Chitosan Enhanced the Growth Rate, Antioxidant Activity, Immunity, Intestinal Morphology and Disease Resistance against Aeromons hydrophila of Juvenile Hybrid Sturgeon (Acipenser baerii♀ × Acipenser schrenckii♂)

Hongsen Xu
Wuhan Polytechnic University
Haoran Sun
Wuhan Polytechnic University
Qianrong Liang
Zhejiang Fisheries Technical Extension Center
Fuguo Liu
University of Aberdeen
Jun Liu
Wuhan Polytechnic University
Denghang Yu (yudenghang1985@163.com)
Wuhan Polytechnic University https://orcid.org/0000-0003-2511-5552

Research Article

Keywords: Acipenser baerii ♀ × A. schrenckii ♂, Chitosan, Aeromonas hydrophila, Antioxidant capacity, Immunity, Intestinal morphology

Posted Date: September 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1636438/v1

Abstract

This study was performed to evaluate the effects of dietary chitosan on hybrid sturgeon (*Acipenser baerii* × *Acipenser schrenckii*). Sturgeons (18.18 ± 0.08 g) were randomly divided into four groups: control (0.00 g/kg), CHI1 (1.00 g/kg), CHI3 (3.00 g/kg) and CHI5 (5.00 g/kg), and fed with corresponding diets for 8 weeks. Then 30 fish from each group were intraperitoneally infected with *A. hydrophila*, and the mortality was recorded for 14 days. The results showed that there were significant differences of FBW, WG, SGR, FI and FCR in sturgeon fed chitosan diets compared to those in fish fed the control diet. Significant enhancement of LZM, ACP, AKP and MPO activities were observed in all fish serum fed the chitosan supplemented groups. Compared to control diet, the CAT, SOD and GSH-Px capacity were significantly increased, and the MDA content was decreased in liver of sturgeons fed chitosan supplemented diets. Moreover, visible enhancement of muscular thickness and goblet cells of fish mid intestine as well as evident increase in the muscular thickness and villus height of fish spiral valve were observed in the chitosan supplemented groups. The challenge test with *A. hydrophila* resulted in mortalities of 100%, 76.67%, 53.33% and 50.00% for hybrid sturgeons fed control, CHI1, CHI3, and CHI5 diets respectively. Taken together, our study revealed that dietary chitosan enhanced growth performance, elevated antioxidant capability and immunity, regulated intestinal morphology, and promoted resistance against *A. hydrophila* in hybrid sturgeon. The optimal dose was 3.00 g chitosan/kg diet for hybrid sturgeon.

1. Introduction

As the living fossil, sturgeon is one of the oldest teleost fishes with high economic and academic significance (G. Xu et al., 2018). At present, all 27 sturgeons were listed as endangered animals. With the increase of commercial purposes and human consumption, sturgeon culture has increased rapidly since 2000, making it the largest culture region in the world (Q. Wei, He, Yang, Zheng, & Li, 2004). Hybrid sturgeons are popular with farmers because of their rapid growth performance and relative resistance to disease (Q. W. Wei, Zou, Li, & Li, 2011). In particular, hybrid sturgeon of *Acipenser baerii* × *Acipenser schrenckii* is the most common hybrid varieties cultured in China (Li, Zou, & Wei, 2009).

With rapid expansion and intensification, the appearances of contagious diseases caused by viruses, bacteria, fungus and parasites have substantially accumulated and account the major limiting factor for sturgeon industry (Di et al., 2018; D. F. Zhang et al., 2015). Traditionally, antibiotics and chemotherapeutics were frequently used to treat this problem. However, the extensive and even overuse of drugs lead to the emergence of drug-resistant pathogens, bioaccumulation of residual antibiotics or chemical substances in tissues, and the destruction of microbiota in the aquatic system (RingØ et al., 2010; Zhou et al., 2018). Thus, it's urgent to search for safe and environmental friendly alternatives, such as administration of functional additives with different health-increasing properties for aquatic animals (Dawood, Koshio, & Esteban, 2018).

Various prebiotics have been developed and applied to serve as alternatives for antibiotics as they provide the stimulating factors of defense mechanism, thus offering protection against pathogenic infection (Akhter, Wu, Memon, & Mohsin, 2015). One of those potential prebiotics is chitosan, which is a cationic polymer obtained from the N-deacetylation of chitin and a promising feed additives with excellent features of biosafety, biodegradability, biocompatibility and bio-adhesion (Kamali Najafabad, Imanpoor, Taghizadeh, & Alishahi, 2016). Chitosan has been widely used in aquaculture industry and showed many physiological functions in fish, such as growth promoting, antioxidation, antimicrobial effect and immune regulation (Abdel-Ghany & Salem, 2020). It has been used in some aquaculture species such as *Misgurnus anguillicaudatus* (Yan, Guo, Dawood, & Gao, 2017), *Misgurnus anguillicaudatus* (Fadl et al., 2020), *Oncorhynchus mykiss* (Khani Oushani, Soltani, Sheikhzadeh, Shamsaie Mehrgan, & Rajabi Islami, 2020), *Carassius auratus gibelio* (Y. Chen et al., 2014), *Paralichthys olivaceus* (Cha, Lee, Song, Lee, & Jeon, 2008), *Oreochromis niloticus* (L.) (El-Naggar et al., 2021) and *Caspian kutum* (Kamali Najafabad et al., 2016). However, there is a limited research regarding the application of chitosan in sturgeon aquaculture.

In this study, we will assess the impacts of dietary chitosan on hybrid sturgeon growth performance, antioxidant activity, immunity, intestinal morphology and resistance to *A. hydrophila* infection. The results will deepen our understanding of the benefits of chitosan on sturgeon and provide practical applying strategies in the sturgeon culture. To our knowledge, this approach has been rarely conducted.

2. Material And Methods

2.1. Preparation of diets

In this research, four isonitrogenous (45% crude protein) and isolipidic (10% crude lipid) experimental diets were prepared (Table 1). The chitosan was supplemented in the basal diets at 1.00, 3.00 and 5.00 g/kg (designated as CHI1, CHI3 and CHI5, respectively), and the diet without chitosan is used as a control. The chitosan was purchased from Sigma-Aldrich, St. Louis, MO, USA and characterized as having a typical composition of 99% chitosan. Feeds were manufactured following the method described in previous research (H. Xu et al., 2022). Briefly, all dietary ingredients were ground into fine power with a hammer mill, blended homogenous using a Hobart-type mixer, and then fully mixed and processed into the form of 1 mm^{-3} diameter pellets. Pellets were heated at 60°C for 12 h in a ventilated oven and then stored in vacuum-packed bags at − 20°C until use.

Table 1
Formulation and proximate composition of the experimental diets

Ingredient (%)	Experimental diets g/kg			
	Control	CHI1	CHI3	CHI5
Fish meal[a]	510.00	510.00	510.00	510.00
Wheat flour[b]	376.00	375.00	373.00	371.00
Premixes[c]	14.00	14.00	14.00	14.00
Soy lecithin[d]	20.00	20.00	20.00	20.00
Fish oil	80.00	80.00	80.00	80.00
Chitosan	0.00	1.00	3.00	5.00
Proximate composition				
Moisture	9.16	9.13	9.09	9.03
Crude protein (% DM)	45.12	45.09	45.15	45.11
Ash (% DM)	6.53	6.56	6.58	6.61
Crude lipid (% DM)	10.21	10.16	10.09	10.12
[a] Fishmeal were purchased from TripleNine Fish product Co., Esbjerg, Denmark.				
[b] Guchan Group, Beijing, China.				
[c] Beijing Enhalor Biotech Ltd. Co. Beijing, China.				
[d] YiHai Kerry Investment Company Limited, Shandong, China.				

2.2. Fish management

A total of 600 disease-free hybrid sturgeons with average body weight of 18.18 ± 0.08 g were obtained from a local breeding farm. Prior to the experiment, all fish were temporarily cultured in fiber glass cylinders for 2 weeks and fed with control diet at 7:00 and 19:00 to acclimate the experimental conditions. Then the fish were randomly selected, batch weighed, and assembled into 12 aquaria (300-L water capacity) with 50 fish per tank in triplicate. During the experiment, fish were fed the allocated diet twice a day at 7:00 and 19:00 (2% body weight) to apparent satiation for 8 weeks. Feces and uneaten feed were removed by siphon. During the experiment, water temperature, Ammonia-N, dissolved oxygen and pH were maintained at 16.5 ± 1.0 ℃, 0.03 ± 0.01 mg/L, 7.75 ± 0.25 mg/L and 7.00 ± 0.20, respectively.

2.3. Sample collection

At the termination of eight-week feeding trial, all sturgeon from each tanks were fasted for 24 h, immersed with tricaine methane sulphonate (MS-222, 100 mg/L) for 10 min, and then batch weighed. Fish (N = 6) were randomly removed from each group and then stored at − 80°C for future investigation of whole-body proximate composition. To get sufficient blood for serum extraction, blood (6 fish/tank) was drawn from caudal veins with a 1-mL sterile syringe and immediately released into micro tubes without anticoagulant. The blood was incubated at room temperature for 2 h and then kept at 4°C overnight. After incubation, the serum was obtained after centrifugation at 1500 *g* for 10 min at 4°C, separated into several aliquots and then stored at − 80°C for further evaluation of biochemical and immunological assays. The mid intestine and spiral valve (N = 6) were removed and cut into small pieces using a scalpel, respectively. Sections of each segment were gently flushed with PBS to remove the contents and immediately fixed in 4% paraformaldehyde solution for histological analysis. Meanwhile, the liver was sampled, washed with sterile PBS, placed into Eppendorf tube and then stored at − 80°C for future analysis.

2.4. Growth performance

The body weights of all 50 fish in each tank were measured before and after the 8-week feeding experiment. Meanwhile, the diet used during the test in each tank was calculated. In addition, the liver and viscera of six fish in each group were also sampled and weighed. Based on recording the weight of sturgeon and amount of ingested diet, the growth performance and feed utilization was assessed as weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), hepatosomatic index (HSI) and viscerasomatic index (VSI) as follows:

WG (g) = FBW (g) – IBW (g)

SGR (%/day) = 100 × (Ln FBW – Ln IBW) / T

FCR = feed intake (g) / WG (g)

HSI (%) = liver weight (g) × 100 / body weight (g)

VSI (%) = viscera weight (g) × 100 / body weight (g)

Where IBW is the initial body weight, FBW is the final body weight and T is the number of days in the feeding period.

2.5. Analysis of diets and fish composition

Both experimental feed and fish chemical analysis were carried out according to the standard methods suggested by Association of Official Analytical Chemist (AOAC) (Horwitz, 2010). Briefly, the Moisture was analyzed by oven heating at 105°C for 6 h. The total nitrogen was tested by Kjeldahl method and converted to crude protein content by multiplying by 6.25. The total lipid content was measured through ether extraction method of Folch et al. (Floch, 1957), and crude ash level of samples was determined by incineration in a combustion oven at 550°C for 4 hrs.

2.6. Analysis of immune parameters in the serum

The levels of acid phosphatase (ACP), alkaline phosphatase (AKP), lysozyme (LZM) and myeloperoxidase (MPO) in serum samples were spectrophotometrically detected using commercial available reagent kits produced by Nanjing Jiancheng Bioengineer Institute (Jiangsu, China) following the instructions of the manufacture. Samples were measured in duplicate.

2.7. Determination of antioxidant enzyme activities in the liver

To detect the antioxidant activities in liver, the frozen pooled tissues were weighted and homogenized in ten volumes of ice-cold PBS with an electric homogenizer and centrifuged at 10000 *g* for 10 min. The supernatants were collected with a pipette and used for antioxidant measurement. The capacities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the levels of malondialdehyde (MDA), were detected using the commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.8. Intestinal morphological analysis

The hematoxylin-eosin (H&E) staining was used to estimate the effect of chitosan on intestine morphological as previous described (H. Xu, Xing, Tang, Sheng, & Zhan, 2020). Briefly, the fixed mid intestine and spiral valve were dehydrated in different grades of ethanol solutions, cleared with xylene and embedded in paraffin wax following the standard histological techniques. The wax blocks were cut into a thickness of 5 μm with Leica rotatory microtome, transferred onto pre-treated slides and then stained with hematoxylin and eosin (H&E) or Alcian blue-periodic acid Schiff (AB-PAS). The morphological structures of tissues were taken with an imaging microscope (BX60, Olympus, Tokyo, Japan) and captured using image acquisition software (CellSens standard, Olympus, Tokyo, Japan). The values of villus height (VH), villus width (VW), muscular thickness (MT) and crypt depth (CD), as well as the amount of goblet cells (GC) was measured and counted as previously reported (Kuebutornye et al., 2020; Standen et al., 2015). In each group, six fish were selected, and in each fish, six sections were examined.

2.9. Challenge test with *A. hydrophila*

The pathogenic strain of *A. hydrophila* was originally isolated from diseased sturgeon ascites and cultured in Luria Bertani broth (LB) for 18 h at 37°C. The LD_{50} for 7 days was determined by intraperitoneally injection of sturgeon with graded doses of *A. hydrophila* (3.0×10^5, 3.0×10^6, 3.0×10^7, 3.0×10^8 CFU/mL) and the LD_{50} on the 7th day was 1.5×10^6 CFU/mL based on the results of the test. The bacteria were obtained by centrifuging at 8000 *g* for 10 minutes and then re-suspending them in the sterile PBS.

After the sample collection, thirty fish from each group were anesthetized with MS222, and intraperitoneally infected with 0.2 mL of suspended *A. hydrophila* (1.5×10^6 CFU/mL). After infection, the

dead fish were recorded over a 14-day period, removed from the tanks immediately, and then confirmed the presence of *A. hydrophila* using light microscope and 16S rRNA gene sequence analysis as described previously (Abarike et al., 2018). The cumulative mortality rate (%) was calculated according to Ref. (Liu et al., 2017). The relative percent of survival (RPS) was calculated according to the following formula (Amend, 1981): RPS = {1 - (% mortality in treated fish / % mortality in control fish)} × 100.

2.10. Statistical analysis

All experimental data were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23.0 software for windows (IBM, Armonk, NY, USA). The effects of different concentrations of chitosan on the growth performance, antioxidant activity, immune parameters and intestinal morphology were analyzed with one-way analysis of variance (ANOVA) followed Duncan's multiple comparison test. The statistical significance was defined as $P < 0.05$. GraphPad prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was employed to plot figures.

3. Results

3.1. Growth performance and feed utilization

The growth performance and feed utilization of hybrid sturgeon fed with diets containing different concentrations of chitosan were showed in Table 2. After the 8-week feeding experiment, the growth performance parameters (FBW, WG and SGR) of hybrid sturgeon in the chitosan supplement groups were significantly higher ($P < 0.05$) than those in the control group, and those in CHI3 group was highest. On the other hand, the index of FCR exhibited significant decrease ($P < 0.05$) in the chitosan supplement groups and the lowest value was observed in fish from CHI3 group. It should be noticed that there was no significant effect ($P > 0.05$) on the levels of HIS and VIS when fed with diets containing chitosan.

Table 2
Growth performance of the hybrid sturgeon fed with diets containing different levels of chitosan for 8 weeks.

Parameters	Control	CHI1	CHI3	CHI5
IBW (g)	17.81 ± 0.65	17.90 ± 0.48	17.82 ± 0.64	17.84 ± 0.59
FBW (g)	34.88 ± 1.56^{a}	39.23 ± 1.05^{b}	42.24 ± 0.93^{c}	40.26 ± 1.08^{bc}
WG (g)	16.73 ± 1.48^{a}	20.99 ± 0.94^{b}	24.08 ± 0.93^{c}	22.09 ± 1.10^{bc}
SGR (%/day)	1.16 ± 0.07^{a}	1.36 ± 0.04^{ab}	1.51 ± 0.04^{b}	1.42 ± 0.05^{b}
FI (g/fish)	22.80 ± 0.52^{a}	26.32 ± 0.13^{c}	27.56 ± 0.12^{d}	25.47 ± 0.13^{b}
FCR	1.37 ± 0.15^{a}	1.25 ± 0.05^{b}	1.14 ± 0.04^{c}	1.15 ± 0.05^{bc}
VSI (%)	10.26 ± 0.28	10.04 ± 0.43	10.17 ± 0.27	10.07 ± 0.33
HSI (%)	4.60 ± 0.11	4.65 ± 0.34	4.46 ± 0.06	4.54 ± 0.13

Values in same line with different superscripts shows significant difference ($P < 0.05$). IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio, HIS: Hepatosomatic index, VSI: Viscerasomatic index.

3.2. Body composition

The effects of experimental diets on the whole body composition were presented Table 3. There were no remarkable differences in the whole body moisture, crude protein, crude ash and crude lipid of fish among all the groups ($P > 0.05$).

Table 3
Proximate chemical analysis of the whole body of hybrid sturgeon fed different levels of dietary chitosan for 8 weeks.

Treatments	Proximate composition of fish body (%, on weight basis)			
	Moisture	Crude protein	Ash	Crude lipid
Control	71.58 ± 1.34	12.36 ± 0.27	5.07 ± 0.12	11.23 ± 0.19
CHI1	69.86 ± 1.25	12.45 ± 0.36	5.12 ± 0.18	12.14 ± 1.21
CHI3	70.69 ± 1.18	12.56 ± 0.38	5.24 ± 0.23	12.31 ± 1.05
CHI5	71.56 ± 1.37	12.57 ± 0.33	5.33 ± 0.15	11.43 ± 0.96

Data were expressed as mean ± SD (n = 6).

3.3. Effects of dietary chitosan on serum non-specific immunity of hybrid sturgeon

The activities of LZM, ACP, AKP and MPO in fish serum after feeding with chitosan supplements and control diets for eight weeks were presented in Fig. 1. The results showed that the ACP and MPO activities of chitosan dietary groups were markedly higher ($P < 0.05$) than those from the control group, and the highest level was observed in fish from CHI3 group. The LZM activity for hybrid sturgeon was significantly increased ($P < 0.05$) with the concentration of chitosan, and that in CHI5 group exhibited the maximum value. However, there was no significant change found between CHI1 and CHI3 groups ($P > 0.05$). The AKP activity of fish serum in all treatment groups were significantly improved ($P < 0.05$) in comparison to those from control group.

3.4. Effects of dietary chitosan on antioxidant capacity in the liver of hybrid sturgeon

The potential effects of dietary chitosan on SOD, CAT, GSH-Px and MDA in fish liver were showed in Fig. 2. The SOD activity of hybrid sturgeon in CHI1, CHI3 and CHI5 groups were significantly higher ($P < 0.05$) than the control, and the highest SOD activity was observed in CHI3 group. Levels of CAT and GSH-Px exhibited significant enhancement ($P < 0.05$) in the fish liver from chitosan supplement groups than those from control group. Comparatively, there was no significant difference found among CHI1, CHI3 and CHI5 groups. However, the content of MDA exhibited remarkable decrease ($P < 0.05$) with the concentration of chitosan, and the lowest level was observed in fish liver from the CHI5 group.

3.5. Effects of dietary chitosan on mid-intestine morphology of hybrid sturgeon

After eight-week feeding experiment, the potential role of dietary supplemented chitosan on morphology indexes including the height of VH, VW, MT and CD and the amount of GC in the mid-intestine of fish were studied (Fig. 3). H&E staining revealed that sturgeon fed the different concentrations of chitosan diet have no inflammatory change in the mid intestine compared to the fish cultured with control diet (Fig. 3A). Considering the histochemical characters of mucins in goblet cells from the mid-intestine, results exhibit a variable richness of acid glycoproteins which were stained bright blue by AB-PAS (Fig. 3B). Moreover, there was no remarkable difference on the levels of VH, VW and CD in fish mid-intestine among CHI1, CHI3, CHI5 and control groups (Fig. 3C, 3D, 3F). When fish were fed with diets supplemented dietary chitosan level at 3.00 g/kg and 5.00 g/kg, muscle thickness exhibited significant value ($P < 0.05$) as compared to the fish in control and CHI1 groups (Fig. 3E). Additionally, significantly higher ($P < 0.05$) goblet cells measurements were observed in the CHI1, CHI3 and CHI5 groups as compared to the control group (Fig. 3G).

3.6. Effects of dietary chitosan on spiral valve morphology of hybrid sturgeon

The effects of dietary supplemented chitosan on spiral valve morphological parameters, such as the values of VH, VW, MT and CD, and the amount of GC in hybrid sturgeon were examined and analyzed by H&E and AB-PAS staining (Fig. 4). Results revealed that there were no evident spiral valve

histopathological changes in all experimental groups (Fig. 4A). Based on the histochemical analysis of mucins in spiral valve goblet cells, a variable amount of acid glycoproteins was found (Fig. 4B). The inclusion of chitosan significantly ($P < 0.05$) promoted the value of VH to the control group (Fig. 4C). Additionally, the MT of hybrid sturgeon fed with CHI1, CHI3 and CHI5 diets were extremely ($P < 0.05$) higher than those of fish from control group (Fig. 4E). However, the indexes of VW and CD as well as GC count of fish were not detectably ($P > 0.05$) affected by the experimental diets (Fig. 4D, F, G).

3.7. Chitosan protected hybrid sturgeon against *A. hydrophila* infection

Dietary supplemented different concentrations of chitosan affected the resistance against *A. hydrophila* of sturgeon and the cumulative mortality rates were shown in Fig. 5. After challenging with *A. hydrophila* for 14 days, the cumulative mortalities in chitosan dietary groups were significantly ($P < 0.05$) decreased than the control group, that is, 100%, 76.67%, 53.33% and 50.00% for hybrid sturgeon fed control, CHI1, CHI3, and CHI5 diets respectively. The RPS was highest in the CHI5 group (50.00%), followed by CHI3 (46.67%) and CHI1 (23.33%) groups.

4. Discussion

In aquaculture, growth promotion is a character of particular significance, because it directly connected with the productivity and profitability of enterprises (Serpil Mişe Yonar, 2019). Chitosan is a positive growth promoter and have been regarded as an essential prebiotic for the growth of aquatic animals, such as gibel carp (*Carassius auratus gibelio*) (Y Chen et al., 2014), cobia (*Rachycentron canadum*) (Geng et al., 2011b)and tiger shrimp (*Penaeus monodon*) (Niu et al., 2015). In this research, hybrid sturgeon fed with 1.00 g/kg, 3.00 g/kg and 5.00 g/kg dietary chitosan showed remarkable increase of growth performance evidenced by higher FBW, WG and SGR and lower FCR compared to the fish fed control diet. However, it is worth to note that when chitosan supplemented in diet reached 5.00 g/kg, the growth performance of hybrid sturgeon showed a comparatively decrease though the deference was not significant. The result is consistent with previous research which reported that the sea bass (*Dicentrarchus labrax*) feed with 0.5-4.0 g/kg chitosan have enhanced growth performance, and the optimal supplemental level was 1.0 g/kg (Zaki, Shatby, & Shatby, 2015). However, the significantly higher dietary concentration of chitosan was necessary in other fish species. For example, the growth character of grey mullet (*Mugil cephalus*) has a liner relation with the concentration of chitosan, and the fish fed on diets supplemented with dietary chitosan at concentrations of 10 and 15 g/kg showed significant growth performance compared with those fed with diets containing 5 g/kg chitosan (Akbary & Younesi, 2017). Similarly in European carp (*Cyprinus carpio*), the inclusion of chitosan at a high level of 20 g/kg enhanced the growth rates (Maqsood, Singh, Samoon, & Khansaheb Balange, 2010). The discrepancy in the optimal level of dietary chitosan partial due to differences among fish species or experimental conditions.

The quality of an intestine is determined by biological factors like values of muscle thickness and intestinal villus height as well as numbers of goblet cells (Banan Khojasteh, 2012). The absorption of nutrients by the intestine defines a host's growth performance, which has a directly relation to its morphology (Pirarat et al., 2011). The increment in VL and VW induces the enhancement of intestinal absorption surface area for nutrients (Kuebutornye et al., 2020). Muscular layer mainly play a role in peristaltic motion and increased MT level commonly implies an improvement of intestinal function associated with digestion (F. Chen & Wang, 2013). The increase in crypt depth is coincidence with an improvement production rate of crypt-cell and an overall stimulation of cell renewal in the intestine that has universally been connected with a decreased digestive and absorptive activity (Jiménez et al., 2020; Pluske, Williams, & Aherne, 1996). Goblet cells produce and secrete biologically active substances, generally considered as mucus and glycoproteins components, which have been reported to exert an important role in gut immune systems (Blomberg, Henriksson, & Conway, 1993; Knoop & Newberry, 2018). Moreover, the produced substances cover on the surface of gastrointestinal epithelium where by intestine barrier function is enhanced (Deplancke & Gaskins, 2001). This study showed that dietary inclusion of chitosan increased the value of muscle thickness and amount of goblet cells in the mid intestine of fish, suggesting the improvement of nutrient utilization and immunity. In sturgeon, the supreme nutrient absorption occurred in spiral valve (Caimi et al., 2020), this segment was therefore considered. In the current research, no significant differences of VH, VW and CD were observed in the spiral valve of sturgeon among all experimental groups. However, evident increase of MT and the number of acid mucin producing goblet cells were recorded in chitosan dietary groups compared to the control group, indicating the positive effect of chitosan on spiral valve absorption and disease resistance.

The innate immune system, first line of defense for fish immunity, exerts a key role in preventing invaded pathogens and initiating adaptive immune responses (Magnadóttir, 2006). Lysozyme, referred to as N–acetylmuramide glycanohydrolase or muramidase, is an important bacteriolytic enzyme known to controls microorganism colonization and proliferation by destroying cell wall polysaccharides, resulting in cell wall lyse and bacterial death (Saurabh & Sahoo, 2008; Z.-H. Zhang et al., 2020). Previous studies revealed that chitosan supplementation increased the lysozyme capacity in juvenile loach (*Misgurnus anguillicaudatus*) (Yan et al., 2017), cobia (*Rachycentron canadum*) (Geng et al., 2011a), and gibel carp (*Carassius auratus gibelio*) (Y. Chen et al., 2014). In this study, the serum LZM activity was increased with the concentration of dietary chitosan and the highest value was observed in the CHI3 group which implied the dietary chitosan might improve the immunity of the hybrid sturgeon. ACP and AKP, two kinds of phosphatase capable of hydrolyzing organic phosphate esters, play pivotal roles in non-specific immune system of animals (M. Chen, Chen, Tian, Liu, & Niu, 2020; Tseng et al., 2009) and have been considered as a symbol of macrophage stimulation for the capacity to intracellularly digest antigens in the immune system of animals (Yin, Gong, Ke, & Li, 2015). In this research, as dietary chitosan increased, the activities of ACP and AKP exhibited the tendency of initially increased and then decreased. This was similar with the results in previous studies which demonstrated that the ACP and AKP activities have the similar tendency after fed diets supplement with prebiotics (Q.-Q. Chen et al., 2016; Jia et al., 2017). MPO is a particularly vital enzyme function in the elimination of microorganisms (X. Chen et al., 2020), which

could utilize hydrogen peroxide to generate hypochlorous acid (Dalmo, Ingebrigtsen, & Bøgwald, 1997) and subsequently eliminate invading pathogens through destroying a variety of target substances (Johnston Jr, 1978). The current investigation displayed that the activities of MPO in serum showed an improvement as the concentration of chitosan increased from 1.00 to 3.00 g/kg, then declined when the supplement level reached 5.00 g/kg, indicating that dietary chitosan supplementation within a reasonable range could elevate the immune system and may be able to prevent sturgeon from harmful microorganisms. Various studies have demonstrated that prebiotics elevated the MPO activity in fish (Mohammadi, Rafiee, El Basuini, Abdel-Latif, & Dawood, 2020; G. Xu et al., 2018). In general, the increase of LZM ACP, AKP and MPO activities in the serum, suggesting that dietary chitosan could promote the innate immunity capacity of sturgeon.

The balance between generation and clearance of reactive oxygen species (ROS) is essential for sustaining the dynamics of normal metabolism in the fish body (Ibrahim et al., 2021). Under normal physiological conditions, cells generate ROS. Meanwhile, the body is enclosed by a complex network of antioxidant system that represent as SOD, CAT and GSH-Px to eliminate excessive ROS that may exert serious impairment of DNA and other macromolecules (Aliko, Qirjo, Sula, Morina, & Faggio, 2018; Regoli & Giuliani, 2014). The enzyme SOD, first line defense against oxidative stress, exerts the role in catalyzing toxic superoxide anions produced in the tissues through cellular metabolism or reactions to O_2 and H_2O_2, which would be then transformed into H_2O and O_2 by CAT and GSH-Px to complete the detoxification process prior to attacking the cellular components (Jamalzad Falah, Rajabi Islami, & Shamsaie Mehrgan, 2020; Yu, 1994). Therefore, analysis of those enzyme activities can offer a reflection of the antioxidant function in animals, as also can serve as indicator of oxidative stress (C.-N. Zhang et al., 2013). The oxidative stress lead to the production of excessive ROS in cellular components which could induce lipid peroxidation and accumulation of lipid peroxides in cell (Ferreira, Moradas-Ferreira, & Reis-Henriques, 2005; S. M. Yonar, Sakin, Yonar, Ispir, & Kirici, 2011). MDA as a highly toxic substance produced by lipid peroxidation can be used to elevate the degree of lipid peroxidation and cell damage in fish (Buege & Aust, 1978). The present study illustrated that dietary chitosan could improve the antioxidant capacity where activities of SOD, CAT, and GSH-Px upregulated significantly, while values of MDA downregulated significantly. These results are indicative to the enhancement of antioxidant activity and the alleviation of oxidative stress. Similar results were observed in previous study which showed that chitosan supplementation induced an apparent increase of enzymatic (SOD, CAT and GSH-Px activities) antioxidant and a significant decrease in the MDA level (El-Naggar et al., 2021; Niu et al., 2015). The antioxidant activity of chitosan can be due to free radical-scavenging activities through the donation of hydrogen or one pair of electrons and its abilities of chelating metal ions (S. B. Lin, Chen, & Peng, 2009; Ngo & Kim, 2014).

Feeding fish with supplemented prebiotics and then challenging them with pathogenic microorganism is one of the most valuable way to investigate the potential function of the supplements in terms of resistance to pathogen infections (Xing, Xu, Tang, Sheng, & Zhan, 2019). The decrease of cumulative mortality rate or increase of relative percent survival reflected promotion of fish immunity and resistance

to bacteria. In this research, it is mentioned that dietary supplement with chitosan relatively improved the resistant of hybrid sturgeon against *A. hydrophila*, where the dose of 5.00 g/kg diet recorded the highest protection. The increased protection was in line with the results previously reported in gibel carp (*Carassius auratus gibelio*) (Y. Chen et al., 2014), rainbow trout (*Oncorhynchus mykiss*) (Ahmed et al., 2021) and koi (*Cyprinus carpio koi*) (S. M. Lin et al., 2012), which revealed that fish administrated with prebiotics induced lower cumulative mortality rate or higher relative percent survival rate in the challenge experiment. The positive results may be explained by stimulation of antioxidant activity and non-specific immunity after administration of chitosan.

5. Conclusions

In conclusion, results of the present study indicated that feeding hybrid sturgeon with diets containing chitosan for 8 weeks has an increasing effect on growth performance, antioxidant activity, immunity and intestinal morphology as well as the resistance of fish against *A. hydrophila*. Considering the economic benefits and production performance, the optimal dietary chitosan concentration for hybrid sturgeon could be recommend as 3.00 g/kg diet.

Declarations

Author contribution

Hongsen Xu: Conceptualization, Software, Writing-original draft, Writing-review & editing, Funding acquisition. **Haoran Sun:** Methodology, Data curation, Software, Writing-original draft, Project administration. **Qianrong Liang:** Writing-review & editing. **Fuguo Liu:** Investigation, Writing-review & editing. **Jun Liu:** Conceptualization, Writing-review & editing. **Denghang Yu:** Conceptualization, Writing-review & editing, Supervision, Validation, Funding acquisition.

Funding

This study was supported by the Natural Science Foundation of Hubei Province (grant no. 2021CFB265), National Natural Science Foundation of China (grant no. 31902389), Engineering Research Center of Feed Protein Resources on Agricultural By-products, Ministry of Education (grant no. DKXY2020015) and Key Laboratory of Special Aquatic Formula Feed (grant no. TMKJZ1706 and TMKJZ1912).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Declarations

Ethics approval

Procedures for animal care and handling were conducted in accordance with the ethical standards and the guidelines of “Regulations for the Administration of Affairs Concerning Experimental Animals” documented by the State Science and Technology Commission of Hubei Province. These investigations were also approved by the Ethics Committee for Animal Care and Experiments at Wuhan Polytechnic University.

Consent to participate

All the authors participated to prepare the manuscript in all stages.

Consent for publication

All the authors approved to submit the present manuscript to Fish Physiology and Biochemistry.

Conflicts of interest

All authors declare that they have no competing interests.

References

1. Abarike, E. D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., . . . Kuebutornye, F. K. (2018). Effects of a commercial probiotic BS containing Bacillus subtilis and Bacillus licheniformis on growth, immune response and disease resistance in Nile tilapia, Oreochromis niloticus. *Fish & Shellfish Immunology, 82*, 229-238.
2. Abdel-Ghany, H. M., & Salem, M. E. (2020). Effects of dietary chitosan supplementation on farmed fish; a review. *Reviews in Aquaculture, 12*(1), 438-452. doi:10.1111/raq.12326
3. Ahmed, F., Soliman, F. M., Adly, M. A., Soliman, H. A. M., El-Matbouli, M., & Saleh, M. (2021). Dietary Chitosan Nanoparticles: Potential Role in Modulation of Rainbow Trout (Oncorhynchus mykiss) Antibacterial Defense and Intestinal Immunity against Enteric Redmouth Disease. *Marine Drugs, 19*(2). doi:10.3390/md19020072
4. Akbary, P., & Younesi, A. (2017). Effect of dietary supplementation of Chitosan on growth, hematology and innate immunity of grey Mullet (Mugil cephalus). *Veterinary Researches & Biological Products, 30*(3), 194-203.
5. Akhter, N., Wu, B., Memon, A. M., & Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: A review. *Fish Shellfish Immunol, 45*(2), 733-741. doi:10.1016/j.fsi.2015.05.038

6. Aliko, V., Qirjo, M., Sula, E., Morina, V., & Faggio, C. (2018). Antioxidant defense system, immune response and erythron profile modulation in gold fish, Carassius auratus, after acute manganese treatment. *Fish Shellfish Immunol, 76*, 101-109. doi:10.1016/j.fsi.2018.02.042
7. Amend, D. F. (1981). Potency testing of fish vaccines. *Fish biologics: serodiagnostics and vaccines*, 447-454.
8. Banan Khojasteh, S. M. (2012). The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. *Int. J. of Aquatic Science, 3*(2), 71-88.
9. Blomberg, L., Henriksson, A., & Conway, P. (1993). Inhibition of adhesion of Escherichia coli K88 to piglet ileal mucus by Lactobacillus spp. *Applied and Environmental Microbiology, 59*(1), 34-39.
10. Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310): Elsevier.
11. Caimi, C., Gasco, L., Biasato, I., Malfatto, V., Varello, K., Prearo, M., . . . Gai, F. (2020). Could Dietary Black Soldier Fly Meal Inclusion Affect the Liver and Intestinal Histological Traits and the Oxidative Stress Biomarkers of Siberian Sturgeon (Acipenser baerii) Juveniles? *Animals, 10*(1). doi:10.3390/ani10010155
12. Cha, S.-H., Lee, J.-S., Song, C.-B., Lee, K.-J., & Jeon, Y.-J. (2008). Effects of chitosan-coated diet on improving water quality and innate immunity in the olive flounder, Paralichthys olivaceus. *Aquaculture, 278*(1-4), 110-118. doi:10.1016/j.aquaculture.2008.01.025
13. Chen, F., & Wang, H. (2013). Study on histological structure of intestine in Gymnocypris przewalskii with different age. *Prog. Vet. Med, 1*, 34-37.
14. Chen, M., Chen, X.-Q., Tian, L.-X., Liu, Y.-J., & Niu, J. (2020). Enhanced intestinal health, immune responses and ammonia resistance in Pacific white shrimp (Litopenaeus vannamei) fed dietary hydrolyzed yeast (Rhodotorula mucilaginosa) and Bacillus licheniformis. *Aquaculture Reports, 17*, 100385.
15. Chen, Q.-Q., Liu, W.-B., Zhou, M., Dai, Y.-J., Xu, C., Tian, H.-Y., & Xu, W.-N. (2016). Effects of berberine on the growth and immune performance in response to ammonia stress and high-fat dietary in blunt snout bream Megalobrama amblycephala. *Fish & Shellfish Immunology, 55*, 165-172.
16. Chen, X., Xie, J., Liu, Z., Yin, P., Chen, M., Liu, Y., . . . Niu, J. (2020). Modulation of growth performance, non-specific immunity, intestinal morphology, the response to hypoxia stress and resistance to Aeromonas hydrophila of grass carp (Ctenopharyngodon idella) by dietary supplementation of a multi-strain probiotic. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 231*, 108724.
17. Chen, Y., Zhu, X., Yang, Y., Han, D., Jin, J., & Xie, S. (2014). Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance in gibel carp (C arassius auratus gibelio). *Aquaculture Nutrition, 20*(5), 532-546.
18. Chen, Y., Zhu, X., Yang, Y., Han, D., Jin, J., & Xie, S. (2014). Effect of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and

disease resistance in gibel carp (Carassius auratus gibelio). *Aquaculture Nutrition, 20*(5), 532-546. doi:10.1111/anu.12106

19. Dalmo, R., Ingebrigtsen, K., & Bøgwald, J. (1997). Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of Fish Diseases, 20*(4), 241-273.
20. Dawood, M. A. O., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture, 10*(4), 950-974. doi:10.1111/raq.12209
21. Deplancke, B., & Gaskins, H. R. (2001). Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *The American journal of clinical nutrition, 73*(6), 1131S-1141S.
22. Di, J., Zhang, S., Huang, J., Du, H., Zhou, Y., Zhou, Q., & Wei, Q. (2018). Isolation and identification of pathogens causing haemorrhagic septicaemia in cultured Chinese sturgeon (Acipenser sinensis). *Aquaculture Research, 49*(11), 3624-3633. doi:10.1111/are.13830
23. El-Naggar, M., Salaah, S., El-Shabaka, H., Abd El-Rahman, F., Khalil, M., & Suloma, A. (2021). Efficacy of dietary chitosan and chitosan nanoparticles supplementation on health status of Nile tilapia, Oreochromis niloticus (L.). *Aquaculture Reports, 19*. doi:10.1016/j.aqrep.2021.100628
24. Fadl, S., El-Gammal, G. A., Abdo, W. S., Barakat, M., Sakr, O. A., Nassef, E., . . . El-Sheshtawy, H. S. (2020). Evaluation of dietary chitosan effects on growth performance, immunity, body composition and histopathology of Nile tilapia (Oreochromis niloticus) as well as the resistance to Streptococcus agalactiae infection. *Aquaculture Research, 51*(3), 1120-1132. doi:10.1111/are.14458
25. Ferreira, M., Moradas-Ferreira, P., & Reis-Henriques, M. (2005). Oxidative stress biomarkers in two resident species, mullet (Mugil cephalus) and flounder (Platichthys flesus), from a polluted site in River Douro Estuary, Portugal. *Aquatic Toxicology, 71*(1), 39-48.
26. Floch, J. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem., 226*, 497-509.
27. Geng, X., Dong, X. H., Tan, B. P., Yang, Q. H., Chi, S. Y., Liu, H. Y., & Liu, X. Q. (2011a). Effects of dietary chitosan and Bacillus subtilis on the growth performance, non-specific immunity and disease resistance of cobia, Rachycentron canadum. *Fish Shellfish Immunol, 31*(3), 400-406. doi:10.1016/j.fsi.2011.06.006
28. Geng, X., Dong, X. H., Tan, B. P., Yang, Q. H., Chi, S. Y., Liu, H. Y., & Liu, X. Q. (2011b). Effects of dietary chitosan and Bacillus subtilis on the growth performance, non-specific immunity and disease resistance of cobia, Rachycentron canadum. *Fish & Shellfish Immunology, 31*(3), 400-406. doi:10.1016/j.fsi.2011.06.006
29. Horwitz, W. (2010). *Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs/edited by William Horwitz*: Gaithersburg (Maryland): AOAC International, 1997.
30. Ibrahim, R. E., Amer, S. A., Farroh, K. Y., Al-Gabri, N. A., Ahmed, A. I., El-Araby, D. A., & Ahmed, S. A. (2021). The effects of chitosan-vitamin C nanocomposite supplementation on the growth

performance, antioxidant status, immune response, and disease resistance of Nile tilapia (Oreochromis niloticus) fingerlings. *Aquaculture, 534*, 736269.

31. Jamalzad Falah, F., Rajabi Islami, H., & Shamsaie Mehrgan, M. (2020). Dietary folic acid improved growth performance, immuno-physiological response and antioxidant status of fingerling Siberian sturgeon, Acipenser baerii (Brandt 1896). *Aquaculture Reports, 17*. doi:10.1016/j.aqrep.2020.100391
32. Jia, E., Li, Z., Xue, Y., Jiang, G., Li, X., Liu, W., & Zhang, D. (2017). Effects of dietary fructooligosaccharide on the growth, antioxidants, immunity and disease resistance of Chinese mitten crab. *Aquaculture, 481*, 154-161.
33. Jiménez, M. J., Berrios, R., Stelzhammer, S., Hohmann, M., Verri Jr, W., & Bracarense, A. P. F. (2020). Ingestion of organic acids and cinnamaldehyde improves tissue homeostasis of piglets exposed to enterotoxic Escherichia coli (ETEC). *Journal of Animal Science, 98*(2), skaa012.
34. Johnston Jr, R. (1978). *Oxygen metabolism and the microbicidal activity of macrophages.* Paper presented at the Federation Proceedings.
35. Kamali Najafabad, M., Imanpoor, M. R., Taghizadeh, V., & Alishahi, A. (2016). Effect of dietary chitosan on growth performance, hematological parameters, intestinal histology and stress resistance of Caspian kutum (Rutilus frisii kutum Kamenskii, 1901) fingerlings. *Fish Physiol Biochem, 42*(4), 1063-1071. doi:10.1007/s10695-016-0197-3
36. Khani Oushani, A., Soltani, M., Sheikhzadeh, N., Shamsaie Mehrgan, M., & Rajabi Islami, H. (2020). Effects of dietary chitosan and nano-chitosan loaded clinoptilolite on growth and immune responses of rainbow trout (Oncorhynchus mykiss). *Fish Shellfish Immunol, 98*, 210-217. doi:10.1016/j.fsi.2020.01.016
37. Knoop, K. A., & Newberry, R. D. (2018). Goblet cells: multifaceted players in immunity at mucosal surfaces. *Mucosal immunology, 11*(6), 1551-1557.
38. Kuebutornye, F. K. A., Wang, Z., Lu, Y., Abarike, E. D., Sakyi, M. E., Li, Y., . . . Hlordzi, V. (2020). Effects of three host-associated Bacillus species on mucosal immunity and gut health of Nile tilapia, Oreochromis niloticus and its resistance against Aeromonas hydrophila infection. *Fish Shellfish Immunol, 97*, 83-95. doi:10.1016/j.fsi.2019.12.046
39. Li, B. R., Zou, Y., & Wei, Q. (2009). Sturgeon aquaculture in China: status of current difficulties as well as future strategies based on 2002–2006/2007 surveys in eleven provinces. *Journal of Applied Ichthyology, 25*(6), 632-639.
40. Lin, S. B., Chen, S. H., & Peng, K. C. (2009). Preparation of antibacterial chito-oligosaccharide by altering the degree of deacetylation of β-chitosan in a Trichoderma harzianum chitinase-hydrolysing process. *Journal of the Science of Food and Agriculture, 89*(2), 238-244.
41. Lin, S. M., Mao, S. H., Guan, Y., Luo, L., Luo, L., & Pan, Y. (2012). Effects of dietary chitosan oligosaccharides and Bacillus coagulans on the growth, innate immunity and resistance of koi (Cyprinus carpio koi). *Aquaculture, 342*, 36-41. doi:10.1016/j.aquaculture.2012.02.009
42. Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., . . . Zheng, Y. (2017). Dietary administration of Bacillus subtilis HAINUP40 enhances growth, digestive enzyme activities, innate immune responses

and disease resistance of tilapia, Oreochromis niloticus. *Fish & Shellfish Immunology, 60*, 326-333.

43. Magnadóttir, B. (2006). Innate immunity of fish (overview). *Fish & Shellfish Immunology, 20*(2), 137-151.
44. Maqsood, S., Singh, P., Samoon, M. H., & Khansaheb Balange, A. (2010). Effect of dietary chitosan on non-specific immune response and growth of Cyprinus carpio challenged with Aeromonas hydrophila. *International Aquatic Research, 2*(2), 77-85.
45. Mohammadi, G., Rafiee, G., El Basuini, M. F., Abdel-Latif, H. M. R., & Dawood, M. A. O. (2020). The growth performance, antioxidant capacity, immunological responses, and the resistance against Aeromonas hydrophila in Nile tilapia (Oreochromis niloticus) fed Pistacia vera hulls derived polysaccharide. *Fish Shellfish Immunol, 106*, 36-43. doi:10.1016/j.fsi.2020.07.064
46. Ngo, D.-H., & Kim, S.-K. (2014). Antioxidant effects of chitin, chitosan, and their derivatives. *Advances in food and nutrition research, 73*, 15-31.
47. Niu, J., Li, C. H., Tian, L. X., Liu, Y. J., Chen, X., Wu, K. C., . . . Lin, H. Z. (2015). Suitable dietary chitosan improves the growth performance, survival and immune function of tiger shrimp, Penaeus monodon. *Aquaculture Research, 46*(7), 1668-1678. doi:10.1111/are.12321
48. Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., & Maita, M. (2011). Modulation of intestinal morphology and immunity in nile tilapia (Oreochromis niloticus) by Lactobacillus rhamnosus GG. *Research in veterinary science, 91*(3), e92-e97.
49. Pluske, J., Williams, I., & Aherne, F. (1996). Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Animal Science, 62*(1), 131-144.
50. Regoli, F., & Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Marine environmental research, 93*, 106-117.
51. RingØ, E., Olsen, R. E., Gifstad, T. Ø., Dalmo, R. A., Amlund, H., Hemre, G. I., & Bakke, A. M. (2010). Prebiotics in aquaculture: a review. *Aquaculture Nutrition, 16*(2), 117-136. doi:10.1111/j.1365-2095.2009.00731.x
52. Saurabh, S., & Sahoo, P. K. (2008). Lysozyme: an important defence molecule of fish innate immune system. *Aquaculture Research, 39*(3), 223-239. doi:10.1111/j.1365-2109.2007.01883.x
53. Standen, B., Rodiles, A., Peggs, D., Davies, S., Santos, G., & Merrifield, D. (2015). Modulation of the intestinal microbiota and morphology of tilapia, Oreochromis niloticus, following the application of a multi-species probiotic. *Applied Microbiology and Biotechnology, 99*(20), 8403-8417.
54. Tseng, D.-Y., Ho, P.-L., Huang, S.-Y., Cheng, S.-C., Shiu, Y.-L., Chiu, C.-S., & Liu, C.-H. (2009). Enhancement of immunity and disease resistance in the white shrimp, Litopenaeus vannamei, by the probiotic, Bacillus subtilis E20. *Fish & Shellfish Immunology, 26*(2), 339-344.
55. Wei, Q., He, J., Yang, D., Zheng, W., & Li, L. (2004). Status of sturgeon aquaculture and sturgeon trade in China: a review based on two recent nationwide surveys. *Journal of Applied Ichthyology, 20*(5), 321-332.
56. Wei, Q. W., Zou, Y., Li, P., & Li, L. (2011). Sturgeon aquaculture in China: progress, strategies and prospects assessed on the basis of nation-wide surveys (2007-2009). *Journal of Applied Ichthyology,*

27(2), 162-168. doi:10.1111/j.1439-0426.2011.01669.x

57. Xing, J., Xu, H., Tang, X., Sheng, X., & Zhan, W. (2019). A DNA Vaccine Encoding the VAA Gene of Vibrio anguillarum Induces a Protective Immune Response in Flounder. *Front Immunol, 10*, 499. doi:10.3389/fimmu.2019.00499
58. Xu, G., Xing, W., Li, T., Ma, Z., Liu, C., Jiang, N., & Luo, L. (2018). Effects of dietary raffinose on growth, non-specific immunity, intestinal morphology and microbiome of juvenile hybrid sturgeon (Acipenser baeri Brandt female symbol x A. schrenckii Brandt male symbol). *Fish Shellfish Immunol, 72*, 237-246. doi:10.1016/j.fsi.2017.11.001
59. Xu, H., Su, Y., Zhang, L., Tian, T., Xu, R., Sun, H., . . . Liu, J. (2022). Effects of dietary galactooligosaccharide on growth, antioxidants, immunity, intestinal morphology and disease resistance against Aeromons hydrophila in juvenile hybrid sturgeon (Acipenser baerii × A. schrenckii). *Aquaculture Reports, 23*, 101097.
60. Xu, H., Xing, J., Tang, X., Sheng, X., & Zhan, W. (2020). The effects of CCL3, CCL4, CCL19 and CCL21 as molecular adjuvants on the immune response to VAA DNA vaccine in flounder (Paralichthys olivaceus). *Developmental & Comparative Immunology, 103*. doi:10.1016/j.dci.2019.103492
61. Yan, J., Guo, C., Dawood, M. A. O., & Gao, J. (2017). Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in Misgurnus anguillicaudatus. *Benef Microbes, 8*(3), 439-449. doi:10.3920/BM2016.0177
62. Yin, F., Gong, H., Ke, Q., & Li, A. (2015). Stress, antioxidant defence and mucosal immune responses of the large yellow croaker Pseudosciaena crocea challenged with Cryptocaryon irritans. *Fish & Shellfish Immunology, 47*(1), 344-351.
63. Yonar, S. M. (2019). Growth performance, haematological changes, immune response, antioxidant activity and disease resistance in rainbow trout (Oncorhynchus mykiss) fed diet supplemented with ellagic acid. *Fish & Shellfish Immunology, 95*, 391-398.
64. Yonar, S. M., Sakin, F., Yonar, M. E., Ispir, U., & Kirici, M. (2011). OXIDATIVE STRESS BIOMARKERS OF EXPOSURE TO DELTAMETHRIN IN RAINBOW TROUT FRY (Oncorhynchus mykiss). *Fresenius Environmental Bulletin, 20*(8), 1931-1935.
65. Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiological reviews, 74*(1), 139-162.
66. Zaki, M., Shatby, E., & Shatby, E. (2015). Effect of chitosan supplemented diet on survival, growth, feed utilization, body composition & histology of sea bass (Dicentrarchus labrax). *World Journal of Engineering and Technology, 3*(04), 38.
67. Zhang, C.-N., Li, X.-F., Xu, W.-N., Jiang, G.-Z., Lu, K.-L., Wang, L.-N., & Liu, W.-B. (2013). Combined effects of dietary fructooligosaccharide and Bacillus licheniformis on innate immunity, antioxidant capability and disease resistance of triangular bream (Megalobrama terminalis). *Fish & Shellfish Immunology, 35*(5), 1380-1386.
68. Zhang, D. F., Ji, C., Zhang, X. J., Li, T. T., Li, A. H., & Gong, X. N. (2015). Mixed mycobacterial infections in farmed sturgeons. *Aquaculture Research, 46*(8), 1914-1923. doi:10.1111/are.12346

69. Zhang, Z.-H., Chen, M., Xie, S.-W., Chen, X.-Q., Liu, Y.-J., Tian, L.-X., & Niu, J. (2020). Effects of dietary xylooligosaccharide on growth performance, enzyme activity and immunity of juvenile grass carp, Ctenopharyngodon idellus. *Aquaculture Reports, 18*. doi:10.1016/j.aqrep.2020.100519
70. Zhou, L., Limbu, S. M., Shen, M., Zhai, W., Qiao, F., He, A., . . . Zhang, M. (2018). Environmental concentrations of antibiotics impair zebrafish gut health. *Environ Pollut, 235*, 245-254. doi:10.1016/j.envpol.2017.12.073

Figures

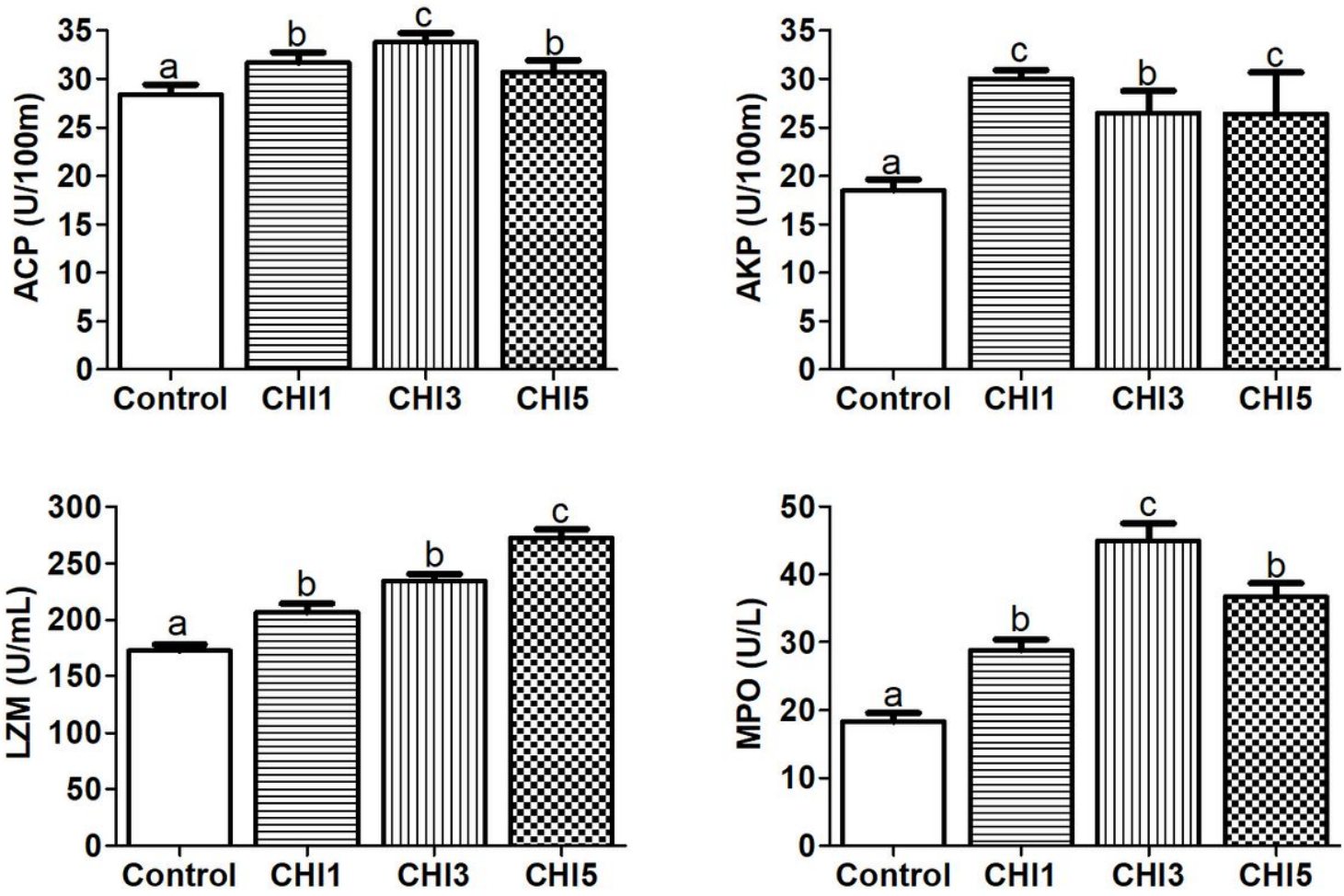

Figure 1

Activities of immune related enzyme in serum of hybrid sturgeon fed diets with different levels of chitosan. Values are presented as means ± SD of six individual fish (N = 6). Different letters above the bars indicate the statistical differences ($P < 0.05$).

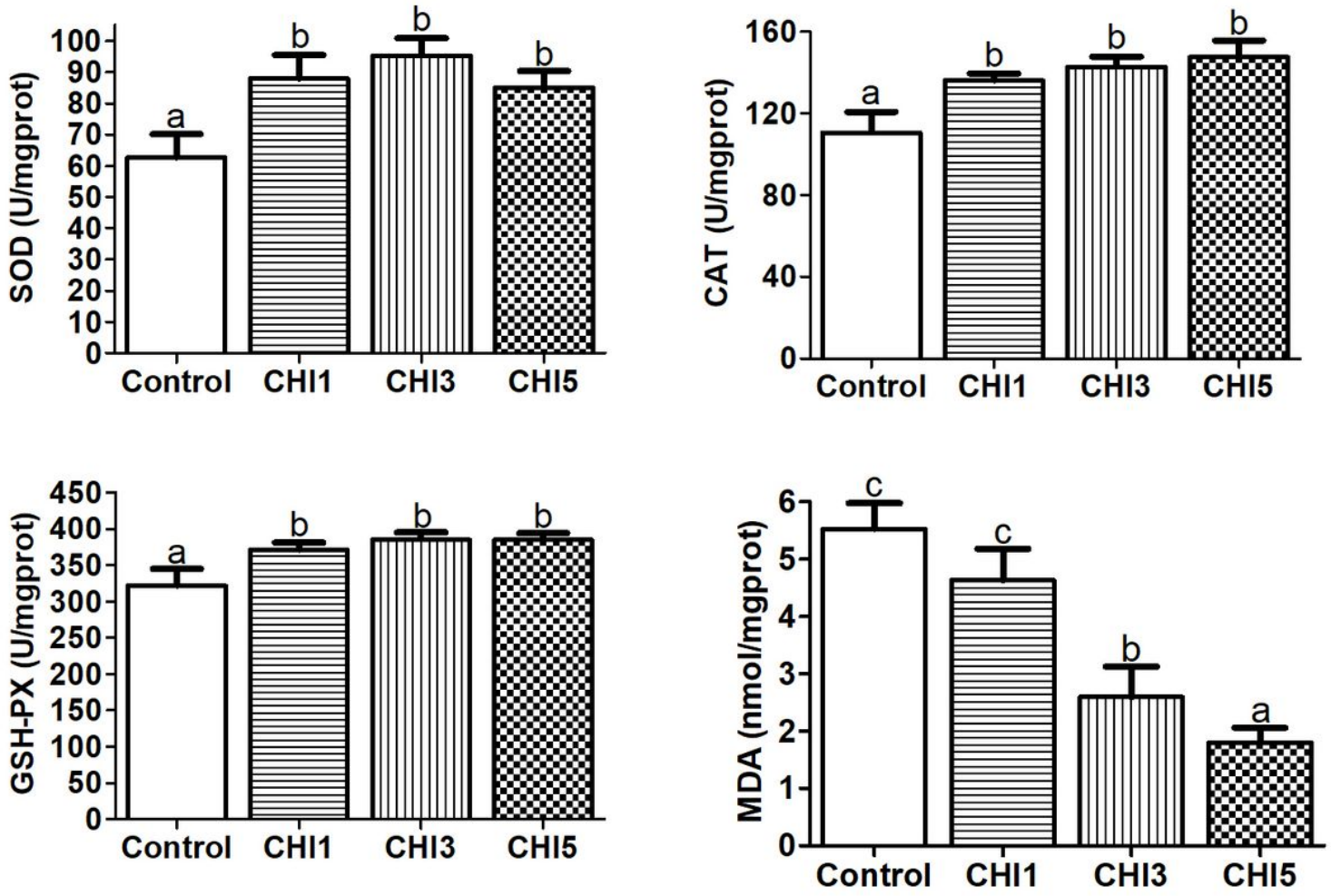

Figure 2

Antioxidant responses of hybrid sturgeon fed with the chitosan at different concentrations in the 8-week feeding trial. Antioxidant parameters assayed were the following: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and malondialdehyde (MDA). Values are presented as mean ± SD of six individual fish (N = 6). Bars assigned with different letters are significantly different ($P < 0.05$).

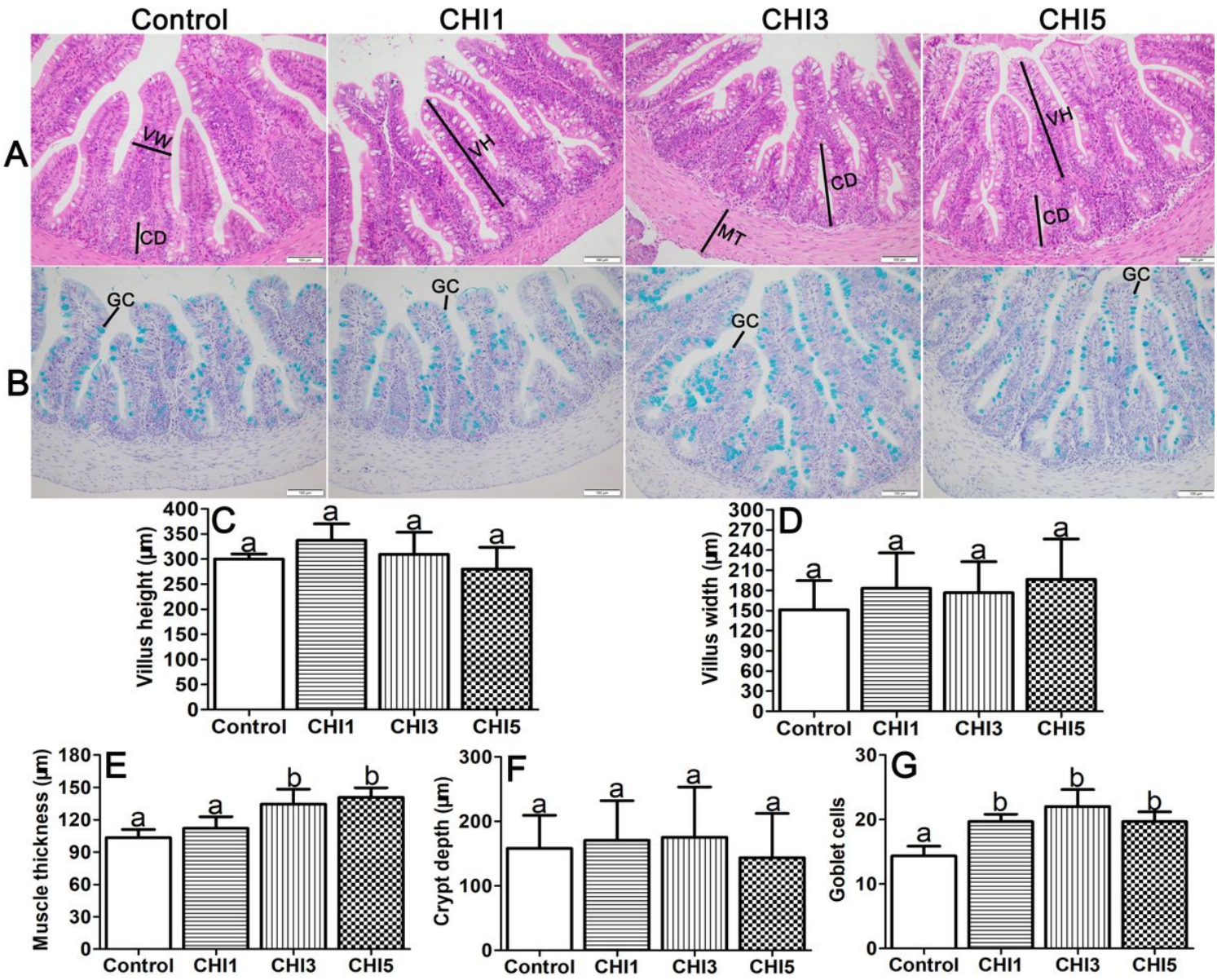

Figure 3

Histological examination of the mid-gut morphology after 8-week feeding trial. (A) H&E staining. (B) AB-PAS staining. (C) villus length. (D) villus width. (E) muscle thickness. (F) crypt depth. (G) goblet cells. VH: villus height, VW: villus width, MT: muscle thickness, CD: crypt depth, GC: goblet cells. All values are expressed as the mean ± SD of six replicates, and mean values with different letters differ at the level of $P < 0.05$. Magnification, × 200.

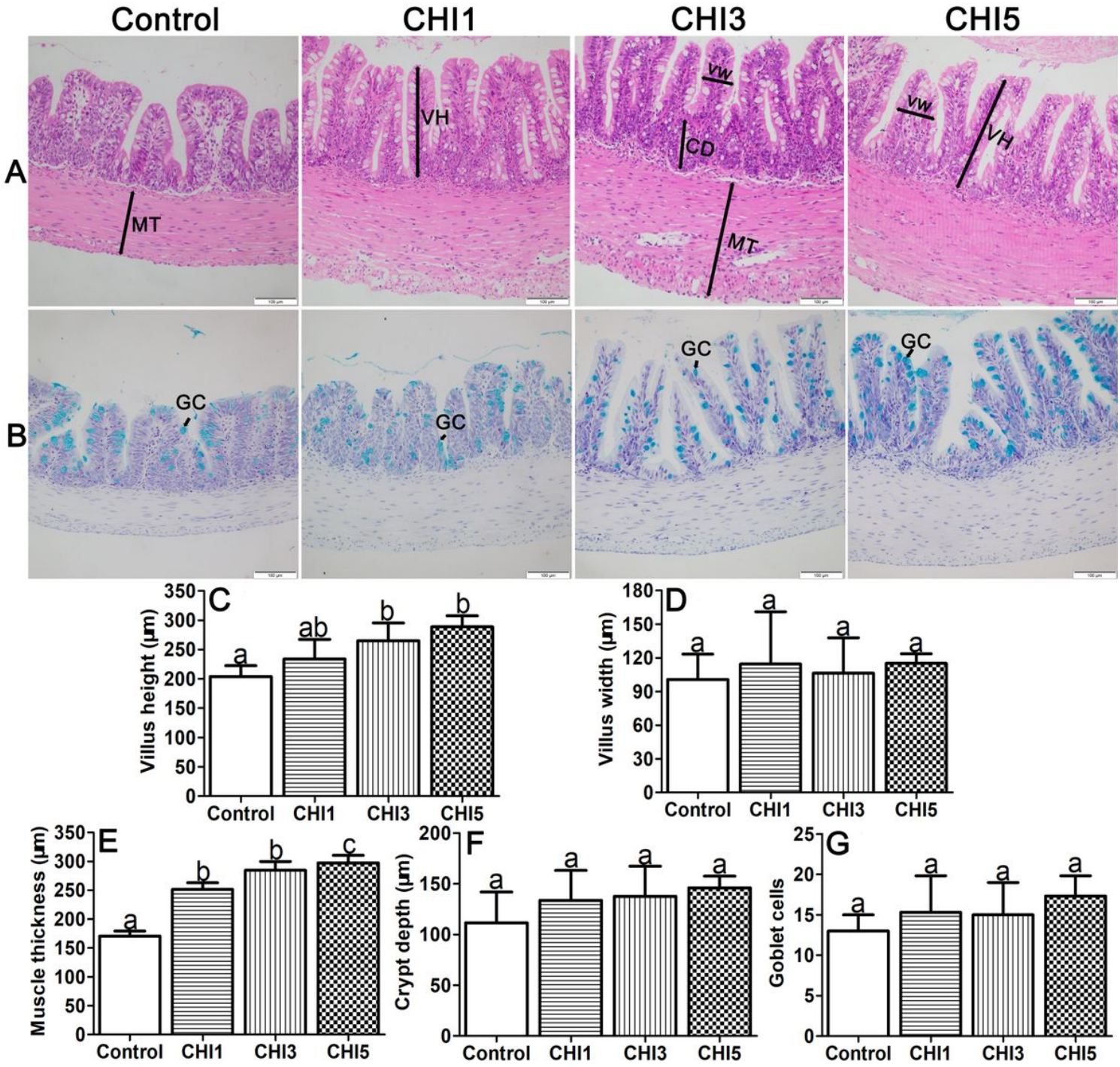

Figure 4

Histological evaluation of the spiral valve morphology after 8-week feeding trial. (A) H&E staining. (B) AB-PAS staining. (C) villus length. (D) villus width. (E) muscle thickness. (F) crypt depth. (G) goblet cells. VH: villus height, VW: villus width, MT: muscle thickness, CD: crypt depth, GC: goblet cells. All values are expressed as the mean ± SD of six replicates, and mean values with different letters represent significant differences ($P < 0.05$). Magnification, × 200.

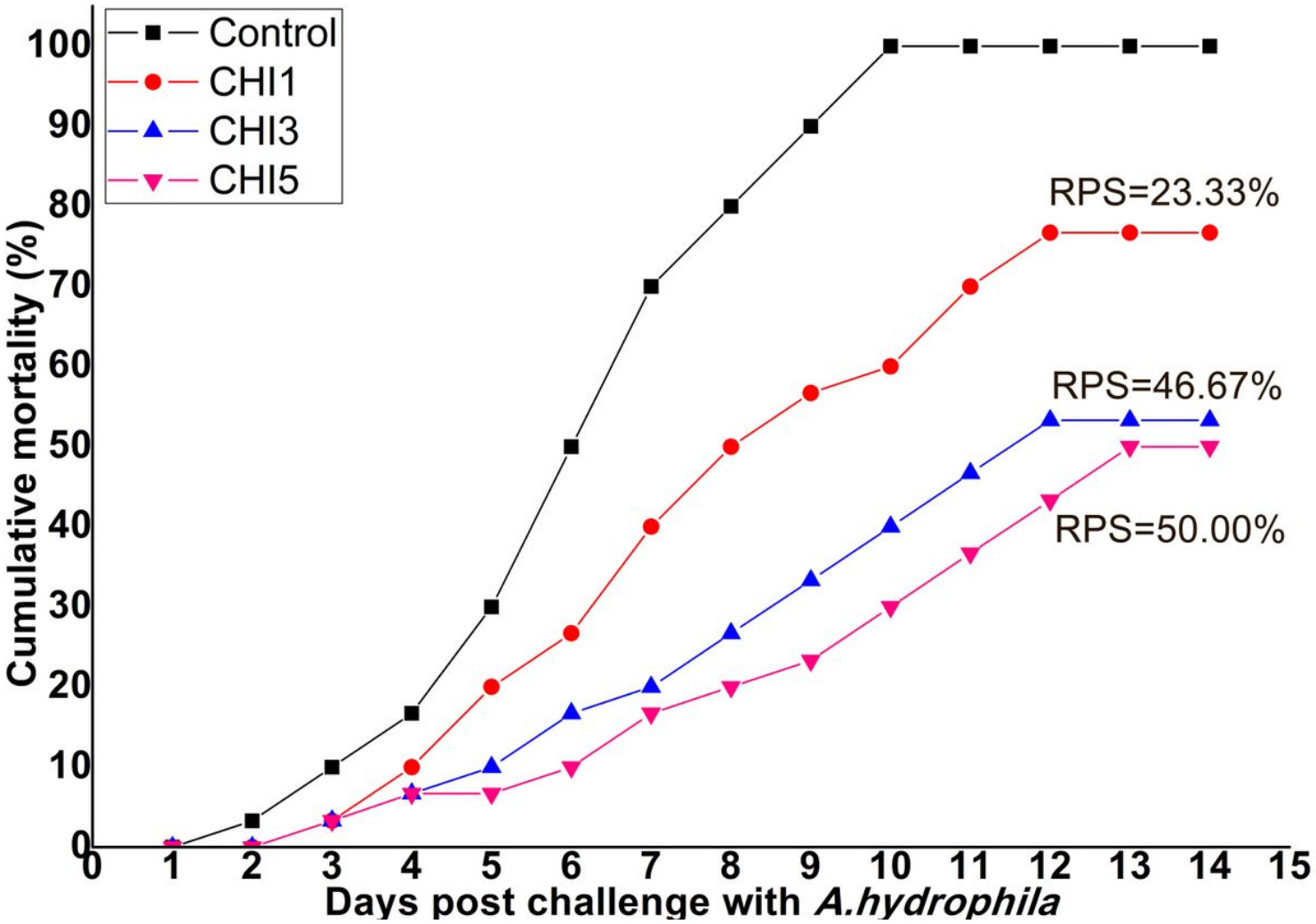

Figure 5

Cumulative mortality and relative percentage survival (RPS) of sturgeon in experimental groups post challenged with live *A. hydrophila*. RPS was calculated with the control group as the control.