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Effects of Se-chitosan on the growth performance and intestinal health of the loach *Paramisgurnus dabryanus* (Sauvage)

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ABSTRACT

To determine the effects of Se-chitosan on the growth performance and intestinal health of the loach *Paramisgurnus dabryanus*, 450 fish (initial mean weight: 5.0 ± 0.2 g) were randomly allocated to 15 PVC tanks and fed diets containing 0 (group C), 0.6 (group T_1), 1.2 (group T_2), 1.8 (group T_3), or 2.4 (group T_4) mg/kg Se-chitosan for 60 days. No statistically significant differences were found in growth parameters, including final average weight, specific growth rate (SGR), weight gain (WG), and survival rate between the control and experimental groups. Acid phosphatase (ACP), alkaline phosphatase (ALP), and lysozyme (LZM) activity levels were significantly affected (increasing to ~1389 U/mL, 96I U/L, and 744 U/mL, respectively) when > 1.2 mg/kg Se-chitosan was added to the loach diet. The immunoglobulin M (IgM) content increased with increasing levels of Se-chitosan, and was significantly higher in the T_4 group than in the control group. Following 16S rRNA sequencing, 296 operational taxonomic units were identified across the control and T_3 groups. Alpha diversity analysis showed that species richness and diversity increased with increasing levels of Se-chitosan. Se-chitosan supplementation increased the abundance of *Bactroidetes*, *Cyanobacteria*, and *Firmicutes*, while decreasing the abundance of *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*. Thus, Se-chitosan supplementation might enhance the intestinal health of the loach, and it might therefore be useful as an immunostimulator in loach aquaculture.

1. Introduction

The loach, *Paramisgurnus dabryanus* (Cypriniformes: Cobitidae), is endemic to China and is widely distributed throughout the middle and lower reaches of the Yangtze and Zhujiang Rivers, as well as in the inland waters of Taiwan (Dong et al., 2014). As one of the most common edible fishes in China, Korea, and Japan, *P. dabryanus* aquaculture has recently expanded dramatically, especially in China. The production reached about 520,000 tons in 2016 (China Fishery Statistical Yearbook, 2017), as farmers and researchers have become increasingly aware that the loach has a high nutritional value, a rapid growth rate, a high tolerance to harsh environments, and a high market value (You et al., 2010).

Selenium (Se) is an essential nutrient required for normal growth, physiological function, and cellular metabolism in fish (Nastova et al., 2014). Se plays an important role in the regulation of the antioxidant enzyme glutathione peroxidase (GPx), which protects both cellular and subcellular membranes from oxidative damage (Lyons et al., 2007). Usually, Se is added as a supplement to the diets of farmed fish as an inorganic mineral salt, typically sodium selenite (Na_2SeO_3), or as an organic compound such as selenocysteine (Se-Cys) or selenomethionine (Se-Met) from Se-enriched yeast (Lin, 2014; Yang, 2014; Lee et al., 2016). However, sodium selenite is toxic at high concentrations, so the misuse of this salt might poison the fish (Wang et al., 2012; Berntssena et al., 2017). In contrast, organic forms of Se, such as Se-enriched yeast, are highly efficient and non-toxic (Ilham et al., 2016; Kong et al., 2017). However, the preparation of Se-enriched yeast is complicated and the cost is high (Chen et al., 2009).

Chitosan is a cationic polysaccharide derived from chitin, which is a natural polymer of *N*-acetyl glucosamine commonly found in crustacean and insect exoskeletons, as well as in fungal cell walls (Jolles and Muzzarelli, 1999). Chitosan has also been associated with increased fish growth performance, as it improves immune function (Sakai et al., 1992; Dananjaya et al., 2016). A number of studies have suggested that chitosan has additional biological properties, acting as an immunoadjuvant and a bacteriostatic agent (Kiruba et al., 2013; Abu-Elala et al., 2015; Sun et al., 2016; Wang et al., 2018). Chitosan is also an excellent carrier of other ingredients, such as vitamins, unsaturated fatty acids, and phytochemicals (Chen and Subirade, 2005; Shaw et al., 2007; Hu et al., 2008).

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Se-chitosan is an immunostimulant that has been well studied in terrestrial livestock (Lyons et al., 2007; Ibrahim et al., 2011; Zhao et al., 2013; Qin et al., 2015). Se-chitosan is prepared by combining chitosan and Na_2SeO_3. Se-chitosan improves the intestinal health of roosters (Gao et al., 2013), and Farrer's scallops (Wang et al., 2007). However, little is known about the effects of Se-chitosan on intestinal immune function in fish.

Fish intestinal health is strongly associated with immune function (Kim and Austin, 2006). Impaired intestinal immunity leads to pathogen translocation, inflammation, and enteritis, possibly resulting in fish death (Molinari et al., 2003). Therefore, enhancing and developing fish intestinal immunity is of great importance for aquaculture (Rombout et al., 2011). In fish, intestinal immunity is closely associated with antimicrobial compounds, such as lysozyme and acid phosphatase (Gao et al., 2017). Therefore, the objective of this study was to evaluate Se-chitosan as a potential dietary supplement for *P. dabryanus*. Our evaluation was based on intestinal health, as indicated by immunological parameters (alkaline phosphatase, acid phosphatase, immunoglobulin M, and lysozyme) and microflora composition, as well as growth performance indicators (i.e., weight gain, specific growth rate, and feed conversion ratio) and survival rate.

2. Materials and methods

2.1. Experimental design

Experimental fish were obtained from a commercial fish farm (Zhoushan Tai Dao Qiu Ye Co., Ltd., Zhejiang Province, China). Before the experiment began, all fish were kept in 300 L cylindrical fiberglass tanks for 14 days. During this period the loaches were fed a control diet to apparent satiation twice daily (at 08:00 and 18:00). At the beginning of the experiment, 450 juvenile loaches of similar size (initial mean weight: 5.0 ± 0.2 g) and without obvious injuries were evenly divided among 15 20 L PVC tanks containing aerated, dechlorinated fresh water. Triplicate tanks of loaches were hand fed the control and experimental diets to visual satiation twice daily (at 08:00 and 18:00). Water temperature was maintained at 25 ± 1 °C, dissolved oxygen at 6.0–7.0 mg/L, pH at 7.3 ± 0.2, total ammonia–nitrogen below 0.2 mg/L, and nitrite below 0.06 mg/L. We changed 30% of the water volume daily. The experiment lasted for 60 days.

2.2. Diet preparation and feeding

Se-chitosan was prepared by combining Na_2SeO_3 and chitosan in the medical laboratory of Zhejiang Ocean University, Zhoushan city, Zhejiang province, China. In brief, 1.0 g chitosan was added to 100 mL 1% acetic acid to make the chitosan solution. Then 0.4 g Na_2SeO_3 was added to the chitosan solution and allowed to react for 2 h. The mixture was filtered to remove insoluble substances and 70% ethyl alcohol was added. After 12 h of alcohol precipitation, the solution was filtered. The filtrate was washed, dried at a low temperature, and ground.

Diet formulations and proximate compositions are presented in Table 1. All ingredients were blended thoroughly in a mixer. Microelement additives were premixed before being added to the dry feed mix. Pellets were made, dried, ground, and sieved in a laboratory pellet mill through a 2.0 mm die. Ground feed was dried in an oven at 40 °C for 48 h, and then stored at −20 °C in sealed plastic bags. The crude protein, crude lipid, ash, and moisture in the diets were determined following the methods of the AOAC (1995). Crude protein was determined using the Kjeldahl method (N × 6.25), after being analyzed with an Auto Kjeldahl System (K358/K355; BUCHI, Flawil, Switzerland). Crude lipid was measured with a Soxhlet extraction using a Soxtec system HT (E-816; BUCHI, Flawil, Switzerland). Ash was determined by combustion at 550 °C in a muffle furnace for 12 h. Phosphorus content was determined with the phosphorus vanadium molybdate yellow colorimetric method. Calcium was determined using ethylenediamine tetraacetate disodium (EDTA-2Na).

Table 1
Formulation and proximate composition of the experimental feeds (all amounts given in g/kg unless otherwise stated).

Ingredients	Treatment groups				
	C	T1	T2	T3	T4
	Se content in each group (mg/kg)				
	0	0.11	0.23	0.34	0.45
Fish meal	210	210	210	210	210
Soybean meal	360	360	360	360	360
Wheat bran	70	70	70	70	70
Rapeseed meal	40	40	40	40	40
Peanut oil	20	20	20	20	20
Corn	170	170	170	170	170
Monocalcium phosphate	18	18	18	18	18
Vitamin premix[a]	1.2	1.2	1.2	1.2	1.2
Mineral premix[b]	0. 8	0. 8	0. 8	0. 8	0. 8
Sodium carboxymethyl cellulose	10	10	10	10	10
Wheat meal	100	99.4	98.7	98.2	97.7
Seleno-chitosan (mg/kg)	0	0.6	1.2	1.8	2.4
Proximate composition (% dry matter)					
Crude protein	33.5	33.4	33.6	33.4	33. 5
Crude lipid	4.6	4.5	4.6	4.5	4.7
Ash	7.4	7.5	7.3	7.5	7.6
Phosphate	1.6	1.8	1.7	1.5	1.5
Calcium	2.1	1.9	1.8	1.9	1.9

[a] Vitamin premix provided per kg of feed: Vitamin A 12,500,000 IU; Vitamin D 2,000,000 IU; Vitamin E 7000 IU; Vitamin K 2000 mg; Vitamin B_1 800 mg; Vitamin B_2 2500 mg; Vitamin B_6 800 mg; Vitamin B_{12} 10 mg; niacin 3000 mg; pantothenic acid 10,000 mg; folic acid 300 mg; biotin 20,000 mg; and VC 20,000 mg.

[b] Minerals provided per kg of feed: Mn 19 mg; Mg 230 mg; Co 0.1 mg; I 0.25 mg; Fe 140 mg; Cu 2.5 mg; and Zn 65 mg.

Experimental diets were prepared using increasing levels of Se-chitosan in the loach feed (Table 1). The amount of Se-chitosan added to each diet was 0 mg/kg (diet C, control), 0.6 mg/kg (diet T_1), 1.2 mg/kg (diet T_2), 1.8 g/kg (diet T_3), and 2.4 mg/kg (diet T_4). The actual Se content in the diets were 0 mg/kg (diet C), 0.11 mg/kg (diet T_1), 0.23 mg/kg (diet T_2), 0.34 mg/kg (diet T_3), and 0.45 mg/kg (diet T_4), as determined with 2,3-diaminonaphthalene fluorometry using an atomic absorption spectrometer (HG-AFS; Varian Inc., Palo Alto, USA).

Loaches were fed 5–7% of wet body weight per day. Remaining feed was siphoned after half an hour. Rations were adjusted daily to slightly exceed satiation, based on the amount of uneaten food left in the tank. Uneaten food was collected, dried, and weighed to calculate the feed conversion ratio (FCR). Survival rate, weight gain (WG), specific growth rate (SGR), and FCR were calculated as follows:

Survival rate (%) = 100 × (final number of loach/initial number of loach),
SGR (%/day) = 100 × [ln (final mean body weight) − ln (initial mean body weight)]/time (days),
FCR = total dry feed consumption/net weight gain (%), and
WG (%) = 100 × (final total weight-initial total weight)/initial total weight.

2.3. Measurement of immunological parameters

At the end of the 60-day feeding trial, all fish were starved for 24 h, then bulk weighted by tank. We randomly selected 10 loaches from each tank for the measurement of intestinal immunological parameters. Fish were prepared for sampling as described previously (Gao et al., 2017). Briefly, selected fish were killed with an overdose of tricaine methanesulfonate (MS-222, 150 mg/L; Sigma-Aldrich, Shanghai,