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The Effects of Replacing Fish Meal with Enzymatic Soybean Meal on the Growth Performance, Whole-Body Composition, and Health of Juvenile Gibel Carp (*Carassius auratus gibelio*)

Adolphe Uyisenga [1], Hualiang Liang [1,2], Mingchun Ren [1,2], Dongyu Huang [1,2], Chunyu Xue [1,3], Heng Yin [3] and Haifeng Mi [3,*]

[1] Wuxi Fisheries College, Nanjing Agriculture University, Wuxi 214081, China; uyado05@gmail.com (A.U.); lianghualiang@ffrc.cn (H.L.); renmc@ffrc.cn (M.R.); huangdongyu1995@163.com (D.H.); 2022213004@stu.njau.edu.cn (C.X.)
[2] Key Laboratory of Integrated Rice-Fish Farming Ecology, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China
[3] Tongwei Agricultural Development Co., Ltd., Key Laboratory of Nutrition and Healthy Culture of Aquatic, Livestock and Poultry, Ministry of Agriculture and Rural Affairs, Healthy Aquaculture Key Laboratory of Sichuan Province, Chengdu 610041, China; yinh@tongwei.com
* Correspondence: mihf@tongwei.com

Abstract: Fish meal (FM) constitutes the main, expensive component in aquatic diets. However, the supply of FM is no longer sufficient to sustain global aquaculture production. This study had the primary goal of assessing if the replacement of FM with enzymatic soybean meal (ESBM) can affect the performance of growth and immunological response in juvenile Gibel carp. Juvenile fish with an initial weight of 45.02 ± 0.03 g were arbitrarily assigned to 18 fish cages of 1 m^3 each, then fed with diets of different levels of ESBM (0% (control group), 4%, 8%, 12%, 16%, and 20%) for 159 days. These diets corresponded, respectively, to the replacement levels of 0% (control group), 20%, 40%, 60%, 80%, and 100% FM by ESBM. For the parameters of growth and whole-body composition, no obvious differences were found between the control group and other replacement levels ($p > 0.05$). Similarly, none of the replacement levels showed significant effects for alanine transaminase (ALT), total cholesterol (TC), alkaline phosphatase (ALP), and glucose (GLU) levels ($p > 0.05$). Malondialdehyde (MDA) levels, as well as the activities of superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in plasma, were not significantly affected at all replacement levels, according to the findings of this study ($p > 0.05$). The replacement level of 60% significantly increased the activities of catalase (CAT), whereas the replacement levels of 20% and 100% markedly decreased the activities of this enzyme ($p < 0.05$). Hepatic and intestinal tissues in this study did not show obvious alterations at all levels of replacement.

Keywords: enzymatic soybean meal; Gibel carp (*Carassius auratus gibelio*); growth performance; replacement levels; health

Key Contribution: ESBM can completely replace FM meal in the diet of Gibel carp.

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1. Introduction

Aquatic animal protein significantly contributes to global food security and the supply of animal protein [1]. With an astounding production of 2.75 million tons in 2020, the Gibel carp (*Carassius auratus gibelio*) is among the major freshwater fish species in the world [2]. This fish species is mostly cultivated in China and has a number of desirable qualities, including good growth rates, excellent taste, suitability for culture systems, and a high level of stress tolerance [3].

Feeding costs can account for up to 70% of an aquaculture enterprise's overall operating costs [4]. Protein is the most expensive resource in aquafeeds. Fish meal is the ideal

source of protein in aquafeeds because it has a balanced amino acid profile, which is rich in vitamins, minerals, and other nutrients that are vital for fish [5]. Normally, the commercial diet for Gibel carp contains 15–20% fish meal, which is a lot for an omnivorous fish [6]. On the other hand, as fish meal is an expensive ingredient in the commercial diet and a premium source of protein, its price has been rising quickly alongside the development of intensive aquaculture [7]. As a result, the world's fish-meal yield is not sufficient to meet the feeding industry's demand for production. Finding alternative protein sources is therefore essential because it can reduce the cost of fish feeds. Soybean is one of the best and most affordable options to use as a substitute for fish meal in commercial aquafeeds. It has been proven to have the best amino acid profile of all the protein-rich plant feeds for satisfying the needs of fish in terms of essential amino acids [8–10]. However, studies have shown that antinutritional factors (ANFs) like tannins, phytic acid, and trypsin inhibitors can have a negative impact on fish growth performance, intestinal health, and immune response [11–13]. Due to these ANFs, soybean meal needs to be treated to improve its utilization rate by fish. Different processing methods have been employed to minimize or remove ANF components in feeds made from soybean, although some of these procedures often have some defect such as protein loss, commercial viability, and environmental sustainability [14]. With different advantages associated with enzymatic hydrolysis, this treatment method is the most efficient and extensively used technique to upgrade the value of plant-origin proteins [15]. Enzymatic preprocessing of foods can successfully eliminate the antinutritional components in soybean meal and enhance nutrient digestibility [16], with a significant beneficial impact of preprocessing seen on several fish species' growth performance [17].

ESBM has been applied with excellent results to the farming of other animals like poultry and pork; however, little information is available on enzymatic soybean meal when used to replace fish meal in fish diets. Hence, the assessment of the effects of replacing FM with ESBM on the growth performance and immune response in juvenile Gibel carp was the main aim of this feeding experiment.

2. Materials and Methods

2.1. Experimental Diets

The commercial diet formula design of Gibel carp was adopted in the experimental feed, and the FM replacement levels were 0% (control group), 20%, 40%, 60%, 80%, and 100% with ESBM, which were, respectively, 0%, 4%, 8%, 12%, 16%, and 20% of the diet (Table 1). The raw feed ingredients were combined with additives, calcium dihydrogen phosphate, and amino acids. After that, the mixture was crushed using a 60-mesh sieve. Next, an appropriate amount of water was added, and the expanded feed was granulated into pellets of 1.0–1.5 mm using an expanding feed machine and lastly sun-dried for use.

2.2. Fish and Experimental Rearing Conditions

The fish breeding base of the Freshwater Fisheries Research Centre (FFRC) provided the juvenile Gibel carp. The experiment was conducted in the water-flow concrete fish pond of 2000 square meters and 3 m depth, and the water condition was controlled; dissolved oxygen (DO) was maintained at >5.0 mg/L, while pH was kept at 7.5–8.2 and aerators were used to maintain a good level of DO. For acclimatization to the experimental conditions, the fish were kept under observation for two weeks before the start of the experiment. During that period, the fish were fed with commercial feed (obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China; crude protein 35%, crude lipid 7%) to acclimate to the experimental environment, and the feeding frequency was three times (7:30, 12:30, and 5:30 p.m.) daily to apparent satiation each time. The health, size, and weight of the juvenile Gibel carp were the same, and the average initial weight (IW) was 45.02 $\pm$ 0.03 g. Six groups of fish were fed with diets of different FM replacement levels. The feeding frequency was three times (7:30, 12:30, and 5:30 p.m.) daily to apparent satiation each time, and the amount of feed in each cage was recorded to enable the calculation of growth parameters at the end

of the experiment. There were three experimental replicates in each group, with 15 test fish for each, and 18 cages (1 m × 1 m × 1 m) were used as outdoor pond cages. The experiment's duration was 159 days.

Table 1. Formulation and composition of experimental fish diet.

Raw Material (%)	Replacing FM with ESBM Levels					
	0	**20%**	**40%**	**60%**	**80%**	**100%**
Fish meal [1]	15	12	9	6	3	0
Chicken meal [1]	5	5	5	5	5	5
Soybean meal [1]	15	15	15	15	15	15
Enzymatic soybean meal [2]	0	4	8	12	16	20
Cottonseed meal [1]	5.5	5.5	5.5	5.5	5.5	5.5
Rapeseed meal [1]	24	24	24	24	24	24
Wheat meal [1]	14.33	14.33	14.33	14.33	14.33	14.33
Rice bran [1]	15.65	13.64	11.62	9.60	7.57	5.55
Soybean oil	0.67	1.29	1.92	2.55	3.18	3.81
Monocalcium phosphate	2.00	2.33	2.66	3.00	3.33	3.66
Vitamin premix for omnivorous fish [3]	0.2	0.2	0.2	0.2	0.2	0.2
Trace element premix for omnivorous fish [4]	2	2	2	2	2	2
Lysine (98.5%)	0.30	0.33	0.36	0.39	0.43	0.46
DL-methionine	0.10	0.13	0.16	0.18	0.21	0.24
Vitamin C phosphates	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2
Proximate Composition (dry basis)						
Moisture (%)	8.56	8.51	8.47	8.51	8.55	8.47
Crude protein (%)	35.31	35.26	35.32	35.31	35.32	35.33
Gross energy (MJ/kg)	15.31	15.29	15.28	15.26	15.22	15.21
Crude lipid (%)	7.83	7.79	7.81	7.83	7.84	7.83
Crude fiber (%)	7.62	7.35	7.08	6.81	6.53	6.26
P (%)	1.47	1.45	1.46	1.48	1.46	1.48

[1] Fish meal, chicken meal, rapeseed meal, cottonseed meal, soybean meal, wheat meal, and rice bran were received from Wuxi Tongwei Feedstuffs Ltd. Fish-meal composition (%, dry matter): arginine, 3.17; histidine, 1.44; isoleucine, 2.55; leucine, 4.22; lysine, 4.32; methionine, 1.66; phenylalanine, 2.36; threonine, 2.43; valine, 2.93; tryptophan, 0.66; moisture, 8.92; crude protein, 60.30; crude lipid, 7.67; crude ash, 14.30; crude fiber, 1.01; phosphorus, 2.88. [2] Enzymatic soybean meal was provided by Jiangsu Fuhai Biotechnology CO., LTD (Nantong, China). ESBM composition (%, dry matter): arginine, 2.96; histidine, 1.25; isoleucine, 2.36; leucine, 3.36; lysine, 3.00; methionine, 0.68; phenylalanine, 2.30; threonine, 1.82; valine, 2.33; tryptophan, 0.61; moisture, 6.01; crude protein, 49.70; crude lipid, 2.41; crude ash, 6.83; crude fiber, 3.21; phosphorus, 0.31. [3] Vitamin premix and trace element premix for omnivore fish were purchased from Wuxi Hanove animal health product Co., Ltd. in Wuxi, China. Vitamin premix (IU or mg/kg of premix): vitamin A, 800,000 IU; vitamin D3, 150,000–250,000 IU; vitamin E, 4500 IU; vitamin K3, 600 mg; thiamin, 800 mg; riboflavin, 800 mg; calcium pantothenate, 2000 mg; pyridoxine HCl, 2500 mg; cyanocobalamin, 8 mg; biotin, 16 mg; folic acid, 400 mg; niacin, 2800 mg; inositol, 10,000 mg; vitamin C, 10,000 mg. [4] Trace element premix (g/kg of premix): magnesium sulphate, 1.0–1.5; ferrous sulphate, 15–30; zinc sulphate, 8–13.5; cupric sulphate, 0.35–0.8; and manganese sulphate, 2–6; rice chaff and zeolite were used as a carrier.

2.3. Collection of Samples

Before sampling, fish were kept under starvation for 24 h to allow the evacuation of the digestive system. Prior to the sample collection, the total number of fish in every cage was assessed and the weight was measured to determine the growth parameters. MS-222 (100 mg/L) was used to anesthetize the collected fish. From every cage, five fish were arbitrarily taken for analysis of whole-body composition. Three fish were randomly selected from each cage for sampling and sedated with 100 mg/L MS-222; then, the blood samples were taken through the caudal vein and centrifuged (3000 rpm, 10 min, at 4 °C) for separating from the plasma. The liver and intestine samples were also taken for tissue structure examination. The collected intestine and hepatic samples were kept at −80 °C for further examination.

2.4. Chemical Analysis

Moisture content, crude protein, crude lipid, and ash were determined by taking as a reference the protocols established by the Association of Official Analytical Chemists [18]. The methods and testing equipment of plasma biochemical indices and antioxidant indices are shown in Table 2, which displays the specific procedures, kits, and equipment used in this study.

Table 2. Main methods and analyzing equipment.

Item	Methods and Testing Equipment
Moisture	Dried to constant weight in an oven at 105 °C.
Protein	Determined by Hanon K1100 auto Kjeldahl apparatus (Jinan Hanon Instruments Co., Ltd., Jinan, China).
Lipid	Determined by Hanon SOX606 auto fat analyzer (Jinan Hanon Instruments Co., Ltd., Jinan, China).
Ash	Determined by burning at 550 °C for 5 h in an XL-2A intelligent muffle furnace (Hangzhou Zhuochi Instruments Co., Ltd., Hangzhou, China).
Alanine transaminase (ALT) Total cholesterol (TC) Glucose (GLU) Alkaline phosphatase (ALP) Albumin (ALB) Triglyceride (TG) Aspartic transaminase (AST) Total protein (TP)	All plasma parameters were determined by assay kits (Mindray Bio Medical Co., Ltd., Shenzhen, China) with a Mindray BS-400 automatic biochemical analyzer (Mindray Medical International Ltd., Shenzhen, China).
Total antioxidant capacity (T-AOC) Superoxide dismutase (SOD) Catalase (CAT) Glutathione (GSH) Glutathione peroxidase (GPx) Malondialdehyde (MDA)	All hepatic antioxidant parameters and MDA levels were tested according to instructions of assay kits purchased from Jian Cheng Bioengineering Institute (Nanjing, China).

2.5. Hematoxylin and Eosin (HE) Staining

Hematoxylin and eosin (HE) staining was used to assess the hepatic and intestinal histology, referring to a previous study [19]. The tissue was first fixed with 4% formaldehyde, washed with water, dehydrated with ethanol, made transparent with methyl salicylate, and immersed in paraffin, then cut on a microtome at a thickness of 5μm, followed by HE staining. Finally, sample photomicrographs were taken before the tissue was examined under a microscope.

For the intestine, the tissue slices were placed on the device using the panoramic slice scanner, and they were then slowly moved in front of the scanner's lens. The tissue information from the tissue slices was scanned and imaged as they moved, creating a folder that comprised all of the tissue information from the tissue slices. CaseViewer2.4 scanning software was used to select the target area of the tissue for imaging. During imaging, the whole field of view was filled with the tissue as far as possible to ensure that the background light of each photo was consistent. Following imaging, the lengths of the villous epithelium and mucosal layer were measured at five different locations within each segment using Image-Pro Plus 6.0 analysis software, and the matching number of goblet cells were counted. The number of goblet cells per unit length = the number of goblet cells/the length of the villous epithelium.

2.6. Statistics Analysis

The tissue's target area for imaging was chosen using CaseViewer2.4 scanning software. The lengths of the villous epithelium and mucosal layer were measured at five different locations within each segment using Image-Pro Plus 6.0 analysis software, and the matching

number of goblet cells were counted. In addition, data are mean value ± standard deviation. Data analysis was carried out using the statistical software for social sciences (SPSS) for Windows version 22.0. To determine whether there were any significant differences between the means of the various treatments, one-way analysis of variance (ANOVA) was utilized, and Tukey's multiple range statistics was used to show where the means were different ($p < 0.05$).

3. Results

3.1. Growth Performance

The results (Table 3) reveal that none of the growth parameters at all replacement levels was significantly affected compared to the control group ($p > 0.05$).

Table 3. Growth parameters and physical indices of Gibel carp fed with the experimental diets over 159 days [1].

Parameters	Replacement Levels						
	0%	**20%**	**40%**	**60%**	**80%**	**100%**	***p* Value**
[2] IW (g)	45.00 ± 0.23	45.02 ± 0.13	44.98 ± 0.12	45.05 ± 0.13	45.05 ± 0.23	45.00 ± 0.15	0.99
[3] FW (g)	229.89 ± 4.62	231.44 ± 0.51	226.62 ± 3.57	231.95 ± 2.85	230.45 ± 1.17	229.03 ± 2.24	0.31
[4] FCR	1.27 ± 0.03	1.21 ± 0.02	1.22 ± 0.02	1.20 ± 0.08	1.26 ± 0.08	1.30 ± 0.05	0.21
[5] SGR (%/d)	1.03 ± 0.011	1.03 ± 0.003	1.02 ± 0.010	1.03 ± 0.008	1.02 ± 0.006	1.02 ± 0.008	0.61
[6] WGR (%)	410.86 ± 9.35	414.13 ± 2.54	403.80 ± 7.93	414.87 ± 6.86	411.56 ± 4.82	408.97 ± 6.18	0.41
[7] VSI (%)	5.77 ± 1.12	6.23 ± 1.04	6.89 ± 0.66	6.60 ± 0.92	6.64 ± 0.34	6.68 ± 0.81	0.27
[8] SR (%)	97.78 ± 3.85	100.00	97.78 ± 3.85	95.56 ± 7.70	97.78 ± 3.85	97.78 ± 3.85	0.90
[9] HSI (%)	4.19 ± 0.96	4.53 ± 0.71	5.36 ± 1.19	4.97 ± 0.91	5.28 ± 0.40	5.21 ± 0.88	0.16
[10] CF (g/cm^3)	2.57 ± 0.31	2.62 ± 0.07	2.74 ± 0.20	2.64 ± 0.14	2.50 ± 0.11	2.47 ± 0.07	0.09

[1] Data are mean values ± standard deviation (n = 3). [2] IW: initial weight. [3] FW: final weight. [4] Feed conversion ratio (FCR) = total feed intake in dry matter basis (g) ÷ weight gain (g). [5] Specific growth rate (SGR, %/d) = [ln (mean final weight)-ln (mean initial weight) ÷ days] × 100. [6] Weight gain rate (WGR, %) = [final weight (g)-initial weight (g) × 100] ÷ initial weight (g). [7] Viscerosomatic index (VSI, %) = Viscera weight (g) ÷ body weight (g) × 100, [8] Survival rate (SR, %) = (final number of fish ÷ initial number of fish) × 100. [9] Hepatosomatic index (HSI, %) = Hepatopancreas weight (g) ÷ body weight (g) × 100. [10] Condition factor (CF, g/cm^3) = (body weight ÷ standard length^3) × 100.

3.2. Whole-Body Composition

The results (Table 4) indicate that none of the whole-body composition indices at all replacement levels were affected compared to the control group ($p > 0.05$).

Table 4. Whole-body composition of Gibel carp fed with the experimental diets over 159 days [1].

Parameter (% Wet Matter)	Replacement Levels						
	0%	**20%**	**40%**	**60%**	**80%**	**100%**	***p* Value**
Moisture	75.39 ± 0.40	76.12 ± 0.84	75.78 ± 0.59	75.59 ± 0.90	76.00 ± 0.83	75.72 ± 0.94	0.87
Crude protein	16.83 ± 0.78	16.61 ± 0.31	16.11 ± 0.59	17.10 ± 0.84	16.69 ± 0.61	16.72 ± 0.61	0.83
Crude lipid	1.84 ± 0.10	1.70 ± 0.04	1.66 ± 0.12	1.64 ± 0.16	1.61 ± 0.02	1.66 ± 0.10	0.16
Crude ash	4.77 ± 0.12	4.69 ± 0.24	4.65 ± 0.37	4.55 ± 0.13	4.32 ± 0.12	4.58 ± 0.35	0.37

[1] All data are mean values ± standard deviation (n = 3 × 3).

3.3. Plasma Biochemical Indices

Table 5 shows the results of the plasma biochemical parameters obtained at the end of this study. Plasma alanine transaminase (ALT), total cholesterol (TC), glucose (GLU), alkaline phosphatase (ALP), and albumin (ALB) levels of the fish under study were not significantly affected in all treatments ($p > 0.05$). The level of plasma triglyceride (TG) was reduced proportionally as the level of ESBM increased. Compared with the control group, the replacement level of 100% significantly increased the AST activity ($p < 0.05$). In addition,

the replacement level of 80% significantly increased the content of TP compared to the control group ($p < 0.05$).

Table 5. Biochemical parameters of blood plasma in Gibel carp fed the experimental diets over 159 days [1].

Parameter	Replacement Levels						
	0%	20%	40%	60%	80%	100%	*p* Value
ALB (g/L)	11.13 ± 0.82	11.11 ± 0.84	11.53 ± 1.46	10.75 ± 0.94	12.66 ± 2.26	11.74 ± 1.64	0.14
ALT (U/L)	4.60 ± 1.53	4.43 ± 0.69	5.15 ± 0.73	4.24 ± 0.79	4.69 ± 1.25	5.19 ± 1.19	0.41
AST (U/L)	100.28 ± 14.17 ^{a}	98.94 ± 12.41 ^{a}	108.85 ± 24.13 ^{ab}	111.81 ± 10.83 ^{ab}	114.61 ± 12.82 ^{ab}	123.19 ± 11.79 ^{b}	0.02
TC (mmol/L)	5.73 ± 0.63	5.50 ± 0.35	5.60 ± 0.50	5.40 ± 0.59	5.73 ± 0.23	5.46 ± 0.48	0.65
TG (mmol/L)	1.91 ± 0.23 ^{c}	1.70 ± 0.11 ^{ab}	1.76 ± 0.15 ^{bc}	1.57 ± 0.13 ^{ab}	1.63 ± 0.20 ^{ab}	1.51 ± 0.18 ^{a}	0.01
GLU (g/L)	6.11 ± 1.64	6.92 ± 1.21	5.62 ± 1.21	5.85 ± 1.04	6.64 ± 1.29	6.94 ± 0.78	0.15
TP (g/L)	36.74 ± 1.15 ^{a}	36.85 ± 2.02 ^{a}	37.74 ± 3.57 ^{ab}	36.30 ± 2.35 ^{a}	40.92 ± 4.43 ^{b}	38.79 ± 4.63 ^{ab}	0.04
ALP (U/L)	12.21 ± 4.71	9.58 ± 3.57	12.15 ± 4.99	10.14 ± 3.09	12.13 ± 4.38	11.88 ± 4.12	0.68

[1] All data are mean values ± standard deviation (n = 3 × 3). On the same row, different superscripted values represent significant differences ($p < 0.05$).

3.4. Results of Plasma Antioxidants Indices

The findings of plasma antioxidant indices are shown in Table 6. Malondialdehyde (MDA) levels, as well as the activities of superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) in plasma, were not significantly affected at all replacement levels, according to the findings of this study ($p > 0.05$). The highest activity of catalase (CAT) was observed in the group at the 60% replacement level, which was significantly higher than the control group ($p < 0.05$). Compared with the control group, 20% replacement showed a significant increase in the content of glutathione (GSH) ($p < 0.05$). Furthermore, the activities of glutathione peroxidase (GPx) were significantly increased in the groups at the 40% and 60% replacement levels compared to the control group ($p < 0.05$).

Table 6. Antioxidant parameters in blood plasma of Gibel carp fed with the experimental diets over 159 days [1].

Parameters	Replacement Levels						
	0%	20%	40%	60%	80%	100%	*p* Value
T-AOC (U/mL)	0.38 ± 0.02	0.40 ± 0.03	0.37 ± 0.05	0.38 ± 0.02	0.39 ± 0.02	0.38 ± 0.03	0.49
SOD (U/mL)	22.22 ± 1.07	22.80 ± 0.92	22.46 ± 0.96	22.71 ± 0.46	22.82 ± 0.95	22.07 ± 1.37	0.56
CAT (U/mL)	21.79 ± 7.49 ^{abc}	13.79 ± 6.65 ^{a}	30.78 ± 7.47 ^{cd}	34.66 ± 9.76 ^{d}	25.90 ± 11.50 ^{bcd}	17.55 ± 10.05 ^{ab}	0.01
GSH (mg/L)	30.56 ± 9.93 ^{ab}	51.24 ± 20.15 ^{c}	36.84 ± 23.21 ^{abc}	47.92 ± 21.33 ^{bc}	30.28 ± 8.71 ^{ab}	26.30 ± 9.25 ^{a}	0.04
GPx (μmol/L)	104.70 ± 42.39 ^{a}	118.16 ± 36.44 ^{a}	178.74 ± 10.42 ^{c}	165.08 ± 41.42 ^{bc}	119.09 ± 44.16 ^{a}	128.95 ± 30.09 ^{ab}	0.01
MDA (nmol/mL)	14.17 ± 5.50	12.52 ± 3.34	12.77 ± 4.24	13.80 ± 2.47	13.16 ± 4.17	9.88 ± 2.40	0.30

[1] All data are mean values ± standard deviation (n = 3 × 3). On the same row, different superscripted values represent significant differences ($p < 0.05$).

3.5. Results of Hepatic Tissue Structure

As shown in Figure 1, the hepatocytes at 0%, 20%, 40%, 60%, 80%, and 100% replacement levels were arranged in double rows into plates, and the plates were scattered around the central veins. The hepatocytes were irregular polygons with centered nuclei and vacuolated cytoplasm (black arrows). There was no obvious expansion of hepatic sinusoids and no obvious inflammatory cell infiltration.

3.6. Results of Intestine Tissue Structure

Among all replacement levels of FM with ESBM, none showed a significantly changed villous epithelium length, number of goblet cells, or number of goblet cells per unit length. In addition, as displayed in the following Figure 2, the structure of each layer of intestinal tissue at 0%, 20%, 40%, 60%, 80%, and 100% replacement levels was clear. The intestinal villi were abundant and arranged regularly, the mucosal epithelial cells were not exfoliated, and many goblet cells were seen without obvious abnormalities (Table 7). This indicates

that the structure of intestinal tissue of Gibel carp is not damaged when FM is completely replaced with ESBM in the diet.

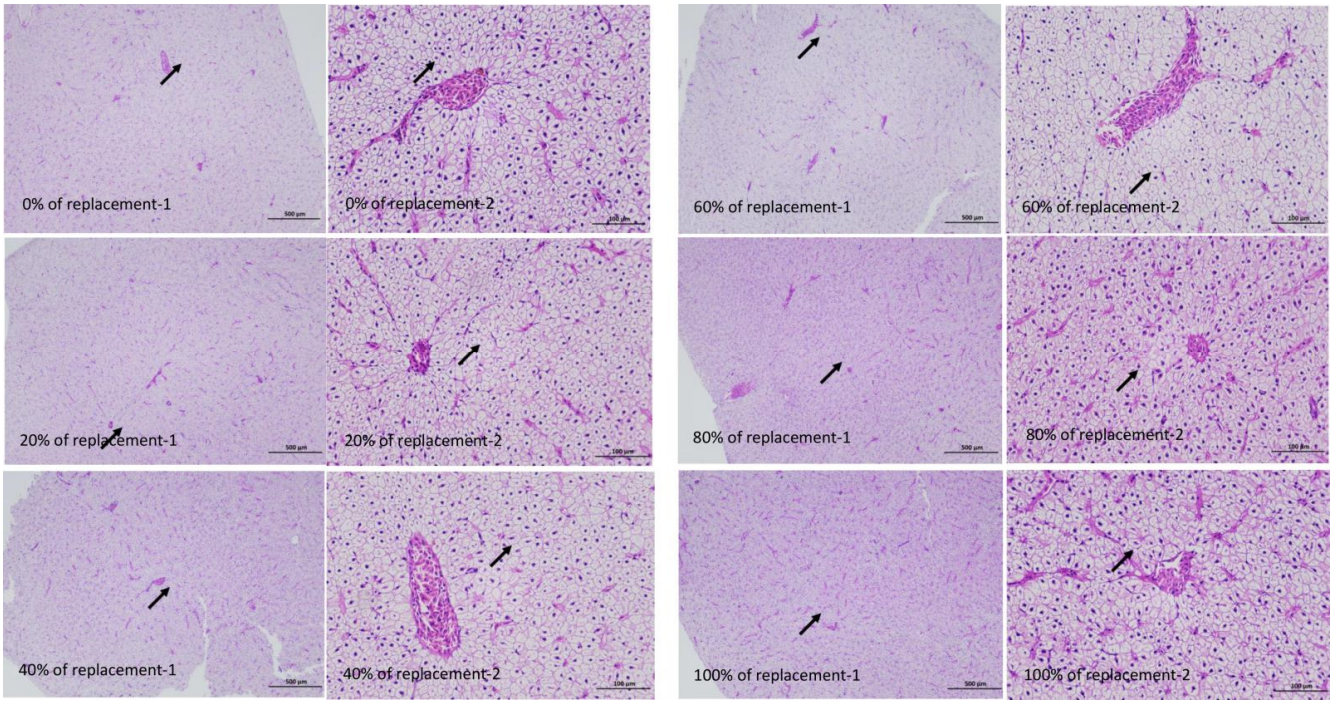

Figure 1. Sections of hepatic tissue structure in Gibel carp fed with the experimental diets over 159 days. The hepatocytes were arranged in double rows into plates, and the plates were scattered around the central veins. The hepatocytes were irregular polygons with centered nuclei and vacuolated cytoplasm (black arrow). The number 1 represents 40× and the number 2 represents 200× observation multiples in each image.

Table 7. Intestinal tissue structure in Gibel carp fed with the experimental diets over 159 days [1].

Replacement Levels (%)	Thickness of Mucosal Layer (mm)	Length of the Villous Epithelium (mm)	Number of Goblet Cells	Number of Goblet Cells per Unit Length (Cell/mm)
0	0.48 ± 0.14	0.54 ± 0.12	14.93 ± 7.19	27.66 ± 10.52
20	0.33 ± 0.05	0.46 ± 0.02	12.90 ± 6.65	27.81 ± 13.26
40	0.41 ± 0.03	0.55 ± 0.03	13.00 ± 5.30	23.74 ± 8.95
60	0.52 ± 0.22	0.69 ± 0.30	15.47 ± 5.67	22.98 ± 3.33
80	0.40 ± 0.05	0.55 ± 0.03	11.47 ± 3.65	20.70 ± 6.37
100	0.43 ± 0.07	0.61 ± 0.13	24.13 ± 7.71	39.70 ± 9.21
p Value	0.58	0.64	0.23	0.19

[1] Data are presented as mean value ± standard deviation (n = 3 × 3).

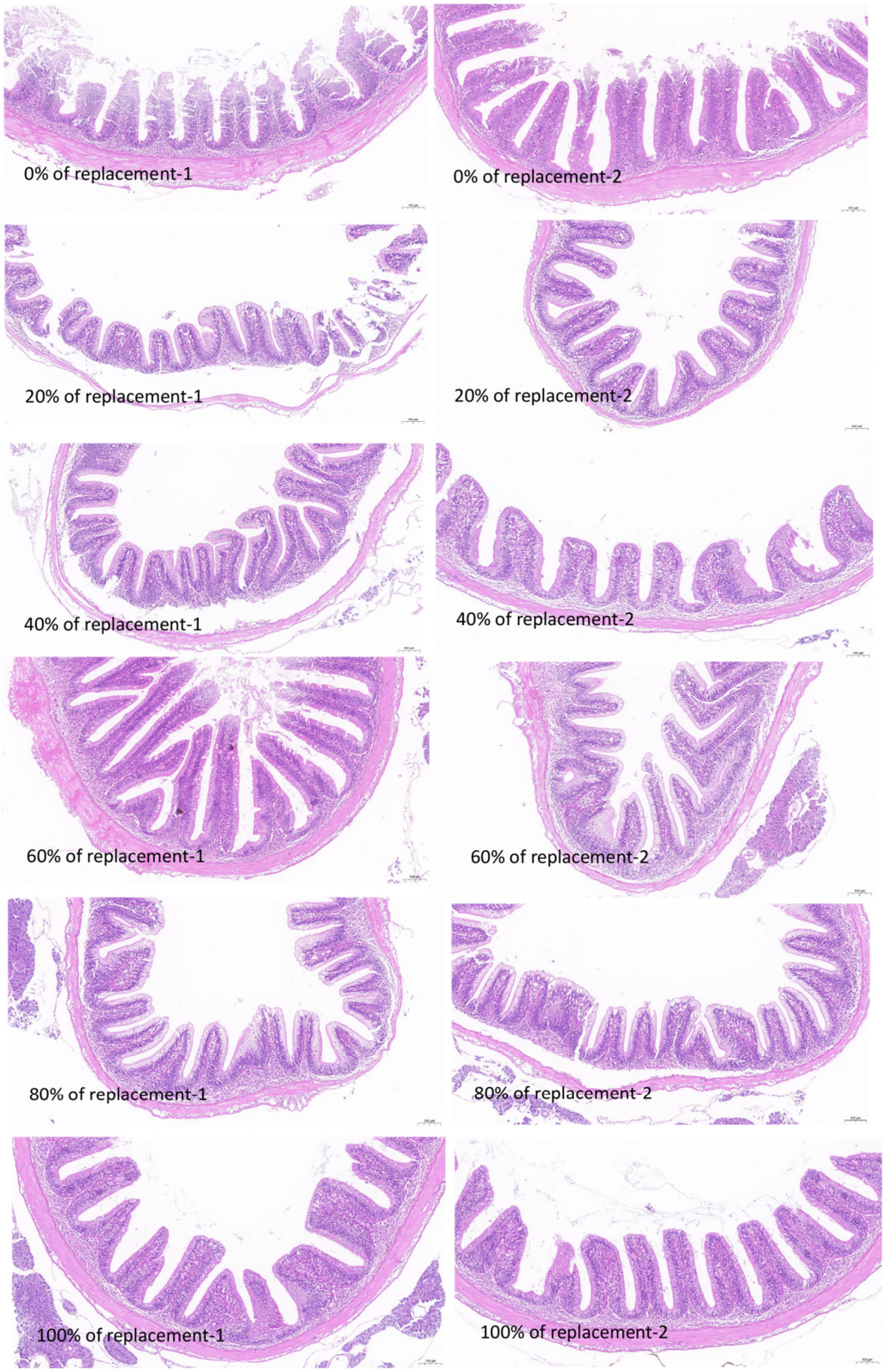

Figure 2. Sections of intestinal tissue structure in Gibel carp fed with the experimental diets over 159 days. The 1 and 2 in each group represent different intestines at 10× viewing multiples.

4. Discussion

Due to the rising demand for FM and the rapid expansion of the aquafeed industry, the price of FM has climbed significantly during the past years [20]. Cheap plant proteins have

typically been employed as FM replacements in aquafeed diets to promote the aquaculture industry [21]. The results of this study show that the growth performance of Gibel carp is not significantly affected, including FW, FCR, SGR, and WGR, when the FM is entirely replaced with ESBM. This indicates that ESBM is a good alternative for FM in the diet of Gibel carp. Liu et al. [22] also provided a similar suggestion after studying the effect of the substitution of FM with ESBM in the feed of juvenile largemouth bass (*Micropterus salmoides*). The capacity of ESBM to replace FM in aquatic diets is correlated to the increase in nutritional status and reduction in antinutritional factors when soybean is treated with enzymes. However, the study performed on rainbow trout (*Oncorhynchus mykiss*) fry suggests that the replacement level of FM with ESBM should be 50% [23]. This dissimilarity may come from different fish species on which the studies were conducted. Furthermore, the SR was not significantly affected at all the replacement levels. Samira et al. [23] found similar results in the study carried out on rainbow trout fry fed with ESBM (HP310) as a replacement for FM in the diet. Fan et al. [24] also gave the same suggestion in their experiment conducted on Pacific white shrimp (*Litopenaeus vannamei*), where FM was replaced with ESBM. The important indicators that represent the body lipid, lean, and growth of fish include the CF, HSI, and VSI [25]. The fish's nutritional status can be determined using the HSI, which is an indirect indicator of hepatic glycogen content. Lower values may indicate stress, and both the HSI and VSI are used to determine whether energy is being diverted away from organ or tissue growth in order to evade stress [26]. In the current study, no significant differences in the HSI and VSI were found at all replacement levels; therefore, total replacement of FM with ESBM did not disturb glycogen and carbohydrate energy reserves. Liu et al. [22] also suggested similar results in juvenile largemouth bass.

Whole-body composition can indicate directly and indirectly an animal's growth [27,28]. The results of this study showed that the moisture, crude lipids, protein, and ash contents of juvenile Gibel carp were not significantly affected by any replacement levels. Similar results for moisture, crude protein, and ash contents after replacing FM with SBM were also found in the hybrid of *Carassius auratus gibelio* ♀ × *Cyprinus carpio* ♂ [29]. Furthermore, Samad et al. [30] suggested that replacement of FM with fermented soybean meal had no significant impact on the crude protein and ash contents of Japanese seabass (*Lateolabrax japonicus*). However, previous studies have stated that soybean can reduce crude lipid content in rainbow trout fry [23]. Additionally, the study of Duan et al. [31] confirmed that the replacement of FM with fermented soybean lessens the level of crude lipid in the fish body of hybrid snakehead (*Channa argus* × *Channa maculate*). These dissimilarities could be caused by differences in fish species, experimental setups, and methods of soybean processing. Plasma biochemical parameters reflect fish metabolism and health status [32]. Information on the state of fish health and protein metabolism is detected using plasma TP and ALB [33]. In addition, fish immunity is also correlated with plasma TP and ALB [34]. The replacement of FM with graded levels of ESBM did not significantly affect the plasma ALB level compared to the control group. However, the 80% replacement level of FM with ESBM was significantly improved compared to the control group. In contrast, it was reported by Ajani et al. [35] that the level of plasma TP is reduced when the FM is completely replaced by soybean with or without methionine fortification in the diet of Nile tilapia (*Oreochromis niloticus*). This difference can be caused by the presence of ANFs in raw soybean and the difference in fish species studied; however, enzymolysis was shown to effectively reduce the levels of these ANFs in this study. Fish plasma GLU is regarded as a stress indicator, and its level reflects the energy produced by a stressor [36]. In this study, the level of plasma glucose was not significantly modified at all replacement levels, which indicates that total replacement of FM with ESMB does not significantly disturb the energy homeostasis of Gibel carp. Ye et al. [37] also reported the same results of plasma glucose in juvenile obscure puffer (*Takifugu obscurus*). The findings of this study also demonstrated that, except at the 40% replacement level, other replacement levels significantly decreased the level of TG in comparison to the control group. On the other hand, TC was not significantly modified by different replacement levels. As the dietary

ESBM level grew, the body's overall crude lipid content decreased, which is correlated to the declining trend of plasma TC and TG. These results agree with the previous report that replacement of fish meal with defatted and fermented soybean meals could lower the level of TC compared to FM in pompano (*Trachinotus blochii*) [38], which is mainly caused by the presence of high concentrations of estrogenic isoflavones [39]. ALT and ALP are enzymes that contribute to the metabolism of protein; the level of these enzymes may indicate fish health status [40]. At all replacement levels, there were no significant modifications in the level of plasma ALT and ALP compared to the control group, which showed that the liver and protein metabolism of Gibel carp were not impaired by the entire replacement (100%) of FM with ESBM. T-AOC is a frequently used analyte to assess the antioxidant defense for combatting against the free radicals caused by a given disease [41]. There was no significant difference in the activity of T-AOC between the control group and all replacement levels. Lin et al. [25] also found similar results in juvenile pompano fish (*Trachinotus ovatus*). The level of MDA in fish plasma can be utilized as an indication of oxidative damage because it is one of the byproducts of cell membrane lipid peroxidation and is regarded as an indirect biomarker of lipid peroxidation [42]. All replacement levels of FM with ESBM in this study had no significant influence on the plasma MDA level compared to the control group. This indicated that the replacement (FM with ESBM) of these ingredients did not cause damage to the health of Gibel carp. Different results have been observed in studies of other fish species like redlip mullet (*Liza haematocheila*) [7], where FM was replaced with soybean, and Japanese seabass [31], where FM was replaced with fermented soybean. These contradictory findings could be the result of different fish species and various soybean-processing techniques. SOD, CAT, and GPx are crucial indicators of the body's capacity to defend against oxidative cell damage [27]. Catalase is a typical enzyme that may be found in almost all living things that are exposed to oxygen, including bacteria, plants, and mammals. It helps hydrogen peroxide break down into water and oxygen. It is a crucial enzyme in preventing reactive oxygen species (ROS) from oxidatively harming the cell.

In this study, no significant differences were observed in SOD activity between the control group and all other replacement levels. This indicated that the full replacement (100%) of FM with ESBM did not significantly disturb the mitochondrial oxidative metabolism of fish cells and did not cause oxidative stress in Gibel carp. GPx eliminates hydrogen peroxide and lipid peroxides generated during metabolism; in addition, it also catalyzes the reduction in glutathione. In this study, the 40 and 60% replacement levels of FM by ESBM significantly improved the activities of GPx; however, lower and higher levels of substitution did not affect the activities of GPx. Huaxing et al. [25] also reported the same results in juvenile pompano fed on soy protein peptide. Furthermore, at the replacement level of 60%, CAT activity was significantly improved compared to the control group. Liu et al. [22] also found the change in CAT activity in juvenile largemouth bass when the FM was replaced by enzyme-treated soybean in diets. As a result, ESBM is capable of producing small peptides with features that increase the body's antioxidant capacity by neutralizing free radicals, preventing lipid peroxidation, and chelating metal ions [22]. A perfect indicator of the fish's good nutrition is the liver and intestine's histological condition [43]. The findings of this feeding experiment showed that none of the replacement levels affected the hepatic and intestinal tissue structure in Gibel carp. These results indicated that ESBM could be used as an FM replacement without causing problems in food digestion, nutrient absorption, and metabolism in Gibel carp. Muhammad et al. [43] and Fotini et al. [44], respectively, stated that fermented soybean meal may contribute to the histological, morphological, and functional alterations in African catfish (*Clarias gariepinus*) liver tissues, and processed soybean resulted in the accumulation of lipids within hepatocytes of gilthead sea bream (*Sparus aurata*). The dissimilarities may be due to the difference in fish species and the low content of antinutritional factors in ESBM [23,28,45].

5. Conclusions

In general, ESBM could completely replace FM in ration formulation for juvenile Gibel carp's diets without causing adverse impacts on growth performance, antioxidant capacity, and the structure of liver and intestine tissues. Furthermore, the partial replacement could even improve the antioxidant capacity of juvenile Gibel carp.

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