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 T1), 1.2 (group T2), 1.8 (group T3), or 2.4 (group T4) 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 T4 group than in the control group. Following 16S rRNA sequencing, 296 operational taxonomic units were identified across the control and T3 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.

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).

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 Na2SeO3. 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.

METHODOLOGY

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. 30% of the water volume was changed daily. The experiment lasted for 60 days.

Se-chitosan was prepared by combining Na2SeO3 and chitosan. 1.0 g chitosan was added to 100 mL 1% acetic acid to make the chitosan solution. Then 0.4 g Na2SeO3 was added to the chitosan solution and allowed to react for 2 h. 70% ethyl alcohol was added. Diet formulations and proximate compositions are presented in Table 1. 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.

Experimental diets were prepared using increasing levels of Se-chitosan in the loach feed (Table 1).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.

Immunological enzymes, alkaline phosphatase (ALP), acid phosphatase (ACP), lysozyme (LZM), and immunoglobulin M (IgM) were determined with ELISA kits (kits 48T/hj-17,285, 48T/hj-17,426, 48T/hj-17,234, and 48T/hj-17,367, respectively; Shanghai Changjin Biotechnology Co., Ltd., Shanghai, China), following the manufacturer's instructions.

Intestinal samples were homogenized ready for microbial DNA extraction. Microbial DNA was extracted using the E.Z.N.A. Soil DNA Kit (50) (Omega, USA) following the manufacturer's instructions. For each sample, DNA was extracted in triplicate then harmonized together. Hiseq 2500 sequencing platform and paired-end technology were provided by Genepioneer Biotechnologies (Nanjing) Co., Ltd, China.

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

Table 2
Effects of Se-chitosan concentration on the growth parameters of the loach *Paramisgurnus dabryanus*

Treatments	Growth parameters				
	Final average weight (g)	WG (%)[1]	SGR (%/d)[2]	FCR[3]	Survival rate (%)[4]
C	14.89 ± 0.45[a]	1.92 ± 0.11[a]	1.79 ± 0.09[a]	1.59 ± 0.09[a]	92.0 ± 2.4[a]
T1	15.16 ± 0.49[a]	2.06 ± 0.26[a]	1.87 ± 0.09[a]	1.58 ± 0.09[a]	93.2 ± 3.9[a]
T2	15.69 ± 0.65[a]	2.21 ± 0.1[a]	1.94 ± 0.07[ab]	1.46 ± 0.07[ab]	92.1 ± 2.9[a]
T3	16.18 ± 0.79[a]	2.29 ± 0.27[a]	1.98 ± 0.05[a]	1.42 ± 0.05[b]	93.1 ± 3.4[a]
T4	15.52 ± 0.34[a]	2.20 ± 0.13[a]	1.94 ± 0.06[a]	1.46 ± 0.06[ab]	92.0 ± 2.3[a]

RESULTS

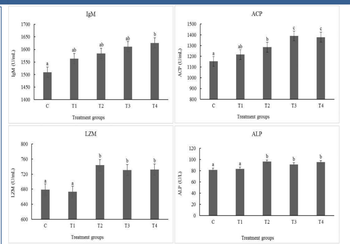

Fig. 1. IgM, LZM, ACP and ALP of loach. Bars indicate means ± standard deviations of three replicates, with 10 fish per replicate. Bars labeled with different lowercase letters are significantly different (P < .05)A.

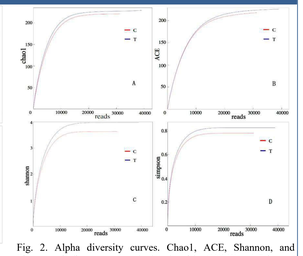

Fig. 2. Alpha diversity curves. Chao1, ACE, Shannon, and Simpson indices were calculated with QIIME v1.7.0 and visualized in R v3.2. OTU richness rarefaction curves were calculated with Mothur v1.27.0, based on an OTU distance of 0.03. T=T3. The T3 group was supplemented with 1.8 mg Se-chitosan/kg feed and C was the control group without Se-chitosan supplementation.

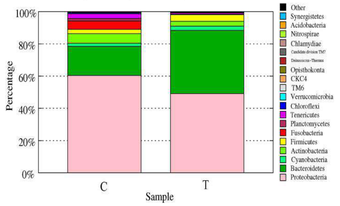

Fig.3.Distribution of the bacterial phyla between samples. T=T3; C=control. The T3 group was supplemented with 1.8 mg Se-chitosan/kg feed. The control group (C) was not fed Se-chitosan supplements.The x-axis represents the sample group, and he y-axis represents the abundance of the bacterial Phyla. Different colors represent different phyla. Floras with low abundance were merged into the other' groups.

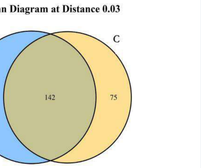

Fig. 4. Venn diagram showing unique and shared OTUs (at 3% distance).T=T3. The T3 group was supplemented with 1.8 mg Se-chitosan/kg feed. The control group (C) was not fed Se-chitosan supplements..

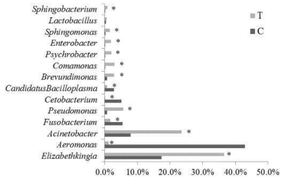

Fig. 5. Composition of bacterial genera in the C and T groups. T=T3. The T3 group was supplemented with 1.8 mg Se-chitosan/kg feed. The control group (C) was not fed Se-chitosan supplements Fisher's exact test was used to analyze differences in rank abundance difference between the two groups. We considered P < .05 statistically significant. Asterisks next to the bars indicate those that are significantly differentially abundant from the controls. Only the 10 most abundant bacterial genera are shown.

DISCUSSION

We observed that Se-chitosan had little effect on growth performance based on WG, SGR, and survival rate after 60 days of Se-chitosan treatment. These observations were consistent with those in *Caspian kutum* (*Rutilus frisii kutum*) fingerlings (Najafabad et al., 2016). Increased Se-chitosan levels led to decreased FCR. However, because some nutrition was likely lost during pellet manufacture and the feeding process (Helland et al., 1996), feed intake may have been artificially inflated, decreasing FCR. In addition, Se-chitosan had no obvious effects on other growth parameters, suggesting that the effects of Se-chitosan on FCR may be lower than indicated by our results.

Our results indicated that Se-chitosan was not only necessary for normal body function but also that appropriate supplementation with Se-chitosan improved ACP, ALP, IgM, and LZM levels, indicating an enhancement of the overall immunity of P. dabryanus. The activity of ACP, ALP, and LZM was increased by Se-chitosan supplementation. IgM content increased significantly compared to the control group when Se-chitosan supplementation was ≥2.4 mg/L. Previous studies have shown that chitosan boosted the immune systems of various organisms (including fish), consistent with our LZM results (Ranjan et al., 2014; Li et al.,2015). The immunological parameters evaluated here apparently performed better with increasing levels of Se-chitosan supplementation. It may be that Se increases LZM cell proliferation, elevating serum lysozyme activity, as has been shown in pacu fish (Biller-Takahashi et al., 2015; Takahashi et al., 2017). Se-chitosan regulates the immune response by enhancing innate immunity and modulating pathogen-induced inflammation through Toll-like receptor-regulated signaling pathways (Ninawe and Selvin, 2009; Rombout Jan et al., 2011). Hoffmann (2007) and Mansour et al. (2017) suggested that Se affected immune function by regulating thyroid hormone metabolism.

The species richness and diversity of the intestinal microflora increased in the T3 group as compared to the control group. Se and chitosan have been reported to stimulate microbial diversity (Kasaikina et al., 2011; Li et al., 2011). We observed high levels of the beneficial bacteria Lactobacillus in the T3 group (Fig. 5). This was consistent with a previous study, which showed that Lactobacillus fecal counts were higher in weanling piglets fed selenium-enriched probiotics (Lv et al., 2015). Se-chitosan-enriched diets also increased Lactobacillus content in broiler chickens (Gao et al., 2013). However, we did not observe an obvious pattern in the common pathogenic bacteria. For example, opportunistic pathogenic bacteria Aeromonas was less abundant in the T3 group, while Enterobacter was more abundant. Most of bacteria that were more common in the T3 group were Proteobacteria, including the genera Acinetobacter, *Pseudomonas, Comamonas*, *Psychrobacter, Sphingomonas*, and *Brevundimonas*.

CONCLUSIONS

Se-chitosan acts as an immunomodulator when used as a dietary supplement's-chitosan may influence fish intestinal health by stimulating immunological parameters and affecting the diversity of the microflora in the intestine. However, more studies are needed to further investigate this compound and the associated mechanisms that affect immunity.

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