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Research Article

Effect of dietary *Bdellovibrio* powder on non-specific immunity, antioxidant ability and disease resistance of Chinese mitten crab *Eriocheir sinensis*

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Abstract

The predatory *Bdellovibrio* is considered one of the most promising probiotics in aquaculture. Yet the effect of dietary *Bdellovibrio* probiotics on Chinese mitten crab *Eriocheir sinensis* has never been evaluated. In the present study, a fifty-day feeding trial was conducted to assess the effects of dietary *Bdellovibrio* powder at a recommended final dose of 7.5 g kg^{-1} diets on non-specific immunity, antioxidant ability and disease resistance of *E. sinensis*. The results indicated that compared with the control, the activities of lysozyme, superoxide dismutase, catalase, acid phosphatase, and alkaline phosphatase were significantly increased in the serum and hepatopancreas of *E. sinensis* fed with *Bdellovibrio* powder-supplemented diets (p<0.05). Besides, a significant reduction of malondialdehyde level was also observed in the serum and hepatopancreas of *Bdellovibrio* powder-fed crabs (p<0.05). In addition, the test crabs fed diets with *Bdellovibrio* powder for fifty days exhibited significantly (p<0.05) higher protection against aeromonasis, showing a relative percentage survival of 81.25% against *Aeromonas veronii* challenge for seven days. To conclude, dietary supplementation of *Bdellovibrio* powder for fifty days can increase immunity, antioxidant capability and resistance of *E. sinensis* to *A. veronii* infection.

Keywords: Antioxidant ability, *Bdellovibrio* powder, Disease resistance, *Eriocheir sinensis*, Non-specific immunity

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Introduction

The Chinese mitten crab, *Eriocheir sinensis,* is an economically important freshwater crustacean which is widely cultivated in China and other East Asian countries (He *et al*., 2014), and has grown to dominate the crab aquaculture production due to its high market value (Wei *et al*., 2014). Especially in China, with the rapid development of farming techniques, the production of Chinese mitten crabs has reached over 775,000 tons in 2020 (Wang *et al*., 2021). However, this industry has been seriously affected by bacterial diseases (Wang, 2011), which are usually caused by *Aeromonas veronii*, *A. punctata* and *A. hydrophila* (Shen *et al*., 2000; Cao *et al*., 2015; Zhou *et al*., 2019). In order to treat bacterial diseases, a wide range of antibiotics are widely used in Chinese mitten crab aquaculture (Fang *et al.*, 2019). However, the widespread and frequent use of antibiotics has resulted in the residues of antimicrobials and the development of antibiotic resistance, which can present a hazard to public health (Aly and Albutti, 2014). Thus, new preventive approaches must be explored to minimize antimicrobial use in crab aquaculture and reduce economic losses due to bacterial diseases.

Bdellovibrio species have been considered as potential agents for controlling aquatic pathogens (Sockett and Lambert, 2004), and their intrinsic bacteriolysis makes them interesting probiotics in aquaculture (Qi *et al*., 2009). At present, *Bdellovibrio* probiotics have shown important roles in health management in aquaculture (Bondad-Reantaso *et al*., 2012). Many studies have proved that the oral administration of *Bdellovibrio* liquid preparations can significantly enhance the immune activities of *Carassius auratus gibelio*, and *Fenneropenaeus chinensis* (Xu *et al*., 2007; Liang *et al*., 2009), and their application in rearing waters can greatly promote growth and survivals in *Haliotis diversicolor aquatilis*, *Haliotis discus hannai*, *Litopenaeus monodon*, *Scophthalmus maximus* (Chen *et al*., 2011; Li *et al*., 2014; Guo *et al*., 2016; Guo *et al*., 2017). However, little has been documented on the effect of dietary *Bdellovibrio* preparations on Chinese mitten crabs.

In aquaculture practices, the efficacy of commercial *Bdellovibrio* liquid preparations has been significantly impaired due to the massive loss of viable cells during long-term room temperature storage (Cao *et al*., 2013). Thus, *Bdellovibrio* powder with good storage stability undoubtedly has more potential for commercial applications (Cao *et al*., 2019). In the present study, a fifty-day feeding trial was conducted on Chinese mitten crabs by supplementation of *Bdellovibrio* powder at the recommended final dose of 7.5 g kg^{-1} diets (Cao *et al*., 2014) to the basal diets, and the effects of dietary *Bdellovibrio* powder on non-specific immunity, antioxidant ability and resistance to *A. veronii* infection in *E. sinensis* were evaluated. To our knowledge, this is the first report on the effect of dietary *Bdellovibrio* probiotics on Chinese mitten crabs, and the findings of this study can be used as a

reference for disease control and health management in crab aquaculture.

Materials and methods

Bdellovibrio powder

Bdellovibrio powder, encapsulated by spray drying with 20 g L^{-1} of gelatin as the coating polymer under the spray flow of 750 L h^{-1}, feed rate of 12 mL min^{-1} and air inlet temperature of 140°C according to Cao *et al*. (2019), was produced by Shanghai Bio-Green Co., Ltd, and was tested to contain 4.0×10^7 PFU g^{-1} by counting the plaque forming units (PFU) on double-layer agar plates after a 10-fold serial dilution in sterile distilled water (Jurkevitch., 2012; Saraoui *et al*., 2018).

Experimental diets

A commercial pellet diet was purchased from Huai'an Dajiang Aquatic Feed Co., Ltd., Jiangsu, China, and was used as a basal diet. The experimental diets were prepared as described by Abarike *et al*. (2018) by supplementing *Bdellovibrio* powder to the basal diets at the recommended final dose of 7.5 g kg^{-1} diets (Cao *et al*., 2014). Briefly, the basal pellets were crushed into powder using a feed miller and divided into two portions afterwards. One portion was used as the basal diet, and for the other portion, *Bdellovibrio* powder was supplemented at the final dose of 7.5 g kg^{-1} diets to make experimental diets. Diets were mixed homogeneously in a helical mixer and sterile distilled water added to make it suitable for cold press extrusion, were then remade into pellets which were dried under aseptic conditions at 16°C using air conditioning, and were stored in clean plastic bags at 4°C until use as recommended by Shen *et al*. (2010).

Crab culture

Chinese mitten crabs (♂, 73.6±1.4 g in weight) were obtained from Lianyungang Damao Aquaculture Professional Cooperative, Jiangsu, China, and were acclimated to experimental conditions and fed with the commercial pellet diets for two weeks. They were examined to be in healthy status by a careful examination of the external appearance, gut condition, and physical behavior according to Liu *et al*. (2013) and precise detection of no presence of parasites, viruses, and bacterial pathogens in a few sampled animals as described by Wu (2015), Yang *et al*. (2018), and Biswas *et al.* (2012). After acclimation, 720 healthy crabs were randomly distributed into six tanks (5.0 m × 5.0 m ×1.0 m) (120 crabs per tank) with 4500 L aerated reservoir water as recommended by Wu *et al*. (2011) at 25°C. Meanwhile, twelve tiles were placed at the bottom of each tank as shelters, as recommended by Fu *et al*. (2017), to avoid cannibalism. Water hyacinth *Eichhornia crassipes* were placed in each tank and covered 40% of the water surface to purify water. The experimental tanks divided into control and treatment groups (three tanks per group), and test crabs in the control and treatment groups were respectively fed with the basal and experimental *Bdellovibrio* powder-supplemented diets twice a day (8:00 and 17:00) at 3% of the total body weight (Fu *et al*., 2017) for

fifty days. During the experimental period, the water quality parameters were pH 7.2-8.0, 6.0-9.0 mg L^{-1} of dissolved oxygen, 0.05-0.40 mg L^{-1} of ammonia and 0.001-0.030 mg L^{-1} of nitrite, which were all within the safe range for *E. sinensis* (Zhang and Li, 2002).

Sample collection and analytical determinations

Eight test crabs were randomly sampled from each tank at intervals of ten days to collect their hemolymph and hepatopancreas samples for non-specific immunity and antioxidant ability analysis using commercial assay kits as recommended by Feng *et al*. (2019). Samples were analyzed as follows: (Ⅰ). The hemolymph of the test crabs was respectively extracted from the root of the third periopod, which was broken off, and was centrifuged at 5,000 rpm at 4°C for 10 min after 24 h of storage at 4°C. Then, the supernatant serum was collected to test the lysozyme (LSZ), superoxide dismutase (SOD), catalase (CAT), acid phosphatase (ACP), alkaline phosphatase (AKP) activities and malondialdehyde (MDA) content by analysis kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions according to Zheng *et al*. (2019). (Ⅱ). The hepatopancreas was respectively dissected from the sampled crabs, rinsed in sterile normal saline and stored at -80°C as recommended by Shen *et al*. (2010), was homogenized with 9 volumes of normal saline (1:9, w/v) using a glass homogenizer, and was then centrifuged at 5,000 rpm at 4°C for 10 min to collect the supernatant to test the LSZ, SOD, CAT, ACP, AKP activities and MDA content by assay kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions according to Liu *et al*. (2020).

Challenge test

In order to assess the effect of dietary *Bdellovibrio* powder on the disease resistance of crabs, a seven-day *A. veronii* infection test was conducted after the fifty-day feeding trial, and was carried out in twelve glass aquaria (76 cm×50 cm×48 cm) supplied with 60 L aerated reservoir water as recommended by Wu *et al*. (2011) with an initial pH of 7.20, 6.0 mg L^{-1} of dissolved oxygen, 0.05 mg L^{-1} of total ammonia, and 0.001 mg L^{-1} of nitrite at 25°C throughout the experiment. Prior to the challenge, *A.veronii* HXH1, previously isolated from the hepatopancreas of diseased *E. sinensis* and identified phenotypically and molecularly (Zhou *et al*., 2019), was obtained from the National Pathogen Collection Center for Aquatic Animals, China. The suspension of *A. veronii* strain HXH1 was prepared as described by Xu *et al*. (2014). The experimental aquaria divided into one negative control (Ctrl-N), one positive control (Ctrl-P), one negative treatment (Treat-N) and one positive treatment (Treat-P) group (three aquaria per group). Each aquarium in the control and treatment groups was stocked with ten crabs selected randomly from the

corresponding control and treatment tanks at the end of the above-mentioned feeding trial. Immediately thereafter, the crabs in the Ctrl-P and Treat-P groups were challenged by injection at the root of the third periopod (Xu *et al*., 2002) with *A. veronii* HXH1 at 3.44×10^3 CFU g^{-1} body weight (Zhou *et al*., 2019), and those in the Ctrl-N and Treat-N groups exposed to the same experimental conditions were injected with the equal volume of sterile normal saline. During the challenge test, dead crabs were immediately removed and the hepatopancreas was sampled to re-isolate and identify the pathogen to confirm if the mortality was caused by the challenge strain. Besides, ultrathin sections were prepared from the hepatopancreas of experimentally dead crabs for light microscopic examination as described by Zhou *et al*. (2012). Briefly, the hepatopancreas was fixed in Boun's solution, followed by dehydration in a graded series of alcohol (75%, 85%, 90%, 95% and 100%) (30 min in each dehydrating agent), cleared by xylol, embedded with paraffin, stained with hematoxylin and eosin, and then observed under a light microscope (Eclipse E100, Nikon). Relative percentage survivals were calculated according to Baulny *et al*. (1996).

Statistical Analysis

Statistical analysis was carried out using the statistical software SPSS 15.0 (SPSS, Inc.) to observe the difference in each assay. All of the data are presented as the mean±standard deviation (SD) for the indicated number of each assay. Differences were considered statistically significant at $p<0.05$ using analysis of variance according to Duncan's test.

Results

Effect of dietary Bdellovibrio powder on non-specific immunity of E. sinensis

The effect of dietary *Bdellovibrio* powder on the serum immune parameters of *E. sinensis* is shown in Figure 1. As compared with the control, the LSZ, ACP, AKP, SOD, and CAT activities in the serum of *E. sinensis* were significantly increased after ten days of feeding with *Bdellovibrio* powder-supplemented diets, and were finally increased by 26.42% ($p<0.05$), 14.52% ($p<0.05$), 27.85% ($p<0.05$), 26.90% ($p<0.05$), 32.53% ($p<0.05$) after fifty days of feeding with diets containing *Bdellovibrio* powder. In addition, the serum MDA content of *Bdellovibrio* powder-fed crabs was also dramatically reduced, with 17.12% ($p<0.05$) lower than that in the control after fifty days of culture. These data indicate that dietary *Bdellovibrio* powder could boost the non-specific immunity of Chinese mitten crabs.

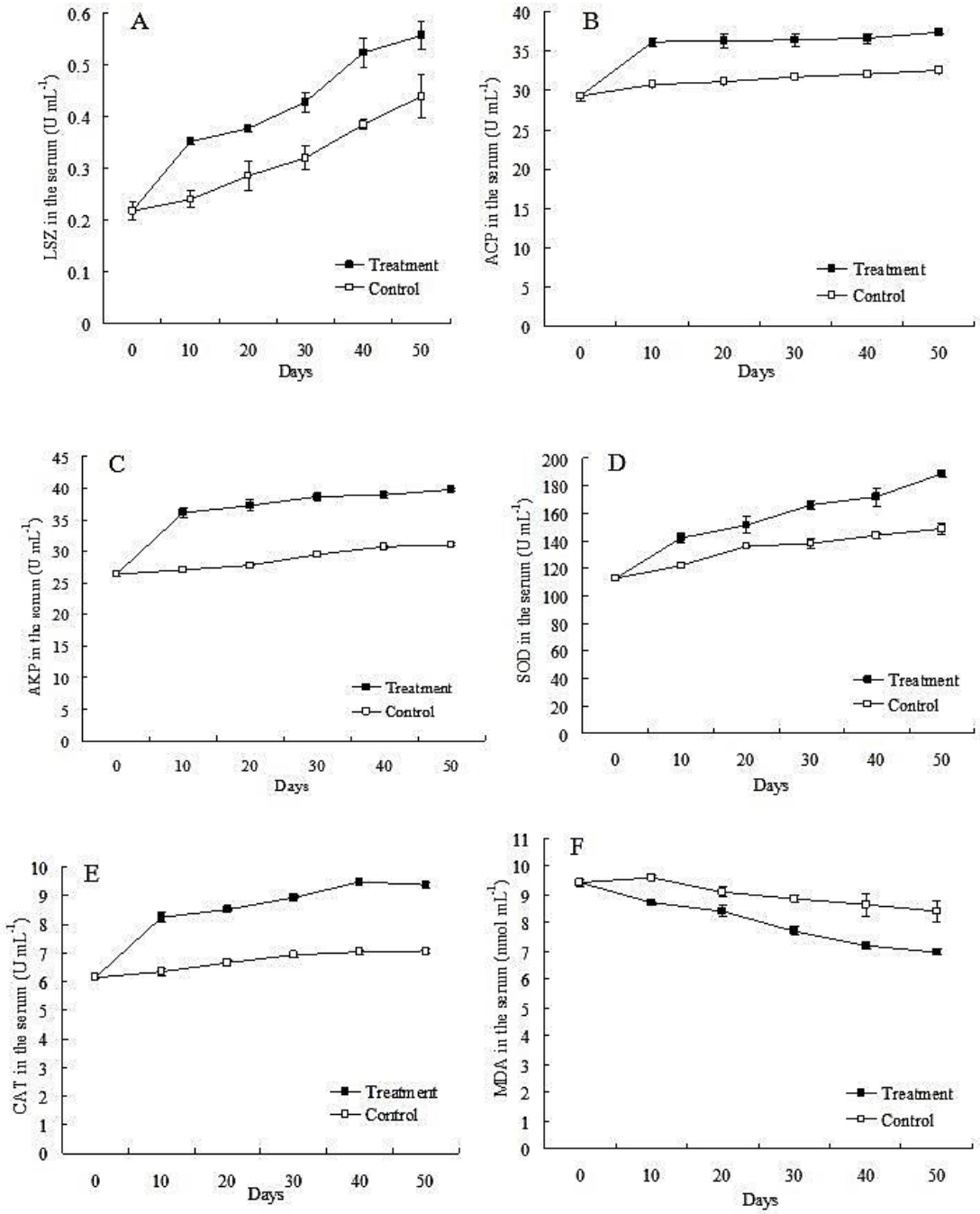

Figure 1: Effect of dietary *Bdellovibrio* powder on the non-specific immune parameters LSZ (A), ACP (B), AKP (C), SOD (D), CAT (E) and MDA (F) in the serum of *E. sinensis*. Control, crabs fed with the basal diets; Treatment, crabs fed with *Bdellovibrio* powder-supplemented diets. LSZ, lysozyme; ACP, acid phosphatase; AKP, alkaline phosphatase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. Eight crabs were sampled from each of three replicate tanks at all time-points. Data are presented as the mean ± SD. Asterisks represent significant differences ($p<0.05$) compared with the control according to Duncan's test.

Effect of dietary Bdellovibrio powder on antioxidant ability of E. sinensis

The effect of dietary *Bdellovibrio* powder on the hepatopancreatic antioxidant parameters of *E. sinensis* is shown in Figure 2.

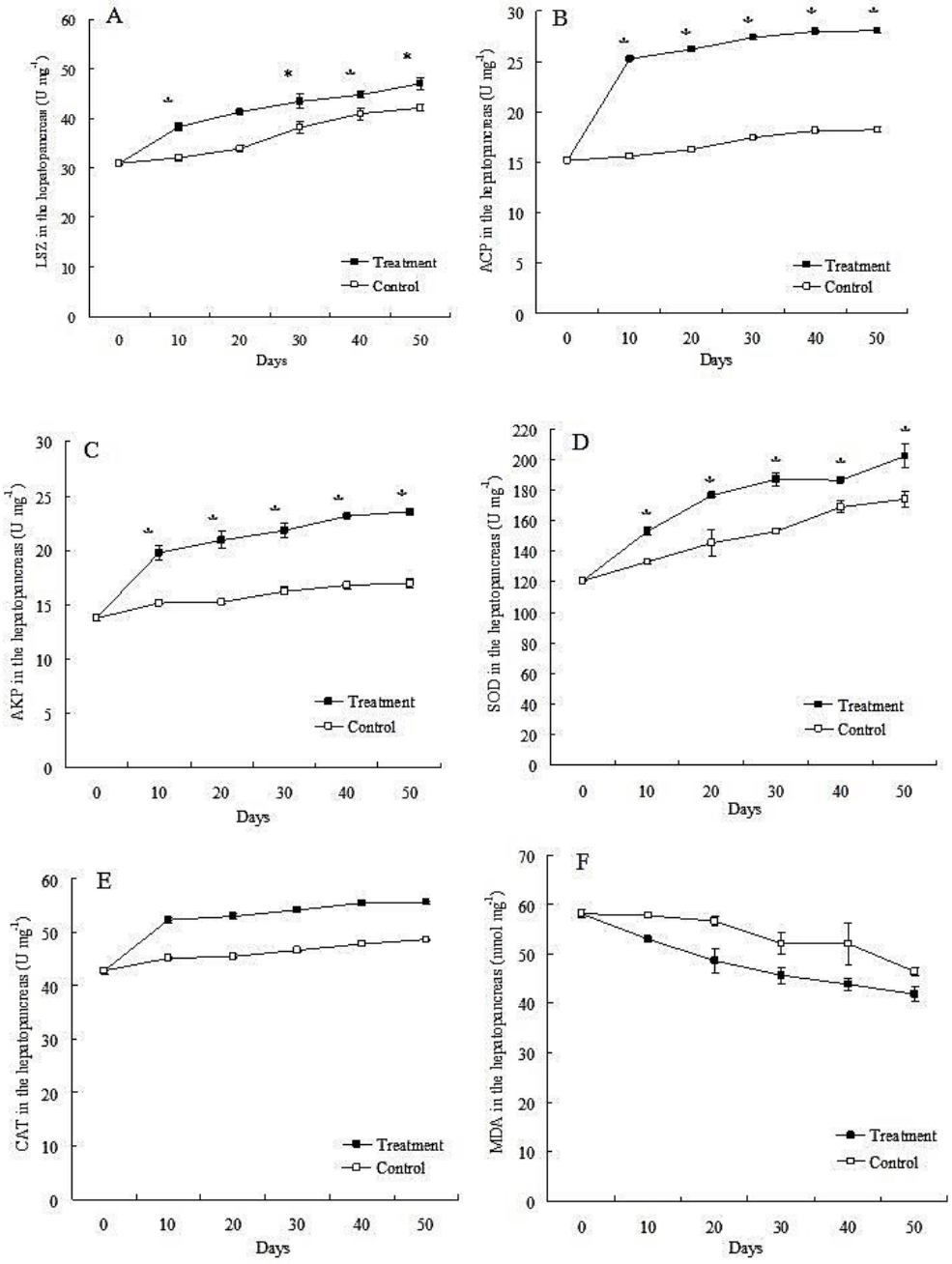

Figure 2: Effect of dietary *Bdellovibrio* powder on the antioxidant parameters LSZ (A), ACP (B), AKP (C), SOD (D), CAT (E) and MDA (F) in the hepatopancreas of *E. sinensis*. Control, crabs fed with the basal diets; Treatment, crabs fed with *Bdellovibrio* powder-supplemented diets. LSZ, lysozyme; ACP, acid phosphatase; AKP, alkaline phosphatase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. Eight crabs were sampled from each of three replicate tanks at all time-points. Data are presented as the mean ± SD. Asterisks represent significant differences (p<0.05) compared with the control according to Duncan's test.

The result showed that dietary *Bdellovibrio* powder could increase the hepatopancreatic antioxidant capability of Chinese mitten crabs. In comparison with the control, the LSZ, ACP, AKP, SOD, and CAT activities in the hepatopancreas of crabs fed with *Bdellovibrio* powder-supplemented diets were significantly raised, and were finally increased by 11.44% (p<0.05), 54.11% (p<0.05), 38.89% (p<0.05), 16.18% (p<0.05), 14.33% (p<0.05) after fifty days of culture. Besides, the significant reduction of the hepatopancreas MDA content was also observed in *Bdellovibrio* powder-fed crabs, with 9.84% (p<0.05) lower than the control after fifty days of feeding.

Effect of dietary Bdellovibrio powder on resistance to A. veronii infection in E. sinensis

The effect of dietary *Bdellovibrio* powder on the resistance to *A. veronii* infection in *E. sinensis* is shown in Figure 3. The result showed that the test crabs in the positive control group challenged with *A. veronii* HXH1 died gradually over time, and exhibited the sign of dark-yellow hepatopancreas (Fig. 4), with the hepatopancreas cells showing irregularly arranged and local necrosis (Fig. 5). However, the test crabs in the positive treatment group died six days after the challenge, and showed a relative percentage survival of 81.25% against *A. veronii* challenge for seven days. In addition, the challenge strain, confirmed by phenotypic and molecular identification (data not shown), was re-isolated from the experimentally dead crabs, and no visible changes or death were observed in the test *E. sinensis* in the negative control and negative treatment groups, further indicating the challenge strain. These data indicate that dietary *Bdellovibrio* powder confers significant protection of the test crabs against *A. veronii* infection.

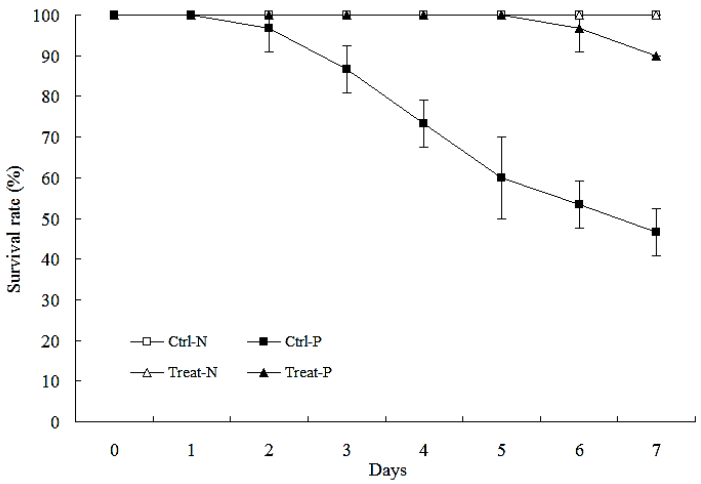

Figure 3: Effect of dietary *Bdellovibrio* powder on resistance to *A. veronii* infection in *E. sinensis*. Ctrl-N, unchallenged crabs fed the basal diets; Ctrl-P, challenged crabs fed the basal diets; Treat-N, unchallenged crabs fed *Bdellovibrio* powder-containing diets; Treat-P, challenged crabs fed *Bdellovibrio* powder-containing diets. Ten crabs were sampled from each of three replicate tanks. Data are presented as the mean ± SD. Asterisks represent significant differences (p<0.05) compared with the others according to Duncan's test.

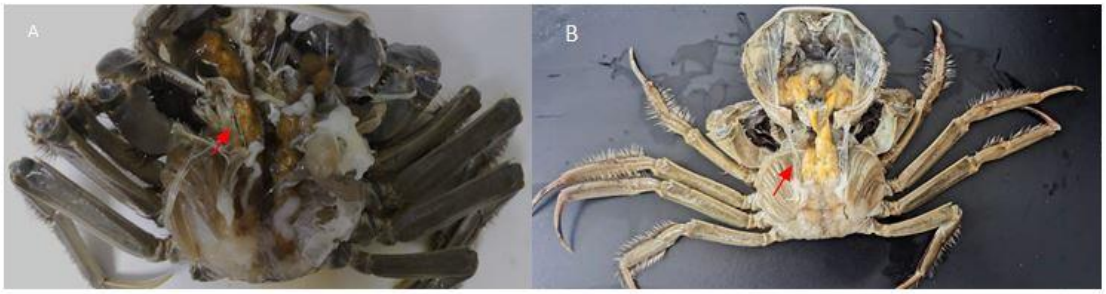

Figure 4: Gross signs of experimentally infected *E. sinensis*. (A). Experimentally infected crab. Arrow shows the dark-yellow hepatopancreas. (B). Healthy crab. Arrow shows the normal orange hepatopancreas.

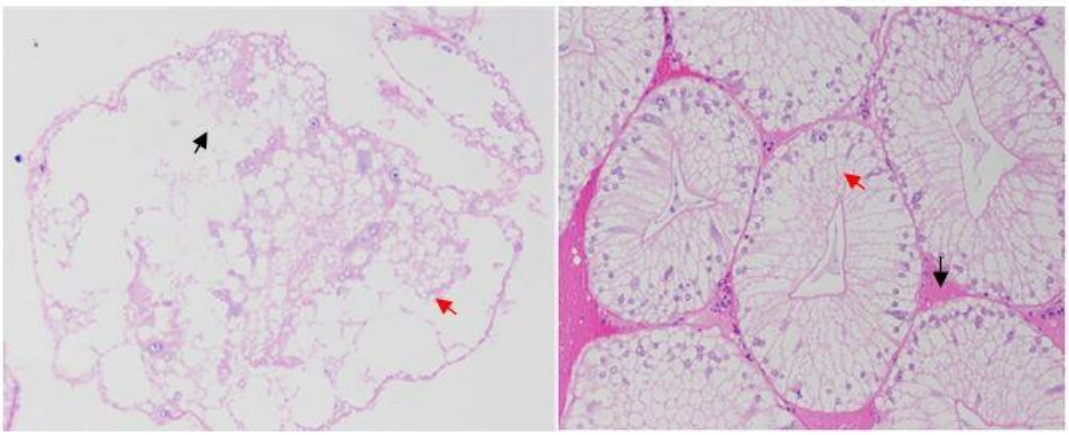

Figure 5: Histopathological changes in the hepatopancreas of experimentally infected *E. sinensis.* (A). the irregularly-arranged hepatopancreas cells (red arrow) with local necrosis (black arrow) in the experimentally infected crab (200×). (B). the regularly-arranged hepatopancreas cells (red arrow) with distinct nucleus (black arrow) in the control healthy hepatopancreas (200×).

Discussion

The use of probiotics is a common practice in crab aquaculture, and has played an important role in health management. For example, Chen *et al*. (2003) demonstrated that the addition of *Bdellovibrio* in rearing water could significantly enhance the growth and survival rate of *E. sinensis*. Thus, more attention should be given for the use of probiotics in crab aquaculture. In the present study, a fifty-day feeding trial was conducted to assess the effects of dietary *Bdellovibrio* powder at a recommended final dose of 7.5 g kg^{-1} diets on non-specific immunity, antioxidant ability and disease resistance of *E. sinensis*. To our knowledge, this is the first report of *Bdellovibrio* powder as a promising feed additive in Chinese mitten crab aquaculture.

Crustaceans, as everyone knows, only have the innate immune system mainly related to their hemolymph (Yang *et al*., 2020), which contains a variety of immune and antioxidant parameters such as LSZ, ACP, AKP, SOD, CAT, and MDA (Ma *et al*., 2010). Several studies have demonstrated that such hematological parameters are important indicators for evaluating the ability of immune response in

crustaceans (Wei *et al*., 2014; Fu *et al*., 2017; She *et al.*, 2019). In our study, the LSZ, ACP, AKP, SOD, and CAT activities in the serum of crabs fed *Bdellovibrio* powder-supplemented diets were significantly higher than those fed the basal diets, indicating that dietary *Bdellovibrio* powder could significantly improve the immunity of *E. sinensis*. Similar findings were also made by Chen *et al.* (2011) and Chen *et al*. (2021) that *Bdellovibrio* could stimulate innate immunity of abalone, *Haliotis diversicolor aquatilis,* and mandarin fish, *Siniperca chuatsi*. Besides, the *Bdellovibrio* powder-fed crabs in our study showed a significant reduction in serum MDA content. This is in agreement with the findings by Shen *et al.* (2010), Fu *et al*. (2017), and Nandi *et al*. (2017) that the serum MDA content is opposite to the activity pattern of LSZ, ACP, AKP, SOD and CAT. Furthermore, the change curves of serum immune parameters in the control crabs followed those in the treatment crabs in our study. Similar findings were also observed in *E. sinensis* treated by *A. hydrophila* microcapsule vaccine (Liu *et al.*, 2009). This is probably due to the proper health management that maintains the normal serum immunity of crabs (Xia *et al.*, 2009).

In crustaceans, reactive oxygen species (ROS) are indispensable for normal cell functions such as anti-pathogens, and play an important role in cell defense (Wu *et al.*, 2017). However, excessive ROS can induce oxidative damage to the hepatopancreas (Valavanidis *et al*., 2006), which is a vital tissue involved in metabolism and detoxication (Hong *et al.*, 2019). Thus, it is important to activate the antioxidant defense system to prevent oxidative damage caused by excessive ROS (Zhang *et al*., 2021). The hepatopancreas LSZ, ACP, AKP, SOD, CAT, and MDA activities are important marker parameters of the antioxidant defense system in crabs, which are directly related to the degree of hepatopancreas health (Sun *et al*., 2017; Zhang *et al*. 2021). In our study, the activities of LSZ, ACP, AKP, SOD, and CAT in the hepatopancreas of *Bdellovibrio* powder-fed crabs were significantly enhanced, and the hepatopancreas MDA level was dramatically reduced, indicating that *Bdellovibrio* powder could improve the health status of hepatopancreas by activating the anti-oxidation system in hepatopancreas and enhancing the antioxidant ability. This is probably because *Bdellovibrios* from the powder can modulate intestinal microbiota and reduce the populations of potential pathogens in the gut by producing active ingredients such as lysozyme (Xu *et al*., 2007; Harding *et al.*, 2020), which may, in turn, increase the antioxidant enzyme-substrate (ATP) contents and metabolites associated with energy biosynthesis and immune system (Tsakiris *et al*., 2004; Zhang *et al*., 2019).

A. veronii is considered as a major aquaculture pathogen with multiple virulence factors such as aerolysin, lipopolysaccharide, bundle-forming pilus (Song *et al*., 2004; Hadi *et al*., 2012; Turska-Szewczuk *et al*., 2012),

which has resulted in significant economic losses in the culture of Chinese mitten crabs (Fang *et al*., 2008; Jiang *et al*., 2016). Therefore, more attention should be given to preventing *A. veronii* infections. Currently, probiotics are an effective preventive measure against *A. veronii* infections in aquaculture. Faeed *et al*. (2016) found that dietary *Entrococcus faecium* could enhance the resistance to *A. veronii* infection in zander *Sander lucioperca*. Zhang *et al*. (2018) reported that oral immunization with recombinant *Lactobacillus casei* expressing OmpAI confered significant protection against *A. veronii* challenge in common carp, *Cyprinus carpio*. Chen *et al*. (2018) also demonstrated that grass carp, *Ctenopharyngodon idellus*, fed probiotics increased survival after the challenge with *A. veronii*. In our study, significant protection was also found in the test crabs fed with *Bdellovibrio* powder-supplemented diets for fifty days. This is probably attributed to the improvement in the immune responses and antioxidant capabilities induced by *Bdellovibrios*, which could further prime the crabs to resist bacterial infections (Zhang and Zhou, 2005).

In conclusion, the present study, for the first time, demonstrated that dietary supplementation of *Bdellovibrio* powder for fifty days could enhance the non-specific immunity, antioxidant capability and resistance of Chinese mitten crabs against *A. veronii* infection.

Acknowledgements

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References

Abarike, E.D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., Tang, J., Jun, L. and Kuebutornye F.K.A., 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 and Shellfish Immunology*, 82, 229-238. https://doi.org/10.1016/j.fsi.2018.08.037

Aly, S.M. and Albutti, A., 2014. Antimicrobials use in aquaculture and their public health impact. *Journal of Aquaculture Research and Development*, 5(**4**), 247. https://doi.org/10.4172/2155-9546.1000247

Baulny, M.O.D., Quentel, C., Fournier, V., Lamour, F. and Gouvello, R.L., 1996. Effect of long-term oral administration of β-glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms*, 26, 139–147. https://doi.org/10.3354/dao026139

Biswas, G., Korenaga, H., Nagamine, R., Kono, T., Shimokawa, H., Itami, T. and Sakai, M., 2012. Immune stimulant effects of a nucleotide-rich baker's yeast extract

in the kuruma shrimp, *Marsupenaeus japonicus*. *Aquaculture*, 366-367, 40-45. https://doi.org/10.1016/j.aquaculture.2012.09.001

Bondad-Reantaso M.G., Arthur J.R. and Subasinghe R.P., 2012. Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production.FAO Fisheries and Aquaculture Technical Paper 547, Rome, Italia.

Cao, H., He, S., Ou, R., Hou, S., Gao, X. and Yang, X., 2013. Progress on *Bdellovibrio bacteriovorus* used in aquaculture. *Progress in Veterinary Medicine*, 34, 86-90. https://doi.org/10.16437/j.cnki.1007-5038.2013.01.030

Cao, H., Yan, H., Li, G., Zhang, X., Sun X. and Gong, Z., 2014. A case of diagnosis and control of tremor disease in the Chinese mitten crab (*Eriocheir sinensis*). *Scientific Fish Farm*, 30(**2**), 64-64.

Cao, H., Wen, L., Bai, J., Zhang, F. and He S., 2015. Isolation, identification and antimicrobial susceptibility of *Aeromonas punctata* from *Eriocheir sinensis*. *Progress in Veterinary Medicine*, 36(**8**), 110-113. https://doi.org/10.16437/j.cnki.1007-5038.2015.08.025

Cao, H., Wang, H., Yu, J., An, J. and Chen, J., 2019. Encapsulated *Bdellovibrio* powder as a potential bio-disinfectant against whiteleg shrimp-pathogenic vibrios. *Microorganisms*, 7, 244. https://doi.org/10.3390/microorganisms7080244

Chen, J., Hu, G., Wu, W. and Qu, J., 2003. Studies on the implications of the beneficial bacteria in *Eriocheir sinensis* farming. *Journal of Shanghai Fisheries University*, 12(**3**), 271-273.

Chen, X., Lin, A. and Cai, J., 2011. Effects of *Bdellovibrio*-and-like organism on non-specific immunity and disease resistance of *Haliotis diversicolor aquatilis*. *Modern Food Science and Technology*, 27(**5**), 502-505. https://doi.org/10.13982/j.mfst.1673-9078.2011.05.009

Chen, J., Cao, X., Liu, L. and Cao X., 2018. Immunomodulation with probiotics against *Aeromonas veronii* in grass carp (*Ctenopharyngodon idellus*). *Israeli Journal of Aquaculture - Bamidgeh*, 70, 1479. https://doi.org/10.46989/001c.20940

Chen, X., Yi, H., Liu, S., Zhang Y., Su, Y., Liu, X., Bi, S., Lai, H., Zeng, Z., and Li, G., 2021. Promotion of pellet-feed feeding in mandarin fish (*Siniperca chuatsi*) by *Bdellovibrio bacteriovorus* is influenced by immune and intestinal flora. *Aquaculture*, 542, 736864. https://doi.org/10.1016/j.aquaculture.2021.736864

Faeed, M., Kasra Kermanshahi, R., Pourkazemi, M. and Darboee, M., 2016. Effect of the probiotic *Entrococcus faecium* on hematological and non-specific immune parameters and disease resistance in zander (*Sander*

lucioperca). *Iranian Journal of Fisheries sciences*, 15(**4**), 1581-1592. http://hdl.handle.net/1834/12134

Fang, H., Chen, C., Zhang, X., Gong, Y. and Ge, M., 2008. Examination of the pathogenic *Aeromonas veronii* isolated from crab *Eriocheir sinensis*. *Chinese Journal of Zoonoses*, 24(**1**), 45-49. https://doi.org/10.3724/SP.J.1011.2008.00335

Fang, H., Yu, J., Wang, Z., Ding, C., Xia, W., Zhao, C., Yuan, H., Wang, Z., Wang, S., and Cui, Y., 2019. Contamination characteristics and ecological risk assessment of antibiotics in typical *Eriocheir sinensis* aquaculture environments of Jiangsu province. *Journal of Ecology and Rural Environment*, 35(**11**), 1436-1444. https://doi.org/10.19741/j.issn.1673-4831.2018.0889

Feng, D., Wang, X., Li, E., Bu, X., and Chen, L., 2019. Dietary aroclor 1254-induced toxicity on antioxidant capacity, immunity and energy metabolism in Chinese mitten crab *Eriocheir sinensis*: amelioration by vitamin a. *Frontiers in Physiology*, 10, 722. https://doi.org/10.3389/fphys.2019.00722

Fu, L., Zhou, G., Pan, J., Li, Y., Lu, Q., Zhou, J. and Li, X., 2017. Effects of *Astragalus* polysaccharides on antioxidant abilities and non-specific immune responses of Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture International*, 25, 1333-1343. https://doi.org/10.1007/s10499-017-0117-2

Guo, Y., Yan, L. and Cai, J., 2016. Effects of *Bdellovibrio* and like organisms on survival and growth performance of juvenile turbot, *Scophthalmus maximus*. *Journal of the World Aquaculture Society*, 47(**5**), 633-645. https://doi.org/10.1111/jwas.12304

Guo, Y., Pan, Q., Yan, S., Chen, Y., Li, M., Chen, D., Han, H., Wu, B. and Cai J., 2017. *Bdellovibri*o and like organisms promoted growth and survival of juvenile abalone *Haliotis discus hannai* Ino and modulated bacterial community structures in its gut. *Aquaculture International*, 25(**4**), 1625-1643. https://doi.org/10.1007/s10499-017-0138-x

Hadi, N., Yang, Q., Barnett, T.C., Tabei, S.M., Kirov, S.M. and Shaw, J.G., 2012. Bundle-froming pilus locus of *Aeromonas veronii* bv. *sobria*. *lnfection and Immunity*, 80(**40**), 1351-1360. https://doi.org/10.1128/iai.06304-11

Harding, CJ., Huwiler, S.G., Somers, H., Lambert, C., and Lovering, A.L., 2020. A lysozyme with altered substrate specificity facilitates prey cell exit by the peri-plasmic predator *Bdellovibrio bacteriovorus*. *Nature Communications*, 11, 4817. https://doi.org/10.1038/s41467-020-18139-8

He, J., Wu, X., Li, J., Huang, Q., Huang, Z. and Cheng Y., 2014. Comparison of the culture performance and profitability of

wide-caught and captive pond-reared Chinese mitten crab (*Eriocheir sinensis*) juveniles reared in grow-out ponds: Implications for seed selection and genetic selection programs. *Aquaculture*, 434, 48-56. https://doi.org/10.1016/j.aquaculture.2014.07.022

Hong, Y., Huang, Y., Yan, G., Pan, C. and Zhang, J., 2019. Antioxidative status, immunological responses, and heat shock protein expression in hepatopancreas of Chinese mitten crab, *Eriocheir sinensis* under the exposure of glyphosate. *Fish and Shellfish Immunology*, 86, 840-845. https://doi.org/10.1016/j.fsi.2018.12.020

Jiang, G., Qian, C., Gu, X., Wei, B., Xu, L. and Song, X., 2016. The etiological agents of bacterial septicemia in *Eriocheir sinensis* and *Procambarus clarkia*. *Journal of Aquaculture*, 37(**4**), 46-51. https://doi.org/10.3969/j.issn.1004-2091.2016.04.010

Jurkevitch, E., 2012. Isolation and classification of *Bdellovibrio* and like organisms. *Current Protocols in Microbiology*, 26, 7B.1.1-7B.1.20. https://doi.org/10.1002/9780471729259.mc07b01s00

Li, H., Chen, C., Sun, Q., Liu, R. and Cai, J., 2014. *Bdellovibrio* and like organisms enhanced growth and survival of *Penaeus monodon* and altered bacterial community structures in its rearing water. *Applied and Environmental Microbiology*, 80(**20**), 6346-6354. https://doi.org/10.1128/aem.01737-14

Liang, S., Fang, W., Wang, K., and Jia, X., 2009. Effects of *Bdellovibrio* sp. Bdh5221 on intestinal microflora and non-specific immunity in crucian carp *Carassius auratus gibelio*. *Journal of Dalian Fisheries University*, 24(**2**), 157-161. https://doi.org/10.16535/j.cnki.dlhyxb.2009.02.010.

Liu, Y., Li, Y., Wang, Y., Chen, W. and Sun, H., 2009. Effect of *Aeromonas hydrophila* microcapsule vaccine on immune function of *Eriocheir sinensis*. *Feed Industry*, 8, 24-27. https://doi.org/10.3969/j.issn.1001-991X.2009.08.008

Liu, J., Gu, Z., Yang, Y., Jia Y., Zhang, A. and Yuan, J., 2013. Technical specification for relaease and enhancement of Chinese mitten-handed crab (*Eriocheir sinensis*). DB 33/T 909-2013. Zhejiang Bureau of Quality and Technical Supervision, China.

Liu, J.D., Liu, W.B., Zhang, D.D., Xu, C.Y., Zhang, C.Y., Zheng, X.C. and Chi, C., 2020. Dietary reduced glutathione supplementation can improve growth, antioxidantcapacity, and immunity on Chinese mitten crab, *Eriocheir sinensis*. *Fish and Shellfish Immunology*, 100, 300. https://doi.org/10.1016/j.fsi.2020.02.064

Ma, Z., Zhang, F., and Jing, A., 2010. Overview and graph theory of the immune system of crustacean. *Agricultural Science and*

Technology, 11(**8**), 19-23, 38. http://www.en.cnki.com.cn/Article_en/CJFDTOTAL-HNNT201008011.htm

Nandi, A., Banerjee, G., Dan, S.K., Ghosh, K. and Ray, A.K., 2017. Probiotic efficiency of *Bacillus* sp. in *Labeo rohita* challenged by *Aeromonas hydrophila*: assessment of stress profile, haemato-biochemical parameters and immune responses. *Aquaculture Research*, 48, 4334-4335. https://doi.org/10.1111/are.13255

Qi, Z., Zhang, X., Boon, N. and Bossier, P., 2009. Probiotics in aquaculture of China - current state, problems and prospect. *Aquaculture*, 290(**1-2**), 15-21. https://doi.org/10.1016/j.aquaculture.2009.02.012

Saraoui, T., Leroi, F., Chevalier, F., Cappelier, J.M., Passerini, D. and Pilet, M.F., 2018. Bioprotective effect of *Lactococcus piscium* CNCMI-4031 against *Listeria monocytogenes* growth and virulence. *Frontiers in Microbiology*, 9, 1564. https://doi.org/10.3389/fmicb.2018.01564

She, Q., Han, Z., Liang, S., Xu, W., Li, X., Zhao, Y., Wei, H., Dong, J., and Li, Y., 2019. Impacts of circadian rhythm and melatonin on the specific activities of immune and antioxidant enzymes of the Chinese mitten crab (*Eriocheir sinensis*). *Fish and Shellfish Immunology*, 89, 345-353. https://doi.org/10.1016/j.fsi.2019.04.011

Shen, J., Yin, W., Qian, D., Liu, W., Cao, Z., Shen, Z., Wu, Y. and Zhang, N.C., 2000. Studies on the pathogens of bacterial diseases of *Eriocheir sinensis*. *Journal of Fishery Sciences of China*, 7(**3**), 89-92. https://kns.cnki.net/kcms/detail/detail. aspx?FileName= HZNY200005019andDbName=CJFQ2000

Shen, W.Y., Fu, L.L., Li, W.F. and Zhu, Y.R., 2010. Effect of dietary supplementation with *Bacillus subtilis* on the growth, performance, immune response and antioxidant activities of the shrimp (*Litopenaeus vannamei*). *Aquaculture Research*, 41, 1691-1698. https://doi.org/10.1111/j.1365-2109.2010.02554.x

Sockett, R.E. and Lambert, C., 2004. *Bdellovibrio* as therapeutic agents: a predatory renaissance? *Nature Reviews Microbiology*, 2, 669-675. https://doi.org/10.1038/nrmicro959

Song, T., Toma, C., Nakasone, N. and Iwanaga, M., 2004. Aerolysin is activated by metalloprotease in *Aeromonas verionii* biovar *sobria*. *Journal of Medical Microbiology*, 53, 477-482. https://doi.org/10.1099/jmm.0.05405-0

Sun, P., Jin, M., Ding, L., Lu, Y., Yuan, Y., Ma, H., and Zhou, Q., 2017. Effect of dietary soybean lecithin and cholesterol on growth, antioxidant status and fatty acid composition of juvenile swimming crab, *Portunus trituberculatus*. *Israeli Journal of Aquaculture*

Bamidgeh, 69, 1-11. https://doi.org/10.46989/001c.21034

Tsakiris, S., Schulpis, K.H., Marinou, K. and Behrakis, P., 2004. Protective effect of L-cysteine and glutathione on the modulated suckling rat brain Na^+, K^+-ATPase and Mg^{2+}-ATPase activities induced by the *in vitro* galactosaemia. *Pharmacological Research*, 49(**5**), 475-479. https://doi.org/10.1016/j.phrs.2003.11.006

Turska-Szewczuk, A., Lindner, B., Pekala, A., Palusinska-Szysz, M., Choma, A., Russa, R. and Holst, O., 2012. Structural analysis of the O-specific polysaccharide from the lipopolysaccharide of *Aeromonas veronii* bv. *Sobria* strain K49. *Carbohydrate Research*, 353, 62-68. https://doi.org/10.1016/j.carres.2012.03.007

Valavanidis, A., Vlahogianni, T., Dassenakis, M., and Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*, 64, 178-189. https://doi.org/10.1016/j.ecoenv.2005.03.013

Wang, D., and Wu, F., 2021. China Fishery Statistics Yearbook. *China Agriculture Press*, Beijing, China, pp. 34.

Wang, W., 2011. Bacterial diseases of crabs: a review. *Journal of Invertebrate Pathology*, 106(**1**), 18-26. https://doi.org/10.1016/j.jip.2010.09.018

Wei, J., Yu, N., Tian, W., Zhang, F., Wu, Q., Li, E., Zhang, M., Du, Z., Qin, J. and Chen, L., 2014. Dietary vitamin B12 requirement and its effect on non-specific immunity and disease resistance in juvenile Chinese mitten crab *Eriocheir sinensis*. *Aquaculture*, 434, 179-183. https://doi.org/10.1016/j.aquaculture.2014.08.010

Wu, X., Wang, Z., Cheng, Y., Zeng, C., Yang, X. and Lu, J., 2011. Effects of dietary phospholipids and highly unsaturated fatty acids on the precocity, survival, growth and hepatic lipid composition of juvenile Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture Research*, 42(**3**), 457-468. https://doi.org/10.1111/j.1365-2109.2010.02643.x

Wu, S., 2015. Aquatic animal parasitology. China Agriculture Press, Beijing, China.

Wu, Y.S., Lee, M.C., Huang, C.T., Kung, T.C., Huang, C.Y., and Nan, F.H., 2017. Effects of traditional medical herbs "minor bupleurum decoction" on the non-specific immune responses of white shrimp (*Litopenaeus vannamei*). *Fish and Shellfish Immunology*, 64, 218-225. https://doi.org/10.1016/j.fsi.2017.03.018

Xia, S., Li, Y., Wang, W., Wang, H., and Wang, M., 2009. Effects of self pollution factors on shrimp and crab health and its mechanism and control. *Fisheries Science*, 28(**6**), 355-360.

https://10.3969/j.issn.1003-1111.2009.06.014

Xu, H., Shu, M., Zhan, X. and Wang, S., 2002. Identification of *Vibrio parahemolyticus* isolated from cultured *Eriocheir sinensis* and pathogenicity of its extracellular products. *Journal of Fisheries of China*, 26(**4**), 357-362. https://doi.org/10.1007/s11769-002-0026-8

Xu, Q., Li, J., Liu, Q. and Wang, Q., 2007. Effects of *Bdellovbrio bacteriovorus* and *Rhodotorula glutinis* on growth and non-specific immune competence of *Fenneropenaeus chinensis*. *Marine Fisheries Research*, 28(**5**), 42-47.http://kns.cnki.net/kcms/detail/detail.aspx?FileName=HYSC200705008andDbName=CJFQ2007

Xu, X., Peng, S., Cui, K., Ding, Z., Cai, Y. and Zhang, Q., 2014. Identification of bacterial pathogens isolated from the diseased *Xiphophorus hellerii*. *Freshwater Fisheries*,44(**1**),66-74. http://kns.cnki.net/kcms/detail/detail.aspx?FileName=DSYY201401013anddDbName=CJFQ2014

Yang, X., Song, X., Zhang, C., Pang, Y., Song, Y., Cheng Y., Nie, L., and Zong, X., 2020. Effects of dietary melatonin on hematological immunity, antioxidant defense and antibacterial ability in the Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture*, 529, 735578. https://doi.org/10.1016/j.aquaculture.2020.735578

Yang, Z., Zhang, Y., Hu, K., Liu, L., Cai, H., Zhang, F. and Yang, X., 2018. Etiological and histopathological study on hepatopancreatic necrosis syndrome in *Eriocheir sinensis*. *Acta Hydrobiologica Sinica*, 42(**1**), 17-24. https://kns.cnki.net/kcms/detail/detail.aspx?FileName=SSWX201801003andDbName=CJFQ2018

Zhang, C., Wang, X., Wang, C., Song, Y., Pan, J., Shi, Q., Qin, J., and Chen, L., 2021. Gamma-aminobutyric acid regulates glucose homeostasis and enhances the hepatopancreas health of juvenile Chinese mitten crab (*Eriocheir sinensis*) under fasting stress. *General and Comparative Endocrinology*, 303, 113704. https://doi.org/10.1016/j.ygcen.2020.113704

Zhang, D., Kang, Y., Chen, L., Siddiqui, S.A., Wang, C., Qian, A. and Shan, X., 2018. Oral immunization with recombinant *Lactobacillus casei* expressing OmpAI confers protection against *Aeromonas veronii* challenge in common carp, *Cyprinus carpio*. *Fish and Shellfish Immunology*, 72, 552-563.https://doi.org/10.1016/j.fsi.2017.10.043

Zhang, L. and Li, J., 2002. Techniques for culture and enhancement of *Erincheir Sinensis*. Jingdun Press, Beijing, China.

Zhang, L. and Zhou, W., 2005. Effect of *Bdellovibrio bacteriovorus* on growth and immunizatoin of *Ctenopharyngodon Idellus*. *Grain*

and Feed Industry, 4, 36-37.https://kns.cnki.net/kcms/detail/detail.aspx?FileName=LSYS20050400FandDbName=CJFQ2005

Zhang, R., Jiang, Y., Zhou, L., Chen, Y., Wen, C., Liu, W., and Zhou, Y., 2019. Effects of dietary yeast extract supplementation on growth, body composition, non-specific immunity, and antioxidant status of Chinese mitten crab (*Eriocheir sinensis*). *Fish and Shellfish Immunology*, 86, 1019-1025.https://doi.org/10.1016/j.fsi.2018.12.052

Zheng, X., Chi, C., Xu, C., Liu, J. and Liu, W., 2019. Effects of dietary supplementation with icariin on growth performance, antioxidant capacity and non-specific immunity of Chinese mitten crab (*Eriocheir sinensis*). *Fish and Shellfish Immunology*, 90, 264-273. https://doi.org/10.1016/j.fsi.2019.04.296

Zhou, H., Huang, X., An, J., Cao, H. and Yang, X., 2019. Isolation, identification and antibiotic susceptibility of pathogenic *Aeromonas veronii* in *Eriocheir sinensis* and its histopathological observations. *Journal of Southern Agriculture*, 50(**8**), 1851-1859.https://kns.cnki.net/kcms/detail/detail.aspx?FileName=GXNY201908029andDbName=CJFQ2019

Zhou, J., Fang, W., Yang, X., Zhou, S., Hu, L., Li, X., Qi, X., Su, H. and Xie, L., 2012. A nonluminescent and highly virulent *Vibrio harveyi* strain is associated with "bacterial white tail disease" of *Litopenaeus vannamei* shrimp. *PLoS ONE*, 7, e29961. https://doi.org/10.1371/journal.pone.0029961