fishes

MDPI

Article

The Effects of Dietary Fermented Soybean Residue on the Growth, Antioxidant Capacity, Digestive Enzyme Activities, and Microbial Compositions of the Intestine in Furong Crucian Carp (Furong Carp♀× Red Crucian Carp♂)

Hongquan Wang, Zheming Zhang, Feilong Li, Liang Hu, Tiaoyi Xiao, Yurong Zhao * and Mengxi Yang *

Hunan Engineering Technology Research Center of Featured Aquatic Resources Utilization, Fisheries College, Hunan Agricultural University, Changsha 410128, China; whq@hunau.net (H.W.); 695789515@stu.hunau.edu.cn (Z.Z.); 13431629695@163.com (F.L.); hl@stu.hunau.edu.cn (L.H.); tiaoyixiao@hunau.edu.cn (T.X.)

* Correspondence: zyr@hunau.edu.cn (Y.Z.); ymx@hunau.edu.cn (M.Y.)

Abstract: An 8-week feeding experiment aimed to investigate the effects of fermented soybean residue in diets on the growth performance, serum antioxidant parameters, intestinal digestive capacity, and microbial composition of the hindgut of Furong crucian carp. The feeds were formulated to contain 0%, 6%, 12%, 18%, and 24% fermented soybean residue (CON, FSR6, FSR12, FSR18, and FSR24, respectively), respectively, to form five diets (crude protein: 33%; crude lipid: 5%). The results showed that incorporating 6% fermented soybean residue into the diet significantly increased the weight gain rate (WGR) and specific growth rate (SGR) and decreased the feed coefficient rate ($p < 0.05$). Through regression analysis of WGR and SGR, the optimal levels of the dietary fermented soybean residue of Furong crucian carp were determined to be 6.78% and 7.06%. Incorporating fermented soybean residue into the diet decreased the lipid content of the whole body and the levels of glucose, total cholesterol, and triglyceride in the serum of Furong crucian carp. The inclusion of 6% and 12% fermented soybean residue in the diet markedly increased the antioxidant capacity, intestinal amylase activity, and intestinal villous height of Furong crucian carp ($p < 0.05$). At the 6% level, fermented soybean residue significantly increased the abundance of *Romboutsia* and *Clostridium_sensu_stricto_1* while significantly decreasing the abundance of *Vibrio* ($p < 0.05$), indicating that a 6% level of fermented soybean residue was beneficial for intestinal health. In conclusion, incorporating 6–7% fermented soybean residue into the diet of Furong crucian carp was recommended.

Keywords: Furong crucian carp; fermented soybean residue; growth performance; antioxidant capacity; intestinal microbiota

Key Contribution: An 8-week feeding experiment aimed to investigate the effects of fermented soybean residue in diets on the growth performance, serum antioxidant parameters, intestinal digestive capacity, and microbial composition in the hindgut of Furong crucian carp.

check for updates

Citation: Wang, H.; Zhang, Z.; Li, F.; Hu, L.; Xiao, T.; Zhao, Y.; Yang, M. The Effects of Dietary Fermented Soybean Residue on the Growth, Antioxidant Capacity, Digestive Enzyme Activities, and Microbial Compositions of the Intestine in Furong Crucian Carp (Furong Carp♀× Red Crucian Carp♂). *Fishes* **2024**, *9*, 138. https://doi.org/10.3390/fishes9040138

Academic Editor: Francisco Javier Alarcón López

Received: 29 February 2024
Revised: 8 April 2024
Accepted: 10 April 2024
Published: 16 April 2024

1. Introduction

The exploration of plant-based ingredients as a protein source continues to garner significant attention within the aquafeed industry. Soybean residue is the byproduct obtained during the production of soy-based products like soy milk and soybean curd. Soybean residue is nutritionally valuable, containing abundant bioactive compounds such as saponins, phytosterols, and isoflavones [1,2]. However, unprocessed wet soybean residue is susceptible to microbial contamination [3]. The lipoxidase in soybean residue can oxidize unsaturated fatty acids, resulting in the development of a beany odor [4].

Furthermore, soybean residue contains antinutritional factors, which can impact digestion and absorption in animals [1]. Consequently, a considerable portion of generated soybean residue is discarded as agricultural waste [5].

Soybean residue fermentation involves processes such as grinding, steam sterilization, and microbial inoculation, followed by a designated fermentation period [6]. The fermentation of soybean residue can effectively enhance its nutritional composition and palatability while reducing levels of anti-nutritional factors [7–9]. The utilization of fermented soybean residue as a feed resource for aquatic animals is an economical and environmentally friendly choice. In largemouth bass (*Micropterus salmoides*), substituting 40 g/kg of soybean meal with fermented soybean residue significantly increased the specific growth rate (SGR) of fish. As the level of fermented soybean residue increased, largemouth bass exhibited a significant decrease in their hepatosomatic index (HSI), viscera ratio, and liver lipid content [10]. In African catfish (*Clarias gariepinus*), similar results were observed, with the replacement of 50% of fish meal using fermented soybean residue showing the highest specific growth rate [11]. Zulhisyam et al. [12] reported that fermented soybean residue altered the probiotic profile in the intestine of African catfish.

Furong crucian carp is obtained through the hybridization of Furong carp (*Cyprinus carpio* Furong) as the maternal parent and red crucian carp (*Carassius auratus* red var.) as the paternal parent, and it is extensively cultured in China. Furong crucian carp offers the advantages of rapid growth, excellent flesh quality, and strong stress resistance [13]. So far, there is no existing research on the utilization of fermented soybean residue in Furong crucian carp. In the present study, various proportions of fermented soybean residue were employed as substitutes for rapeseed meal and rice bran in their feed. Despite the nutritional similarity between rapeseed meal and soybean meal, the practical application of rapeseed meal is constrained due to its elevated levels of harmful substances and numerous anti-nutritional factors such as glucosinolates, sinapine and its derivatives, tannin, etc. [14]. Moreover, the price of rice bran has also been consistently rising. Therefore, the substitution of rapeseed meal and rice bran with fermented soybean residue proves advantageous in reducing aquaculture costs and enhancing economic benefits.

2. Materials and Methods

2.1. Ethical Statement

This study obtained approval from the Ethics Committee of Hunan Agricultural University, Changsha, China. The study's procedures were meticulously conducted in strict adherence to applicable laws and guidelines.

2.2. Experimental Design

The fermented soybean residue was supplied by Hunan Junhui International Agriculture and Animal Husbandry Co., Ltd. (Changsha, China). The fermented soybean residue was generated through a 72-h fermentation process with lactic acid bacteria. The nutrient contents measured after drying were 20.31% crude protein, 11.12% crude fat, 21.14% crude fiber, and 4.5% ash. The feeds were formulated to contain 0%, 6%, 12%, 18%, and 24% fermented soybean residue (CON, FSR6, FSR12, FSR18, and FSR24, respectively), respectively, to form five isonitrogenous (crude protein: 33%) and isolipidic (crude lipid: 5%) diets. Following complete grinding, the raw materials were sieved through a screen with a 0.425 mm aperture, weighed, blended, pelletized, dried, and then stored at 4 °C until required. The formula and composition of the diets are displayed in Table 1.

Table 1. Ingredients and proximate composition of experimental diets (% dry matter).

Ingredients	CON	FSR6	FSR12	FSR18	FSR24
Fish meal [a]	6.00	6.00	6.00	6.00	6.00
Soybean meal [b]	26.00	26.00	26.00	26.00	26.00
Rapeseed meal [c]	22.00	20.15	18.27	16.40	14.50
Meat meal	4.00	4.00	4.00	4.00	4.00
Rice bran	8.00	6.00	4.00	2.00	0
Flour	18.00	18.00	18.00	18.00	18.00
Soybean oil	0.80	0.65	0.53	0.40	0.30
Fermented soybean residue [d]	0	6.00	12.00	18.00	24.00
Wheat bran	10.00	8.00	6.00	4.00	2.00
$Ca(H_2PO_4)_2$	1.30	1.40	1.60	1.80	1.90
Bentonite	1.90	1.80	1.60	1.40	1.30
Premix [e]	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition					
Moisture	9.88	10.04	10.15	10.23	10.21
Crude protein	33.86	33.49	34.11	33.56	32.70
Crude fat	5.10	5.08	5.05	5.05	5.02
Ash	10.30	9.96	9.59	9.69	9.83
Gross energy (KJ/g)	14.43	14.46	14.50	14.47	14.44

Notes: [a] Fish meal: 52% crude protein; [b] soybean meal: 44.2% crude protein; [c] rapeseed meal: 36% crude protein; and [d] fermented soybean residue: 20.31% crude protein. [e] The premix provided the following per kg of diet: VA: 2000 IU; VD_3: 400 IU; VE: 100 IU; VK_3: 10 mg; VB_1: 5 mg; VB_2: 10 mg; calcium pantothenate: 40 mg; nicotinic acid: 100 mg; VB_6: 10 mg; biotin: 1 mg; folic acid: 5 mg; VB_{12}: 0.02 mg; VC: 300 mg; inositol: 200 mg; antioxidant: 1000 mg; betaine: 1500 mg; NaCl: 3000 mg; Cu (as copper sulfate): 6 mg; Fe (ferrous sulfate): 160 mg; Mn (manganese sulfate): 33 mg; Zn (zinc sulfate): 120 mg; I (potassium iodide): 0.3 mg; Se (sodium selenite): 0.2 mg; and Co (cobalt sulfate): 5 mg.

2.3. Fish and Experimental Conditions

The experiment was carried out at the aquaculture facility located at Hunan Agricultural University. The Furong crucian carp used for the experiment were obtained from the Xiangyin Fisheries Science Institute, Yueyang, Hunan, China. A total of 750 healthy fish (25.00 ± 2.08 g) were randomly allocated into twenty-five cages (1.5 m^3) suspended in a cement pool. The experiment comprised five treatments, each with five replicates (cages) per treatment. All fish were fed CON for 14 days, following which each group was fed diets of CON, FSR6, FSR12, FSR18, and FSR24, respectively. The feeding trial lasted for 56 days. The fish were fed to apparent satiation twice (8:30 and 16:30) daily. Throughout this duration, the water temperature was 27 ± 5 °C. The pH, dissolved oxygen, nitrite, and NH4-N were 7.7 ± 0.2, 7.5 ± 1.5 mg/L, <0.005 mg/L, and <0.2 mg/L, respectively.

2.4. Collecting Samples

At the trial's conclusion, a 24-h fasting period was observed for the fish in each cage before individual counting and weighing. Nine fish per cage were randomly picked and anesthetized using eugenol (Macklin Biochemical Technology Co., Ltd., Shanghai, China). Following the measurement of their body length and weight, four fish per cage (20 fish per treatment) were chosen for a whole-fish proximate analysis. For the remaining five fish per cage (25 fish per treatment), after collecting blood samples, the serum was separated. Following dissection, the viscera and liver were isolated and weighed. The hindgut contents of each fish were collected to identify their intestinal microbiota composition. The foregut was collected to identify intestinal digestive enzymes, and the midgut was prepared for intestinal slices.

2.5. Analyzing the Composition of Diets and Fish

The crude protein, crude lipid, and moisture of the experimental diets and whole fish were analyzed using standard methods [15].

2.6. Serum Biochemical and Antioxidant Parameters

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and the levels of albumin (ALB), total protein (TP), globulin (GLB), total cholesterol (TC), glucose (GLU), and triglyceride (TG) in serum were measured using a BS450 automatic biochemical analyzer (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

The total antioxidant capacity (T-AOC) (A015-1-2), the total superoxide dismutase activity (T-SOD) (A001-1-1), and the content of malondialdehyde (MDA) (A003-1-1) in serum were analyzed using detection kits purchased from Nanjing Jiancheng (Nanjing, China).

2.7. Intestinal Digestive Enzymes

The activities of amylase (C016-1-1), trypsin (A080-2-2), and lipase (A054-1-1) were measured using detection kits obtained from Nanjing Jiancheng. For the determination of amylase activity, iodine–starch colorimetry was utilized [16]. Tissue samples were accurately weighed and mixed with nine times their volume of saline solution. The mixture was homogenized in an ice bath, followed by centrifugation at 2500 rpm for 10 min to collect the supernatant. In a test tube, 100 μL of the supernatant was combined with 500 μL of 0.4 mg/mL of soluble starch in a 20 mM phosphate buffer. After incubation at 37 °C for 7.5 min, the reaction was halted by adding 500 μL of a 0.01 M iodine solution, followed by the addition of 3000 μL of distilled water. In blank samples, 100 μL of distilled water was added after the iodine solution. The absorbance of the reaction mixture was measured at 660 nm. One unit of amylase activity was defined as the amount of amylase in 100 mL of supernatant that hydrolyses 10 mg of the substrate (starch) in 30 min at 37 °C.

For the determination of trypsin activity, the method described by Gong et al. [17] was used. The preparation method of the sample supernatant was the same as that of amylase. The reaction was initiated by mixing a 3 mL substrate solution (67 mM sodium phosphate buffer with 0.25 mM N-α-Benzoyl-L-Arginine ethyl ester) and 0.2 mL of supernatant in quartz cuvettes (1 cm light path). The increase in the A253 nm value was recorded for 5 min. One unit (U) of enzyme activity was defined as a ΔA253 nm of 0.001 per min at a pH of 7.6 and 25 °C.

Lipase activity was evaluated using a modified method described by Lessinger et al. [18]. Tissue samples were accurately weighed and mixed with four times their volume of saline solution. The mixture was homogenized in an ice bath and then centrifuged at 2500 rpm for 10 min to collect the supernatant. An oil emulsion was prepared containing 35 mM of Na-deoxycholate, 8 mL of olive oil, 100 mM of $CaCl_2$, and ultrapure water to a final volume of 250 mL. This emulsion served as the substrate for lipase hydrolysis, leading to a reduction in turbidity. The supernatant was thoroughly mixed with the oil emulsion. Absorbance measurements were taken before and after incubating the mixture in a 37 °C water bath for 10 min. Lipase activity was expressed as units per gram of protein (U/g protein), with one unit defined as the consumption of 1 μmol of substrate per minute at 37 °C.

2.8. Intestinal Histomorphology

The intestinal tissues underwent dehydration using a gradient of ethanol concentrations that increased progressively, followed by equilibration in xylene and embedding in paraffin (PPDT-12C1, Ceike, Shanghai, China). Sections were cut to 4 μm using a paraffin microtome (RM2235, Leica Camera AG, Stuttgart, Germany), stained with hematoxylin and eosin, mounted with neutral resin, and examined under a light microscope (E600, Nikon Corporation, Tokyo, Japan). Villous height (Vh), muscularis thickness (Mt), and goblet cell (GC) numbers were recorded using image analysis software (version 1.7) (Olympus CellSens Standard, Tokyo, Japan).

2.9. The Intestinal Microbial Composition

DNA was extracted from the hindgut contents, and its purity and quality were subsequently assessed. PCR reactions were conducted to amplify the V3–V4 hypervariable

region of the bacterial 16S rRNA gene. The PCR products were submitted to Beijing Ovisen Genetech Co., Ltd. (Beijing, China), for paired-end sequencing using the Illumina-MiSeq PE300 high-throughput sequencing platform. Sequences were clustered into Operational Taxonomic Units (OTUs) with a 97% sequence similarity threshold. Venn diagrams were generated based on the differential OTUs in each group. The Mothur software (Version 1.31.2) was employed for an alpha diversity analysis. Statistical comparisons of the taxa abundance at the phylum and genus levels among groups were conducted using the Metastats Program.

2.10. Statistical Analysis

Data were expressed as means ± standard errors. Statistical analysis was conducted using the SPSS 22.0 software. Statistical comparisons between diets were carried out using one-way ANOVA and Tukey's multiple range tests. Significance was considered at $p < 0.05$.

3. Results

3.1. Growth Performance and Physical Parameters

As shown in Table 2, incorporating fermented soybean residue into the diet significantly affected the final body weight (FBW), weight gain rate (WGR), SGR, feed coefficient rate (FCR), and feed intake (FI) of Furong crucian carp ($p < 0.05$) without exerting a notable influence on their survival rate (SR) ($p > 0.05$). As the amount of dietary fermented soybean residue increased, FBW, WGR, and SGR initially increased and then decreased. Compared to the CON, the FBW, WGR, and SGR in the FSR6 showed increases, reaching their maximum, while exhibiting decreases in the FSR18 and FSR24 groups. FCR and FI showed an initial decrease, followed by a gradual increase with rising levels of fermented soybean residue. In comparison to the CON, the FSR6 group exhibited a notable reduction in FCR ($p < 0.05$), while the FSR18 and FSR24 groups showed increases. No significant variations were noted among the diets regarding the condition factor (CF) and HSI ($p > 0.05$). In comparison to the CON, the FSR12, FSR18, and FSR24 groups exhibited a notable reduction in the viscerosomatic index (VSI) ($p < 0.05$).

Table 2. Effects of dietary fermented soybean residue on growth performance and physical parameters of Furong crucian carp.

Parameters	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
IBW (g)	24.99 ± 0.01	24.99 ± 0.01	25.04 ± 0.04	24.99 ± 0.01	25.01 ± 0.02	0.333
FBW (g)	104.35 ± 1.58 ^{ab}	108.34 ± 1.36 ^{a}	105.23 ± 1.18 ^{ab}	100.42 ± 1.38 ^{b}	91.79 ± 1.23 ^{c}	<0.001
WGR (%)	317.52 ± 6.28 ^{ab}	333.49 ± 5.51 ^{a}	320.16 ± 5.17 ^{ab}	301.88 ± 5.50 ^{b}	267.07 ± 4.75 ^{c}	<0.001
SGR (%/day)	2.51 ± 0.03 ^{ab}	2.57 ± 0.02 ^{a}	2.52 ± 0.02 ^{ab}	2.44 ± 0.02 ^{b}	2.28 ± 0.02 ^{c}	<0.001
FCR (%)	1.62 ± 0.03 ^{ab}	1.54 ± 0.03 ^{c}	1.60 ± 0.02 ^{ab}	1.74 ± 0.05 ^{b}	1.93 ± 0.04 ^{a}	<0.001
FI (g/fish/d)	1.06 ± 0.01 ^{bc}	1.03 ± 0.01 ^{c}	1.06 ± 0.01 ^{bc}	1.10 ± 0.01 ^{b}	1.18 ± 0.01 ^{a}	<0.001
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	98.66 ± 0.43	100 ± 0.00	0.062
CF (g/cm^3)	2.85±0.04	2.84±0.05	2.76±0.05	2.78±0.06	2.85±0.07	0.631
VSI (%)	11.12 ± 0.36 ^{a}	10.36 ± 0.21 ^{ab}	10.04 ± 0.34 ^{b}	10.02 ± 0.19 ^{b}	9.98 ± 0.21 ^{b}	0.018
HSI (%)	2.24 ± 0.15	1.95 ± 0.13	2.12 ± 0.16	1.92 ± 0.08	1.81 ± 0.14	0.186

Note: Values are means ± SEMs. Values in the same row with distinct letters indicate significant statistical differences ($p < 0.05$). IBW: initial body weight; FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FCR: feed coefficient rate; FI: feed intake; SR: survival rate; CF: condition factor; VSI: viscerosomatic index; and HSI: hepatosomatic index.

Quadratic curve fitting was performed separately for WGR and SGR based on the levels of dietary fermented soybean residue, and regression equations were established (Figure 1). The optimal levels of dietary fermented soybean residue were determined to be 6.78% and 7.06%, corresponding to the highest WGR and SGR, respectively.

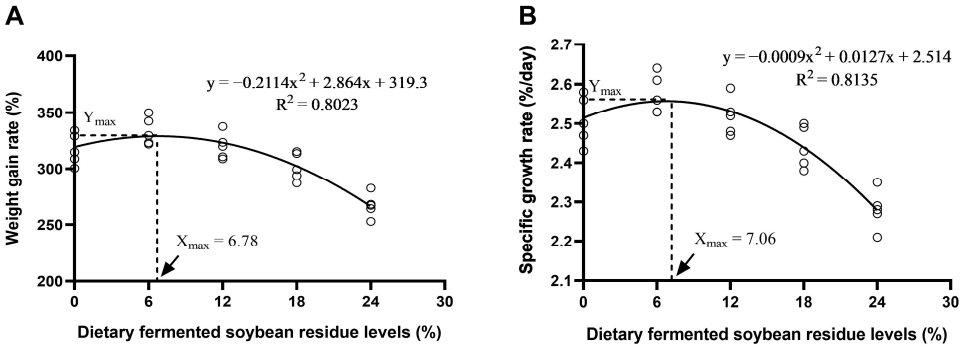

Figure 1. The optimum dietary fermented soybean residue level based on the multiple regression analysis. (**A**) the optimal dietary fermented soybean residue level based on the multiple regression analysis of weight gain rate. (**B**) the optimal dietary fermented soybean residue level based on the multiple regression analysis of specific growth rate. Hollow circles represent different replicates (cages) for each treatment group.

3.2. Whole Body Composition

As shown in Table 3, no notable distinctions were identified in crude protein and moisture among the groups ($p > 0.05$). However, the inclusion of fermented soybean residue led to a reduction in the crude lipid content, in contrast to the CON.

Table 3. Effects of dietary fermented soybean residue on the body composition of Furong crucian carp (% wet weight).

Parameters	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
Moisture	73.58 ± 0.23	74.87 ± 0.32	74.47 ± 0.29	74.78 ± 0.51	74.05 ± 1.14	0.543
Crude protein	16.91 ± 0.05	17.00 ± 0.29	16.88 ± 0.23	16.95 ± 0.12	17.78 ± 0.50	0.167
Crude lipid	6.83 ± 0.20 ^{a}	5.65 ± 0.27 ^{b}	5.74 ± 0.21 ^{ab}	4.83 ± 0.25 ^{b}	5.02 ± 0.34 ^{b}	<0.001

Note: Values are means ± SEMs. Values in the same row with distinct letters indicate significant statistical differences ($p < 0.05$).

3.3. Serum Biochemical Parameters

Table 4 indicates that the addition of fermented soybean residue significantly influenced the levels of TC, GLU, and TG in serum ($p < 0.05$) without exerting a notable influence on AST, ALT, TP, ALB, or GLB ($p > 0.05$). As the dietary fermented soybean residue levels increased, the GLU levels showed a decreasing trend, significantly decreasing in the FSR24 group, in contrast to the CON ($p < 0.05$). Compared to the CON group, TC and TG levels initially declined and subsequently rose with the increasing levels of fermented soybean residue. Compared to the CON group, the FSR18 group exhibited a noteworthy reduction in TC levels ($p < 0.05$). In contrast to the CON group, the FSR18 group exhibited a significant reduction in TG levels ($p < 0.05$).

Table 4. Effects of dietary fermented soybean residue levels on serum biochemical parameters of Furong crucian carp.

Parameters	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
AST (U/L)	230.14 ± 65.45	214.11 ± 35.92	148.53 ± 33.08	173.99 ± 29.71	150.26 ± 21.91	0.504
ALT (U/L)	31.44 ± 2.99	27.84 ± 3.31	26.96 ± 2.64	26.30 ± 4.47	23.13 ± 3.16	0.547
TP (g/L)	27.88 ± 0.10	27.95 ± 0.73	27.01 ± 0.65	25.37 ± 1.31	27.27 ± 1.15	0.283
ALB (g/L)	13.61 ± 0.47	13.08 ± 0.79	12.35 ± 0.44	11.86 ± 0.51	12.45 ± 0.69	0.300
GLB (g/L)	14.20 ± 0.49	14.60 ± 0.75	14.40 ± 0.24	13.60 ± 0.98	15.00 ± 0.63	0.665

Table 4. *Cont.*

Parameters	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
GLU (mg/dL)	5.63 ± 0.22 ^{a}	4.99 ± 0.41 ^{ab}	4.88 ± 0.45 ^{ab}	4.90 ± 0.37 ^{ab}	3.80 ± 0.44 ^{b}	0.041
TC (mmol/L)	4.34 ± 0.10 ^{a}	4.10 ± 0.16 ^{ab}	4.09 ± 0.13 ^{ab}	3.51 ± 0.27 ^{b}	4.12 ± 0.21 ^{ab}	0.049
TG (mmol/L)	3.35 ± 0.20 ^{a}	2.89 ± 0.22 ^{ab}	2.80 ± 0.04 ^{ab}	2.43 ± 0.15 ^{b}	3.27 ± 0.21 ^{a}	0.008

Note: Values are means ± SEMs. Values in the same row with distinct letters indicate significant statistical differences ($p < 0.05$). AST: aspartate aminotransferase; ALT: alanine aminotransferase; TP: total protein; ALB: albumin; GLB: globulin; GLU: glucose; TC: total cholesterol; and TG: triglyceride.

3.4. Serum Antioxidant Parameters

As depicted in Figure 2, incorporating fermented soybean residue into the diet significantly affected the serum T-AOC and MDA levels in Furong crucian carp ($p < 0.05$) with no significant impact on T-SOD ($p > 0.05$). In contrast to the CON group, a notable rise in serum T-AOC was observed in the FSR6 group ($p < 0.05$), while the FSR12 and FSR18 groups showed no significant alterations ($p > 0.05$). The MDA levels in the FSR6, FSR12, and FSR18 groups exhibited no statistically significant alterations when compared to the CON group ($p > 0.05$), whereas the MDA content in the FSR24 group markedly increased ($p < 0.05$).

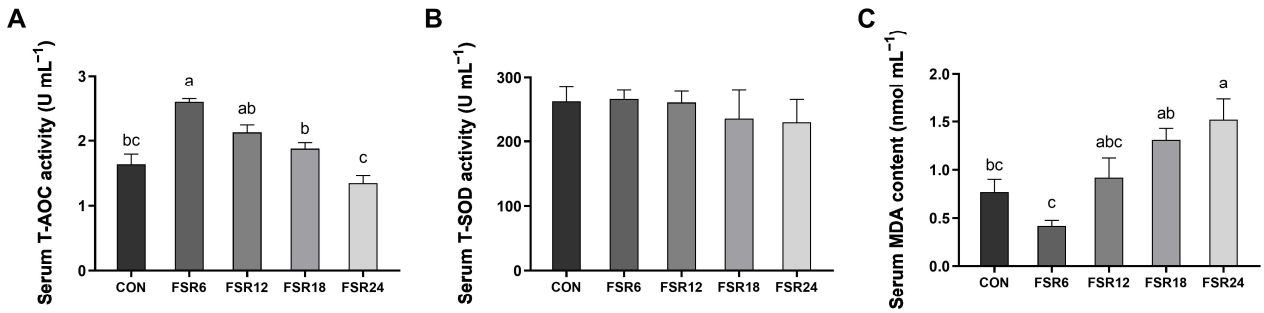

Figure 2. Effects of dietary fermented soybean residue on serum antioxidant parameters of Furong crucian carp. (**A**) T-AOC, total antioxidant capacity; (**B**) T-SOD, total superoxide dismutase; and (**C**) MDA, malondialdehyde. Values with distinct letters indicate significant statistical differences ($p < 0.05$).

3.5. Intestinal Digestive Enzyme Activities

As depicted in Table 5, incorporating fermented soybean residue into the diet significantly affected the activities of amylase in the intestine of Furong crucian carp ($p < 0.05$) without notable variances noted in trypsin or lipase activities ($p > 0.05$). Relative to the CON group, the FSR6 and FSR12 groups displayed a noteworthy increase in amylase activity ($p < 0.05$), whereas no significant alteration was observed in the FSR18 and FSR24 groups ($p > 0.05$).

Table 5. Effects of dietary fermented soybean residue on intestinal digestive enzyme activities of Furong crucian carp.

Parameters	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
Amylase (U/mg)	1.19 ± 0.15 ^{b}	1.93 ± 0.32 ^{a}	1.69 ± 0.06 ^{a}	1.23 ± 0.18 ^{b}	1.02 ± 0.07 ^{b}	0.027
Trypsin (U/mg)	7.50 ± 2.20	8.10 ± 3.18	7.75 ± 4.76	5.41 ± 1.23	7.52 ± 3.02	0.973
Lipase (U/g)	9.14 ± 0.90	6.63 ± 1.84	6.30 ± 1.12	8.91 ± 1.53	7.05 ± 1.42	0.502

Note: Values are means ± SEMs. Values in the same row with distinct letters indicate significant statistical differences ($p < 0.05$).

3.6. Intestinal Histomorphology

Figure 3 indicates that the addition of fermented soybean residue significantly influenced the Mt and Vh of the intestine in Furong crucian carp ($p < 0.05$) with no significant impact on the number of GC ($p > 0.05$). Relative to the CON group, the FSR12 group exhibited a marked increase in Mt ($p < 0.05$), while the other groups showed no significant alterations ($p > 0.05$). In comparison to the control group, the Vh showed a significant rise in the FSR6, FSR12, and FSR18 groups ($p < 0.05$), whereas no notable changes were detected in the FSR24 group ($p > 0.05$).

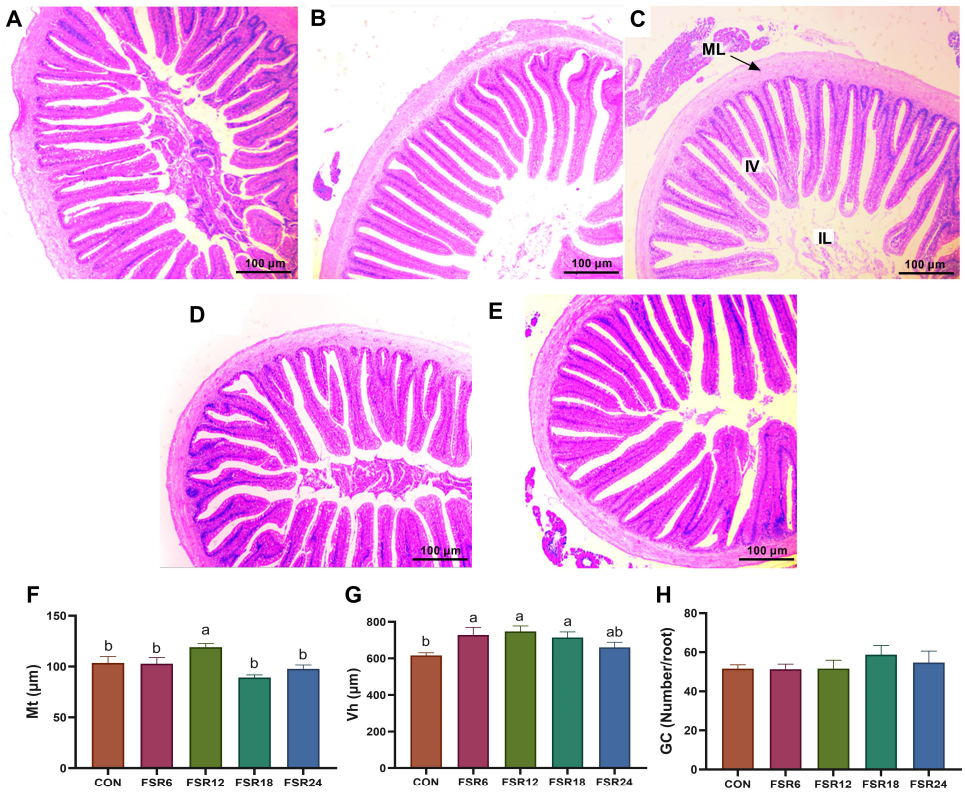

Figure 3. Effects of dietary fermented soybean residue on the intestine histomorphology of Furong crucian carp. (**A**–**E**) intestinal morphology. The scale bar is 100 μm. ML: muscularis layer; IV: intestinal villi; IL: ileum. (**F**) Mt, muscularis thickness; (**G**) Vh, villous height; and (**H**) GC, goblet cells. Values with distinct letters indicate significant statistical differences ($p < 0.05$).

3.7. Intestinal Microbiota Composition

Table 6 reveals that there were no significant disparities among the groups in observed species and the PD whole tree ($p > 0.05$). The addition of fermented soybean residue did not result in significant changes in the Shannon and Simpson indices when compared to the CON group ($p < 0.05$). The Shannon index was significantly higher in the FSR18 group compared to the CON, FSR6, and FSR24 groups ($p < 0.05$). Additionally, the Simpson index showed a notable elevation in the FSR18 group when contrasted with the CON, FSR6, and FSR24 groups ($p < 0.05$).

As shown in Figure 4A,B, there were 730 OUTs in the intersection of the five groups, with CON, FSR6, FSR12, FSR18, and FSR24 having 1289, 1806, 1309, 1358, and 1694 OUTs, respectively. At the phylum level, Fusobacteriota, Proteobacteria, Firmicutes, Desulfobacterota, Cyanobacteria, Planctomycetota, Verrucomicrobiota, Bacteroidota, and Chloroflexi composed the top nine dominant phyla of intestinal microbiota (Figure 4C). However, there were no noteworthy variations in the abundance of phyla among the diets ($p > 0.05$). At the genus level, *Cetobacterium*, *Aeromonas*, ZOR0006, *Vibrio*, *Romboutsia*, *Photobacterium_sp._JCM_19050*, *Dielma*, *Paeniclostridium*, LD29, *Candidatus_Competibacter*,

Clostridium_sensu_stricto_1 comprised the predominant bacterial genera in the intestine of Furong crucian carp (Figure 4D). As shown in Figure 4E–G, in comparison with the CON, the relative abundances of *Romboutsia* and *Clostridium_sensu_stricto_1* in the intestinal microbiota of Furong crucian carp in the FSR6 group significantly increased, whereas there was a notable reduction in the relative abundance of *Vibrio* ($p < 0.05$). In comparison to the control group, the relative abundance of *Vibrio* significantly rose in the FSR12 group and markedly declined in the FSR24 group ($p < 0.05$).

Table 6. Effects of dietary fermented soybean residue on the alpha diversity of hindgut microbial community of Furong crucian carp.

Items	CON	FSR6	FSR12	FSR18	FSR24	*p*-Value
Observed species	556.00 ± 54.61	600.16 ± 51.44	782.20 ± 144.62	817.58 ± 112.84	573.34 ± 95.68	0.315
PD whole tree	41.93 ± 2.78	47.04 ± 4.51	54.03 ± 7.74	57.88 ± 5.72	43.52 ± 5.56	0.339
Shannon	2.31 ± 0.43 ^{b}	2.25 ± 0.18 ^{b}	3.27 ± 0.37 ^{ab}	3.94 ± 0.50 ^{a}	2.10 ± 0.31 ^{b}	0.009
Simpson	0.51 ± 0.08 ^{b}	0.49 ± 0.04 ^{b}	0.62 ± 0.05 ^{ab}	0.76 ± 0.06 ^{a}	0.43 ± 0.05 ^{b}	0.007

Note: Values are means ± SEMs. Values in the same row with distinct letters indicate significant statistical differences ($p < 0.05$).

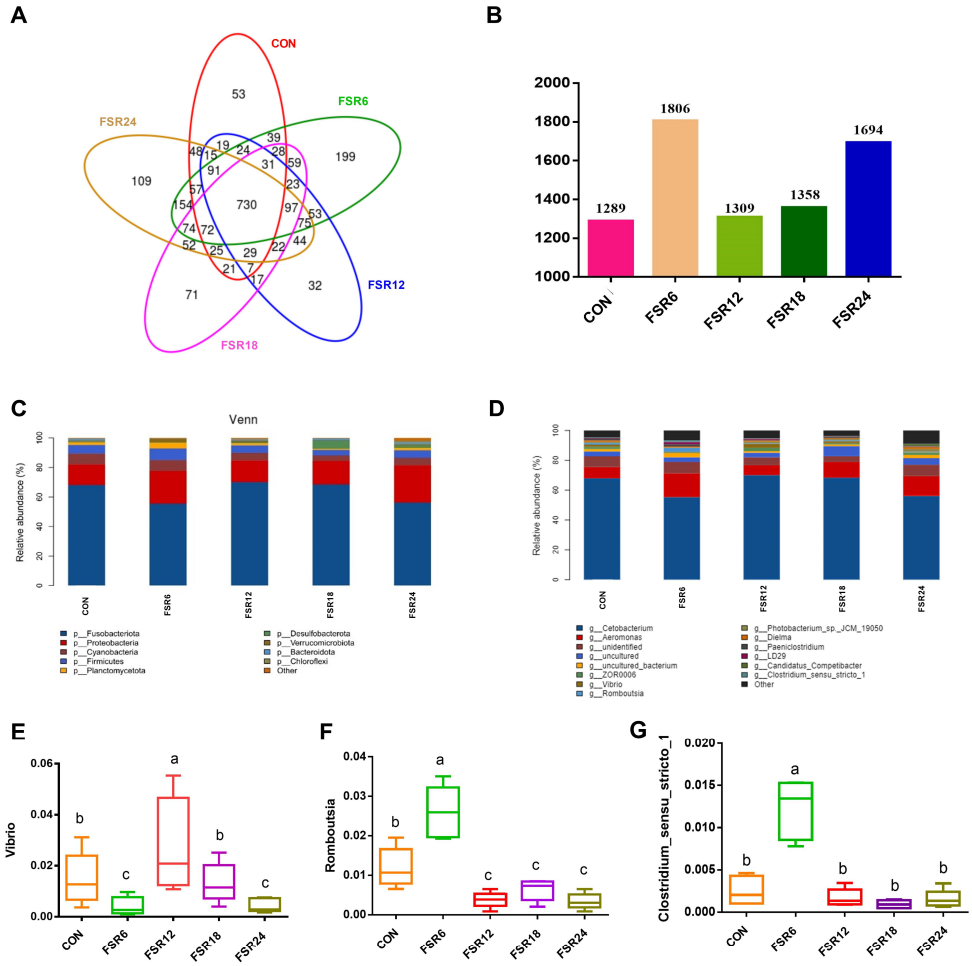

Figure 4. Effects of dietary fermented soybean residue on the intestinal microbiota composition of Furong crucian carp. (**A**) Venn diagram of OTUs of Furong crucian carp fed different diets; (**B**) the OTUs of Furong crucian carp fed different diets; (**C**) the top 10 most abundant taxa at the phylum level; and (**D**) the top 15 most abundant taxa at the genus level. (**E**–**G**) relative abundances of *Vibrio*, *Romboutsia*, and *Clostridium_sensu_stricto_1*. Values with distinct letters indicate significant statistical differences ($p < 0.05$).

4. Discussion

After fermentation, soybean residue exhibited an increase in crude protein content accompanied by an effective reduction in anti-nutritional factors [7]. Additionally, soluble dietary fiber, polyphenol isoflavones, and other active substances increased [6], potentially promoting the growth of the animals. Accordingly, the findings from this study indicated that incorporating 6.78% to 7.06% fermented soybean residue into the diet significantly enhanced the WGR and SGR of Furong crucian carp. A study reported that, through a regression analysis of SGR, it was determined that the optimal inclusion level of fermented soybean residue in the diet of *Jian* carp (*Cyprinus carpio* var. Jian) is 10.2% [19]. In an investigation of largemouth bass, the optimal growth performance was observed when fermented soybean residue, at a level of 9%, replaced 4% of soybean meal in the diet [10]. In this study, the inclusion of fermented soybean residue at 12% resulted in an increase in the FCR for Furong crucian carp, accompanied by marked decreases in WGR and SGR. Similar to the findings of this study, the earlier study found that a high level of fermented soybean residue in the diet is detrimental to the growth of African catfish [20]. In this study, in conjunction with the analysis of microbial communities, it was observed that, when the fermentation of soybean residue reached 12%, there was a significant increase in the abundance of *Vibrio*, while beneficial bacteria such as *Romboutsia* and *Clostridium_sensu_stricto_1* significantly decreased. Previous studies have reported that certain species of *Vibrio* act as opportunistic pathogens in aquaculture [21]. Therefore, the observed decline in the growth of Furong crucian carp when soybean residue substitution levels exceeded 12% may be associated with a decrease in the fish's resistance to pathogens.

Certain components in soybeans, including protein, isoflavones, and dietary fiber, have been documented to impact cholesterol metabolism [22,23]. The results of the present study indicated that incorporating fermented soybean residue into the diet notably reduced the lipid content in Furong crucian carp. Additionally, with the increasing level of fermented soybean residue, the serum GLU, TC, and TG levels of Furong crucian carp significantly decreased. This suggested that the fermented soybean residue in the diet promoted the carbohydrate and lipid metabolism in Furong crucian carp. In largemouth bass, as the level of fermented soybean residue replacing soybean meal increased, there was a decrease in liver fat content, as well as a reduction in plasma GLU, TG, and TC levels [10]. In Syrian hamsters, Villanueva et al. [24] noted a substantial decrease in the TC and TG concentrations in plasma and the liver by adding fermented soybean residue to the high-fat diet. It was suggested that the dietary fiber and protein, being the primary constituents of fermented soybean residue, might have influenced cytochrome P450 14α-sterol demethylases and peroxisome proliferator-activated receptor alpha in the liver, thereby regulating lipid metabolism. In this study, the precise mechanism through which fermented soybean residue promoted carbohydrate and lipid metabolism remained to be further investigated.

A study employing the yeast *Yarrowia lipolytica* to ferment soybean residue demonstrated substantial increases in lipid, succinate, and free amino acid levels, consequently enhancing the antioxidant capacity [9]. Furthermore, the increase in phenolic compounds and isoflavone content in fermented soybean residue also contributed to its antioxidant properties [25]. MDA serves as an indicator reflecting the extent of damage induced via oxygen free radicals, SOD neutralizes superoxide radicals, and T-AOC reflects the overall antioxidant capacity of a biological system. In the present study, incorporating 6% fermented soybean residue into the diet resulted in the highest serum SOD and T-AOC and the lowest MDA content. This indicated that 6% fermented soybean residue enhanced the antioxidant capacity of Furong crucian carp. Similarly, enhanced antioxidant capacities were also observed in black sea bream (*Acanthopagrus schlegeli*) and white shrimp (*Litopenaeus vannamei*) fed diets containing fermented soybean meal [26,27].

The activities of digestive enzymes in the intestine reflect the ability of fish to digest and absorb nutrients. In the present study, 6% fermented soybean residue significantly improved the amylase activity of the intestine of Furong crucian carp, which might partially explain the improved growth performance using 6% fermented soybean residue. In African

catfish, the substitution of 50% fermented soybean residue for fish meal significantly upregulated the activity of intestinal amylase [20]. Soybean-derived peptides are small protein fragments that could be generated via lactic acid–bacteria-mediated fermentation [28,29]. The variations in soybean-derived peptides might be the reason why incorporating fermented soybean residue into the diet could influence the intestinal digestive activities in Furong crucian carp. Jiang et al. [30] found that supplementing the diet with bioactive peptides derived from soybeans enhanced the alpha-amylase and trypsin activities in the intestine of Chinese mitten crab (*Eriocheir sinensis*).

Mt, Vh, and GC are important indicators for evaluating intestinal structural characteristics. In the present study, incorporating 6% fermented soybean residue into the diet significantly increased the Vh in the intestine of Furong crucian carp. When the level of fermented soybean residue in the diet reached 12%, the Mt in the intestine of Furong crucian carp showed a significant increase. In the study on African catfish, it was demonstrated that the substitution of 25% fishmeal with fermented soybean residue (at a level of 9% in the diet) markedly increased the Vh in the intestine [11]. In the investigation carried out by Refstie et al. [31], it was observed that the pathological changes in the intestine induced via soybean meal were less prominent in fish that were fed fermented soybean white flakes, as opposed to those fed non-fermented soybean white flakes. Research studies have indicated that diverse antinutritional components present in soybeans, such as saponins, play a role in the development of morphological alterations in the intestines of salmonids [32,33]. On one hand, fermentation possesses the capacity to decrease or eliminate anti-nutritional components in soybean residue. On the other hand, some literature, including studies by Feng et al. [34] and Tellez et al. [35], has reported a direct correlation between Vh and intestinal microbial populations.

It was noted that the predominant phyla in the hindgut of different treatments for Furong crucian carp were consistent, comprising Fusobacteriota, Proteobacteria, Cyanobacteria, and Firmicutes, respectively. This pattern resembled the dominant microbial composition observed in the intestinal tract of tilapia (*Oreochromis niloticus*) [36]. In this study, the dominant bacterial genera in the hindgut of Furong crucian carp included the *Cetobacterium*, *Aeromonas*, and ZOR0006. Among them, *Cetobacterium* is overwhelmingly dominant, with abundances exceeding 50% in all treatment groups. *Cetobacterium* is a core microbial group in the intestinal tract of herbivorous fish. This genus not only facilitates cellulose hydrolysis but also engages in the fermentation of proteins and carbohydrates, producing vitamin B_{12} [37]. Abundant *Cetobacterium* demonstrated the ability to use dietary fiber in the diet of Furong crucian carp. In the present study, 6% fermented soybean residue significantly increased the relative abundances of *Romboutsia* and *Clostridium_sensu_stricto_1* in the intestine of Furong crucian carp. *Romboutsia* represents a novel class of probiotics that could potentially enhance the host's intestinal health. A significant reduction in its presence may serve as an indicator of dysbiosis within the host's intestinal microbiota [38]. In addition, *Romboutsia* possesses the capability to metabolize diverse carbohydrates into beneficial compounds such as short-chain fatty acids, oligosaccharides, and other prebiotics [39]. The changes in the abundance of *Romboutsia* noted in this study could be linked to the content of soybean saponin in fermented soybean residue [40]. Microbial species belonging to Clostridiales were tightly correlated with the generation of short-chain fatty acids and demonstrated distinct anti-inflammatory properties [41]. A study also reported that the inclusion of microbial fermented and enzymatically hydrolyzed soybean meal in the diet increased the abundance of *Clostridium_sensu_stricto_1* in the intestine of weaned piglets [42]. The predominant representation of *Vibrio* within the Proteobacteria phylum suggested its potential role as an intestinal pathogen, contributing to the occurrence of vibriosis outbreaks [43]. In the present study, incorporating 6% fermented soybean residue into the diet could reduce the abundance of *Vibrio* in the intestines of Furong crucian carp, increase the abundance of beneficial bacteria such as *Romboutsia* and *Clostridium_sensu_stricto_1*, promote the metabolism of carbohydrates and fatty acids, and contribute to intestinal health.

5. Conclusions

In the present study, incorporating 6% fermented soybean residue into the diet of Furong crucian carp resulted in a significant improvement in both WGR and SGR, accompanied by a reduction in the FCR. By conducting regression analysis on WGR and SGR, the study identified the optimal dietary levels of fermented soybean residue for Furong crucian carp to be 6.78% and 7.06%. Incorporating fermented soybean residue into the diet decreased the lipid content of the whole body and the levels of TC, GLU, and TG in the serum of Furong crucian carp. The inclusion of 6% and 12% fermented soybean residue in the diet markedly increased the antioxidant capacity, intestinal amylase activity, and intestinal Vh of Furong crucian carp. At the 6% level, fermented soybean residue significantly increased the abundance of *Romboutsia* and *Clostridium_sensu_stricto_1* while significantly decreasing the abundance of *Vibrio*, indicating that a 6% level of fermented soybean residue was beneficial for intestinal health. In conclusion, incorporating 6–7% fermented soybean residue into the diet of Furong crucian carp is recommended.

Author Contributions: H.W.: data curation and writing—original draft. Z.Z.: project administration and data curation. F.L.: formal analysis and validation. L.H.: data curation. T.X.: supervision and funding acquisition. Y.Z.: methodology and supervision. M.Y.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Innovation of Key Technologies for the Facility Aquaculture Utilization of Famous Indigenous Fish Species in Yunnan (Grant number 2022xczx-357) and the National Natural Science Foundation of China (Grant number 32303026). The APC was funded by the project of Design and Breeding of Novel Fish Varieties Resistant to Diseases and Stress (Grant number 2023ZD0406503).

Institutional Review Board Statement: This study obtained approval from the Ethics Committee of Hunan Agricultural University, Changsha, China (Approval Code: 202109020; Approval Date: 20 September 2021).

Data Availability Statement: The data produced in this study can be obtained from the corresponding author upon reasonable request.

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

References

1. Li, S.; Zhu, D.; Li, K.; Yang, Y.; Lei, Z.; Zhang, Z. Soybean curd residue: Composition, utilization, and related limiting factors. *Int. Sch. Res. Not.* **2013**, *2013*, 423590. [CrossRef]
2. Mok, W.K.; Tan, Y.X.; Lee, J.; Kim, J.; Chen, W.N. A metabolomic approach to understand the solid-state fermentation of okara using *Bacillus subtilis* WX-17 for enhanced nutritional profile. *AMB Express* **2019**, *9*, 60. [CrossRef] [PubMed]
3. Wali, A.; Nishino, N. Bacterial and fungal microbiota associated with the ensiling of wet soybean curd residue under prompt and delayed sealing conditions. *Microorganisms* **2020**, *8*, 1334. [CrossRef]
4. Wang, B.; Zhang, Q.; Zhang, N.; Bak, K.H.; Soladoye, O.P.; Aluko, R.E.; Fu, Y.; Zhang, Y. Insights into formation, detection and removal of the beany flavor in soybean protein. *Trends Food Sci. Technol.* **2021**, *112*, 336–347. [CrossRef]
5. Choi, I.S.; Kim, Y.G.; Jung, J.K.; Bae, H.-J. Soybean waste (okara) as a valorization biomass for the bioethanol production. *Energy* **2015**, *93*, 1742–1747. [CrossRef]
6. Vong, W.C.; Liu, S. Changes in volatile profile of soybean residue (okara) upon solid-state fermentation by yeasts. *J. Sci. Food Agric.* **2017**, *97*, 135–143. [CrossRef] [PubMed]
7. Jiang, K.; Tang, B.; Wang, Q.; Xu, Z.; Sun, L.; Ma, J.; Li, S.; Xu, H.; Lei, P. The bio-processing of soybean dregs by solid state fermentation using a poly γ-glutamic acid producing strain and its effect as feed additive. *Bioresour. Technol.* **2019**, *291*, 121841. [CrossRef] [PubMed]
8. Li, S.; Chen, Y.; Li, K.; Lei, Z.; Zhang, Z. Characterization of physicochemical properties of fermented soybean curd residue by *Morchella esculenta*. *Int. Biodeterior. Biodegrad.* **2016**, *109*, 113–118. [CrossRef]
9. Vong, W.C.; Yang, K.L.C.A.; Liu, S.-Q. Okara (soybean residue) biotransformation by yeast *Yarrowia lipolytica*. *Int. J. Food Microbiol.* **2016**, *235*, 1–9. [CrossRef]
10. Jiang, Y.; Zhao, P.-F.; Lin, S.-M.; Tang, R.-J.; Chen, Y.-J.; Luo, L. Partial substitution of soybean meal with fermented soybean residue in diets for juvenile largemouth bass, *Micropterus salmoides*. *Aquac. Nutr.* **2018**, *24*, 1213–1222. [CrossRef]

11. Kari, Z.A.; Kabir, M.A.; Mat, K.; Rusli, N.D.; Razab, M.K.A.A.; Ariff, N.S.N.A.; Edinur, H.A.; Rahim, M.Z.A.; Pati, S.; Dawood, M.A.; et al. The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). *Aquac. Rep.* **2021**, *21*, 100815. [CrossRef]
12. Zulhisyam, A.K.; Kabir, M.A.; Munir, M.B.; Wei, L.S. Using of fermented soy pulp as an edible coating material on fish feed pellet in African catfish (*Clarias gariepinus*) production. *Aquac. Aquar. Conserv. Legis.* **2020**, *13*, 296–308.
13. Wang, J.L.; Wu, Y.A.; Li, C.W. Conventional freshwater-fish new hybrids, *Furong crucian* carp. *Sci. Fish Farming* **2010**, *5*, 42.
14. Negawoldes, T.Y. Review on nutritional limitations and opportunities of using rapeseed meal and other rape seed by-products in animal feeding. *J. Nutr. Health Food Eng.* **2018**, *8*, 43–48.
15. AOAC. *Official Methods of Analysis of Official Analytical Chemists International*, 16th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1995.
16. Vâlsănescu, T.; Mateescu, M.A.; Schell, H.D.; Enache, E.; BenŢia, T.; Scânteie, L.; Zarchievici, V.; Rotaru, C. All-reagent test tablets and method for rapid and selective α-amylase iodometric determination. *Anal. Biochem.* **1985**, *146*, 299–306. [CrossRef] [PubMed]
17. Gong, J.S.; Li, W.; Zhang, D.D.; Xie, M.F.; Yang, B.; Zhang, R.X.; Li, H.; Lu, Z.M.; Xu, Z.H.; Shi, J.S. Biochemical characterization of an arginine-specific alkaline trypsin from *Bacillus licheniformis*. *Int. J. Mol. Sci.* **2015**, *16*, 30061–30074. [CrossRef] [PubMed]
18. Lessinger, J.M.; Dourson, J.L.; Férard, G. Importance of standardization of lipase assays by using appropriate calibrators. *Clin. Chem.* **1996**, *42*, 1979–1983. [CrossRef]
19. Jiang, Y.; Zhao, P.F.; Chen, Y.J.; Luo, Q.; Li, H.; Lin, S.M. Effects of fermented soybean residue on growth performance, plasma biochemical indexes and antioxidant capacity of *Jian* carp. *J. Anim. Nutr.* **2018**, *30*, 1387–1395.
20. Kari, Z.A.; Kabir, M.A.; Dawood, M.A.; Razab, M.K.A.A.; Ariff, N.S.N.A.; Sarkar, T.; Pati, S.; Edinur, H.A.; Mat, K.; Ismail, T.A.; et al. Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). *Aquaculture* **2022**, *546*, 737418. [CrossRef]
21. Austin, B. Vibrios as causal agents of zoonoses. *Vet. Microbiol.* **2010**, *140*, 310–317. [CrossRef]
22. Lichtenstein, A.H.; Jalbert, S.M.; Adlercreutz, H.; Goldin, B.R.; Rasmussen, H.; Schaefer, E.J.; Ausman, L.M. Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. *Arter. Thromb. Vasc. Biol.* **2002**, *22*, 1852–1858. [CrossRef] [PubMed]
23. Matsumoto, K.; Watanabe, Y.; Yokoyama, S.-I. Okara, soybean residue, prevents obesity in a diet-induced murine obesity model. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 720–727. [CrossRef] [PubMed]
24. Villanueva, M.; Yokoyama, W.; Hong, Y.; Barttley, G.; Rupérez, P. Effect of high-fat diets supplemented with okara soybean by-product on lipid profiles of plasma, liver and faeces in Syrian hamsters. *Food Chem.* **2011**, *124*, 72–79. [CrossRef]
25. Shi, M.; Yang, Y.; Guan, D.; Wang, Y.; Zhang, Z. Evaluation of solid-state fermentation by *Ganoderma lucidum* using soybean curd residue. *Food Bioprocess Technol.* **2013**, *6*, 1856–1867. [CrossRef]
26. Azarm, H.M.; Lee, S.-M. Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquac. Res.* **2014**, *45*, 994–1003. [CrossRef]
27. Lin, Y.-H.; Mui, J.-J. Comparison of dietary inclusion of commercial and fermented soybean meal on oxidative status and non-specific immune responses in white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* **2017**, *63*, 208–212. [CrossRef] [PubMed]
28. Barati, M.; Javanmardi, F.; Jazayeri, S.M.H.M.; Jabbari, M.; Rahmani, J.; Barati, F.; Nickho, H.; Davoodi, S.H.; Roshanravan, N.; Khaneghah, A.M. Techniques, perspectives, and challenges of bioactive peptide generation: A comprehensive systematic review. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1488–1520. [CrossRef] [PubMed]
29. Singh, B.P.; Vij, S. In vitro stability of bioactive peptides derived from fermented soy milk against heat treatment, pH and gastrointestinal enzymes. *LWT* **2018**, *91*, 303–307. [CrossRef]
30. Jiang, K.; Liu, B.; Sun, C.; Zhou, Q.; Zheng, X.; Liu, M.; Xu, G.; Jin, W.; Tian, H.; Hu, H. Promotion of improved intestinal barrier health by soybean-derived bioactive peptides in Chinese mitten crab (*Eriocheir sinensis*) fed a low fishmeal diet. *Br. J. Nutr.* **2023**, *131*, 974–986. [CrossRef]
31. Refstie, S.; Sahlström, S.; Bråthen, E.; Baeverfjord, G.; Krogedal, P. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture* **2005**, *246*, 331–345. [CrossRef]
32. Iwashita, Y.; Suzuki, N.; Yamamoto, T.; Shibata, J.-I.; Isokawa, K.; Soon, A.H.; Ikehata, Y.; Furuita, H.; Sugita, T.; Goto, T. Supplemental effect of cholyltaurine and soybean lecithin to a soybean meal-based fish meal-free diet on hepatic and intestinal morphology of rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.* **2008**, *74*, 1083–1095. [CrossRef]
33. Knudsen, D.; Urán, P.; Arnous, A.; Koppe, W.; Frøkiær, H. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. *J. Agric. Food Chem.* **2007**, *55*, 2261–2267. [CrossRef] [PubMed]
34. Feng, J.; Liu, X.; Xu, Z.R.; Wang, Y.Z.; Liu, J.X. Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. *Poult. Sci.* **2007**, *86*, 1149–1154. [CrossRef] [PubMed]
35. Santos, F.S.d.L.; Tellez, G.; Farnell, M.B.; Balog, J.M.; Anthony, N.B.; Pavlidis, H.O.; Donoghue, A.M. Hypobaric hypoxia in ascites resistant and susceptible broiler genetic lines influences gut morphology. *Poult. Sci.* **2005**, *84*, 1495–1498. [CrossRef] [PubMed]
36. Bereded, N.K.; Abebe, G.B.; Fanta, S.W.; Curto, M.; Waidbacher, H.; Meimberg, H.; Domig, K.J. The impact of sampling season and catching site (wild and aquaculture) on gut microbiota composition and diversity of Nile tilapia (*Oreochromis niloticus*). *Biology* **2021**, *10*, 180. [CrossRef] [PubMed]

37. Tsuchiya, C.; Sakata, T.; Sugita, H. Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Lett. Appl. Microbiol.* **2008**, *46*, 43–48. [CrossRef] [PubMed]
38. Mangifesta, M.; Mancabelli, L.; Milani, C.; Gaiani, F.; De'angelis, N.; De'angelis, G.L.; van Sinderen, D.; Ventura, M.; Turroni, F. Mucosal microbiota of intestinal polyps reveals putative biomarkers of colorectal cancer. *Sci. Rep.* **2018**, *8*, 13974. [CrossRef] [PubMed]
39. Zeng, Q.; Li, D.; He, Y.; Li, Y.; Yang, Z.; Zhao, X.; Liu, Y.; Wang, Y.; Sun, J.; Feng, X.; et al. Discrepant gut microbiota markers for the classification of obesity-related metabolic abnormalities. *Sci. Rep.* **2019**, *9*, 13424. [CrossRef]
40. Li, P.; Zhao, Y.; Yan, S.; Song, B.; Liu, Y.; Gao, M.; Tang, D.; Guo, Y. Soya saponin improves egg-laying performance and immune function of laying hens. *J. Anim. Sci. Biotechnol.* **2021**, *12*, 126. [CrossRef]
41. Martin-Gallausiaux, C.; Marinelli, L.; Blottière, H.M.; Larraufie, P.; Lapaque, N. SCFA: Mechanisms and functional importance in the gut. *Proc. Nutr. Soc.* **2021**, *80*, 37–49. [CrossRef]
42. Tang, J.; Li, W.; Zhou, Q.; Fang, Z.; Lin, Y.; Xu, S.; Feng, B.; Zhuo, Y.; Jiang, X.; Zhao, H.; et al. Effect of heating, microbial fermentation, and enzymatic hydrolysis of soybean meal on growth performance, nutrient digestibility, and intestinal microbiota of weaned piglets. *J. Anim. Sci.* **2023**, *101*, skad384. [CrossRef] [PubMed]
43. Egidius, E. Vibriosis: Pathogenicity and pathology. A review. *Aquaculture* **1987**, *67*, 15–28. [CrossRef]