The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1818814, 10 pages
 https://doi.org/10.46989/001c.39683

Effects of selenium adaptation on intestinal morphology, antioxidant-relate genes expression and intestinal microflora of grass carp (*Ctenopharyngodon idella*)

Zong zhen Li[1]*, You xin Xu[1], Fa jun Li[1], Hong tao Ren[2]

[1]*Jia Sixie College of Agriculture, Weifang University of Science and Technology, Shouguang 262700, China*
[2]*Animal Science and Technology College, Henan University of Science and Technology, Luoyang 471003, China*

(Received Sep 29, 2022; Accepted Oct 18, 2022; Published Oct 26, 2022)

Keywords: antioxidant-related gene, grass carp, intestinal morphology, selenium adaptation

Abstract

In the study, the effects of selenium on intestinal tissue morphology, antioxidant-related genes, and intestinal flora of grass carp (*Ctenopharyngodon idella*) were studied. For this purpose, 180 healthy grass carps (20.0±2.0 g) were randomly divided into three groups with three replicates each: the corresponding amount of anhydrous sodium selenite was added to make experimental water solutions of different concentrations, including 0 μg/L Se^{4+} (control group), 200 μg/L Se^{4+} group and 300 μg/L Se^{4+} group. The experiment was carried out for 42 days. The obtained results showed that: at the end of the experiment, the 200 μg/L Se^{4+} adaptation can have beneficial effects on the intestinal villi height and goblet cells. The CuZnSOD and CAT genes mRNA levels of grass carp intestine were strongly upregulated in the 200ug/L Se^{4+} group. 200ug/L selenium could increase the expression level of the Hsp70 gene in the intestinal tract of grass carp after 42 days of adaptation. At the genus level, the most abundant sequence in the gut of Se-treated grass carp was *Pseudomonas*, while *Aeromon*, *Flavobacterium,* and *Defluviimonas* were more abundant in the control group. In conclusion, this study demonstrates that 200ug/L Se^{4+} selenium adaptation can positively affect gut morphology and antioxidant responses and can alter the gut microbiota structure of grass carp. The results will provide a theoretical basis for further research on the effect of selenium on aquatic animals.

* Corresponding author. e-mail: zzhli2020@163.com

Introduction

Grass carp is one of the most important aquaculture species. It has the advantages of delicious meat and fast growth and is intensively cultured. However, deteriorating water quality due to intensive farming resulted in excessive breeding densities, making grass carp more susceptible to environmental stress and pathogens (Pei et al., 2019). In the long-term evolution process, aquatic animals can adapt to the changing external environment through the body's physiological, biochemical, and molecular reactions (Shang et al., 2021).

Selenium (Se) is an essential micronutrient that can maintain the body's regular metabolism and play an indispensable role in anti-oxidant, immunoregulation, anti-inflammatory and anti-viral, and Selenium deficiency leads to susceptibility to disease and decreased immunity in animals (Khan et al., 2016). Selenium protects the animal body from oxidative stress mainly by forming thioredoxin reductase and glutathione peroxidase. Therefore, selenium supplementation can effectively promote the physiological health and growth, and reproduction of fish (Yu et al., 2005; Hoffmann and Berry, 2008). However, the current research on selenium primarily focuses on the effects of adding selenium in feed to fish. At present, Se added in feed have been reported in many fish, for example, Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), crucian carp (*Carassius auratus*), grouper (*Epinephelus malabaricus*) and Snub-Nosed Dart (*Trachinotus Blochii*) (Hilton et al.,1980; Gatlin and Wilson 1984; Lin and shiau, 2005; Hancz et al., 2012; Zhu et al., 2017; Lee et al., 2016; Sang et al., 2016). Selenium plays an essential physiological role in animals. Both deficiency and excess will cause adverse effects on animals. Lack of selenium in animals will lead to adverse symptoms in the body. Studies have reported that selenium deficiency in Atlantic salmon manifests increased mortality, growth retardation, decreased glutathione transferase activity, and muscle wasting (Poston et al., 1976). However, selenium has specific toxicity, and excessive intake will cause harm to the body (Saffari et al., 2018).

So far, there are few reports on the long-term adaptation of grass carp to selenium solution. In this study, we assessed histological changes in the gut of grass carp. We analyzed the antioxidant-relate gene expression and some types of gut microbiota after adaptation to selenium in water. The results will provide a theoretical basis for further research on the effect of selenium on aquatic animals.

Materials and methods

Experimental design

The grass carp used in this experiment was purchased from a local standardized grass carp farm. The purchased fish were first sterilized in a sodium chloride bath (2%, 15 minutes) and then put into three 500L water tanks for 7 days of acclimation, the water quality was detected every day, and the water body was continuously oxygenated. The fish were fed twice a day, and observed the ingestion of grass carp. Then 180 grass carp (20.0±2.0 g) were randomly divided into 3 treatment groups with 3 tanks (800mm×500mm×600mm) in each group. 3 treatment groups including: control group (0μg/L Se^{4+}), 200μg/L Se^{4+}group and 300μg/L Se^{4+}group. The corresponding amount of anhydrous sodium selenite was added to make experimental solutions of different concentrations. Change the test solution every 48 hours to ensure proper Se^{4+} concentration. During the trial, the water was continuously aerated and changed by 50% every 48h, and checked the water quality regularly to make sure that the water quality stayed within the following ranges: water temperature (21.5-26.5°C), PH (7.2-7.8), dissolved oxygen (DO) (5.2-6.8 mg/L), NH_3–N (<0.11mg/L) and the photoperiods followed a natural daylight cycle. During the trial period, grass carp were fed twice daily at 8:30 and 14:30 (3% of body weight per day), and the feeding rate was adjusted every 2 weeks.

At the end of the experiment, grass carp fasted 24 hours in advance. Then 6 fish were randomly selected from each replicate and anesthetized with MS-222 (100 mg/L), of which 3 fish were sacrificed, and the intestinal tissue was fixed for histomorphological analysis. The intestines of the other 3 fish were snap-frozen in liquid nitrogen for gene expression analysis and gut microbiome analysis.

Preparation of tissue slices

The intestines of grass carp collected from the experiment were fixed with 10% formalin solution, dehydrated, embedded, sectioned, and finally stained with hematoxylin and eosin (H&E).

RNA extraction and determination of the expressions of antioxidant-relate genes

Quickly slaughtered grass carp to take the intestine, quick-frozen in liquid nitrogen, and stored in a -80 ^{O}C refrigerator until analysis, using the Trizol method (Trizol Reagen kit, Takara, Japan) to extract total RNA from the intestine, and measuring the RNA concentration and purity with a micro-UV-visible spectrophotometer. 2 μg of total RNA was reverse transcribed into cDNA using PrimeScript RT-PCR kit (Takara, Japan). RT-qPCR was performed on each treated cDNA sample by RT-qPCR (SYBR Green II) on a Thermocycler Dice TP800 Sequence Detection System (Takara, Japan). *β*-actin was used as an internal reference to normalize the mRNA expression levels of the genes in each sample. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Sequences of primers for RT-qPCR are shown in **Table 1**.

Table 1 Sequences of primers for RT-qPCR

Primers	Primer direction	Primer sequence (5'→3')	Tm (℃)	Accession number
HSP70	Forward	CTGCTGGATGTGGCTCCTCTGTC	60	GU475146.1
	Reverse	AAGGTCTGGGTCTGTTTGGTGGG		
CAT	Forward	GAAGTTCTACACCGATGAGG	60	FJ560431
	Reverse	CCAGAAATCCCAAACCAT		
CuZnSOD	Forward	CGCACTTCAACCCTTACA	58	GU901214
	Reverse	ACTTTCCTCATTGCCTCC		
β-actin	Forward	GTCCGTGACATCAAGGAGAAGC	60	DQ211096.1
	Reverse	GGATACCGCAAGACTCCATACCC		

DNA extraction and 16S rRNA gene analysis

The preserved samples were sent to Shanghai Yuanshen Biotechnology Company for DNA extraction, library construction, and high-throughput sequencing of 16S rRNA amplicon. The process is as follows: use 16S V3-V4 variant region primers to amplify the DNA of the extracted samples, and use the TruSeq DNA PCR-Free Sample Preparation Kit for library construction. The constructed library was quantified by Qubit and Q-PCR. After the library was qualified, the NovaSeq6000 was sequenced on the computer.

Statistical analysis

After data were processed by Excel, a one-way analysis of variance (one-way ANOVA) was performed with SPSS 18.0, and multiple comparisons were performed with Duncan's method. Data presented as Mean ± standard deviation (SD), $P< 0.05$ indicates a significant difference.

Results

Growth performance and survival

The growth performance and survival of grass carp following waterborne adaptation to selenium for 42 days are in **Table 2**. Results showed that FBW and SGR in the control group and 200ug/l Se^{4+} group were significantly higher than those of the 300ug/l Se^{4+} group. At the end of the experiment, the survival rates of the control group and the 200ug/l Se^{4+} group were 100%.

Table 2 Effects of selenium adaptation on growth performance and survival of grass carp

Items	Control	200ug/l Se^{4+}	300ug/l Se^{4+}
IBW (g)	20.12±0.35	20.34±0.42	20.23±0.26
FBW(g)	47.45±1.21^{a}	52.62±1.11^{a}	42.37±1.36^{b}
SGR (%)	2.04±0.03^{a}	2.26±0.04^{a}	1.76±0.06^{b}
SR (%)	100.00	100.00	91.67

Note: Values are mean ± S.E.M, and values in the same row with different letters indicate a significant difference ($p < 0.05$). IBW, initial body weight; FBW, final body weight; SR, survival rate; SGR, specific growth rate; SGR== (Ln final weight − Ln initial weight) × 100/days;

Effects of selenium on histopathological changes of intestine

Figure 1 shows the intestinal epithelial layer was orderly, lamina propria dense, and nuclei regular in the control group (**Figure 1A**), whereas the grass carp gut showed lamina propria enlargement in microvilli after exposure to 200 μg/l Se^{4+} and 300 μg/l Se^{4+}; The villus space was increased (**Figure 1B, C**). **Figure 2** displays that the VH (intestinal villi height) and GC (goblet cells) of grass carp treated with 200 μg/L Se^{4}+ were significantly higher than those in the control group and 300 μg/L Se^{4+} groups (P<0.05). However, the intestinal MT (muscular layer thickness) of grass carp in the control group was significantly higher than that in the selenium treatment group (P<0.05).

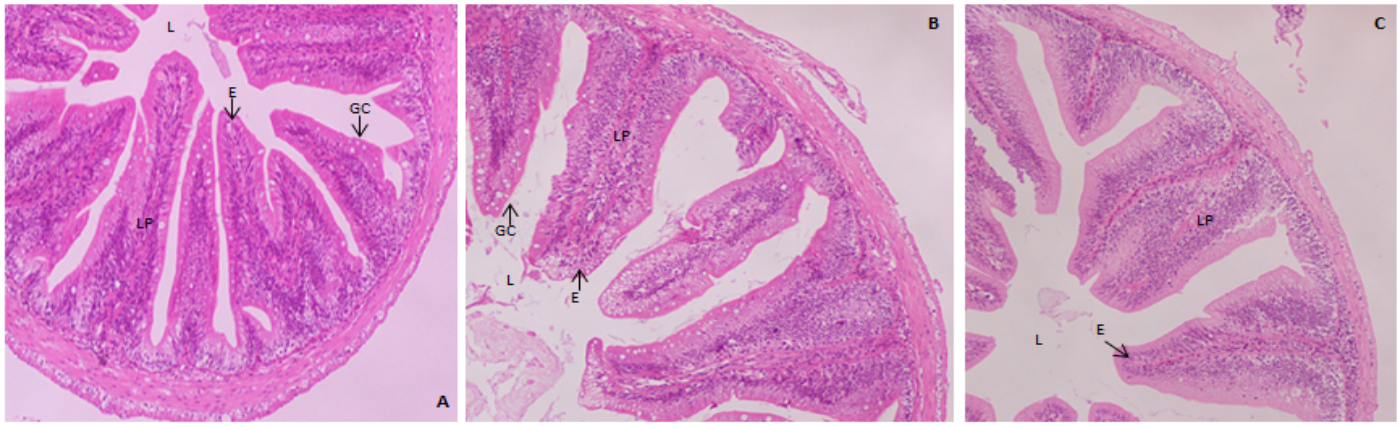

Figure 1 Intestinal morphology in grass carp following waterborne adaptation to selenium for 42 days. The panels include, A: control; B: 200μg/L Se^{4+}; C: 300μg/L Se^{4+}. GC, goblet cells; LP, lamina propria; L, lumen; E, epithelium. H&E stain (100 ×).

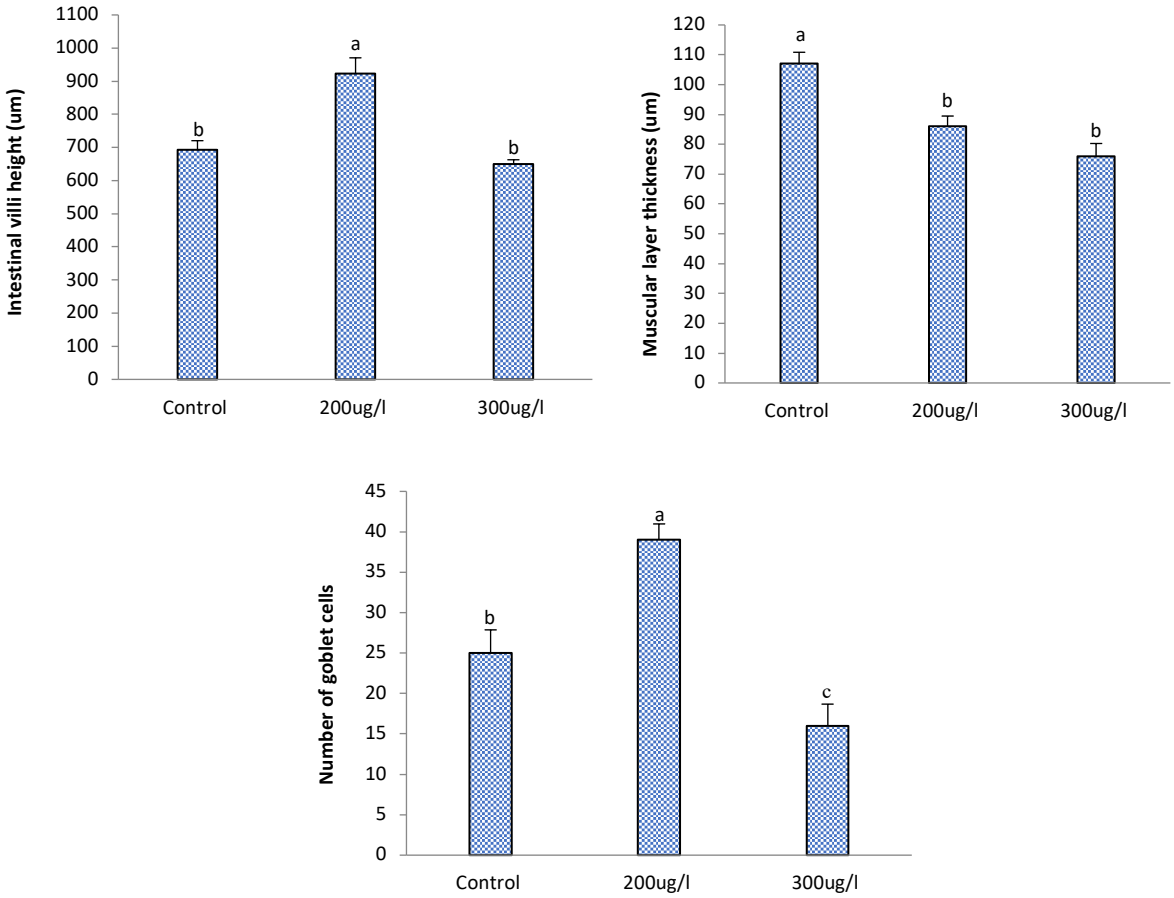

Figure 2 Effect of Intestinal morphology in grass carp following waterborne adaptation to selenium for 42 days.

Expressions of antioxidant-relate genes in the intestine of grass carp

Figure 3 demonstrates the expression levels of CAT, CuZnSOD, and Hsp70 genes in the intestinal tract of grass carp in the 200ug/L Se^{4+} group were significantly higher than those in the control group (P<0.05). In contrast, the gene levels of CAT and Hsp70 in the 200ug/L Se^{4+} group were significantly higher than those in the 300ug/L Se^{4+} group (P<0.05).

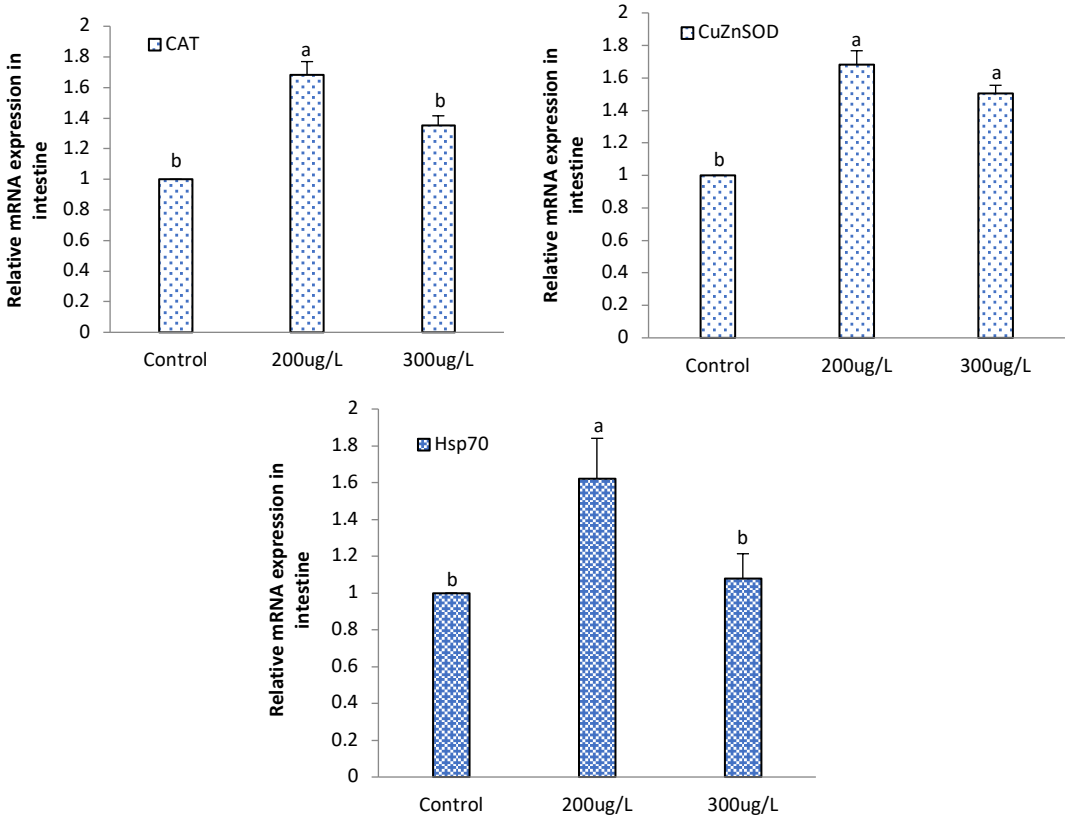

Figure 3 Expressions of antioxidant-relate genes in the intestine of grass carp following waterborne adaptation to selenium for 42 days.

The abundance of differences in microbial taxa at the genus level

Figure 4 compares the control group; Aeromon*, Flavobacterium,* and *Defluviimonas* were the most dominant genus at the genus level. However, *Pseudomonas* is abundant in sequences in the selenium-treated samples. The abundances of *Aeromon, Flavobacterium, and Defluoromonas* were significantly reduced in the selenium-treated group compared with the control group ($P< 0.05$).

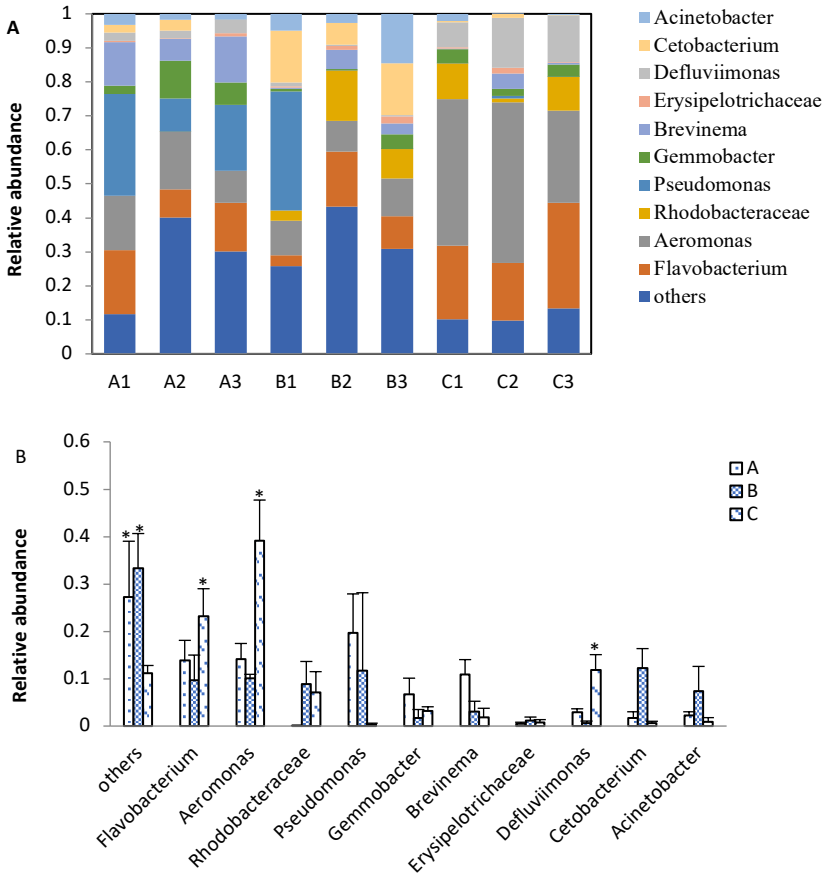

Figure 4 (A) Relative abundance of the top 10 genera in the fecal microbiota between control and experimental groups. (B) Abundance differences in microbial taxa at the genus level between control and experimental groups. * *P*< 0.05 indicates a significant difference between the groups. A: 200ug/L Se^{4+} group; B: 300ug/L Se^{4+} group; C: control group.

Discussion

Selenium is an essential trace element for animals. The information about selenium in aquatic animals studies have focused on the effects of adding a certain amount of selenium to the feed on aquatic animals, including crucian carp (*Carassius auratus gibelio*), Japanese seabass (*Lateolabrax japonicus*), cobia (*Rachycentron canadum*), common carp (*Cyprinus carpio*) and marron (*Cherax cainii-marron*) (Zhou et al., 2009; Liang et al., 2006; Yang et al., 2016; Saffari et al., 2018; Nugroho and Fotedar, 2013). However, little information about the effect of selenium in an aqueous solution on the intestinal function of fish has been reported. As an essential part of fish's digestive system, the development of the intestine is closely related to absorption efficiency. The higher the intestinal fold, the stronger the ability of the intestine to absorb nutrients; the thicker the muscle layer, the stronger the intestinal peristalsis (Lee et al. 2017). In this study, the 200ug/L Se^{4+} treatment group had beneficial effects on both the villus height and the number of goblet cells in the intestinal tract of grass carp. A couple of previous studies also suggest that dietary selenium has a promoting effect on the morphological development of fish intestines (Saffari et al., 2018; Shang et al., 2021). Goblet cells are known to lubricate and protect the mucosa from chemical and physical damage by secreting mucus (Begam and Sengupta, 2015). Healthy and complete intestinal folds can

prevent harmful bacteria from colonizing the intestines, reduce the incidence of enteritis and enhance the disease resistance of fish (Huang et al., 2010).

As an essential antioxidant in animals, selenium can not only improve the antioxidant capacity of animals but also remove excess free radicals, protect cell membranes, and resist lipid peroxidation damage, thereby maintaining the health of the body (Yu et al., 2005; Hoffmann and Berry, 2008; Khan et al., 2016). Environmental stressors affect the antioxidant system of fish, causing oxidative stress in the body (Zhang et al., 2019). Essential micronutrients, such as selenium, are strong antioxidant micronutrients that play a key role in inhibiting oxidative stress and promoting cell stability in fish (Martínez-Álvarez et al., 2005). At present, some studies are only limited to adding selenium in feed to improve the activity of antioxidant enzymes in tissues and organs, and there is no report on the research on improving the activity of antioxidant enzymes by adapting to selenium in water (Jovanovic et al., 1997; Lin and shiau, 2005; Zhu et al., 2017; Lee et al., 2016).

To study the relationship between the molecular mechanism of selenium adaptation and the expression of antioxidant-related genes in the intestine of grass carp (*Ctenopharyngodon idellus*). The results showed that the gene expression levels of CuZnSOD and CAT in the intestine of grass carp were increased in the 200ug/l Se^{4+} Group, suggesting that proper selenium adaptation may enhance the antioxidant capacity of grass carp. Hsp70 has various functions, such as antioxidant and synergistic immunity, which can maintain cell homeostasis and enhance the body's ability to resist stress (Feder and Hofmann, 1999; Daugaard et al., 2007). In this study, the Hsp70 expression level of the intestine was increased in the 200ug/L Se^{4+} group. This study showed that the expression levels of Hsp70 in the intestines of the 200ug/L Se^{4+} group increased. 200ug/L selenium adaptation can increase the expression level of the Hsp70 gene, thereby enhancing stress resistance.

In this study, many fish gut microbes were discovered through high-throughput sequencing and bioinformatics analysis. The most abundant sequence in the selenium-treated samples is *Pseudomonas. The* abundance of *Aeromon, Flavobacterium, and Defluviimonas* decreased significantly in the selenium-treated groups. This study also found that there are many unknown gut microbes in the gut of the experimental grass carp, and the types and functions of these gut microbes need to be further studied. These findings reveal that selenium is closely related to the gut microbiota and may influence the healthy development of the gut.

Conclusion

In conclusion, we demonstrate for the first time the adaptive response of grass carp intestine to selenium. The 200ug/L Se^{4+} solution has a specific promoting effect on the morphological development of grass carp intestines and antioxidant responses. Meanwhile, the 200ug/L Se^{4+} could alter the intestinal microbiota structure of grass carp. The research results will provide a theoretical basis for further in-depth study of the application of selenium in aquaculture.

Acknowledgments

This work was funded by the Shandong Provincial Natural Science Foundation (ZR2021MC061) and; the Science and Technology Project of Weifang University of Science and Technology (2022KJ01).

References

Begam M, Sengupta M, 2015. Immunomodulation of intestinal macrophages by mercury involves oxidative damage and rise of pro-inflammatory cytokine release in the fresh water fish *Channa punctatus Bloch*. *Fish Shellfish Immunol*. 45, 378-385. doi.org/10.1016/j.fsi.2015.04.017

Daugaard M, Rohde M, Jäättelä M, 2007. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett*. 581, 3702-3710. doi.org/10.1016/j.febslet.2007.05.039

Feder M, Hofmann G, 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol.* 61, 243-282. doi.org/10.1146/annurev.physiol.61.1.243

Gatlin DM, Wilson RP, 1984. Dietary selenium requirement of fingerling channel catfish, *J. Nutr.* 114, 627-633.

Hancz C, Mezes M, Balogh K, Biro J, Molnar T, 2012. Improving the Nutritional Value of Nile tilapia fillet by dietary selenium supplementation. *Israeli Journal of Aquaculture - Bamidgeh* 64 (January). https://doi.org/10.46989/001c.20641

Hilton JW, Hodson PV, Slinger SJ, 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*), *J. Nutr.* 110, 2527-2535. https://doi.org/10.1093/jn/110.12.2527

Hoffmann PR, Berry, M.J. 2008. The influence of selenium on immune responses. M*olecular nutrition and food research* 52, 1273-1280. https://doi.org/10.1002/mnfr.200700330

Huang YZ, Lin X, Wang QX, 2010. Effects of astragalus polysaccharide on structure of intestinal villus and intestinal immunocyte of tilapia. *Chinese Journal of Animal Nutrition* 22, 108-116.

Jovanovic A, Grubor–Lajsic G, Djukic N, Gardinovacki G, Matic A, Spasic M, Pritsos CA, 1997. The effect of selenium on antioxidant system in erythrocytes and liver of the carp (*Cyprinus carpio L.*). *Crit. Rev. Food. Sci. Nutr.* 37, 443-448. doi.org/10.1080/10408399709527783

Khan KU, Zuberi A, Nazir S, Fernandes JBK, Jamil Z, Sarwar H, 2016. Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile Tor putitora. *Turk. J. Zool.* 40, 704-712. https://doi.org/10.3906/zoo-1510-5

Lee S, Katya K, Park Y, Won S, Seong M, hamidoghli A, Bai SC, 2017. Comparative evaluation of dietary probiotics Bacillus subtilis WB60 and Lactobacillus plantarum KCTC3928 on the growth performance, immunological parameters, gut morphology and disease resistance in Japanese eel, Anguilla japonica. *Fish Shellfish Immunology* 61, 201-210. https://doi.org/10.1016/j.fsi.2016.12.035

Lee S, Nambi RW, Won S, Katya K, Bai SC, 2016. Dietary selenium requirement and toxicity, levels in juvenile Nile tilapia, *Oreochromis niloticus,* Aquaculture 464, 153-158. https://doi.org/10.1016/j.aquaculture.2016.06.027

Liang MQ, Wang JL, Chang Q, Liu XD, Mai KS, 2006. Effects of dietary Se on growth performance and activities of related enzymes in juvenile Japanese seabass *Lateolabrax japonicas*. *Journal of Fishery Sciences of China* 13, 1017-1022. https://doi.org/10.1360/aps050066

Lin YH, Shiau SY, 2005. Dietary selenium requirements of juvenile grouper, *Epinephelus malabaricus*, *Aquaculture* 250, 356-363. https://doi.org/10.1016/j.aquaculture.2005.03.022

Martínez-Álvarez RMa, Morales AE, Sanz A, 2005. Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish. Biol. Fish* 15 (1-2), 75-88. https://doi.org/10.1007/s11160-005-7846-4

Nugroho RA, Fotedar R, 2015. Effects of dietary organic selenium on immune responses, total selenium accumulation and digestive system health of marron, *Cherax cainii* (Austin, 2002), *Aquaculture research* 46, 16567-1667. https://doi.org/10.1111/are.12320

Pei C, Gao Y, Sun X, Li L, Kong X, 2019. A developed subunit vaccine based on fiber protein VP56 of grass carp reovirus providing immune protection against grass carp hemorrhagic disease. *Fish Shellfish Immunol.* 90, 12-19. https://doi.org/10.1016/j.fsi.2019.04.055

Poston HA, Combs GF, Leibovitz L, 1976. Vitamin E and selenium interrelations in the diet of Atlantic salmon (Salmo salar): gross, histological, and biochemical deficiency signs. *Journal of Nutrition* 106, 892-904. https://doi.org/10.1093/jn/106.7.892

Saffari S, Keyvanshokooh S, Zakeri M, Ali Johari S, Pasha-Zanoosi H, Mozanzadeh MT, 2018. Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). *Fish Physiol Biochem.* 44, 1087-1097. https://doi.org/10.1007/s10695-018-0496-y

Sang HM, Thuy NT, Lam HS, 2016. Effects of Organic Selenium (OS) on Survival, Physiological and Immunological Response of Snub-Nosed Dart (*Trachinotus Blochii* Lacépède, 1801) Fingerlings When Challenged with Bacterial Infection and Copper Exposure. *The Israeli Journal of Aquaculture-Bamidgeh*. https://doi.org/10.46989/001c.20826

Shang X, Yu Pe, Yin YW, Zhang Y, Lu YT, Mao QH, Li YH, 2021. Effect of selenium-rich Bacillus subtilis against mercury-induced intestinal damage repair and oxidative stress in common carp, *Comparative Biochemistry and Physiology, Part C* 239, 108851. https://doi.org/10.1016/j.cbpc.2020.108851

Yang YZH, Nie JQ, Tan BP, Dong XH, Yang QH, Chi SHY, 2016. Effects of Selenium Source and Selenium Level on Growth Performance, Liver and Serum Antioxidant Indices and Selenium Content in Tissues of Juvenile Cobia (*Rachycentron canadum*), *Chinese Journal of Animal Nutrition* 28, 3894-3904.

Yu HJ, Liu JQ, Böck A, Li J, Luo GM, Shen JC, 2005. Engineering glutathione transferase to a novel glutathione peroxidase mimic with high catalytic efficiency Incorporation of selenocysteine into a glutathione-binding scaffold using an auxotrophic expression system. *J. Biol. Chem.* 280, 11930-11935. https://doi.org/10.1074/jbc.M408574200

Zhang W, Xia S, Zhu J, Miao L, Ren M, Lin Y, Ge X, Sun S, 2019. Growth performance, physiological response and histology changes of juvenile blunt snout bream, *Megalobrama amblycephala* exposed to chronic ammonia. Aquaculture 506, 424-436. https://doi.org/10.1016/j.aquaculture.2019.03.072

Zhou XX, Wang YB, Gu Q, Li WF, 2009. Effects of different dietary selenium sources (*selenium nanoparticleand selenomethionine*) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). *Aquaculture* 291, 78-81. https://doi.org/10.1016/j.aquaculture.2009.03.007

Zhu L, Han D, Zhu X, Yang Y, Jin J, Liu H, Xie S, 2017. Dietary selenium requirement for on-growing gibel carp (*Carassius auratus* gibelio var. CAS III), *Aquacult. Res.* 48, 2841-2851. https://doi.org/10.1111/are.13118