. Multiple unpaired *t*-tests (SPSS 19.0) determined differences in the treatment means when assumptions were valid. Data were presented as the mean ± SD; the significance level was set at $p < 0.05$.

3. Results

3.1. Growth Performance and Morphological Parameters

After conducting a 20-week feeding trial, it was observed that the average survival rates in the XG and FG groups were 91.7% and 88.3%, respectively. The difference in survival rates between the two groups was not found to be significant. Moreover, Table 2 demonstrates that there were no significant differences in growth and morphological characteristics between the XG and FG groups, although there were significant differences in the growth rates between males and females in the same feed group ($p < 0.05$). It was noticed that the FG group exhibited a slight decrease in individual weight gain, fullness, hepatic index, and viscera index compared to the XG group.

Table 2. Growth performance of spotted mandarin fish fed two different diets.

Item	Female			Male		
	XG	FG	*p*-Value	XG	FG	*p*-Value
Initial weight (g)	17.19 ± 0.72	16.83 ± 0.73	0.211	17.19 ± 0.72	16.83 ± 0.73	0.211
Final weight (g)	89.85 ± 13.93	80.16 ± 13.98	0.359	71.16 ± 9.05	53.57 ± 11.07	0.115
Weight gain (%)	472.7 ± 81.1	376.3 ± 83.0	0.451	314.0 ± 52.6	218.3 ± 65.8	0.141
Fullness (g/cm)	4.89 ± 0.60	4.79 ± 0.24	0.854	4.13 ± 0.24	3.11 ± 0.98	0.069
Hepatic index (%)	5.90 ± 0.92	5.54 ± 0.51	0.165	5.86 ± 0.69	5.54 ± 0.54	0.443
Viscera index (%)	16.6 ± 1.6	16.2 ± 2.0	0.692	15.6 ± 1.7	13.8 ± 1.2	0.116

Values are the means of triplicate groups and are presented as mean ± SD (n = 3). Values are significantly different at $p < 0.05$.

3.2. Muscle Proximate Composition

As is shown in Table 3, the crude protein and ash content in the XG group were 20.42% and 1.52%, which were 4.49% and 8.98% lower than those in FG group ($p < 0.05$), respectively. In contrast, the crude fat content in the FG group was 1.48%, which was 2.21 times that in the FG group ($p < 0.05$). There was no significant difference between the two groups in terms of moisture content.

Table 3. Muscle composition of spotted mandarin fish fed two different diets (%).

Item	XG	FG	*p*-Value
Moisture	77.12 ± 0.10	77.13 ± 0.15	0.883
Crude protein	20.42 ± 0.08	21.38 ± 0.16	0.001
Crude lipid	1.48 ± 0.08	0.67 ± 0.06	<0.001
Ash	1.52 ± 0.03	1.67 ± 0.06	0.015

Values are the means of triplicate groups and are presented as mean ± SD (n = 3). Values are significantly different at $p < 0.05$.

3.3. Amino Acid Composition

The amino acid compositions of the XG and FG group muscles are shown in Table 4. In the present study, a total of 17 amino acids were identified and qualified in the muscles of the two groups, including 9 essential amino acids (EAAs) and 8 non-essential amino acids (NEAAs) (Table 4). The essential amino acids were threonine, lysine, isoleucine, leucine, phenylalanine, methionine, histidine, and arginine. The lysine content was significantly higher in the XG compared to the FG group ($p < 0.05$) and was the most abundant EAA in the muscles of the two groups. The non-essential amino acids were aspartic acid, alanine, glutamic acid, glycine, serine, cystine, proline, and tyrosine. The two groups had a significant difference in proline content. The proline content found in the XG group was 4.37%, while the amount was 4.68% in the FG group. There were no significant differences in the contents of the other 15 amino acids, EAAs, NEAAs, and sweet amino acids (SAAs). Ratios of EAA/NEAA, EAA/TAA, and SAA/TAA are shown in Figure 1. It was found that EAA/NEAA in the XG group was a little higher than in the FG group, but this difference was not significant difference ($p > 0.05$). However, the ratios of EAA/TAA and EAA/NEAA in the XG and FG groups were both above 0.4 and 0.6, which were in line with the FAO/WHO minimum guidelines, respectively.

Table 4. Amino acid composition percentages in the muscle of spotted mandarin fish fed two diets (%).

Amino Acid Items	XG	FG	*p*-Value
Threonine	4.69 ± 0.01	4.67 ± 0.05	0.522
Lysine	9.55 ± 0.05	9.24 ± 0.03	0.001
Isoleucine	4.34 ± 0.09	4.26 ± 0.03	0.230
Leucine	7.85 ± 0.17	7.80 ± 0.16	0.747
Phenylalanine	4.15 ± 0.03	4.14 ± 0.04	0.690
Valine	4.90 ± 0.05	4.91 ± 0.02	0.779
Methionine	3.21 ± 0.07	3.19 ± 0.03	0.624
Histidine	2.21 ± 0.01	2.20 ± 0.01	0.975
Arginine	7.45 ± 0.07	7.48 ± 0.07	0.616
EAA	48.35 ± 0.39	47.90 ± 0.41	0.239
Aspartic acid *	10.21 ± 0.07	10.07 ± 0.14	0.201
Alanine *	6.91 ± 0.31	6.89 ± 0.15	0.937
Glutamic acid *	15.44 ± 0.17	15.06 ± 0.16	0.051
Glycine *	6.82 ± 0.23	7.58 ± 0.50	0.075
Serine	4.31 ± 0.03	4.33 ± 0.01	0.545
Cysteine	0.31 ± 0.01	0.31 ± 0.01	0.731
Proline	4.37 ± 0.14	4.68 ± 0.02	0.019
Tyrosine	3.27 ± 0.10	3.18 ± 0.07	0.308
NEAA	51.64 ± 0.37	52.10 ± 0.41	0.223
SAA	39.37 ± 0.32	39.60 ± 0.35	0.450

Values are the means of triplicate groups and are presented as mean ± SD (n = 3). Values are significantly different at $p < 0.05$; * sweet amino acids (SAA).

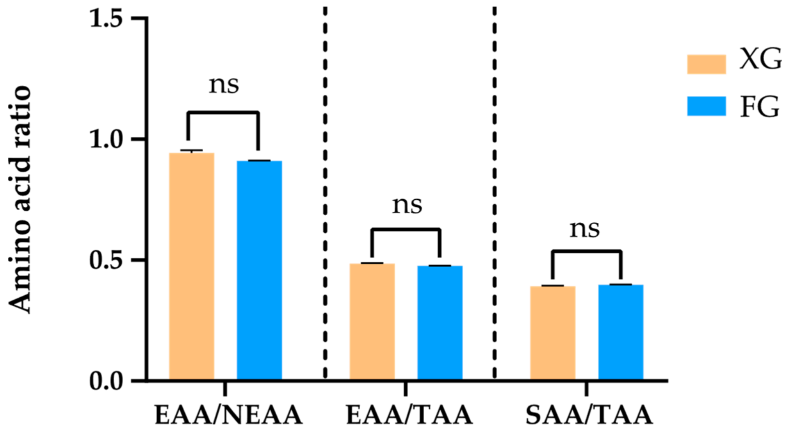

Figure 1. Ratios of EAA/NEAA, EAA/TAA, and SAA/TAA in the muscles of spotted mandarin fish fed on two diets. A significant level was set at $p < 0.05$; ns means non-significant.

As is shown in Table 5, the nutritional qualities of the muscle protein of the XG and FG groups were evaluated using an amino acid ratio score based on the FAO/WHO score model and egg score model. Lysine obtained the highest scores of AAS and CS in the muscle in both the XG and FG groups, and the XG group scored significantly higher than the FG group ($p < 0.05$). In terms of both the AAS and CS scores, the first and second limiting amino acids were valine and methionine in two groups, while the XG group scored a little higher than the FG group.

Table 5. Comparison of EAA compositions in the muscle of spotted mandarin fish fed two diets.

Amino Acid	The FAO/WHO Score Model (mg/g pro)	AAS			The Egg Score Model (mg/g pro)	CS		
		XG	FG	*p*-Value		XG	FG	*p*-Value
Isoleucine	40	0.92 ± 0.03	0.89 ± 0.01	0.244	54	0.68 ± 0.02	0.66 ± 0.01	0.244
Leucine	70	0.95 ± 0.02	0.92 ± 0.01	0.130	86	0.77 ± 0.01	0.75 ± 0.01	0.130
Lysine	55	1.46 ± 0.01	1.40 ± 0.01	0.045	70	1.15 ± 0.01	1.10 ± 0.01	0.045
Threonine	40	0.98 ± 0.01	0.97 ± 0.02	0.405	47	0.83 ± 0.01	0.83 ± 0.01	0.407
Valine	50	0.83 ± 0.01	0.82 ± 0.01	0.663	66	0.63 ± 0.01	0.62 ± 0.01	0.665
Methionine–cysteine	35	0.85 ± 0.02	0.83 ± 0.02	0.192	57	0.52 ± 0.00	0.51 ± 0.01	0.191
Phenylalanine–tyrosine	60	1.05 ± 0.01	1.02 ± 0.02	0.259	93	0.68 ± 0.01	0.66 ± 0.01	0.258

Values are the means of triplicate groups and are presented as mean ± SD (n = 3). Values are significantly different at $p < 0.05$.

3.4. Comparison and Evaluation of the Fatty Acid Composition

The composition of fatty acids in the XG and FG groups are shown in Table 6. A total of 15 fatty acids were detected in the XG group, including 5 saturated fatty acids (SFAs), 3 monounsaturated fatty acids (MUFAs), and 7 polyunsaturated fatty acids (PUFAs). A total of 16 fatty acids were detected in the FG group, including 6 SFAs, 3 MUFAs, and 7 PUFAs. This showed a trend of ΣMUFAs > ΣPUFA > ΣSFA in the content of fatty acids in the FG group, whereas a direction of ΣMUFAs > ΣSFA > ΣPUFA was observed in the content of fatty acids in the XG group. In both groups, palmitic acid (C16:0) scored the highest percentage of SFAs, with 23.07% and 19.48% in the XG and FG groups, respectively. Stearic acid (C18:0) followed, with 5.91% and 5.01% in the XG and FG groups, respectively. Arachidic acid (C20:0) was the only SFA detected in the FG group, at a concentration of 0.23%, but not in the XG group. It was shown that the five other SFAs were significantly lower in the FG group than in the XG group.

Table 6. Fatty acid composition and percentages in the muscle of spotted mandarin fish fed two different diets (%).

Nutrients	Fatty Acids	XG	FG	*p*-Value
Saturated fatty acid (SFA)	C14:0, Myristic acid	3.39 ± 0.04	2.72 ± 0.01	0.002
	C15:0, Pentadecanoic acid	0.81 ± 0.02	0.71 ± 0.01	0.019
	C16:0, Palmitic acid	23.07 ± 0.65	19.48 ± 0.01	0.016
	C17:0, Heptadecanoic acid	0.62 ± 0.01	0.57 ± 0.01	0.029
	C18:0, Stearic acid	5.91 ± 0.09	5.01 ± 0.02	0.005
	C20:0, Arachidic acid	—	0.23 ± 0.01	<0.001
	ΣSFA	33.82 ± 0.65	28.73 ± 0.02	0.008
Monounsaturated fatty acid (MUFA)	C16:1, Palmitoleic acid	8.53 ± 0.27	7.78 ± 0.01	0.058
	C18:1, Oleic Acid	28.00 ± 0.24	30.59 ± 0.01	0.004
	C20:1, Eicosenoic acid	1.37 ± 0.08	1.77 ± 0.01	0.021
	ΣMUFA	37.90 ± 0.06	40.14 ± 0.01	<0.001
Polyunsaturated fatty acid (PUFA)	C18:2, Linoleic acid	14.79 ± 0.12	15.29 ± 0.01	0.028
	C18:3, Linolenic acid	2.09 ± 0.03	1.94 ± 0.02	0.018
	C20:2, Eicosadienoic acid	0.64 ± 0.04	0.85 ± 0.01	0.019
	C20:3, Eicosatrienoic acid	1.02 ± 0.05	1.31 ± 0.01	0.015
	C20:4, Eicosatetraynoic acid	2.85 ± 0.09	3.44 ± 0.01	0.013
	C20:5, Eicosapentaenoic acid EPA	0.81 ± 0.02	0.80 ± 0.01	0.333
	C22:6, Docosahexaenoic acid DHA	6.07 ± 0.24	7.49 ± 0.03	0.014
	ΣPUFA	28.28 ± 0.59	31.13 ± 0.02	0.021
	ΣUFA	66.18 ± 0.65	71.27 ± 0.03	0.008
	EPA + DHA	6.88 ± 0.26	8.29 ± 0.03	0.017
	$\omega-3$	10.00 ± 0.34	11.54 ± 0.01	0.023
	$\omega-6$	17.65 ± 0.22	18.74 ± 0.01	0.019
	$\omega-3/\omega-6$	0.57 ± 0.01	0.61 ± 0.01	0.028
	ΣPUFA/ΣSFA	0.84 ± 0.03	1.08 ± 0.01	0.009

As is shown in Table 6, the oleic acid (C18:1) scored the highest percentage of the MUFAs and total fatty acids, scoring 30.59% and 28.00% in the FG and XG groups, respectively. This was followed by the fatty acid palmitoleic acid, which scored 7.78% and 8.53% in the FG and XG groups, respectively. Moreover, linoleic acid (C18:2) scored the highest percentage out of the PUFAs, at 15.29% and 14.79% in the FG and XG groups, respectively. This was followed by the fatty acid docosahexaenoic acid (DHA), which scored 7.49% and 6.07% in the FG and XG groups, respectively. It was found that almost all of the fatty acids except EPA differed significantly in their content composition between the FG and XG groups ($p < 0.05$). A similar result was observed in the content of ΣMUFAs, ΣPUFAs, and ΣSFAs. Moreover, the unsaturated fatty acids (UFAs) dominated the total extracted lipids in the fatty acid profile in both the FG and XG groups, at 71.27% and 66.18%, respectively. It was shown that MUFAs and PUFAs were greater in the FG group at 40.14% and 31.13%, than the XG group at 37.90% and 28.28% ($p < 0.05$), respectively. The SFAs were significantly lower for the FG group at 28.73% than the XG group at 33.82% ($p < 0.05$). Furthermore, it was found that both the FG and XG groups were rich in MUFAs and PUFAs. The ΣPUFA/ΣSFA ratio was found to be 1.08 in the FG group, which was greater than 0.84 in the XG group ($p < 0.05$). In terms of PUFAs, the FG group had higher levels of $\omega-3$, $\omega-6$, and EPA + DHA than the XG group ($p < 0.05$). The ratios of $\omega-3/\omega-6$ were 0.61 and 0.57 in the FG and XG group, respectively.

Moreover, FG had lower values of atherogenicity (*AI*) and thrombogenicity (*TI*), and a higher hypocholesterolemic/hypercholesterolemic ratio (*HH*) and higher flesh lipid quality (*FLQ*) compared to the XG group ($p < 0.05$), which indicated that the muscle fatty acid profile in the FG group was healthier than that of the XG group (Figure 2).

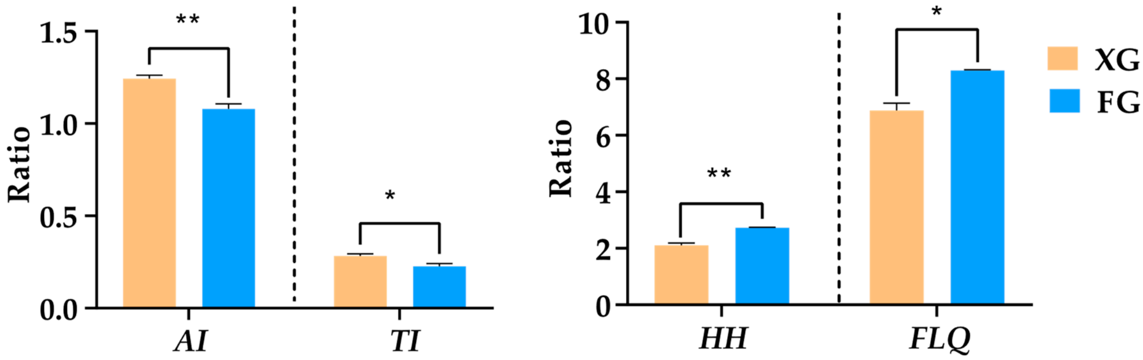

Figure 2. Variations in lipid quality indices of *AI* (atherogenicity index), *TI* (thrombogenicity index), *HH* (hypocholesterolemic/hypercholesterolemic ratio), and *FLQ* (flesh lipid quality) in the muscle of spotted mandarin fish fed two different diets; * significant ($p < 0.05$); ** extremely significant ($p < 0.01$).

4. Discussion

In recent years, the demand for aquatic foods has been increasing; alongside this, the volume of captive fisheries has stabilized and most major fishing areas have reached their maximum potential [17]. Therefore, it is difficult to produce enough iced trash fish or fish meal to meet the growing production needs of the aquafeed industry. Moreover, feeding fish with iced trash fish may cause a decline in natural fish resources, water quality deterioration, disease problems, and environmental pollution [10]. Currently, the majority of spotted mandarin fish in China, over 95%, are still being fed iced trash fish or live bait. This is primarily due to the low success rate of using artificial feed for mandarin fish. In this study, we have achieved a remarkable breakthrough by developing and producing artificial feeds exclusively designed for nourishing spotted mandarin fish. Our findings demonstrate that feeding mandarin fish with artificial feed can result in a survival rate equivalent to that achieved with a diet of iced trash fish. This finding will have a positive impact on the advancement of artificial feed feeding techniques and the domestication of mandarin fish. Feeding fish with formulated diets is important for sustainable aquaculture and environmental protection. Additionally, the palatability and nutrient level of feed affect the growth and development of fish, and determine the success of aquaculture [18]. It was found that there was no significant difference in growth performance and survival rates between the formulated diet and an iced trash fish diet fed to *Lates calcarifer* and *Blotchy rock cod* [19]. Similarly, the weight gain rate and fullness of spotted mandarin fish in the formulated diet group were only slightly lower than those in the iced trash fish diet group, implying that the formulated diet in the present study could replace the iced trash fish diet, which is consistent with the above research. A similar result was found in *Siniperca chuatsi* fed with a formulated diet at the juvenile stage; they had slightly lower growth than those provided with live fish bait [11]. However, there were slight discrepancies that suggested that the formulated diet did not meet the desired level of taste and nutritional balance found in the iced trash fish diet. The amino acid and fatty acid compositions in the iced trash fish diet may be more suitable for the digestion and absorption of mandarin fish, thereby promoting better growth in their natural state. Hence, it is crucial to improve the formulated diets by considering the nutritional requirements of spotted mandarin fish. Additionally, we fed spotted mandarin fish twice a day. This was mainly based on the actual feeding frequency in spotted mandarin fish culture and previous mandarin fish feeding trials [11,20]. This proved that this feeding frequency can adequately explain the influence of different feeding content on the growth of spotted mandarin fish. The visceral and hepatic index in the iced trash fish diet group was slightly greater than in the formulated diet group. This indicated that some immune and digestive organs of the fish were in swollen and unhealthy conditions, which was inappropriate in terms of animal welfare. On the other hand, it represented the lower flesh production rate of spotted mandarin fish, and the discarded visceral parts of fish may cause environmental pollution. Therefore, replacing the ice trash fish diet with a formulated diet when cultivating spotted mandarin fish is promising and feasible.

Protein, fat, and other nutrients in fish muscle can be important indicators of fish quality [21]. It was found that muscle protein and ash contents usually showed an opposite trend compared with fat content in fish [22]. Similarly, the crude fat content of the iced trash fish diet group was higher than that of the formulated diet group, while the crude protein and ash contents decreased. This was consistent with the results seen in *Onychostoma simus* [23], *Epinephelus malabaricus* [24], and *Nibea albiflora* [25]; all these studies showed that the dietary fat and protein levels could positively affect muscle composition. However, the proportion of variations in crude protein and ash in the muscle between the different feed groups was still low, indicating that protein and ash, except lipids, occupied a relatively stable composition in the muscle of mandarin fish feeding on plant-based ingredients as a replacement for animal-based ingredients [26]. However, there was no significant difference in the moisture content between the two groups, which implied that the moisture content of fish remained species-specific and dependent on the aquaculture environment [27].

EAAs are essential for humans and animals. They are important in regulating growth, development, and metabolism [28]. The ratio of EAA to NEAA is an indicator to determine protein consistency. In the present study, nine EAAs were detected in the two different feed groups, and there was no significant difference in the proportion of other essential amino acids except lysine, indicating no noticeable change in the EAA ratios in the fish muscle of the two feed groups. Although lysine significantly differed between the two groups, the difference was only 4.1%, and both groups achieved the levels recommended by the FAO/WHO and egg score models. As we know, the nutritional value of amino acids depends mainly on the content, type, and proportional composition of amino acids. The grading models of the FAO/WHO and whole egg protein can effectively evaluate the level of amino acids in the fish muscle and its nutritional value; furthermore, its calculated value reflects how close the protein quality of the evaluated object is to the standard protein [29]. The closer the value is to 1, the higher the proximity to the standard protein and the higher the amino acid utilization. At the same time, glutamic acid and aspartic acid, two sweet NEAAs, occupied a relatively high proportion in the mandarin fish muscle, remaining stable in both groups without any significant difference. These two NEAAs can increase the umami taste of spotted mandarin fish [30]. Moreover, the EAA/NEAA, EAA/TAA, and SAA/TAA ratios remained stable in both groups and achieved the minimum thresholds set out by the FAO/WHO guidelines. This indicated that both the iced trash fish diet and formulated diet groups can be considered high-quality protein food sources. Therefore, we can shift our attention to a formulated diet that is cheaper to produce and has other ecological benefits [31,32].

According to the fat content of fish muscle, fish can be classified into four types: lean (less than 2% fat), low fat (2–4% fat), medium fat (4–8% fat), and high fat (more than 8% fat) [33]. This study showed that both spotted mandarin fish fed with the iced trash fish and formulated diets can be classified as lean fish based on their fat content. However, the unsaturated fatty acid (UFA) content of mandarin fish in the iced trash fish diet group was lower than in the formulated diet group, indicating that different diets consumed by spotted mandarin fish may result in changes in muscle fatty acid components. Similar results were found in *Ctenopharyngodon idellus* [34], *Epinephelu* [35], and *Micropterus salmoides* [36]. EPA and DHA are important omega-3 polyunsaturated fatty acids, and they all perform many biological functions in animals. Although the EPA and DHA contents of mandarin fish in this study were far lower than in Atlantic salmon [37], they could be increased through the addition of animal ingredients such as insect meal, mussel meal, and poultry by-product meals, which has been confirmed as feasible in a previous study [26]. It is known that increasing the $\omega - 3/\omega - 6$ ratio helps to lower blood lipids, reduce the risk of cancer, and prevent coronary heart disease [38,39]. In the present study, the $\omega - 3/\omega - 6$ ratios of the two groups were close to the WHO-recommended minimum level of 0.6, indicating that the two groups of fish could achieve a balanced ratio of unsaturated fatty acid levels. However, the formulated diet group achieved a higher $\omega - 3/\omega - 6$ ratio than the iced trash fish diet group, suggesting that fish fed on a formulated diet can be transformed into a more suitable product for human consumption through continuous formula optimization [40,41].

It is known that higher atherosclerosis (*AI*) and thrombosis (*TI*) values in the flesh of fish are harmful to human health, while elevated cholesterol to high-cholesterol (*HH*) levels and muscle fat mass (*FLQ*) is beneficial [42,43]. In this study, the *AI* and *TI* indices were lower in the formulated diet group than those in the iced trash fish diet group, but the *HH* and *FLQ* indices were on the contrary, indicating that the muscle fat quality in the formulated diet group was better than in the iced trash fish diet group. Therefore, replacing the iced trash fish diet with a formulated diet for spotted mandarin fish rearing is effective and promising.

5. Conclusions

The results suggest that spotted mandarin fish fed a formulated diet had comparable survival rates, weight gain, and satiety to those fed an iced trash fish diet. Furthermore, there were variations in the levels of crude protein, crude fat, and ash between the two groups, with the formulated diet leading to a better fatty acid composition. This study demonstrates the possibility of replacing the iced trash fish diet with a formulated diet for rearing spotted mandarin fish, although further improvements are needed in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8080393/s1, Table S1: Statistical data comparing the effects of two different types of diets.

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