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Effects of Different Protein Sources on Growth Performance, Muscle Flavor Substances and Quality Structure in Triploid Crucian Carp

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Abstract: A 56-day feeding trial was conducted to evaluate the effect of different dietary protein sources on the growth performance, muscle flavor substances, and quality structure of Triploid Crucian Carp. Three isonitrogenous (32.00%), isolipidic (8.00%), and isoenergetic (18.00 MJ kg^{-1}) practical diets were formulated. These diets consisted of fishmeal as the animal-derived protein source (AP), a combination of soybean meal and rapeseed meal as the plant-derived protein source (PP), and a mixture of fishmeal, soybean meal, and rapeseed meal as the mixed protein source (MP). Each diet was randomly assigned to triplicate tanks of fish and each tank was stocked with 25 fish (11.5 ± 0.4 g). The fish were fed until apparent satiation twice a day. The results showed a significant enhancement in the growth performance of Triploid Crucian Carp in the AP group compared with both the MP and PP groups ($p < 0.05$). Dietary plant derived protein can remarkably reduce the crude lipid content and increase the moisture content of the whole body and the dorsal muscle ($p < 0.05$). The antioxidant ability of fish in the PP group and MP group was better than that in the AP group ($p < 0.05$). Regarding free amino acids composition of muscle, the contents of glycine, methionine, and lysine were significantly enhanced in the AP group, while the histidine content was significantly increased in the PP group ($p < 0.05$). In terms of texture, dietary plant protein significantly improved the muscular hardness, gumminess, and chewiness. The activities of intestinal trypsin and alkaline phosphatase (ALP) of fish in the PP group were significantly higher than that in the AP group ($p < 0.05$). The expression levels of hepatopancreas TOR and IGF1 genes in the PP group were significantly higher than that in the MP group ($p < 0.05$). The present results indicate dietary fishmeal significantly improved the growth performance and muscular flavor glycine content, while dietary plant-based protein increased crude protein content, antioxidant ability, and muscular texture performance of Triploid Crucian Carp.

Keywords: protein source; Triploid Crucian Carp; growth performance; texture; muscle quality

Key Contribution: Dietary fishmeal significantly improved the growth performance and muscular flavor glycine content of Triploid Crucian Carp. Dietary inclusion of plant-based protein significantly increased crude protein content, antioxidant ability, and muscular texture performance of Triploid Crucian Carp.

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1. Introduction

As a superior protein source, fishmeal is extensively utilized in aquatic feed, not only due to its high protein content, comprehensive essential amino acids profile, minimal anti-nutritional factors, and facile digestibility and absorption, but also because it is a rich source of long-chain omega-3 fatty acids, vitamins, and minerals essential for normal animal growth [1,2]. However, the rapid expansion of aquaculture and overfishing have led to the depletion of wild fishery resources, resulting in a scarcity and escalating cost of fishmeal resources [3]. In order to maintain the sustainable development of the fishery industry, there is a growing emphasis placed on exploring alternative protein sources to reduce or substitute fishmeal [4].

It is widely acknowledged that plant-derived raw materials, such as rapeseed meal, soybean meal, palm meal, and peanut meal, have the potential to partially or completely substitute fishmeal protein in the diets of herbivorous and omnivorous fish due to their cost-effectiveness, diverse source availability, and high protein content [5–7]. The substitution of 75% fishmeal with soybean meal had no significant differences in growth, digestive enzyme activities, and antioxidant status in Nile tilapia (*Oreochromis niloticus*) [8]. According to reports, soybean meal could completely replace fishmeal for juvenile benni (*Mesopotamichthys sharpeyi*) [9]. However, most carnivorous and some omnivorous fish species may be adversely affected by increasing levels of dietary plant protein. A study on Chinese sucker (*Myxocyprinus asiaticus*) revealed a significant reduction in the growth, feed intake (FI), and digestibility as the substitution level of soybean meal replacing fishmeal in the diet increased from 40% to 100% [10]. Similarly, as dietary inclusion level of rapeseed meal surpasses exceed 25%, there is a discernible decline observed in growth performance and feed efficiency (FE) of juvenile cobia (*Rachycentron canadum*) [11]. This may be attributed to the presence of numerous antinutritional factors, the imbalance of amino acids, or the poor digestibility, which limit bioavailability of fish [12,13]. Overall, in order to evaluate the applicability of plant protein sources in different fish species and enhance cost-effectiveness and efficiency, it is necessary to investigate the comparative effects of different protein sources on fish growth performance.

The textural parameters of flesh, such as hardness, gumminess, chewiness, adhesiveness, cohesiveness, and springiness are widely recognized as the primary physical indicators for evaluating muscle quality [14]. Flavor substances are also key flesh quality parameters that confer a high market value and consumer demand [15]. Free amino acids (AAs), such as aspartic acid, alanine, histidine, glutamate, and glycine, contribute directly to flavor development in fish, and are important substances of muscle flavor [15,16]. Interestingly, despite extensive research on the utilization of plant-derived proteins as potential substitutes for fishmeal, limited studies have conducted comprehensive assessments of their impact on the textural parameters and flavor related amino acid content of fish muscle quality. A previous study showed that replacing dietary soybean meal and rapeseed meal with faba bean meal decreased crude lipid content but increased collagen, flavor amino acids, and textural properties of the muscle of tilapia [17]. Additionally, in research on mammals, when rapeseed meal and faba beans were used to substitute soybean meal in a grow-finish pig diets, it improved pork color, increased the concentration of free amino acids, and led to reduced warmed-over flavor and flavor attributes [18]. These data suggest that plant protein sources might have a positive effect on improving fish muscle quality, which deserves investigation.

The crucian carp, belonging to the family *Cyprinidae* of the order *Cypriniformes* of *Actinopterygii*, is widely recognized as one of the most economically significant freshwater species in Chinese aquaculture [19]. Triploid Crucian Carp was generated through inter-ploidy crossing between Japanese crucian carp (*Carassius cuvieri*, 2n = 100, ♀) and allotetraploid (4n = 200, ♂), which was obtained by mating red crucian carp (*Carassius auratus* red var., 2n = 100, ♀) with common carp (*Cyprinus carpio* L., 2n = 100, ♂) [20]. The Triploid Crucian Carp is extensively cultivated throughout China due to its robust adaptability, sterility, rapid growth rate, and exceptional muscle quality [20]. The aim

of this study was to investigate the effect of different dietary protein sources on growth performance, antioxidant capacity, intestinal absorption, and muscle quality in Triploid Crucian Carp. The results of this study provide a theoretical basis for the rational utilization of plant-derived protein in aquatic feed, which is essential for sustainable aquaculture development. Additionally, the findings of this study may provide novel insights into the specific nutritional attributes of the protein sources and their potential metabolic impacts on fish.

2. Materials and Methods

2.1. Experimental Diets and Procedures

Three isonitrogenous (32.00%), isolipidic (8.00%), and isoenergetic (18.00 MJ kg^{-1}) practical diets were formulated using fishmeal as the animal derived protein (AP), a mixture of soybean meal and rapeseed meal as the plant derived protein (PP), and a mixture of fish meal and soybean meal with rapeseed meal as the mixed protein (MP). The diet formulations and chemical compositions are shown in Table 1. The feed ingredients were sieved through a 40 mesh sifter, thoroughly mixed, and subsequently extruded into 1.5 mm pellets using a laboratory granulator (SZLH200, Jiangsu Zhengchang Group Co. Ltd., Jiangsu, China). The prepared pellets were finally stored in separate sealed plastic bags at −20 °C until use.

Table 1. Diet formulations and proximate compositions of the experimental diets (% dry matter).

Ingredient	Mixed Protein Group (MP)	Animal Protein Group (AP)	Plant Derived Protein Group (PP)
Fishmeal [1]	12.00	44.40	0.00
Soybean meal [1]	20.00	0.00	37.10
Rapeseed meal [1]	15.00	0.00	15.00
Casein [1]	6.50	0.00	6.50
Fish oil [1]	3.00	1.63	3.50
Soybean oil [1]	3.00	1.63	3.50
Corn starch	16.80	31.00	10.00
Wheat flour	10.00	10.00	10.00
Choline chloride	0.50	0.50	0.50
Premix [2]	3.00	3.00	3.00
CMC [3]	3.00	3.00	3.00
Cellulose	7.20	4.84	7.90
Total	100.00	100.00	100.00
	Proximate composition		
Crude protein	32.68	32.68	32.28
Crude lipid	8.00	7.80	8.66
Moisture	10.05	9.73	9.86
Ash	7.54	10.09	6.27
Gross energy (MJ kg^{-1})	18.06	18.07	18.00

[1] All of these ingredients were purchased from Hunan Zhenghong Science and Technology Develop Co., Ltd., Yueyang, China. [2] Premix (mg/kg diet): Vitamin B12, 0.02; Folic acid, 5; Calcium pantothenate, 50; Inositol, 100; Niacin, 100; Biotin, 0.1; Vitamin B1, 20; Vitamin B2, 20; Vitamin B6, 20; Vitamin A, 11; Vitamin D, 2; Vitamin E, 50; Vitamin K, 10; Vitamin C, 100; cellulose, 3412; $CaH_2PO_4 \cdot 2H_2O$, 7650.6; $FeSO_4 \cdot 7H_2O$, 2286.2; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750.0; $ZnSO_4 \cdot 7H_2O$, 178.0; NaCl, 500.0; $MgSO_4 \cdot 7H_2O$, 8155.6; $NaH_2PO_4 \cdot 2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $MnSO_4 \cdot H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 7H_2O$, 0.91; KI, 1.5; Na_2SeO_3, 0.60; Corn starch, 899.7. [3] CMC: Carboxymethyl cellulose.

2.2. Fish, Cage Culture, and Experimental Procedures

All the Triploid Crucian Carp in this experiment were provided by the Fisheries Research Institute of Hunan Province (Changsha, China). Before the experiment, all of the fish were temporary cultured in two 1500-L circular fiberglass tanks in an indoor recirculating system for two weeks. Then, healthy and similar sized fish (n = 225, initial body weight: 11.5 ± 0.4 g) after 24 h of starvation were batch-weighted, randomly selected, and stocked into nine fiberglass tanks (100 L). Each tank was randomly filled with 25 fish

and a group of three tanks was allocated one of the diets. During the feeding trial, fish were hand-fed to apparent satiation twice daily at 8:30 a.m. and 3:30 p.m., for 56 days. The water temperature was maintained at 24.5 ± 1.0 °C, dissolved oxygen content was kept above 6.5 mg L^{-1}, the concentration of ammonia nitrogen was <0.5 mg kg^{-1}, pH ranged from 7.0 to 7.8, and the experimental fish received a 12-h light-dark cycle (light on 8:00 a.m.).

2.3. Sample Collection

At the end of the feeding trial, Triploid Crucian Carp were fasted for 24 h before sampling. All fish from each tank were rapidly captured, anaesthetized using MS-222 (50 mg L^{-1}, tricaine methane sulphonate, Sigma-Aldrich, St. Louis, MO, USA), and then weighed and counted. Six fish were randomly selected from each tank to collect blood, liver, intestine, and muscle tissue samples, and three other fish were taken to measure condition factor (CF) and viscerasomatic index (VSI). Plasma was collected from the caudal vein using 2.0 mL disposable syringes rinsed with heparin sodium. After centrifugation (3000× *g*, 10 min, 4 °C), plasma samples were separated into 200 µL PCR tubes and stored at −80 °C for future analysis of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and lysozyme (LZM). After blood sampling, fish were immediately dissected on ice to collect the liver, intestine, and muscle tissue samples and stored at −80 °C for further analysis. The isolated intestine samples were used for further analysis of enzymes related to digestion and intestinal development. The muscles were used to analyze nutritional components, free amino acids, flavor nucleotides, and textural parameters. And the liver tissues were used for determining the expression profile of genes related to growth.

Growth performance of fish, such as weight gain rate (WGR), specific growth rate (SGR), feed efficiency (FE), feeding rate (FR), condition factor (CF), and viscerasomatic index (VSI), was calculated based on the following standard formulae:

Weight gain rate (WGR, %) = (final body weight (g) − initial body weight (g))/initial body weight (g) × 100;

Specific growth rate (SGR, %/d) = (ln final body weight (g) − ln initial body weight (g))/number of trial days (d) × 100;

Feed efficiency (FE, %) = (final body weight (g) − initial body weight (g))/dry feed intake (g) × 100;

Feeding rate (FR, %) = total food intake (g)/[number of trial days (d) × (final body weight (g) + initial body weight (g))/2] × 100;

Condition factor (CF, g/cm^3) = individual mass of each fish (g)/body length of each $fish^3$ (cm^3) × 100;

Viscerasomatic index (VSI) = visceral mass/individual mass of each fish × 100.

2.4. Biochemical Composition

Crude protein, crude lipid, moisture, and ash content of the diets, for the whole body and dorsal muscle, were determined according to the method of AOAC [21]. Crude protein of the samples was determined through an 8400 kjeltec azotometer (FOSS Tecator, Haganas, Sweden). Crude lipid content was analyzed via ether extraction using a soxhlet extractor (ST 243 Soxtec TM, FOSS Tecator, Haganas, Sweden). Moisture content was measured by drying the samples at 105 °C for 4 h to constant weight. Ash content was detected after burning in a muffle furnace at 550 °C for 12 h. Gross energy was determined via combustion in an adiabatic microbomb calorimeter (Phillipson Microbomb Calorimeter, Gentry Instructions Inc., Aiken, SC, USA).

2.5. Determination of Amino Acids in Muscle

Muscle samples were pretreated, then the free amino acids were analyzed by an automatic amino acid analyzer (Agilent 1100 Series; Palo Alto, CA, USA) and a high-performance liquid chromatography (Shimadzu Corporation, Kyoto, Japan).

2.6. Assay of Muscle Textural Properties

Three fish were selected from each tank for textural analyses. Texture determination of dorsal muscle samples was performed using a texture analyzer (TMS-PRO, Food Technology Corporation, Sterling, VA, USA). Double compression was applied to construct the texture profile analyses (TPA) on raw fillets (fillet thickness ranged from 1.0 to 1.5 mm). The textural characteristics of each sample, such as chewiness, springiness, hardness, adhesiveness, cohesiveness, and gumminess, were calculated via the force-time curve produced using the computer software Texture Lab Pro (1.18–408, FTC, Sterling, VA, USA).

2.7. Detection of Plasma and Intestinal Enzyme Activity

The activity of plasma and intestinal SOD, CAT, MDA, alkaline phosphatase (ALP), trypsin, and Na^+K^+-ATPase was measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The plasma LZM activity was determined through turbidimetric assay according to the method of Cao et al. [22].

2.8. RNA Isolation and cDNA Synthesis

The total RNAs were extracted from the samples of Triploid Crucian Carp using Trizol reagent, following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The integrity and quality of RNA samples was measured by agarose gel electrophoresis, and confirmed by calculating via the A260/A280 and A260/A230 ratio spectrophotometer (BioPhotometer Eppendorf, Hamburg, Germany). To mitigate the presence of endogenous DNA contamination, 1 g of RNA was digested with DNase I before synthesis. Subsequently, the RNA was subjected to reverse transcription using a PrimeScrip RT kit with gDNA eraser (Takara, Dalian, China).

2.9. Real-Time Polymerase Chain Reaction (PCR) Analysis

Table 2 lists the primer sequences used for quantitative detection of target gene transcription, such as rapamycin (TOR), Insulin-like growth factor 1 (IGF1), ribosomal protein S6 kinase 1 (S6K1), and 4E-binding protein 2 (4E-BP2). β-actin was used as the internal reference for normalization. Based on the TOR, IGF1, S6K1, and 4E-BP2 sequences of crucian carp in the NCBI database, primers specific for each gene were designed using the Primer Premier 5.0 program.

Table 2. Primer sequences used for quantitative real-time PCR.

Acronym	Primer Sequence	Accession No.	Annealing Temp. (°C)
TOR	F: TCAGGGTTGTCAGCGTATTG R: AGGGTTTTATGGGCTAGTGC	KF772613	60
IGF1	F: ATTGCCCGCATCTCATCCTC R: TGACCGCTAGACATCCCCTT	KF813006	60
S6K1	F: CGAGCTGGAGTTAATAGGGTT R: AGGTGACATGCACCATCTATG	KF880601	57
4E-BP2	F: CACTTTATTCTCCACCACCC R: GATGTTGTTAGCCTCATTCCT	KF900277	60
β-actin	F: TTGAGCAGGAGATGGGAACCG R: AGAGCCTCAGGGCAACGGAAA	AB039726.2	60

qPCR analyses were performed based on previous work in our laboratory [23]. Briefly, following the manufacturer's protocol, qPCR was conducted on a Bio-Rad CFX96™ Real-time PCR detection system (Bio-Rad, Hercules, CA, USA). Reactions including 0.8 μL forwards and reverse primer, 2 μL cDNA, 10 μL SYBR® Premix Ex Taq (Tli RNaseH Plus, 2×) (TaKaRa, Dalian, China), and 6.4 μL of ddH_2O were run with the following conditions: initial denaturation at 95 °C for 3 min, followed by a total of 40 cycles consisting of denaturation at 95 °C for 10 s, annealing at 60 °C for 22 s, and extension at 72 °C for 10 s. The baseline was automatically set by quantitative software to maintain consistency.

Analysis of each specimen was conducted in duplicate and the relative mRNA expression level was calculated through the comparative CT method ($2^{-\Delta\Delta Ct}$ method) [24].

2.10. Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS19.0 (SPSS Inc., Chicago, IL, USA) statistical software, followed by Duncan's multiple range test. The differences were deemed statistically significant at a significance level of $p < 0.05$. The results are presented in the form of mean ± standard error (SE).

3. Results

3.1. Growth Performance

The effect of dietary protein sources on the growth performance of Triploid Crucian Carp was evaluated after a 56-day feeding trial, as presented in Table 3. The AP group had the highest final body weight (FBW), weight gain rate (WG), and specific growth rate, followed by the MP group, and the PP group had the lowest numbers ($p < 0.05$). The feed efficiency of the AP group was markedly higher than that of the MP and PP group ($p < 0.05$). The feeding rate (FR) in the AP group was significantly decreased compared with the PP group ($p < 0.05$). Additionally, the condition factor (CF) of fish from the AP group was significantly higher than that in the PP group ($p < 0.05$).

Table 3. Effects of different dietary protein sources on growth performance in Triploid Crucian Carp.

Items	MP	AP	PP
IBW [1]	11.83 ± 0.07	11.69 ± 0.08	11.72 ± 0.11
FBW [2]	24.26 ± 0.33 [b]	27.75 ± 0.28 [c]	22.80 ± 0.33 [a]
WGR [3]	105.11 ± 3.84 [b]	137.32 ± 3.01 [c]	94.57 ± 1.07 [a]
SGR [4]	1.20 ± 0.03 [b]	1.44 ± 0.02 [c]	1.11 ± 0.01 [a]
FE [5]	41.35 ± 2.64 [a]	53.61 ± 0.30 [b]	39.75 ± 1.51 [a]
FR [6]	2.58 ± 0.07 [ab]	2.49 ± 0.01 [a]	2.69 ± 0.04 [b]
CF [7]	1.98 ± 0.08 [ab]	2.08 ± 0.03 [b]	1.88 ± 0.04 [a]
VSI [8]	10.44 ± 0.18	10.49 ± 0.63	9.21 ± 0.38

Values are Means ± SE; Different superscript letters in the same line mean significantly different ($p < 0.05$); Absence of letters indicates no significant difference between treatments. [1] IBW, initial body weight (g); [2] FBW, final body weight (g); [3] WGR, weight gain rate (%); [4] SGR, specific growth rate (% d^{-1}); [5] FE, feed efficiency (%); [6] FR, feeding rate (BW% d^{-1}); [7] CF, condition factor (g cm^{-3}); [8] VSI, viscerasomatic index (%).

3.2. Biochemical Composition

The crude protein content in the whole body did not show any significant difference among the three groups, whereas a significantly higher level of crude protein was observed in the dorsal muscle of the PP group compared with that of the AP group (Table 4) ($p < 0.05$). Crude lipid content in the whole body and dorsal muscle in the PP group was significantly lower than that in the MP and AP groups ($p < 0.05$). However, the moisture content in the whole body and dorsal muscle exhibited an inverse pattern, with significantly higher moisture content observed in the PP group compared with both the MP and AP groups ($p < 0.05$). No significant difference was found for ash content among the three groups ($p > 0.05$).

3.3. Free Amino Acids

A total of 15 free amino acids, including 5 flavor amino acids, were detected in the muscle tissues of Triploid Crucian Carp (Table 5). Among the flavor amino acids, muscular glycine content in the AP group was significantly higher than that in the MP and PP groups ($p < 0.05$). Among non-flavor amino acids, the histidine and proline contents in the muscle in the PP and MP groups were significantly higher than that in the AP group ($p < 0.05$), while the contents of muscular methionine and lysine in the PP and MP groups were significantly lower than that in the AP group ($p < 0.05$). The contents of other amino acids were not significantly different among the three groups ($p > 0.05$).

Table 4. Effects of different dietary protein sources on the proximate composition of the whole fish body and dorsal muscle of Triploid Crucian Carp (% wet weight).

Item	MP	AP	PP
Whole body of fish			
Crude protein	14.34 ± 0.36	15.06 ± 0.07	15.00 ± 0.16
Crude lipid	10.88 ± 0.43 ^{b}	11.37 ± 0.27 ^{b}	9.61 ± 0.31 ^{a}
Moisture	70.65 ± 0.63 ^{ab}	70.09 ± 0.27 ^{a}	71.89 ± 0.21 ^{b}
Ash	2.39 ± 0.03	2.33 ± 0.05	2.49 ± 0.06
Dorsal muscle			
Crude protein	19.47 ± 0.27 ^{ab}	18.80 ± 0.20 ^{a}	20.33 ± 0.34 ^{b}
Crude lipid	1.36 ± 0.12 ^{b}	1.10 ± 0.14 ^{ab}	0.72 ± 0.07 ^{a}
Moisture	78.59 ± 0.48 ^{ab}	77.69 ± 0.81 ^{a}	81.43 ± 1.46 ^{b}
Ash	1.11 ± 0.07	1.20 ± 0.03	1.09 ± 0.19

Values are Means ± SE (n = 6); Different superscript letters in the same line mean significantly different ($p < 0.05$); Absence of letters indicates no significant difference between treatments.

Table 5. Effects of different dietary protein sources on the composition of free amino acids in muscle of Triploid Crucian Carp (mg kg^{-1} dry matter).

Item	MP	AP	PP
Flavor amino acid			
Glycine	256.63 ± 18.03 ^{a}	363.19 ± 13.89 ^{b}	271.4 ± 17.10 ^{a}
Alanine	250.35 ± 20.19	273.42 ± 3.69	254.28 ± 23.30
Glutamic acid	208.89 ± 33.67	189.75 ± 21.47	177.14 ± 11.70
Tyrosine	16.99 ± 4.15	15.19 ± 7.48	11.68 ± 2.05
Aspartic acid	305.27 ± 16.42	201.46 ± 94.55	202.82 ± 85.78
Serine	74.84 ± 3.16	72.38 ± 29.59	66.21 ± 18.44
Other free amino acids			
Histidine	3260.22 ± 75.45 ^{ab}	3033.35 ± 119.37 ^{a}	3433.52 ± 55.73 ^{b}
Arginine	2675.69 ± 230.78	2923.03 ± 18.16	3063.52 ± 43.56
Threonine	115.58 ± 24.07	117.27 ± 16.43	79.75 ± 14.29
Valine	30.82 ± 11.43	25.01 ± 14.21	18.97 ± 4.22
Methionine	35.76 ± 3.05 ^{b}	45.84 ± 5.21 ^{b}	20.64 ± 2.13 ^{a}
Isoleucine	13.91 ± 5.45	10.21 ± 6.78	5.24 ± 1.90
Leucine	27.89 ± 9.70	24.47 ± 13.81	12.83 ± 2.85
Lysine	635.78 ± 272.75 ^{a}	1350.54 ± 178.08 ^{b}	516.26 ± 61.36 ^{a}
Proline	276.93 ± 12.07 ^{b}	193.97 ± 13.86 ^{a}	220.36 ± 19.89 ^{a}

Values are Means ± SE (n = 6); Different superscript letters in the same line mean significantly different ($p < 0.05$); Absence of letters indicates no significant difference between treatments.

3.4. Muscle Texture Properties

The effects of different protein sources on the texture parameters of Triploid Crucian Carp are shown in Table 6. The hardness, gumminess, and chewiness of fish from the PP group exhibited significantly higher values compared with those from the MP and AP groups ($p < 0.05$), while no statistically significant differences were observed in the springiness, adhesiveness, and cohesiveness of fish muscle among the three feeding groups ($p > 0.05$).

3.5. Antioxidant and Intestinal Digestive Enzymes

The plasmic SOD and CAT activity of fish in the MP and PP groups exhibited higher levels compared with those in the AP group, particularly with a significant difference observed between the MP group and AP group ($p < 0.05$) (Figure 1). The MDA content of fish in the AP group was higher than that in the MP and PP groups; conversely, the LZM activity of fish in the AP group was lower than that in the MP and PP groups, although this difference did not reach statistical significance ($p > 0.05$). The ALP and trypsin activity in the intestine of Triploid Crucian Carp in the AP group exhibited significantly lower levels compared with those from the MP and PP groups ($p < 0.05$) (Figure 2). The intestinal

Na^+K^+-ATPase activity in the MP group was significantly lower than that in both the AP and PP groups ($p < 0.05$).

Table 6. Effects of different dietary protein sources on texture parameters of Triploid Crucian Carp.

Item	MP	AP	PP
Springiness (mm)	0.67 ± 0.04	0.70 ± 0.03	0.69 ± 0.03
Hardness (N)	18.65 ± 1.18 ^{a}	17.51 ± 0.91 ^{a}	21.49 ± 0.76 ^{b}
Gumminess (g × mm)	3.10 ± 0.21 ^{a}	2.90 ± 0.20 ^{a}	3.98 ± 0.23 ^{b}
Chewiness (mJ)	2.14 ± 0.25 ^{ab}	2.06 ± 0.20 ^{a}	2.75 ± 0.21 ^{b}
Adhesiveness (N × mm)	0.029 ± 0.006	0.039 ± 0.007	0.027 ± 0.005
Cohesiveness (%)	0.170 ± 0.012	0.167 ± 0.009	0.185 ± 0.007

Values are Means ± SE (n = 12). Different superscript letters in the same line mean significantly different ($p < 0.05$); Absence of letters indicates no significant difference between treatments.

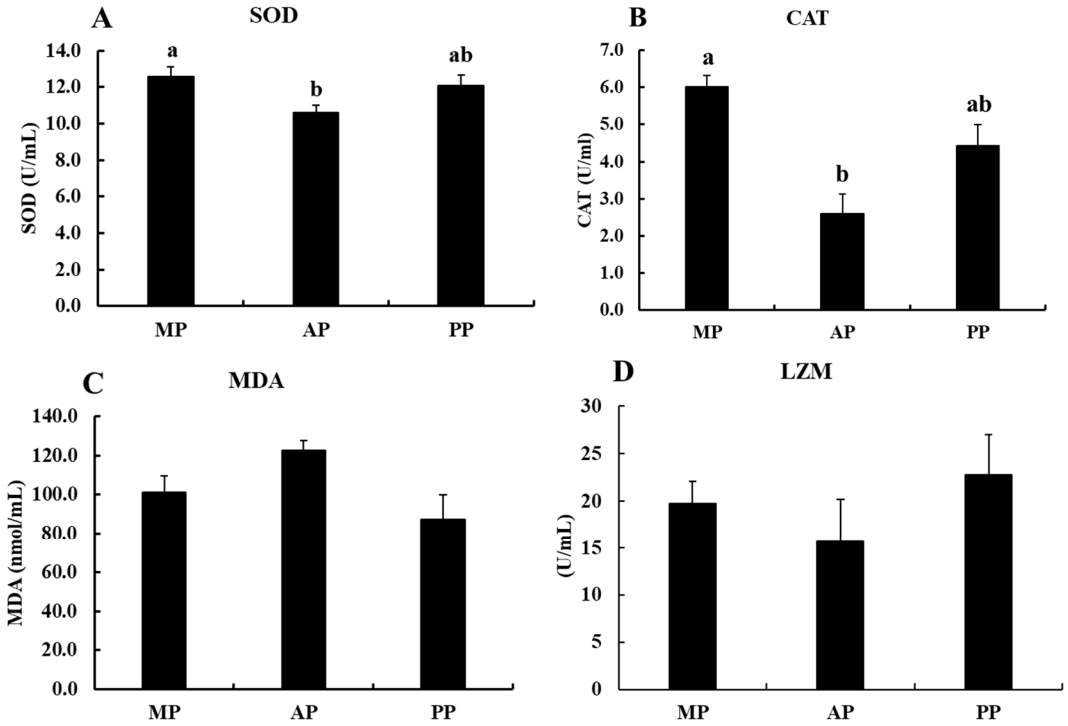

Figure 1. Effects of different dietary protein sources on the activities of plasma antioxidant enzymes in Triploid Crucian Carp. (**A**) SOD, (**B**) CAT, (**C**) MDA, (**D**) LZM. All data are expressed as Means ± SE (n = 6). Bars with different superscript letters mean significant differences between each different protein source group ($p < 0.05$).

3.6. The Expression of Genes in the TOR Signaling Pathway

The effect of different protein sources in feed on the expression of liver related genes in Triploid Crucian Carp is shown in Figure 3. The expression of TOR in the PP group livers was significantly increased compared with the MP group ($p < 0.05$). The expression of IGF1 in the AP and PP group livers was significantly higher than that in the MP group ($p < 0.05$).

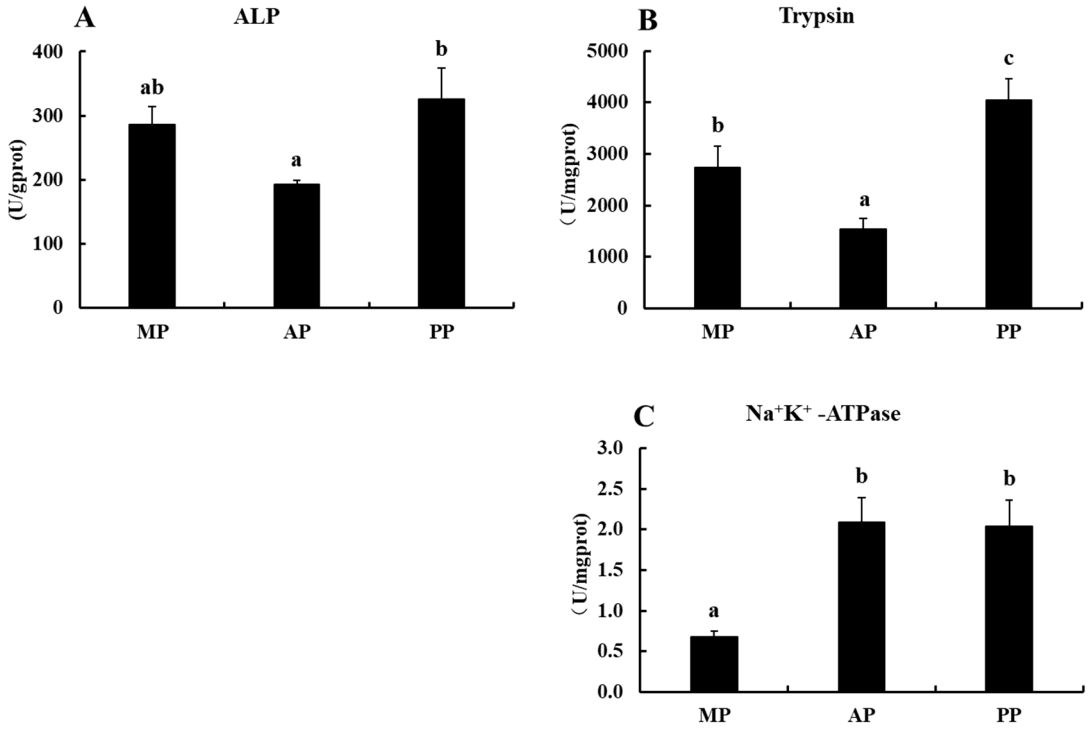

Figure 2. Effects of different dietary protein sources on the activities of intestinal enzymes in Triploid Crucian Carp. (**A**) ALP, (**B**) Trypsin, (**C**) Na^+K^+-ATPase. All data are expressed as Means ± SE (n = 6). Bars with different superscript letters mean significant differences between each different protein source group ($p < 0.05$).

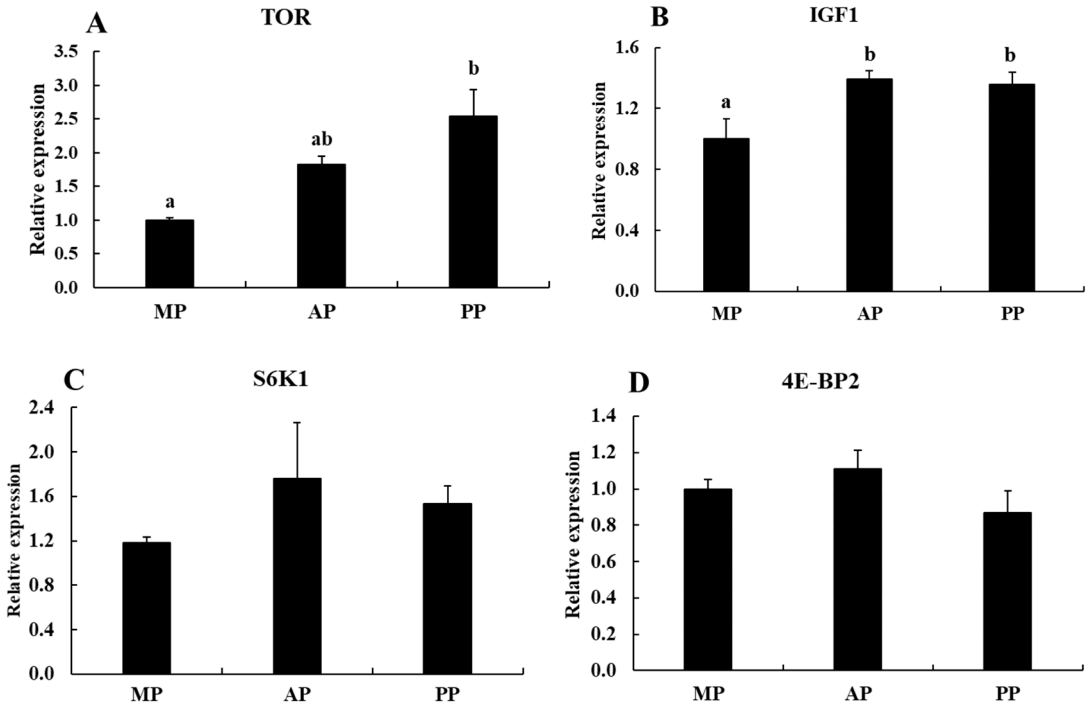

Figure 3. Effects of different dietary protein sources on hepatopancreas gene expressions in Triploid Crucian Carp. (**A**) TOR, (**B**) IGF1, (**C**) S6K1, (**D**) 4E-BP2. All data are presented as Means ± SE (n = 6). Bars with different superscript letters mean significant differences between each different protein source group ($p < 0.05$).

4. Discussion

In the present study, fish fed with the AP diet exhibited superior growth performance in terms of final body weight, WGR, and SGR compared with fish in the MP and PP groups. Similarly, research on Japanese seabass (*Lateolabrax japonicas*) and barramundi (*Lates calcarifer*) showed that dietary inclusion of high levels of a dietary plant protein source (rapeseed meal) significantly reduces fish growth [12,25]. The study conducted by Zhou et al. [26] demonstrated that the growth of blunt snout bream (*Megalobrama amblycephala*) decreased as the level of plant protein (rapeseed meal) in feed exceeded 350.5 g kg^{-1}, indicating a negative correlation between the increase in rapeseed meal replacing fish meal and the growth performance. The deficiency of essential amino acids, such as methionine and lysine, in plant protein sources has been demonstrated to be a significant limiting factor for fish growth due to their involvement in protein synthesis and other crucial physiological functions [17,27,28]. Moreover, this study showed that the feeding rate in the PP group was significantly higher than that in the AP group, while the feeding efficiency in the AP group was significantly higher than that in the PP group. Therefore, the reduced growth could be due to low feeding efficiency, which was mainly caused by imbalanced amino acids composition and antinutrients in the diets, such as glucosinolate, phytate, and tannins [26].

The growth performance of animals is closely correlated with the digestive and absorption function of the intestine [29]. Trypsin, an endogenous digestive enzyme secreted by the pancreas, plays a pivotal role in catalyzing the hydrolysis of peptide bonds within proteins [30]. Alkaline phosphatase (ALP) and Na^+K^+-ATPase are two crucial enzymes involved in the absorption processes of nutrients in aquatic animals, serving as comprehensive indicators for nutrient absorption [31]. A study on Chinese sucker has revealed that the presence of anti-nutritional factors, such as phytic acid and protease inhibitors, in plant protein sources (such as soybean meal), can effectively inhibit both trypsin and alkaline protease activities [10]. Consequently, this inhibition leads to a reduction in the digestibility and utilization of feed protein, ultimately resulting in impaired fish growth performance [32]. However, it is interesting to note that the intestinal ALP and trypsin activities of Triploid Crucian Carp in the PP group was significantly higher than those in the AP group. And there was no significant difference observed in the intestinal Na^+K^+-ATPase activity between the AP and PP groups. This phenomenon may be attributed to the presence of anti-nutritional factors in plant proteins, rendering them resistant to digestion. Consequently, fish require the induction of digestive enzyme secretion to fulfill their digestive requirements.

In Triploid Crucian Carp, the crude lipid of the whole body and dorsal muscle in the plant-based protein group was significantly lower than that in the animal derived protein group. This result is consistent with previous studies conducted on Japanese seabass (*Lateolabrax japonicus*) and obscure puffer (*Takifugu obscurus*) [2,33], which demonstrated a significant reduction in crude lipid content when dietary fishmeal was replaced by soybean meal. El-Sheekh et al. [34] suggested that vegetable ingredients such as Spirulina platensis could suppress excessive lipid accumulation in muscle. In rainbow trout, it has been reported that a high concentration of polyphenol in plant meal could endow it hypolipidemic activity [35]. Moreover, in the present study, the crude protein content in dorsal muscle was significantly higher in the plant-based protein group compared with the animal derived protein group. Also, the expression level of TOR and IGF1 of fish in the PP group was significantly higher than that in the MP group. Some research on fish and certain mammals has confirmed that the accumulation of protein primarily occurs through the activation of the TOR signaling pathway [36]. Similar results have been observed in blunt snout bream (*Megalobrama amblycephala*) where a diet containing 1% or 3% cottonseed meal protein significantly increased the expression of pituitary growth hormone (GH), GH receptor, and liver IGF1 [37]. Therefore, it can be speculated that some unknown promoters in plant meal activated the TOR signaling pathway and promoted protein accumulation in the muscle of Triploid Crucian Carp. Further studies are warranted for the identification of specific promoters in plant meal that activate the TOR signal.

Some previous studies have confirmed that lysine and methionine are the primary limiting amino acids in rapeseed meal and soybean meal [14,38]. Additionally, a dietary deficiency of these limiting amino acids can result in a reduction of their concentration within fish muscles [39,40]. In the current study, the contents of free lysine and methionine in the dorsal muscle of fish in the AP group was significantly elevated compared with those in the MP and PP groups, indicating that lysine and methionine contents in the fishmeal diet were significantly higher than those in the plant-based diet. This result provides further evidence for the conclusion that fish fed a plant meal diets have poorer growth performance than fish fed an animal meal diet [41]. The presence of free amino acids in the muscular tissues of aquatic animals is considered to be a crucial indicator of fish muscle flavor [42]. For example, the amino acids glycine, alanine, serine, and proline are characterized by a sweet taste, aspartate and glutamic acid both have fresh and sweet flavors, while histidine has a bitter taste [16,43]. Among Triploid Crucian Carp, the muscular free glycine content in the AP group was significantly higher compared with that in the MP and PP groups, while the histidine content in the AP group was significantly lower than in the PP group. The content of other flavored amino acids did not show any statistically significant difference. These findings suggest that the consumption of the animal-based diet enhanced the sweet flavor in fish muscles, while the ingestion of the plant-based diet led to a higher accumulation of the bitter amino acid histidine in the dorsal muscles.

The texture characteristics serve as one of the criteria for assessing the quality of fish flesh, and are primarily influenced by factors such as fish species, diet, aquaculture environment, and feeding strategies [40]. Texture properties mainly include hardness, springiness, gumminess, cohesiveness, adhesiveness, and chewiness [14]. Research has shown that an increase in muscle hardness is closely related to a decrease in crude fat content [44]. Chewiness is determined by factors such as hardness, cohesiveness, and springiness [17]. In the current study, the muscular hardness, gumminess, and chewiness of fish in the PP group were significantly higher than those in the AP and MP groups. The observed variations might be attributed to the relatively lower crude lipid content in the muscles of fish in the PP group compared with both the AP and MP groups. In addition, studies on brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) have demonstrated a positive correlation between muscle hardness and muscle fiber density, while revealing a negative correlation with muscle fiber diameter [45,46]. Therefore, the increase in hardness may also be caused by the thinning of muscle fiber bundles in the PP group of Triploid Crucian Carp, which was caused by its poor growth performance.

The SOD and CAT enzymes play a pivotal role in counteracting excessive reactive oxygen species (ROS) and mitigating the stress response, while MDA serves as a direct indicator of lipid peroxidation levels [47]. Also, LZM plays a vital role in the innate immune defense system, capable of eliminating Gram-positive microbes [48]. In this study, fish fed diets containing plant-based meal (the PP and MP groups) exhibited significantly higher levels of SOD and CAT compared with those fed the animal protein (AP) diet. However, there were no significant differences observed in MDA content and LZM activity among the three groups. Similarly, a study on blunt snout bream showed that with dietary fishmeal replaced by plant protein (soybean meal, rapeseed meal, peanut meal, etc.), the glutathione peroxidase (GPx) and CAT activities in the plasma of fish were significantly increased [49]. Also, glutathione reductase (GR) in the liver and muscle of gilthead sea bream (*Sparus aurata*) was enhanced by plant protein inclusion [50]. The antioxidant activity of plant meal may be attributed to its polyphenolic compounds [51]. In a study conducted on grass carp (*Ctenopharyngodon idella*), soy isoflavones, which are a specific type of polyphenol, significantly enhanced the antioxidant performance and immune response of fish [22].

5. Conclusions

The results of this study showed that the Triploid Crucian Carp achieved the best growth performance in the fishmeal group (AP group), followed by the mixed protein

group and the plant protein group. The expression levels of TOR and IGF1 genes remained significantly elevated in both the fish meal group and the plant protein group. Dietary fishmeal could significantly improve the contents of muscular free lysine, methionine, and glycine, thereby enhancing the flavor of fish muscle. The inclusion of plant-based protein in the diet could improve antioxidant capacity by increasing levels of SOD and CAT enzymes, thereby raising their health performance. In addition, plant protein sources could significantly enhance muscular texture characteristics, including hardness, gumminess, and chewiness, thus enhancing the taste of fish muscles. These data provide a basis for further investigation into the specific nutritional attributes of the protein sources and their potential metabolic impacts on fish. In addition, it may also provide a basis for a more detailed exploration of the intricate relationships between dietary protein sources and the resultant changes in growth performance, muscular characteristics, and molecular markers.

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Institutional Review Board Statement: Animal experiments: All procedures did comply with the ARRIVE guidelines and carried out in accordance with U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. This study was approved by the Committee of Laboratory Animal Management and Animal Welfare of Changsha University (Changsha, China), and all of the experimental produces conformed to the Ethical Committee of Changsha University (ethic code number: 2021085).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cao, S.; Mo, P.; Xiao, Y.; Chen, Y.; Shi, Y.; Hu, Y.; Tang, J.; Qu, F.; Luo, M.; Ai, X.; et al. Dietary supplementation with fermented plant meal enhances growth, antioxidant capacity and expression of TOR signaling pathway genes in gibel carp (*Carassius auratus gibelio* var. CAS V). *Aquac. Rep.* **2021**, *19*, 100559. [CrossRef]
2. Zhang, C.; Rahimnejad, S.; Wang, Y.; Lu, K.; Song, K.; Wang, L.; Mai, K. Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. *Aquaculture* **2018**, *483*, 173–182. [CrossRef]
3. Zhou, J.; Chen, Y.; Ji, H.; Yu, E. The effect of replacing fish meal with fermented meal mixture of silkworm pupae, rapeseed and wheat on growth, body composition and health of mirror carp (*Cyprinus carpio* var. *Specularis*). *Aquac. Nutr.* **2017**, *23*, 741–754. [CrossRef]
4. Mamauag, R.E.P.; Koshio, S.; Ishikawa, M.; Yokoyama, S.; Gao, J.; Nguyen, B.T.; Ragaza, J.A. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **2011**, *321*, 252–258. [CrossRef]
5. Hua, K.; Cobcroft, J.M.; Cole, A.; Condon, K.; Jerry, D.R.; Mangott, A.; Praeger, C.; Vucko, M.J.; Zeng, C.; Zenger, K.; et al. The future of aquatic protein: Implications for protein sources in aquaculture diets. *One Earth* **2019**, *1*, 316–329. [CrossRef]
6. Laining, A.; Rachmansyah; Ahmad, T.; Williams, K. Apparent digestibility of selected feed ingredients for humpback grouper (*Cromileptes altivelis*). *Aquaculture* **2003**, *218*, 529–538. [CrossRef]
7. Li, H.; Mai, K.; Ai, Q.; Zhang, L.; Zhang, C.; Zhang, W.; Liufu, Z. Apparent digestibility of selected protein ingredients for larger yellow croaker (*Pseudosciaena crocea*). *Acta Hydrobiol. Sin.* **2007**, *31*, 370–376.
8. Pervin, M.A.; Jahan, H.; Akter, R.; Omri, A.; Hossain, Z. Appraisal of different levels of soybean meal in diets on growth, digestive enzyme activity, antioxidation, and gut histology of tilapia (*Oreochromis niloticus*). *Fish Physiol. Biochem.* **2020**, *46*, 1397–1407. [CrossRef]

9. Mohammadinafchi, F.; Mohammadiazarm, H.; Yavari, V. Evaluation effect of soybean meal and baker'syeast on resistance to anoxia stress and blood biochemical parameters of fingerlings (*Mesopotamichthys sharpeyi Günther*, 1874). *Int. J. Biosci.* **2014**, *5*, 215–222.
10. Yu, D.; Gong, S.; Yuan, Y.; Lin, Y. Effects of replacing fish meal with soybean meal on growth, body composition and digestive enzyme activities of juvenile Chinese sucker (*Myxocyprinus asiaticus*). *Aquac. Nutr.* **2013**, *19*, 84–90. [CrossRef]
11. Luo, Y.; Ai, Q.; Mai, K.; Zhang, W.; Xu, W.; Zhang, Y. Effects of dietary rapeseed meal on growth performance, digestion and protein metabolism in relation to gene expression of juvenile cobia (*Rachycentron canadum*). *Aquaculture* **2012**, *368–369*, 109–116. [CrossRef]
12. Cheng, Z.; Ai, Q.; Mai, K.; Xu, W.; Ma, H.; Li, Y.; Zhang, J. Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass (*Lateolabrax japonicus*). *Aquaculture* **2010**, *305*, 102–108. [CrossRef]
13. Lee, S.M.; Azarm, H.M.; Chang, K.H. Effects of dietary inclusion of fermented soybean meal on growth, body composition, antioxidant enzyme activity and disease resistance of rockfish (*Sebastes schlegeli*). *Aquaculture* **2016**, *459*, 110–116. [CrossRef]
14. Hu, Y.; Hu, Y.; Wu, T.; Chu, W. Effects of high dietary levels of cottonseed meal and rapeseed meal on growth performance, muscle texture, and expression of muscle-related genes in grass carp. *N. Am. J. Aquac.* **2019**, *81*, 235–241. [CrossRef]
15. Xu, J.; Feng, L.; Jiang, W.; Wu, P.; Liu, Y.; Jiang, J.; Kuang, S.; Tang, L.; Zhou, X. Different dietary protein levels affect flesh quality, fatty acids and alter gene expression of Nrf2-mediated antioxidant enzymes in the muscle of grass carp (*Ctenopharyngodon idella*). *Aquaculture* **2018**, *493*, 272–282. [CrossRef]
16. Ma, X.; Feng, L.; Wu, P.; Liu, Y.; Kuang, S.; Tang, L.; Zhou, X.; Jiang, W. Enhancement of flavor and healthcare substances, mouthfeel parameters and collagen synthesis in the muscle of on-growing grass carp (*Ctenopharyngodon idella*) fed with graded levels of glutamine. *Aquaculture* **2020**, *528*, 735486. [CrossRef]
17. Peng, K.; Fu, B.; Li, J.; Zhao, H.; Cao, J.; Huang, W.; Chen, B.; Li, X.; Peng, Z.; Wei, M. Effects of replacing soybean meal and rapeseed meal with faba bean meal on growth performance and muscle quality of tilapia (*Oreochromis niloticus*). *Aquac. Rep.* **2022**, *26*, 101328. [CrossRef]
18. Grabež, V.; Egelandsdal, B.; Kjos, N.P.; Håkenåsen, I.M.; Mydland, L.T.; Vik, J.O.; Hallenstvedt, E.; Devle, H.; Øverland, M. Replacing soybean meal with rapeseed meal and faba beans in a growing-finishing pig diet: Effect on growth performance, meat quality and metabolite changes. *Meat Sci.* **2020**, *166*, 108134. [CrossRef]
19. Mao, Z.; Luo, S.; Zhao, D.; Zhou, X.; Zhang, Z.; Xiao, Y.; Cao, S.; Zhou, Y.; Liu, S.; Tang, J.; et al. Molecular characterization and nutrition regulation of the neutral amino acid transporter ASCT2 in Triploid Crucian Carp. *Fishes* **2021**, *6*, 77. [CrossRef]
20. Cai, L.; Ao, Z.; Tang, T.; Tong, F.; Wei, Z.; Yang, F.; Shu, Y.; Liu, S.; Mai, K. Corrigendum to "Characterization of difference in muscle volatile compounds between triploid and diploid crucian carp" [Aquacult. Rep. 20 (July) (2021) 100641]. *Aquac. Rep.* **2021**, *20*, 100689. [CrossRef]
21. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemist*, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2005.
22. Cao, S.; Xiong, D.; Luo, W.; Tang, J.; Qu, F.; Zhou, Y.; He, Z.; Xie, S.; Liu, Z. Effects of dietary soy isoflavones on growth, antioxidant status, immune response and resistance of juvenile grass carp (*Ctenopharyngodon idella*) to aeromonas hydrophila challenge. *Aquac. Res.* **2020**, *51*, 2472–2482. [CrossRef]
23. Qu, F.; Tang, J.; Hu, R.; Hao, G.; Zhou, Y.; Lu, S.; Zhao, Q.; You, C.; Li, Y.; Zhou, Z.; et al. Molecular characterization and nutritional regulation of specificity protein 1 (Sp1) in grass carp (*Ctenopharyngodon idella*). *Aquac. Nutr.* **2018**, *24*, 940–951. [CrossRef]
24. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-DDCt} method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
25. Ngo, D.T.; Wade, M.N.; Pirozzi, I.; Glencross, B.D. Effects of canola meal on growth, feed utilisation, plasma biochemistry, histology of digestive organs and hepatic gene expression of barramundi (Asian seabass; *Lates calcarifer*). *Aquaculture* **2016**, *464*, 95–105. [CrossRef]
26. Zhou, Q.L.; Habte-Tsion, H.M.; Ge, X.; Xie, J.; Ren, M.; Liu, B.; Miao, L.; Pan, L. Graded replacing fishmeal with canola meal in diets affects growth and target of rapamycin pathway gene expression of juvenile blunt snout bream (*Megalobrama amblycephala*). *Aquac. Nutr.* **2018**, *24*, 300–309. [CrossRef]
27. Lovell, R.T. *Nutrition and Feeding of Fish*; Van Nostrand Reinhold: New York, NY, USA, 1989; 268p.
28. Luo, Z.; Liu, Y.; Mai, K.; Tian, L.; Tan, X.; Yang, H.; Liang, G.; Liu, D. Quantitative L-lysine requirement of juvenile grouper *Epinephelus coioides*. *Aquac. Nutr.* **2006**, *12*, 165–172. [CrossRef]
29. Feng, L.; Feng, L.; Jiang, W.; Liu, Y.; Zhang, L.; Kuang, S.; Ren, H.; Jin, X.; Li, S.; Mi, H.; et al. The beneficial effects of exogenous protease K originated from *Parengyodontium album* on growth performance of grass carp (*Ctenopharyngodon idella*) in relation to the enhanced intestinal digestion and absorption capacities. *Aquaculture* **2023**, *563*, 738929. [CrossRef]
30. Mir, I.N.; Srivastava, P.P.; Bhat, I.A.; Muralidhar, A.P.; Varghese, T.; Gireesh-Babu, P.; Jain, K.K. Expression and activity of trypsin and pepsin during larval development of Indian walking catfish (*Clarias magur*). *Aquaculture* **2018**, *491*, 266–272. [CrossRef]
31. Maulu, S.; Hualiang, L.; Ke, J.; Ren, M.; Ge, X.; Huang, D.; Yu, H. Dietary clostridium autoethanogenum protein modulates intestinal absorption, antioxidant status, and immune response in GIFT (*Oreochromis niloticus*) juveniles. *Aquac. Res.* **2021**, *52*, 5787–5799. [CrossRef]

32. Liu, X.; Han, B.; Xu, J.; Zhu, J.; Hu, J.; Wan, W.; Miao, S. Replacement of fishmeal with soybean meal affects the growth performance, digestive enzymes, intestinal microbiota and immunity of Carassius auratus gibelio♀× Cyprinus carpio♂. *Aquac. Rep.* **2020**, *18*, 100472. [CrossRef]
33. Ye, H.; Xu, M.; Liu, Q.; Sun, Z.; Zou, C.; Chen, L.; Su, N.; Ye, C. Effects of replacing fish meal with soybean meal on growth performance, feed utilization and physiological status of juvenile obscure puffer (*Takifugu obscurus*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2019**, *216*, 75–81. [CrossRef] [PubMed]
34. El-Sheekh, M.M.; Hamad, S.M.; Gomaa, M. Protective effects of spirulina on the liver function and hyperlipidemia of rats and human. *Braz. Arch. Biol. Technol.* **2014**, *57*, 77–86. [CrossRef]
35. Saito, M.; Saito, K.; Kunisaki, N.; Kimura, S. Green tea polyphenols inhibit metalloproteinase activities in the skin, muscle, and blood of rainbow trout. *J. Agric. Food Chem.* **2002**, *50*, 7169–7174. [CrossRef] [PubMed]
36. Seiliez, I.; Panserat, S.; Skiba-Cassy, S.; Fricot, A.; Vachot, C.; Kaushik, S.; Tesseraud, S. Feeding status regulates the polyubiquitination step of the ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*) muscle. *J. Nutr.* **2008**, *138*, 487–491. [CrossRef] [PubMed]
37. Yuan, X.; Jiang, G.; Cheng, H.; Cao, X.; Wang, C.; Dai, Y.; Liu, W. Replacing fish meal with cottonseed meal protein hydrolysate affects growth, intestinal function, and growth hormone/insulin-like growth factor I axis of juvenile blunt snout bream (*Megalobrama amblycephala*). *J. World Aquac. Soc.* **2020**, *51*, 1235–1249. [CrossRef]
38. Savolainen, L.; Gatlin, D.M., III. Evaluation of sulfur amino acid and zinc supplements to soybean-meal-based diets for hybrid striped bass. *Aquaculture* **2010**, *307*, 260–265. [CrossRef]
39. Mai, K.; Wan, J.; Ai, Q.; Xu, W.; Liufu, Z.; Zhang, L.; Zhang, C.; Li, H. Dietary methionine requirement of large yellow croaker (*Pseudosciaena crocea* R). *Aquaculture* **2006**, *253*, 564–572. [CrossRef]
40. Wu, F.; Tian, J.; Yu, L.; Wen, H.; Jiang, M.; Lu, X. Effects of dietary rapeseed meal levels on growth performance, biochemical indices and flesh quality of juvenile genetically improved farmed tilapia. *Aquac. Rep.* **2021**, *20*, 100679. [CrossRef]
41. Liu, T.; Han, T.; Wang, J.; Liu, T.; Bian, P.; Wang, Y.; Cai, X. Effects of replacing fish meal with soybean meal on growth performance, feed utilization and physiological status of juvenile redlip mullet *Liza haematocheila*. *Aquac. Rep.* **2021**, *20*, 100756. [CrossRef]
42. Yang, B.; Jiang, W.; Wu, P.; Liu, Y.; Zeng, Y.; Jiang, J.; Kuang, S.; Tang, L.; Tang, W.; Wang, S.; et al. Soybean isoflavones improve the health benefits, flavour quality indicators and physical properties of grass carp (*Ctenopharygodon idella*). *PLoS ONE* **2019**, *14*, e0209570. [CrossRef]
43. Lin, H.; Fang, J.; Bi, X.; Yu, X.; He, Q.; Liu, P.; Ding, W.; Che, Z. Optimization of the extraction technique of free amino acids from Pixian board-bean paste by response surface methodology and analysis of their taste characteristics. *Sci. Technol. Food Ind.* **2019**, *40*, 56–63.
44. Ginés, R.; Valdimarsdottir, T.; Sveinsdottir, K.; Thorarensen, H. Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). *Food Qual. Prefer.* **2004**, *15*, 177–185. [CrossRef]
45. Bugeon, J.; Lefevre, F.; Fauconneau, B. Fillet texture and muscle structure in brown trout (*Salmo trutta*) subjected to long-term exercise. *Aquac. Res.* **2003**, *34*, 1287–1295. [CrossRef]
46. Johnston, I.; Li, X.; Vieira, V.; Nickell, D.; Dingwall, A.; Alderson, R.; Campbell, P.; Bickerdike, R. Muscle and flesh quality traits in wild and farmed Atlantic salmon. *Aquaculture* **2006**, *256*, 323–336. [CrossRef]
47. Nordberg, J.; Arnér, E.S. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.* **2001**, *31*, 1287–1312. [CrossRef] [PubMed]
48. Saurabh, S.; Sahoo, P.K. Lysozyme: An important defence molecule of fish innate immune system. *Aquac. Res.* **2008**, *39*, 223–239. [CrossRef]
49. Mokrani, A.; Ren, M.; Liang, H.; Yang, Q.; Ji, K.; Kasiya, H.; Ge, X. Effect of the total replacement of fishmeal with plant proteins and supplemental essential amino acids in the extruded diet on antioxidants genes, enzyme activities, and immune response in juvenile blunt snout bream. *Aquac. Int.* **2019**, *28*, 555–568. [CrossRef]
50. Sitjà-Bobadilla, A.; Peña-Llopis, S.; Gómez-Requeni, P.; Médale, F.; Kaushik, S.; Pérez-Sánchez, J. Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). *Aquaculture* **2005**, *249*, 387–400. [CrossRef]
51. Rajan, V.K.; Muraleedharan, K. A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol (Gallic acid). *Food Chem.* **2017**, *220*, 93–99. [CrossRef]