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Research Article

Effects of Three Compound Attractants in Plant Protein Diets on Growth, Immunity, and Intestinal Morphology of Yellow River Carp *Cyprinus carpio* var

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A feeding experiment was conducted to evaluate the effects of three compound attractants on the growth performance, immunity, and intestinal morphology of Yellow River (YR) carp. Five treatment groups were included in the feeding experiment. Group I consisted of a fishmeal-based diet (positive control), and group II was a plant protein diet (negative control). The compound attractants 1 (0.06%dimethyl – beta – propiothetin (DMPT) + 0.22%tangerine peel powder + 0.75%yeast powder), 2 (0.05%garlic powder + 0.06 %DMPT + 0.75%yeast powder), and 3 (0.07%sodium glutamate + 0.22%tangerine peel powder + 0.34%betaine) were added to the plant protein diet, belonging to groups III, IV, and V, respectively. The three different compound attractants significantly improved the weight gain rate, special growth rate, and protein efficiency ratio of Yellow River (YR) carp ($P < 0.05$) compared with the negative control, among which group III performed best. Similarly, group III had significantly higher serum lysozyme (LZM) and acid phosphatase (ACP) activities than the negative control, and groups III, IV, and V depicted significantly higher LZM activities in the liver and gill ($P < 0.05$). Groups III, IV, and V revealed significantly higher superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC) activities in the serum and liver than the negative control ($P < 0.05$), while the malondialdehyde (MDA) content was the opposite ($P < 0.05$). Moreover, the height and width of the plica in the foregut and midgut and the thickness of the midgut muscle layer in group III were significantly higher than those in the negative control ($P < 0.05$). Therefore, adding compound attractants in a plant protein diet can significantly enhance the total antioxidant and immune capacity, along with improved growth performance of YR carp. Similarly, compound attractants significantly elevated the height and width of the intestinal fold within the anterior and middle sections of the intestine, depicting that compound attractants promote intestinal digestion and absorption, thereby reducing intestinal injury. The results from the three experimental groups found that the compound attractants combined with 0.06% DMPT, 0.22% tangerine peel powder, and 0.75% yeast powder were more suitable for the basic plant protein diet of YR carp.

1. Introduction

In China, aquaculture has developed rapidly in the past decade, and the quality and level of protein requirements in aquatic feeds are very high. The protein source in aquatic feed mainly depends on fish and soybean meals. In contrast, the fish meal consumed through aquaculture in China is mainly imported. As a result, the self-produced fish meal proportion is low, leading to the shortage of fish meal resources and higher prices. Therefore, finding unconventional low-cost protein feeds with rich sources for the sustainable development of aquaculture is essential. Plant protein is increasingly used in aquaculture because of its abundant supply, low cost, and good nutritional balance [1]. However, the palatability and biological potency of plant feed have hampered its extensive use in aquaculture. Moreover, the resulting

environmental pollution from aquaculture with the increasing scale and intensification has gradually drawn public attention. Therefore, improving the palatability and attractiveness could essentially substitute protein.

As a nonnutritive feed additive, the food attractant has garnered extensive attention by enhancing the appetite of aquatic animals; improving feed palatability, intake, and utilization efficiency; reducing water pollution; and refining aquaculture benefits. Adding attractants to the plant diet is one of the most effective methods to improve feed intake [2]. Many studies have demonstrated that specific substances can behave as effective attractants for *Oncorhynchus mykiss* [3], *Rachycentron canadum* [4], *Litopenaeus vannamei* [5], *Scophthalmus maximus* [6], and *Oreochromis* sp. [7]. Additionally, these additives include amino acids [3], DMPT [6], nucleotides [8], and natural feeding stimulants [1]. Moreover, previous studies have depicted that compound attractants are more effective in feeding palatability and attractiveness than individual attractants [9, 10].

Yellow River (YR) carp is quite common, and the breeding yield is very high in China, especially in the north. Several results of a single attractant on this fish have been identified ([11]; Sun et al., [12, 13]). However, no study has tried to understand the impact of compound attractants on YR carp. Therefore, this study was aimed at evaluating the effect of three compound attractants on the growth, immunity, and intestinal morphology of YR carp. The DMPT, garlic powder, tangerine peel powder, sodium glutamate, yeast powder, and betaine were selected in this study. Based on the preliminary work, three compound attractants were established, including single and compound maze food attractants. Then, a feeding experiment on YR carp was carried out with the chosen compound food attractant. The most suitable compound attractant for YR carp was comprehensively selected based on growth indexes, blood biochemistry, antioxidation, immunity, and intestinal tissue morphology.

2. Material and Methods

2.1. Trial Design and Diets. Our previous research used the maze feeding induction test to study the feeding induction and application effect of compound attractants. Through the comparative difference analysis of a variety of single and compound attractants, the three best compound attractant ratios were selected in this study. The growth test was divided into five treatment groups. Group I had the fishmeal diet (positive control, PC), and group II had a plant protein (composed of soybean meal, rapeseed meal, cottonseed meal, and peanut meal) diet (negative control, NC). Compound attractants 1 (0.06%dimethyl – beta – propiothetin (DMPT) + 0.22%tangerine peel powder + 0.75%yeast powder), 2 (0.05%garlic powder + 0.06%DMPT + 0.75%yeast powder), and 3 (0.07%sodium glutamate + 0.22%tangerine peel powder + 0.34%betaine) were added to the basic diet of group II and named as groups III, IV, and V, respectively. Table 1 depicts the formula and chemical composition of the test diet. The feed ingredients were crushed and passed through a 300 μM sieve. The pellet feed was manufactured using a twin-screw extruder and dried naturally.

2.2. Test Fish and Feeding. The Yellow River carp purchased from a breeding farm were acclimatized for 14 days. Eight hundred healthy fishes (initial average weight 12.97 ± 0.13 g) were randomly selected and kept in 20 fish tanks (400 L). They were divided into five treatment groups and four repetitions leading to 40 fishes in each tank. After each feeding, the residual food was collected using a fishing net within 30 min, then dried and recorded. In addition, the temperature, pH value, and dissolved oxygen of circulating water were recorded every week. During the test, the water temperature was 25.5 ± 3.0°C, the pH value was 7.4 ± 0.15, and the DO content was about 6.5 mg/L.

2.3. Sample Collection. After the feeding test, five fishes were randomly selected from each tank and refrigerated at -20°C for the fish body composition assay. Five more fishes were taken out from each tank and anesthetized with MS-222 (120 mg/L) for the blood index assay. Blood samples were drawn from the caudal vein with a sterile syringe, then centrifuged at 4000 g for 10 min at 4°C. The serum was taken out and frozen at -80° C. The protein, lipid, and ash contents in feeds, test diets, and fish bodies were determined following the AOAC [14]. The contents of total protein, blood glucose, total cholesterol, triglyceride, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities in the serum were determined using the colorimetric enzymatic method.

2.4. Immune Index Analysis. The lysozyme (LZM) activity was measured using turbidimetry. The freeze-dried lyophilized *Micrococcus lysodeikticus* (0.3 mg/mL) was used as the LZM substrate in 0.05 M sodium phosphate buffer (pH 6.2). The test serum (diluent 1 : 2, 10 μL) was then added to a 200 μL bacterial suspension, and the absorbance recorded at 450 nm was tested after 0.5 and 4.5 min. The unit of LZM activity is the number of enzymes that cause a 0.001 reduction in absorbance per minute. The acid phosphatase (ACP) and alkaline phosphatase (AKP) activities were detected using the test kits. The unit definition of ACP and AKP activities corresponds to 1 mg phenol (mol) production when a 100 mL supernatant sample interacts with the matrix at 37° C for 30 min. The complement 3 (C3) activity was determined based on Ma et al. [15] with a C3 ELISA test kit. The concentration of C3 was expressed as μg per mL. The immunoglobulin M (IgM) in the serum and the specific IgM antibody in the reagent develop an antigen-antibody complex to produce turbidity. The turbidity is directly proportional to IgM in the serum based on a certain amount of antibodies. The IgM content in the serum was calculated by measuring the absorbance value at 340 nm and referring to the calibration curve.

2.5. Antioxidant Index Test. The activities of enzymes such as superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in the serum, liver, and gill tissue were determined with test kits. Xanthine and xanthine oxidase produced superoxide free radicals, which interacted with INT chloride to form red formazan dye.

Table 1: Formulation and proximate chemical composition of test diets (%).

	Group I	Group II	Group III	Group IV	Group V
Composition (%)					
Wheat	29.6	17.2	17.2	17.2	17.2
Fish meal	14.0				
Soybean meal	14.0	22.0	22.0	22.0	22.0
Rapeseed meal	12.0	18.0	18.0	18.0	18.0
Cottonseed meal	12.0	17.0	17.0	17.0	17.0
Peanut meal	12.0	17.0	17.0	17.0	17.0
Soybean oil	3.6	5.6	5.6	5.6	5.6
Calcium hydrogen phosphate	2.0	2.0	2.0	2.0	2.0
Choline chloride (50%)	0.3	0.3	0.3	0.3	0.3
Vitamin premix[1]	0.1	0.1	0.1	0.1	0.1
Mineral premix[2]	0.1	0.1	0.1	0.1	0.1
Sodium chloride	0.3	0.3	0.3	0.3	0.3
L-lysine (98%)	0	0.3	0.3	0.3	0.3
DL-methionine (98%)	0	0.14	0.14	0.14	0.14
Compound attractant 1			1.03		
Compound attractant 2				0.86	
Compound attractant 3					0.65
Proximate chemical composition of trial diets					
Crude protein (%)	34.59	34.59	34.58	34.58	34.59
Crude lipid (%)	6.52	6.91	6.91	6.91	6.91
Gross energy (MJ/kg)	17.62	17.76	17.76	17.76	17.76
Lysine	1.78	1.81	1.81	1.81	1.81
Methionine	0.62	0.63	0.63	0.63	0.63
Tryptophan	0.41	0.42	0.42	0.42	0.42
Histidine	0.87	0.86	0.86	0.86	0.86
Arginine	2.68	2.94	2.94	2.94	2.94
Isoleucine	1.33	1.27	1.27	1.27	1.27
Leucine	2.32	2.20	2.20	2.20	2.20
Threonine	1.23	1.16	1.16	1.16	1.16
Phenylalanine	1.57	1.61	1.61	1.61	1.61
Valine	1.59	1.52	1.52	1.52	1.52

Note: groups I, II, III, IV, and V represent positive control, negative control, compound attractant 1, compound attractant 2, and compound attractant 3, respectively. The following table is the same. [1]Vitamin premix (mg kg^{-1} diet): retinol acetate, 30 mg; cholecalciferol, 5 mg; alpha-tocopherol, 60 mg; ascorbic acid, 600 mg; vitamin K_3, 7 mg; thiamin, 20 mg; riboflavin, 20 mg; pyridoxine HCL, 12 mg; vitamin B_{12}, 0.05 mg; inositol, 100 mg; pantothenic acid, 50 mg; niacin acid, 35 mg; folic acid, 8 mg; and biotin, 0.06 mg. [2]Mineral premix (mg or g/kg diet): KI (1%), 60 mg; $CoCl_2 \cdot 6H_2O$ (1%), 7 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $FeSO_4 \cdot H_2O$, 300 mg; $ZnSO_4 \cdot H_2O$, 200 mg; $MnSO_4 \cdot H_2O$, 60 mg; $Na_2SeO_3 \cdot 5H_2O$ (1%), 60 mg; $MgSO_4 \cdot 7H_2O$, 2600 mg.

The superoxide dismutase activity was determined. A unit of SOD activity is defined as the amount of enzyme that leads to 50% inhibition of color formation at 550 nm. A test kit was used to analyze the CAT activity, which was defined as decomposing the dose of 1 μmol H_2O_2 per second in per mL of coelomic fluid supernatants. The T-AOC activity was determined by measuring the reduction of Fe^{3+} to Fe^{2+} by Benzie and Strain ([16]). Each unit is defined as the amount of enzyme increasing the absorbance by 0.01 per minute. The GSH-Px activity in the serum or tissue sample supernatant was determined following Subramanian et al. [17]. One unit of GSH-Px activity is the amount of enzyme that reduces the glutathione content in the reaction system at 1 mmol per liter per minute. The MDA content was analyzed by thiobarbituric acid test and determined based on the molar extinction coefficient of the red pigment.

2.6. Determination of Intestinal Tissue Morphology

2.6.1. Preparation of Intestinal Tissue Sections. After the feeding experiment, eight fishes (2 in each repetition) were randomly selected from each group for dissection. The anterior, middle, and posterior intestines were washed with deionized water and fixed using 4% paraformaldehyde. The tissue samples were dehydrated and embedded in paraffin. After that, thin slices of the tissue were cut (with a thickness of 5 μm), stained with hematoxylin-eosin, then sealed, observed, and photographed under an optical microscope.

2.6.2. Measurement of Intestinal Tissue Sections. A photographic microscope was used to capture the target area of the tissue 100 times. After imaging, the Image-Pro Plus 6.0 software measured the plica height and width and muscle layer thickness with mm as the standard unit. We measured the height and width of the 10 longest intestinal folds and the thickness of the thickest muscle layer in each section. Plica is a protrusion formed from mucosa and submucosa on the cavity surface. The plica height is the vertical distance from the base of the submucosa to the highest protrusion point. The fold width is the transverse width of the protrusion developed from the mucosa and submucosa on the cavity surface. The muscular layer thickness is the thickness of the circular and longitudinal muscular layer of the intestine. The vertical distance from the base of the submucosa to the serosa is the muscular layer thickness.

2.7. Statistical Analysis. One-way ANOVA was used to analyze all the test data. All the data were presented as means $\pm$ SD ($n = 4$), and the SPSS 22.0 software was used to undergo statistical analyses.

3. Results

3.1. Growth Index. The growth and body mass index are shown in Table 2. The most significant growth was observed in the fishmeal diet ($P < 0.05$). Three different compound attractants significantly elevated the weight gain rate, special growth rate, and PER of YR carp fed the plant protein diet ($P < 0.05$), and group III had the best results. No significant difference was observed in the condition factor of groups III, IV, and V. However, the condition factor of group III was significantly higher than that of the negative control by 17.4% ($P < 0.05$). The lowest hepatopancreatic index was shown in the fish-fed fishmeal diet ($P < 0.05$). The hepatopancreatic indexes of groups III, IV, and V was significantly lower than those of the negative control by 15.2%, 14.2%, and 12.2%, respectively ($P < 0.05$).

3.2. Whole Fish Body Composition. The whole fish body composition of YR carp is shown in Table 3. The highest average crude protein content was revealed in the fish-fed fishmeal basal diet ($P < 0.05$). The crude protein content of group III was significantly higher than that of the plant protein diet group ($P < 0.05$). However, no significant difference was shown among groups III, IV, and V. No significant difference was also observed in the content of moisture, ash, and crude fat in all the groups ($P < 0.05$).

3.3. Biochemical Parameters. The serum biochemical indexes of YR carp are represented in Table 4. The highest content of serum triglyceride (TG) was observed in the fish-fed fishmeal diet ($P < 0.05$), and the lowest TG was in the negative control. Moreover, the TG contents in groups III, IV, and V were 13.84%, 20.54%, and 21.43% higher, respectively, than those in the fish-fed plant protein diet. On the other hand, the lowest ALT activity was seen in the fish-fed fishmeal diet ($P < 0.05$), and the ALT activity in groups III, IV, and V was significantly lower than that in the negative control. No significant difference was observed in other serum indexes among all the groups ($P > 0.05$).

3.4. Immune Parameters

3.4.1. Immune Parameters in Serum. The serum immune indexes of YR carp are depicted in Table 5. The highest serum LZM and ACP contents were observed in the fish-fed fishmeal diet ($P < 0.05$). Furthermore, serum LZM and ACP contents in group III were significantly higher than those in the plant protein diet group ($P < 0.05$). On the other hand, no significant differences were observed in the contents of AKP, C3, and IgM among all the groups ($P > 0.05$).

3.4.2. Immune Parameters in Liver and Gill. The immune indexes of liver and gill tissue of YR carp are represented in Table 6. The highest LZM activity in the liver and gill was observed in the fish-fed fishmeal diet ($P < 0.05$), and the LZM activity in groups III, IV, and V was significantly higher than that in the plant protein diet group ($P < 0.05$). On the other hand, no significant differences were noticed in the ACP and AKP activities among all the groups ($P > 0.05$).

3.5. Antioxidant Parameters

3.5.1. Antioxidant Parameters in Serum. The serum antioxidant indexes of YR carp are depicted in Table 7. Significant differences were observed in serum SOD, CAT, and T-AOC activities among all the groups ($P < 0.05$). The highest SOD, CAT, and T-AOC activities were observed in the fish-fed fishmeal diet ($P < 0.05$). Moreover, SOD, CAT, and T-AOC activities in groups III, IV, and V were significantly higher than those in the plant protein diet group ($P < 0.05$). Conversely, the lowest MDA content was observed in the fish-fed fishmeal diet ($P < 0.05$), and the MDA content of groups III, IV, and V was significantly lower than that of the plant protein diet group ($P < 0.05$).

3.5.2. Antioxidant Parameters in Liver and Gill. The antioxidant indexes of the liver and gill are shown in Table 8. The highest SOD, CAT, GSH-Px, and T-AOC activities were observed in the fish-fed fishmeal diet ($P < 0.05$). Moreover, SOD, CAT, GSH-Px, and T-AOC activities in groups III, IV, and V were significantly higher than those in the plant protein diet group ($P < 0.05$). On the other hand, the lowest MDA content of the liver was observed in the fish-fed fishmeal diet, and the MDA contents in groups III, IV, and V were lower than those in the plant protein diet group ($P > 0.05$). For the gill tissue, the highest SOD activity was observed in the fish-fed fishmeal diet ($P < 0.05$), and the SOD activity of groups III, IV, and V was significantly higher than that of the plant protein diet group ($P < 0.05$).

3.6. Intestinal Morphology

3.6.1. Electron Micrograph of Intestinal Section. As shown in Figure 1, the intestinal mucosal folds of the fish meal diet group (Figure 1(a)) are complete, with high and closely arranged folds, having a large area, and a smooth striated edge on the surface. In the plant protein diet group

Table 2: The effects of compound attractants on the growth and body mass index of YR carp.

Items	Group I	Group II	Group III	Group IV	Group V
IBW (g)	13.01 ± 0.14	12.84 ± 0.12	12.94 ± 0.16	13.09 ± 0.16	13.02 ± 0.20
FBW (g)	60.03 ± 2.58^{a}	45.57 ± 1.92^{c}	51.84 ± 2.45^{b}	52.63 ± 2.75^{b}	52.78 ± 2.60^{b}
FCR	1.12 ± 0.04^{c}	1.49 ± 0.05^{a}	1.29 ± 0.08^{b}	1.38 ± 0.08^{b}	1.37 ± 0.06^{b}
WGR (%)	362.22 ± 19.77^{a}	263.98 ± 9.25^{c}	328.54 ± 8.27^{b}	316.81 ± 2.20^{b}	300.63 ± 4.68^{b}
SGR (%/d)	3.02 ± 0.02^{a}	2.57 ± 0.03^{c}	2.86 ± 0.11^{b}	2.83 ± 0.09^{b}	2.80 ± 0.11^{b}
PER	2.58 ± 0.12^{a}	1.94 ± 0.09^{c}	2.24 ± 0.11^{b}	2.09 ± 0.08^{bc}	2.11 ± 0.08^{bc}
CF (%)	1.73 ± 0.03^{a}	1.32 ± 0.06^{c}	1.55 ± 0.10^{b}	1.51 ± 0.08^{b}	1.52 ± 0.10^{b}
VSI (%)	9.68 ± 0.53	11.24 ± 0.22	10.37 ± 0.40	10.63 ± 0.13	10.75 ± 0.52
HI (%)	1.56 ± 0.06^{c}	1.97 ± 0.09^{a}	1.67 ± 0.12^{b}	1.69 ± 0.03^{b}	1.73 ± 0.14^{b}
Sur (%)	99.00 ± 1.01	99.00 ± 0.90	99.00 ± 1.00	99.00 ± 1.00	99.00 ± 0.89

Data represents means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments. IBW: initial body weight (g); FBW: final body weight (g); FCR: feed conversion ratio = feed intake/(final body weight − initial body weight); WGR: percent weight gain rate (%) = (final body weight − initial body weight) × 100/initial body weight; SGR: specific growth rate (%day^{-1}) = 100 × [(Ln (final body weight) − Ln (initial body weight))/duration (50 days)]; PER: protein efficiency ratio = live weight gain (g)/dry protein intake (g); CF: condition factor (%) = 100 × [body weight of fish (g)/length of fish (cm)^{3}]; VSI: viscera index (%) = 100 × viscera weight (g)/fish weight (g); HI: hepatopancreatic index (%) = 100 × hepatopancreatic weight (g)/fish weight (g); Sur: survival (%) = 100 × (final no.of fish/initial no.of fish).

Table 3: Whole body proximate analysis (% wet basis) in the YR carp fed test diets for 50 days.

Items	Group I	Group II	Group III	Group IV	Group V
Moisture	72.10 ± 3.03	72.46 ± 2.15	72.41 ± 1.40	71.97 ± 1.47	72.96 ± 1.36
Ash	4.23 ± 0.35	4.3 ± 0.20	4.22 ± 0.44	3.98 ± 0.24	4.04 ± 0.08
Crude protein	14.85 ± 0.51^{a}	13.15 ± 0.64^{b}	14.41 ± 0.61^{a}	13.98 ± 0.62^{ab}	13.95 ± 0.53^{ab}
Crude lipid	8.08 ± 0.56	7.38 ± 0.59	7.48 ± 0.85	7.12 ± 0.47	7.24 ± 0.46

Data represents means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 4: The effects of compound attractants on the biochemical indexes of YR carp.

Items	Group I	Group II	Group III	Group IV	Group V
GLU (mmol/L)	5.02 ± 1.29	4.63 ± 0.34	4.72 ± 0.24	4.77 ± 0.20	4.75 ± 0.46
ALB (g/L)	14.58 ± 0.84	15.89 ± 0.56	14.83 ± 0.99	14.57 ± 1.05	14.99 ± 0.91
GLB (g/L)	14.02 ± 1.02	15.63 ± 1.08	14.47 ± 2.09	13.87 ± 1.73	13.83 ± 0.10
TG (mmol/L)	3.26 ± 0.21^{a}	2.24 ± 0.68^{c}	2.55 ± 0.52^{bc}	2.7 ± 0.47^{b}	2.72 ± 0.15^{b}
TC (mmol/L)	4.38 ± 0.65	4.28 ± 0.22	4.25 ± 0.56	4.31 ± 1.11	4.27 ± 0.66
AST (U/L)	29.83 ± 4.59	30.34 ± 4.89	32.59 ± 4.77	32.76 ± 5.63	32.74 ± 6.88
ALT (U/L)	391.59 ± 13.15^{c}	730.26 ± 16.96^{a}	522.29 ± 7.39^{b}	550.71 ± 19.76^{b}	557.74 ± 6.64^{b}

Data represents means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 5: The effects of compound attractants on the immune indexes of YR carp.

Items	Group I	Group II	Group III	Group IV	Group V
LZM (μg/mL)	164.35 ± 0.09^{a}	133.12 ± 0.41^{c}	148.57 ± 0.07^{b}	143.71 ± 0.57^{bc}	141.83 ± 0.26^{bc}
ACP (U/100 mL)	5.79 ± 0.74^{a}	4.28 ± 0.91^{c}	4.80 ± 0.63^{b}	4.42 ± 0.63^{bc}	4.47 ± 1.85^{bc}
AKP (U/100 mL)	7.10 ± 0.03	6.72 ± 0.14	7.06 ± 0.09	7.14 ± 0.10	7.03 ± 0.04
C3 (μg/mL)	60.05 ± 1.34	52.93 ± 0.26	58.36 ± 1.68	55.24 ± 1.73	56.46 ± 2.04
IgM (mg/L)	25.80 ± 0.89	25.82 ± 1.61	24.15 ± 2.69	24.54 ± 1.27	25.92 ± 1.46

Data represents means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 6: The effects of compound attractants on the liver and gill immune indexes of YR carp.

Items	Tissue	Group I	Group II	Group III	Group IV	Group V
LZM (μg/mg)	Liver	17.58 ± 0.59^{a}	15.21 ± 0.27^{c}	16.75 ± 0.14^{b}	16.69 ± 0.26^{b}	16.54 ± 0.07^{b}
	Gill	18.47 ± 1.92^{a}	14.42 ± 1.03^{c}	16.81 ± 1.21^{b}	16.20 ± 0.94^{b}	16.47 ± 1.07^{b}
ACP (U/100 mg)	Liver	0.37 ± 0.09	0.30 ± 0.10	0.34 ± 0.02	0.34 ± 0.06	0.35 ± 0.12
	Gill	0.21 ± 0.03	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.01
AKP (U/100 mg)	Liver	2.56 ± 0.23	2.30 ± 0.27	2.35 ± 0.13	2.36 ± 0.28	2.34 ± 0.24
	Gill	0.17 ± 0.04	0.13 ± 0.02	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.01

Data represents means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 7: The effects of compound attractants on the blood antioxidant indexes of YR carp.

Items	Group I	Group II	Group III	Group IV	Group V
SOD (U/mL)	145.50 ± 8.99^{a}	103.26 ± 9.54^{c}	123.62 ± 8.89^{b}	118.75 ± 7.29^{b}	119.69 ± 5.23^{b}
CAT (U/mL)	8.09 ± 0.17^{a}	5.47 ± 0.61^{c}	6.74 ± 1.09^{b}	6.49 ± 1.45^{b}	6.56 ± 1.30^{b}
GSH (μmol/mL)	197.60 ± 9.43	196.19 ± 1.95	196.08 ± 4.20	194.11 ± 5.32	195.73 ± 3.90
T-AOC (mmol)	0.84 ± 0.04^{a}	0.63 ± 0.04^{c}	0.73 ± 0.04^{b}	0.69 ± 0.05^{b}	0.71 ± 0.05^{b}
MDA (nmol/mL)	4.15 ± 0.67^{c}	6.76 ± 0.36^{a}	5.31 ± 0.37^{b}	5.60 ± 1.05^{b}	5.47 ± 0.62^{b}

Data represent means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

Table 8: The effects of compound attractants on the liver and gill antioxidant indexes of YR carp.

Items	Tissue	Group I	Group II	Group III	Group IV	Group V
SOD (U/mg)	Liver	69.92 ± 1.65^{a}	44.56 ± 1.64^{c}	53.81 ± 2.71^{b}	50.42 ± 4.26^{b}	52.38 ± 3.52^{b}
	Gill	7.71 ± 0.30^{a}	5.32 ± 0.13^{c}	6.24 ± 1.20^{b}	6.12 ± 0.41^{b}	6.16 ± 1.02^{b}
CAT (U/mg)	Liver	7.41 ± 0.77^{a}	5.05 ± 0.02^{c}	6.27 ± 0.03^{b}	6.22 ± 0.04^{b}	6.03 ± 0.04^{b}
	Gill	3.62 ± 0.48	3.47 ± 0.33	3.51 ± 0.15	3.56 ± 0.74	3.54 ± 0.21
GSH (μmol/mg)	Liver	202.01 ± 6.10^{a}	165.58 ± 1.79^{c}	181.60 ± 3.21^{b}	177.25 ± 3.73^{b}	178.80 ± 1.20^{b}
	Gill	585.07 ± 94.26	592.33 ± 46.51	587.37 ± 37.71	602.24 ± 52.61	598.40 ± 67.14
T-AOC (mmol)	Liver	2.86 ± 0.09^{a}	2.21 ± 0.04^{c}	2.53 ± 0.07^{b}	2.33 ± 0.10^{bc}	2.43 ± 0.09^{b}
	Gill	2.30 ± 0.23	2.07 ± 0.14	2.19 ± 0.04	2.21 ± 1.62	2.19 ± 0.37
MDA (nmol/mg)	Liver	0.43 ± 0.04^{b}	0.66 ± 0.04^{a}	0.51 ± 0.01^{ab}	0.54 ± 0.03^{ab}	0.53 ± 0.03^{ab}
	Gill	0.07 ± 0.03	0.11 ± 0.11	0.09 ± 0.01	0.08 ± 0.02	0.09 ± 0.01

Data represent means ± SD ($n = 4$). Values with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments.

(Figure 1(b)), intestinal villi were sparse and short, damaged, and shed. The intestinal morphology of groups III (Figure 1(c)), IV (Figure 1(d)) and V (Figure 1(e)) was between the positive and the negative control group. A small amount of relatively close fracture was there in group III. The tight density of the plica in group IV was lower than that in group III, and plica fracture occurred. There was fold dissolution in group V, and the arrangement of folds was tighter than in group IV.

3.6.2. Height of Intestinal Fold. The intestinal fold height of YR carp is shown in Figure 2. The highest plica height in the anterior and middle intestines was observed in the fish-fed fishmeal diet ($P < 0.05$), and the plica heights in groups III and IV were significantly higher than those in the negative control ($P < 0.05$). In the posterior intestine, the highest plica height was observed in group V. No significant difference was observed in groups I and III ($P > 0.05$). However, they were significantly higher than those in the negative control ($P < 0.05$).

3.6.3. Width of Intestinal Fold. The width of the intestinal fold is shown in Figure 3. The highest plica width in the anterior and middle intestines was observed in group III ($P < 0.05$), the highest intestinal fold width in the posterior intestine was shown in the fish-fed fishmeal diet ($P < 0.05$), and no significant differences were observed among groups II, III, and IV ($P > 0.05$). However, they were significantly higher than those in group V ($P < 0.05$).

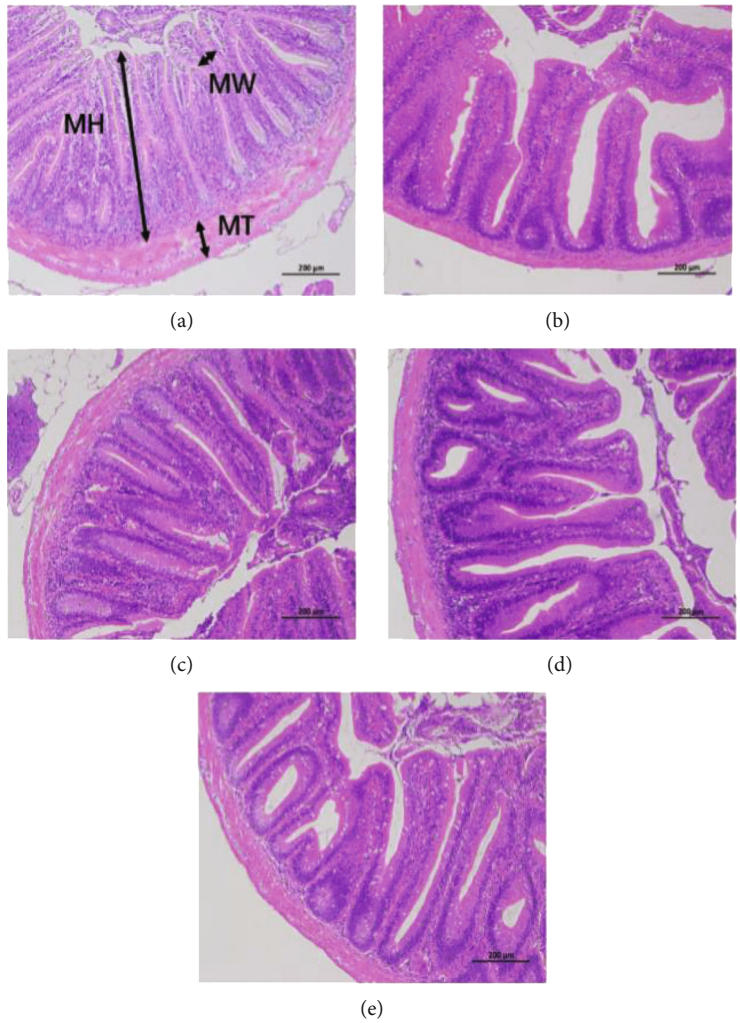

Figure 1: Electron micrograph of the intestinal section of YR carp. (a) The foregut intestinal tissues of group I; (b) the foregut intestinal tissues of group II; (c) the foregut intestinal tissues of group III; (d) the foregut intestinal tissues of group IV; (e) the foregut intestinal tissues of group V. MH: crease height; MT: crease width; MW: muscle layer thickness.

3.6.4. Thickness of Intestinal Muscle Layer. The thickness of the intestinal muscle layer is shown in Figure 4. In the anterior intestine, no significant difference was observed in the thickness of the intestinal muscle layer among all the groups ($P > 0.05$). However, the muscle layer thickness in group III was higher than that in the negative control. In the middle intestine, the highest thickness of the muscle layer was observed in the fish-fed fishmeal diet ($P < 0.05$), and the thickness of the muscle layer in groups III and V was significantly higher than that in the negative control ($P < 0.05$). Finally, in the posterior intestine, the highest thickness of the muscle layer was observed in the fish-fed fishmeal diet ($P < 0.05$).

4. Discussion

Total or partial replacement of fishmeal with plant proteins can reduce the appetite of fish, leading to growth inhibition and histopathological changes. Thus, attractants are used [18] to overcome these problems. This study indicated that adding three distinct kinds of compound attractants to the plant protein diet elevated feed intake and conversion efficiency to improve the growth performance of the YR carp. Ma [19] demonstrated that a 0.5% mixture of disodium 5′-inosinate and sodium glutamate (at ratio 1 : 7) in a fishmeal-free diet could promote feeding behavior and growth performance of juvenile turbot. Chen et al. [20] found that adding 1.0% compound attractant (including betaine, DMPT, and sodium glutamate) to the soybean meal basic diet improved the feeding rate and specific growth rate of *Paralichthys olivaceus*, and no significant difference was observed with the whole fish meal group. Li et al. [21] found that the addition of compound attractants composed of nucleotides, betaine, amino acids, and taurine in the diet significantly increased the weight gain rate and food intake of *Monopterus albus*, and the feed coefficient decreased significantly consistent with the results of this research.

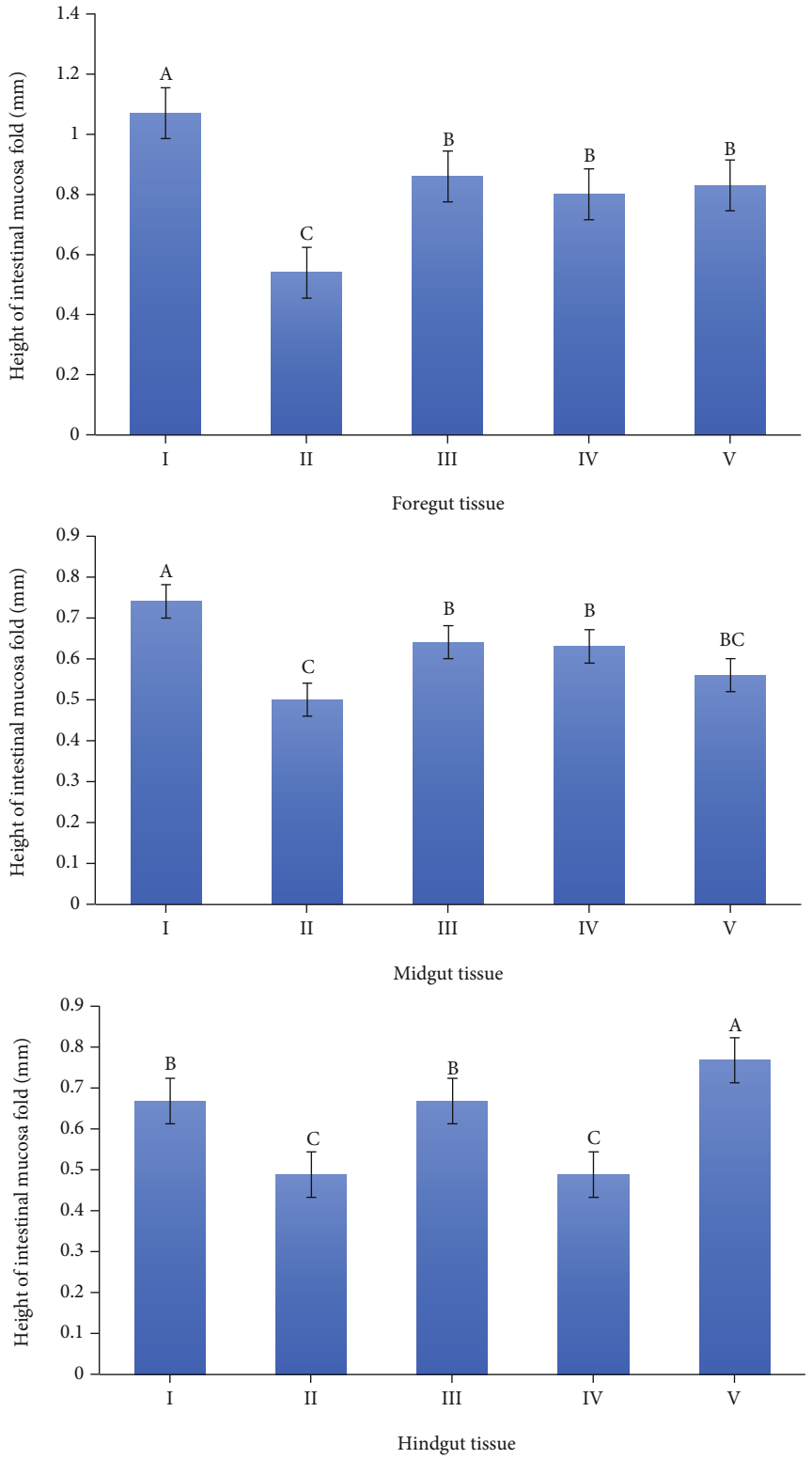

Figure 2: The effects of compound attractants on the height of the intestine fold of YR carp. The value columns with different small letters mean significant difference ($P < 0.05$).

This paper added three different compound attractants to the plant protein diet. Among them, the specific growth rate and weight gain rate of group III (including 0.06% DMPT, 0.22% tangerine peel powder, and 0.75% yeast powder) were higher than those of the other two compound attractants. The main reason for the difference is that compound attractant III has a better effect than that of the other two attractants. Moreover, the effect is related to the

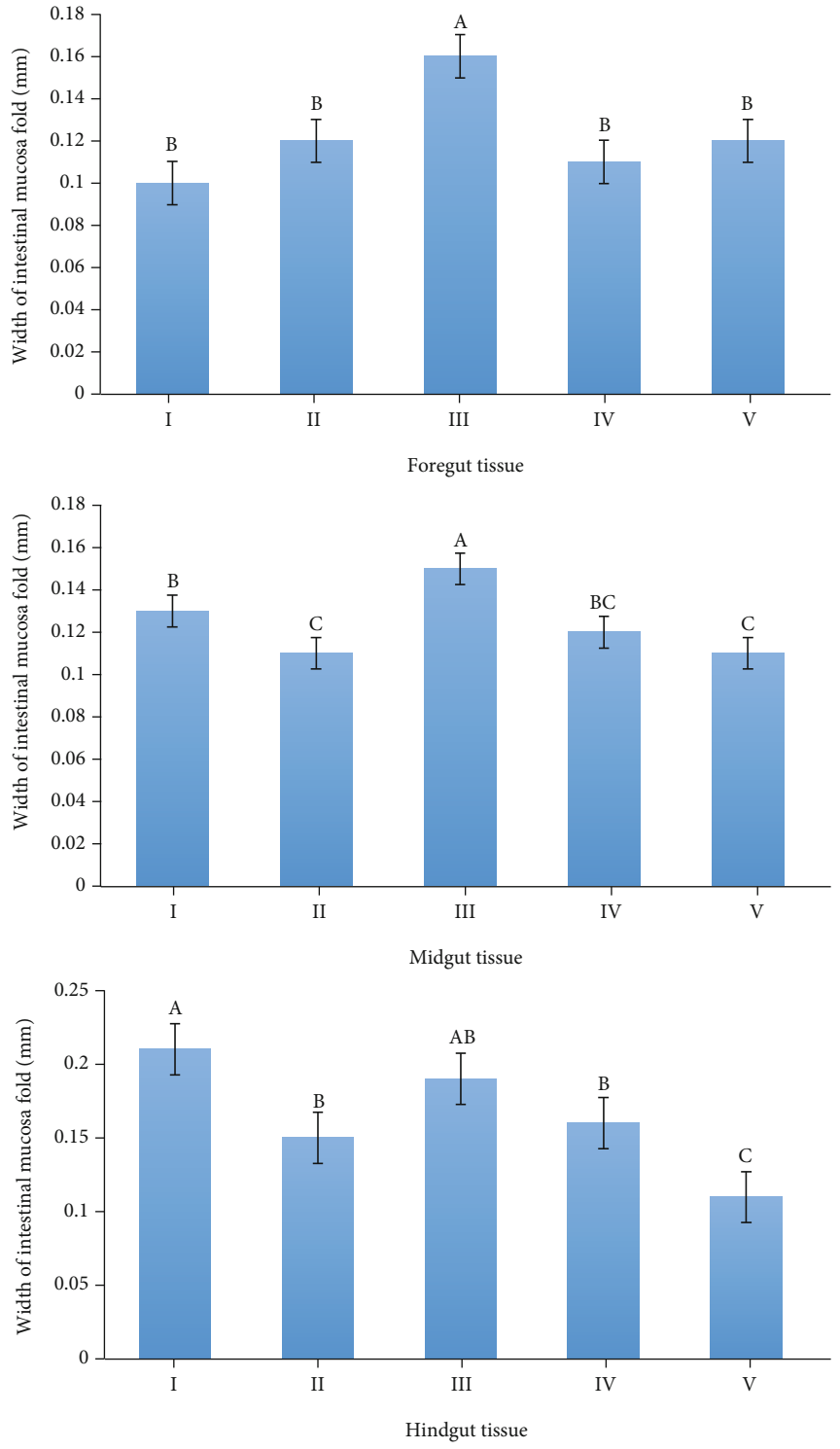

Figure 3: The effects of compound attractants on the width of the intestine fold of YR carp. The value columns with different small letters mean significant difference ($P < 0.05$).

composition and content of the compound attractant. Li et al. [22] found that DMPT has a significant feeding promoting behavior over *Allogynogenetic crucian* carp and tilapia. Zhao et al. [23] also depicted that 0.30 g/kg DMPT can enhance the feeding rate of *Penaeus vannamei*, thus significantly elevating the weight gain rate. Chen et al. [24] identified six Chinese herbal medicines, such as licorice and tangerine peel, that can significantly improve the specific growth rate of crucian carp. Therefore, in this study, group III performs well, indicating that the ratio of the compound attractant is suitable for the feeding and growth of YR carp. In the body indexes, the condition factor of YR carp in

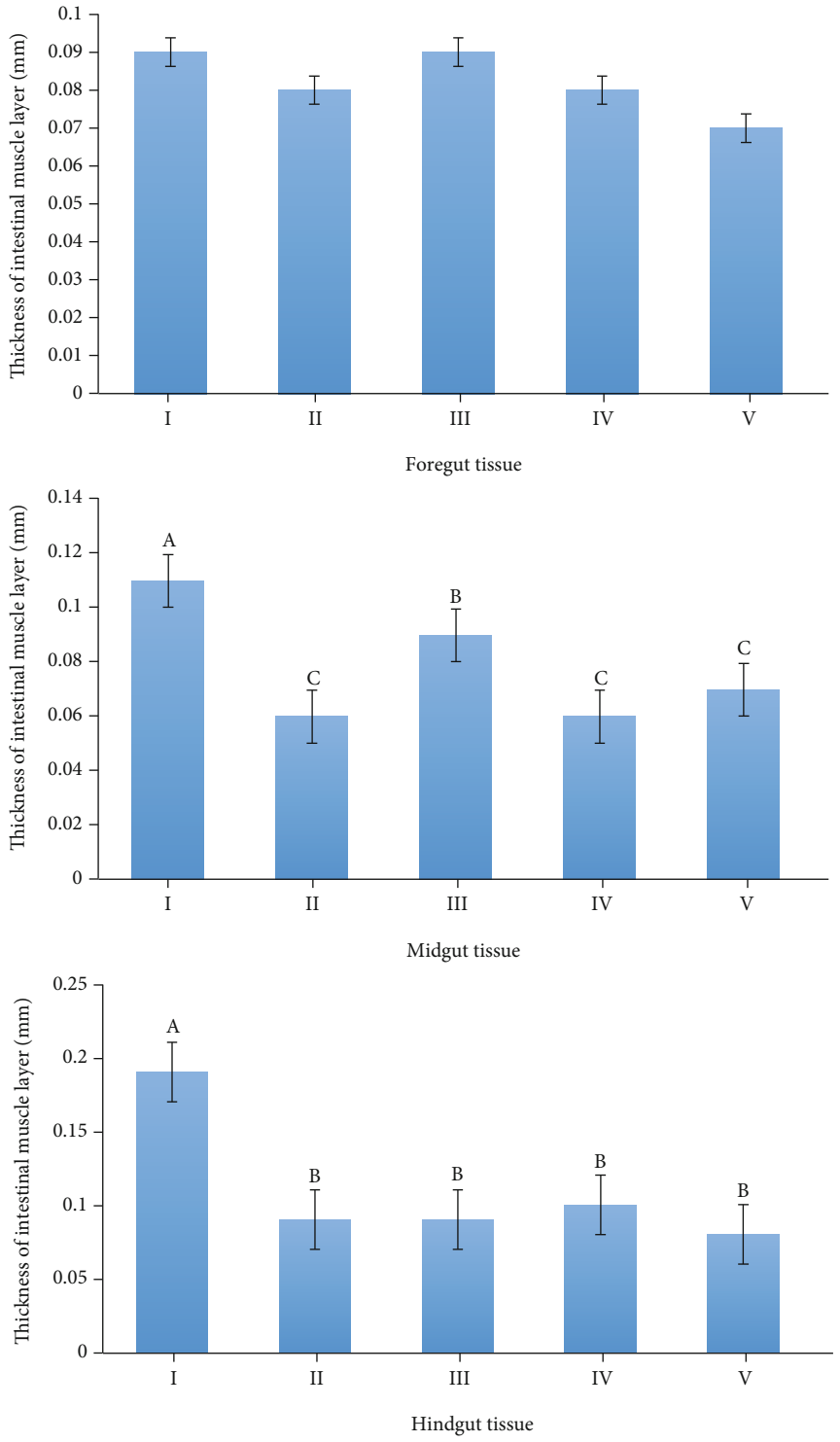

Figure 4: The effects of compound attractants on the thickness of the foregut muscular layer of YR carp. The value columns with different small letters mean significant difference ($P < 0.05$).

groups III, IV, and V was higher than that of fish fed the plant protein diet, indicating that adding compound attractants improves the condition factor of YR carp. Li [25] found that compound attractants (including allicin, DMPT, and TMAO) could significantly enhance the condition factor of GIFT tilapia, which is consistent with our findings. The hepatopancreas index and the visceral ratio primarily reflect the health status of fish. The metabolism in the body of fish will change when its health is relatively poor. As a result, the hepatopancreas is enlarged, and a large amount of fat is

enriched around the hepatopancreas and viscera of the fish, elevating the visceral ratio. The shift in the hepatopancreas index is also pronounced due to the slow change of fish weight [26]. These results indicated that the hepatopancreatic index and visceral body ratio of YR carp in groups III, IV, and V were lower than those in the plant protein diet group. Therefore, adding compound attractants can reduce liver damage caused by plant protein metabolism in the diet. Compared with the negative control group, adding a 0.2% compound attractant (including allicin, DMPT, and TMAO) to the diet can significantly reduce the hepatopancreatic index and the visceral body ratio of GIFT tilapia to improve its health [25]. The components of DMPT, tangerine peel, and garlic powder in the compound attractant playing a positive role could have led to the reduction. As a type of Chinese herbal medicine, the tangerine peel contains nutritional components and functions such as antibacterial and antipathogenic microorganisms. The tangerine peel stores many bioactive substances, enhancing the humoral and cellular immunity in fishes [24]. Allicin in garlic powder has a good antibacterial and anti-inflammatory effect. Studies have shown that the liver protection function of DMPT can improve the health status of animals and reduce the visceral ratio [25]. Thus, adding compound attractants can positively improve fish health, enhance liver protection, and enhance the growth performance of YR carp.

The results of whole fish body composition showed that adding compound attractant 1 in a plant protein diet could significantly elevate the crude protein content of YR fish. Studies have depicted that adding compound attractants to the diet reduced the crude fat content and enhanced the crude protein content of tilapia [25]. Some studies have also shown that compound attractant does not significantly influence the crude protein content in the soybean meal-based diet of *Paralichthys olivaceus*. However, the content is also significantly lower in the fish-fed fishmeal diet [20]. Similar results were observed in the study of loach. Wang [27] found that adding a compound attractant to loach feed can significantly improve its tissue protein content. The possible reason is that part of the fish feed energy is supplied for life activities, mainly transformed into body tissue. The remaining nutrients are utilized for fish growth and are primarily stored as protein and fat in the body. Therefore, we should improve the growth performance of cultured fish and consider how to increase the content of nutrients such as protein in fish to enhance the economic value of aquaculture animals continuously.

Blood glucose is a critical factor in regulating the life activities of animals providing a dynamic balance of energy in tissues and cells. The results showed no significant difference in blood glucose content among all the groups, indicating that adding compound attractants did not significantly affect glucose metabolism levels of fish. The serum protein level is a standard index for evaluating the nutritional status of fish. Protein metabolism is primarily carried out in the liver. Liver damage leads to the decline of protein metabolism. Therefore, the decrease in serum protein content also indicates the degree of liver damage (Prelusky et al., [28]). The serum protein content changes when fishes suffer from hunger, malnutrition, liver injury, or other diseases. Albumin is related to both nutrition and inflammation. An increase in serum albumin levels reflects the existence of systemic inflammation in animals [29]. These results depicted that the highest serum albumin content was demonstrated in fish fed a plant protein diet, indicating no severe inflammatory reaction of YR carp by compound attractant. Serum AST and ALT levels are essential indicators of liver health. The increase of AST and ALT activities in the serum is due to the pathological changes within the liver tissue, increased cell membrane permeability, and entrance of many AST and ALT in the blood. The current study showed that the ALT activity was significantly lower in groups III, IV, and V than in the plant protein diet group. Studies showed no significant difference in the albumin content and AST activity compared with the control group after adding compound attractants (0.04% betaine, 0.02% TMAO, 0.02% DMPT, and 0.02% allicin) to the *Procambarus clarkii* diet. However, it can significantly reduce the serum ALT activity [30]. Chang et al. [31] demonstrated that adding compound attractants (0.02% DMPT, 0.1% sodium glutamate, and 0.1% betaine) did not cause significant differences in the contents of serum albumin, AST, and ALT of *Acipenser schrenckii* than the control group with no adverse effects on its immune system. Our results also depict similar outcomes, which show that adding compound attractants within a specific concentration range will not cause liver function damage of YR carp nor will it lead to a severe imbalance of protein metabolism. Varied test results may be caused by different test animals, attractants formulas, and concentrations.

AKP and ACP are marker enzymes of macrophage lysosomes within the animal immune system. These enzymes can hydrolyze invading pathogens, promote phagocytosis, degrade phagocytes, and play a significant role in immune function [32]. The higher its content, the stronger the immune capability of the animal body. The lysozyme is a nonspecific humoral immune factor widely present among fishes. The lysozyme activity reflects the strength of nonspecific immunity [33]. The complement system in fishes is the first defense mechanism against pathogenic infection, undertaking a significant role from tissue resistance to microbial infection (Wang et al., [27]). The fish will have a specific regulatory ability to adapt to the external environment and nutrient changes, but the regulatory power is limited. The immune indicators in fish will change when it exceeds the regulatory range. The results indicated that the content of serum C3 in groups III, IV, and V was significantly higher than that in the plant protein diet group. Thus, adding compound attractants to the diet improved the immunity of YR carp. Similar results were also shown in the research of feeding attractants on *Monopterus albus*. Li et al. [21] found that adding a 0.1% compound attractant (nucleotide, betaine, amino acid, and taurine) to the feed significantly elevated the concentration of serum C3 and CAT activity of *Monopterus albus*. Therefore, the addition of a compound attractant enhanced the immunity and antioxidant ability of *Monopterus albus*, improved its body health,

and was conducive to the growth of *Monopterus albus*. The current study found that the activities of LZM and ACP in the serum, liver, and gill in groups III, IV, and V were significantly higher than those in the plant protein diet group. It stated that the addition of compound attractants could improve the immune ability of YR carp. Chang et al. [31] observed that adding a compound attractant (0.02% DMPT, 0.1% sodium glutamate, and 0.1% betaine) to sturgeon diet did not change the contents of AKP, ACP, and C3 in the blood, indicating that the compound attractant did not inhibit the immune ability of sturgeon. Cao et al. [34] found that adding 100 mg/kg allicin to the pure feed of *Fugu obscurus* significantly enhanced the activity of lysozyme in the spleen. Thus, the food attractant allicin can improve the nonspecific immunity of fish. The results of this study depicted higher immune indexes of groups III, IV, and V than of the plant protein diet group due to the addition of compound attractants increasing the feeding desire of YR carp. Thus, the food intake is increased, further meeting the nutrients required for the growth and development of the fish. Moreover, DMPT and garlic powder are also immune-stimulating substances, signifying that adding compound attractants to the YR carp diet can reduce the damage caused by the antinutritional factors in plant feed sources.

The antioxidant index of the animal body can directly or indirectly reflect the health state of the animal body. The SOD, CAT, and GSH-PX are widely existing antioxidant enzymes in organisms, effectively removing reactive oxygen free radicals to protect tissues from any damage. MDA is one of the end products of fat oxidation. The body accumulation level reflects the degree of a free radical attack on body cells. These results showed that SOD, CAT, and T-AOC activities in groups III, IV, and V were significantly higher than those in the negative control group. Moreover, the activities of SOD and T-AOC in the liver and SOD in the gill also had similar performance, and the contents of MDA in groups III, IV, and V were lower than those in the plant protein diet group. There are few studies on the above indicators with compound attractants. In studying the antioxidant indexes of aquatic animals with a single attractant, Xu et al. [35] observed that adding different concentrations of yeast culture to the feed can increase the serum SOD activity of *allogynogenetic crucian* carp, significantly reduce the MDA content, and improve the T-AOC activity. The results of this study are consistent with our finding. Xu et al. [36] also depicted that 0.5% or 2.5% garlic powder reduced the serum MDA content of mirror carp and elevated the SOD and LYZ activities in hepatopancreas. The reasons why the addition of compound attractants can enhance the self-immunity of fish body are as follows. The first possible reason for this is that the addition of compound attractants enhances the feeding desire of YR carp, providing sufficient nutrients and enhancing immunity in fishes. The second reason is that DMPT and allicin in the compound attractant have specific antioxidant capacities, putting the body in a better physiological state with improved metabolic functions, thus promoting various enzyme activities [37]. The addition of a compound attractant can encourage YR carp feeding, increase body metabolism, and promote enzyme reaction syntheses. As a result, free radicals and metabolic waste are eliminated from the body, the vitality of phagocytes is enhanced, and the antioxidant capacity of the body is improved.

The intestines of most fish undertake the essential functions of digestion and absorption because only a few carnivorous fish have stomachs, and their intestinal health is closely related to immunity. Therefore, the height and width of the plica and the thickness of the intestinal muscle layer are generally used to measure its digestion and absorption capacity when studying the function of the fish intestine. The current study showed that the plica heights of anterior and middle intestines in groups III and IV were significantly higher than those in the plant protein diet group and lower than those in the fishmeal diet group. In addition, the height of anterior, middle, and posterior intestinal folds in group III was the highest among the three groups treated with a compound attractant. Moreover, the width of intestinal folds in the anterior and middle intestines in group III was also the highest among all the groups. Other experiments also had similar results. For example, Wu et al. [38] found that adding 0.5% glutamine dipeptide to the feed elevated the intestinal villus height and mucosal thickness of grass carp larvae, improving the digestion and absorption capacity of nutrients and the integrity of the intestinal mucosal barrier. Furthermore, studies showed that adding 2% arginine to the feed of hybrid striped bass can increase the fold height of the middle and the end of the intestine [39]. The addition of 0.2% and 0.4% glutamine in the feed enhanced the intestinal villus height and the fold height of juvenile *Pelteobagrus fulvidraco* [40].

The addition of a compound attractant can elevate the height and width of intestinal folds of YR carp, increase the functional surface area of the intestinal mucosa, and improve the absorption rate of nutrients to promote fish growth. The possible reasons are as follows: First, plant proteins such as soybean meal and rapeseed meal in the diet affect the feed palatability, and the antinutritional factors cause inflammation and congestion in the intestinal tissue by the reduction of the protein utilization and inhibition of enzyme activities, damaging the digestive system of fish [41]. Second, compound attractants can enhance food intake, elevate nutrient intake, and improve protein utilization rate. The fish can obtain sufficient nutrition and provide material preparation for synthesizing various digestive enzymes in the intestine. Third, the intestine is also the immune organ of the fish. Therefore, food attractants like orange peel and garlic powder have an antibacterial and anti-inflammatory role, improve the immune and antioxidant activities in fish, and reduce the damage of antinutritional factors in the intestine. Among the three compound attractant treatments, the height and width of intestinal folds in group III (0.06%DMPT + 0.22%tangerine peel powder + 0.75%yeast powder) were significantly higher and wider than those in the other two groups. Therefore, the formula composition of compound attractants in this group was significantly more conducive to the intestinal growth and development of YR carp.

5. Conclusion

Although fish meal is still the best ingredient in aquatic animal feed, the number of wild fish is gradually decreasing due to overfishing, pollution, and lack of regulation in the world. Therefore, it is necessary to further encourage the use of plant protein instead of fish meal. In this paper, the specific growth rate and feed utilization efficiency of YR carp could be substantially enhanced by adding compound attractants to the plant protein diet. These compounds elevated the activities of lysozyme and superoxide dismutase in the serum, liver, and gill; increased the activities of serum acid phosphatase and catalase; enhanced the total antioxidant capacity of fish; reduced the malondialdehyde content in the serum, liver, and gill; and improved the immune capacity of YR carp. Furthermore, the compound attractant significantly elevated the height and width of the intestinal fold, indicating that the compound attractant also promotes intestinal digestion and absorption and reduces intestinal injury. Among the three experimental groups, the supplementation of compound attractants combined with 0.06% DMPT, 0.22% tangerine peel powder, and 0.75% yeast powder is more suitable to the plant protein diet of YR carp. It is suggested to further study the effects of different attractants on different aquatic animals. Replacing fish meal with different plant proteins will make a significant contribution to the sustainability of the growing world's aquaculture industry.

Data Availability

The data that support the finding of this study are available within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Tingting Fang and Xiang Li contributed equally to this work.

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