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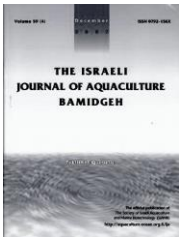

Comparative Analysis of Non-Specific Immunity of *Clarias fuscus*, *Silurus asotus*, and *Silurus meridionalis*

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Keywords: head kidney index; *Clarias fuscus*; *Silurus asotus*; *Silurus meridionalis*; lysozyme activity

Abstract

Clarias fuscus, *Silurus asotus* and *Silurus meridionalis* are economically important species in the aquaculture industry in China. However, comparative analysis of immune indices of *C. fuscus*, *S. asotus,* and *S. meridionalis* has been rarely been conducted. In the present study, head kidney and spleen indices and blood serum immunologically related enzyme activity of *C. fuscus*, *S. asotus,* and *S. meridionalis* were detected. Both head kidney and spleen body ratios were highest in *S. meridionalis*, followed by *S. asotus*, and lowest in *C. fuscus*. This suggests that the degree of lymphocyte proliferation and immune function of *S. meridionalis* might be strongest compared to *S. asotus* and *C. fuscus*. Blood serum lysozyme activity, superoxide dismutase (SOD) activity, and alkaline phosphatase activity, were highest in *S. meridionalis*, followed by *S. asotus*, but lowest in *C. fuscus*. Compared to *S. asotus*, acid phosphatase activity and catalase activity was higher in *C. fuscus*. Both acid phosphatase and catalase activities were highest in *S. meridionalis*, which was in line with lysozyme, superoxide dismutase (SOD), alkaline phosphatase, head kidney and spleen body ratios. This indicated that compared to *S. asotus* and *C. fuscus*, the immune function of *S. meridionalis* was strongest, and might have the greatest resistance to invaders like parasites, bacteria, and viruses. These results provide a theoretical basis for selection of varieties and optimization of germplasm in fish culture production and may be very valuable in future research.

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Introduction

As in higher vertebrates, fish rely on both specific and non-specific mechanisms to protect themselves against invading pathogens (Xia et al., 2017). In fish, the primary areas of non-specific defense are the skin and mucus (Kumar *et al*., 2013). As a first line of defense, various peptides/proteins such as lysozymes, antibodies, complementary factors, and other lytic factors are present in the serum where they prevent colonization of microorganisms, leading to prevention of infection and disease (Das *et al*., 2009).

Clarias fuscus is an economically important fish in China and is distributed widely in south China, Yangtze river, Pearl River, Min River, and even Hainan island (Zhou *et al*., 2015). It is also distributed in Southeast Asia and Africa and forms different geographical population in the aforementioned region. It is an important economic fish also distributed in other regions.

Silurus asotus is classified Siluriformes and Siluridae (Park *et al*., 2004). The fish is a warm water fish inhabiting waters with temperatures ranging from 20-27°C. It is a nocturnal carnivorous fish and preys not only on crustaceans but also on other aquatic animals (Yang *et al*., 2015). Far Eastern catfish grow about 20-50 cm per a year. *S. asotus* live in China, Japan, and all Korean rivers. They are delicious and highly nutritious so are utilized as edible fish (Park *et al*., 2004).

Silurus meridionalis is a carnivorous forager that is characterized by a size, faster growth rate, and stronger disease resistance than other carnivorous fish in the same waters. It can survive with several months of food deprivation (Fu et al., 2011; Zhang and Xie 2000). This species is widely distributed in the Changjiang and Jialing rivers in China, where food supply fluctuates dramatically throughout the year (Zeng *et al*., 2014).

There are interspecific and intraspecific differences in non-specific immunity of fish. *C. fuscus*, *S. asotus,* and *S. meridionalis* are economically important species in the aquaculture industry in China. However, comparative study of non-specific immune indices of *C. fuscus*, *S. asotus,* and *S. meridionalis* has rarely been undertaken. The aim of the present study was to compare the non-specific immune indices of *C. fuscus*, *S. asotus,* and *S. meridionalis* to provide a theoretical basis for selection of varieties and optimization of germplasm in fish culture production, which would be valuable for research and application.

Materials and Methods

Experimental fish

C. fuscus, *S. asotus,* and *S. meridionalis* (body weight: 450-455g) were collected from a breeding base of Hunan University of Arts and Science. Prior to beginning the experiments, fish were acclimatized and quarantined in plastic tanks in aerated freshwater at 24±2°C for two weeks. The experiment was approved by the Institutional Animal Care and Use Committees (IACUC) of Hunan University of Arts and Science, Changde, China.

Head kidney and spleen indices

The fish were weighed and then sacrificed after collecting blood samples. The head kidney and spleen were removed and weighed, and head kidney and spleen indices were calculated.

Head kidney index = weight of head kidney (g) / body weight (g) × 100%

Spleen index = weight of spleen (g) / body weight (g) × 100%

Blood sampling

Samples of ten fish were collected from each species, and 1 mL blood was collected from the caudal vein of each fish with syringes and needles rinsed with heparin. The blood sample was centrifuged at 1000 g for 5 min to separate the blood serum that was stored at -20 °C for CAT, AKP, ACP, T-SOD, and lysozyme activity tests.

Lysozyme activity

Lysozyme activity was measured using the turbidity assay. Lysozyme standard product powder (80,000 U/mg) was used as a standard and 1 mg lyophilized micrococcus lysodeikticus in sodium phosphate buffer (pH 5.75) was used as substrate. 20 µL plasma sample was added to 2 mL of substrate and the reduction in the transmittance at 530 nm was determined after 20 s and 8 min incubation. One unit of lysozyme activity was defined as the increase in transmittance of 0.001 per min.

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical-dependent reactions using the Ransod Kit (Randox, Crumlin, UK). The reaction mixture (1.7ml) contained xanthine (0.05 mM) and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT, 0.025 mM) dissolved in CAPS 50 mM (pH 10.2) and EDTA (0.94 mM). In the presence of xanthine oxidase (80 U l1, 250 µl), superoxide and uric acid were produced. The superoxide radical then reacted with INT to produce a red formazan dye. Optical density was measured at 505 nm, and the rate of reaction was calculated by the absorbance readings 30 s and 3 min after adding xanthine oxidase. A reference standard SOD was supplied with the Ransod Kit. One unit of SOD was defined according to the amount required to inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units/ml.

Catalase, Alkaline phosphatase, and Acid phosphatase activity

In the experiments, we measured catalase activity (CAT), alkaline phosphatase activity (AKP), and acid phosphatase activity (ACP) of blood serum. Catalase activity, alkline phosphatase activity, and acid phosphatase activity were determined using the Diagnostic Reagent Kits purchased from Nanjing Jian Cheng Bioengineering Institute (China).

Statistical analysis

Data are presented as mean value ± standard error (SE); mean values of immune indices of *C. fuscus*, *S. asotus,* and *S. meridionalis* were compared using the one-way analysis of variance by Duncan's test of STATISTICA software package (Version 6.0, Statsoft, Inc.). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Results

Head kidney and spleen indices of *C. fuscus*, *S. asotus,* and *S. meridionalis* were shown in Figs. 1 & 2. Head kidney body ratio was highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus* (Fig. 1). Spleen body ratio of *S. meridionalis* was highest, and the spleen body ratio of *S. asotus* was higher than *C. fuscus* (Fig. 2).

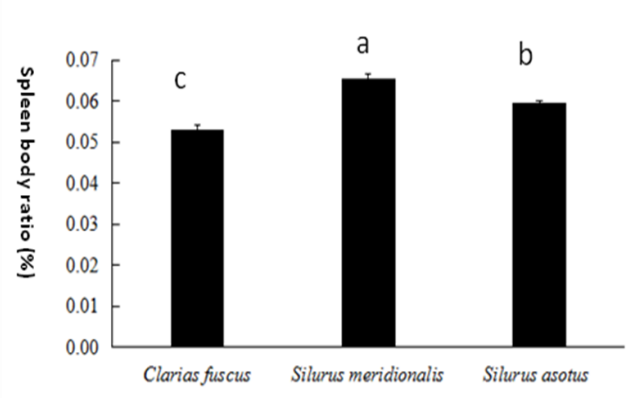

Fig. 1. Head kidney body ratio of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

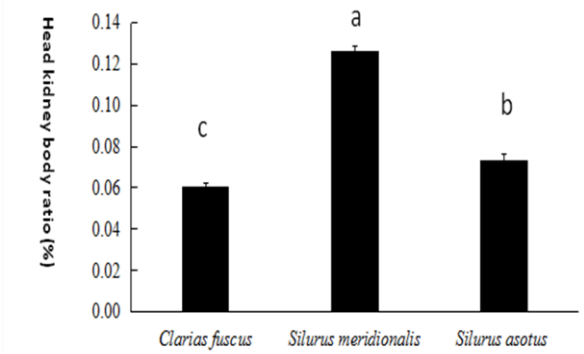

Fig. 2. Spleen body ratio of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Lysozyme activity of *C. fuscus*, *S. asotus,* and *S. meridionalis* was shown in Fig. 3. Lysozyme activity was 2.75 U/ml in *C. fuscus*, 9.75U/ml in *S. meridionalis,* and 8 U/ml in *S. asotus.* Lysozyme activity was highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus* (Fig. 3).

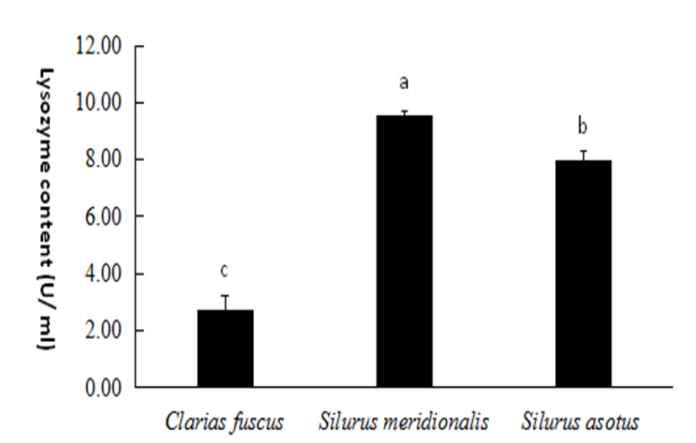

Fig. 3. Lysozyme activity of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Superoxide dismutase (SOD) activity of *C. fuscus*, *S. asotus,* and *S. meridionalis* was shown in Fig. 4. Superoxide dismutase (SOD) activity was 56.35 U/ml in *C. fuscus*, 64.65 U/ml in *S. meridionalis* and 60.15 U/ml in *S. asotus.* Superoxide dismutase (SOD) activity of *S. meridionalis* was highest, and superoxide dismutase (SOD) activity of *S. asotus* was higher than *C. fuscus* (Fig. 4).

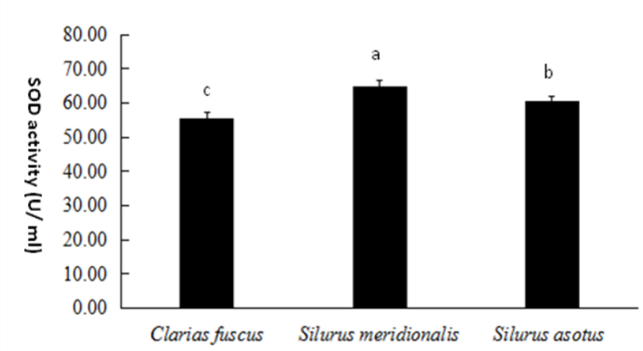

Fig. 4. Superoxide dismutase (SOD) activity of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Catalase activity (CAT) of *C. fuscus*, *S. asotus,* and *S. meridionalis* is shown in Fig. 5. Catalase activity (CAT) was 2.41 U/ml in *C. fuscus*, 5.62 U/ml in *S. meridionalis* and 1.92 U/ml in *S. asotus.* Catalase activity (CAT) was highest in *S. meridionalis*, followed by *C. fuscus*, and was lowest in *S. asotus* (Fig. 5).

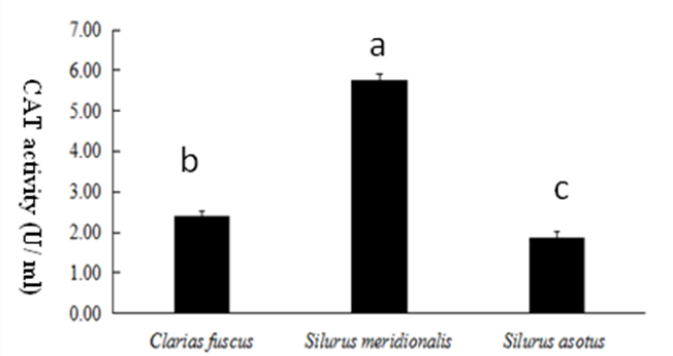

Fig. 5. Catalase activity (CAT) of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Alkaline phosphatase activity (AKP) of *C. fuscus*, *S. asotus,* and *S. meridionalis* is shown in Fig. 6. Alkaline phosphatase activity (AKP) was 2.21 U/ml in *C. fuscus*, 5.18 U/ml in *S. meridionalis* and 4.91 U/ml in *S. asotus.* Alkaline phosphatase activity (AKP) was highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus* (Fig. 6).

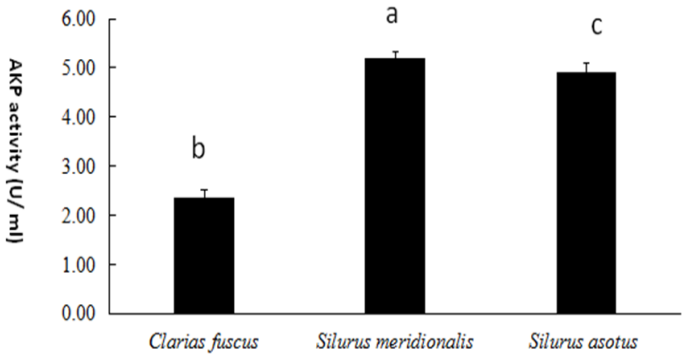

Fig. 6. Alkaline phosphatase activity (AKP) of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Acid phosphatase activity (ACP) of *C. fuscus*, *S. asotus,* and *S. meridionalis* was shown in Fig. 7. Acid phosphatase activity (ACP) was 1.68 U/ml in *C. fuscus*, 1.83 U/ml in *S. meridionalis* and 0.42 U/ml in *S. asotus.* Acid phosphatase activity (ACP) was highest in *S. meridionalis*, followed by *C. fuscus*, and was lowest in *S. asotus* (Fig. 7).

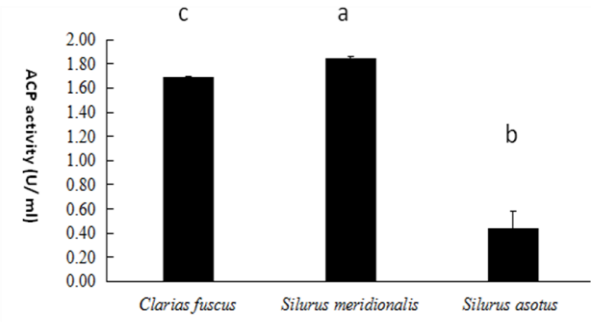

Fig. 7. Acid phosphatase activity (ACP) of *Clarias fuscus*, *Silurus asotus* and *Silurus meridionalis.* Data are presented as mean ± SE (n =10). Differences were determined by one-way analysis of variance (ANOVA). Different letters above bars represented significant difference at the levels of $p<0.05$, and same letters above bars indicated no significant difference.

Discussion

Immune organs are important parts of the immune system. As the histology foundations, they are vital in maintaining normal immune function (Qiao *et al*., 2013). The head kidney morphology and function were similar to the mammalian bone marrow, which was a major hematopoietic organ and site of production of antibodies and other immune cells in teleost fish (Xia et al., 2015). Head kidney is the major immune organ in fish and plays an important role in immune response of teleostei (Rijkers *et al*., 1980). The spleen is the last genesia-organ in the development of the lymphatic system of teleosts (Manning and Turner, 1994). To some extent changes in weight of head kidney and spleen indicate the developmental status of these organs which is important for the immunity of fish (Wang *et al*., 2006). In the present study, both head kidney and spleen body ratios were highest in *S. meridionalis*, followed by *S. asotus*, and lowest in *C. fuscus.* This indicated that the degree of lymphocyte proliferation and immune function of *S. meridionalis* was strongest compared to *S. asotus* and *C. fuscus.*

Neutrophils are considered the source of lysozyme and the enzyme appears to be much more bactericidal than lysozyme of higher vertebrates (Ellis, 2017). Lysozyme

activity functions as a primary defense factor of non-specific humoral immunity in preference to cellular defense mechanisms (Basha *et al*., 2013). Fish serum lysozyme is believed to be of leukocyte origin (Kumar *et al*., 2013). Lysozyme plays an important role in innate immunity by lysis of bacterial cell wall and thus stimulates the phagocytosis of bacteria (Ellis, 1990). Its ability to disrupt the cell walls of certain pathogens makes lysozyme a natural antagonist to harmful invaders like parasite, bacteria and virus. Lysozyme occurs prominently in fish serum and mucus (Wang *et al*., 2006). Lysozyme activity may be enhanced at relatively low concentrations of pollutants, and it has been proved that lysozyme activity was induced by relatively low dosage of mercury exposure (Low and Sin, 1996). The serum lysozyme activity was significantly increased in low dose of MCs group (Qiao *et al*., 2013).

The serum lysozyme is used as an indicator of innate immune response in fish (Tort *et al*., 2003). An increased level is considered to be a natural protective mechanism in fish (Ingram, 1980). In the present study, blood serum lysozyme activity was highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus.* We found that the blood serum of *S. meridionalis* had potentially strong resistance to harmful invaders like parasites, bacteria, and viruses compared to *S. asotus* and *C. fuscus.*

Superoxide dismutase (SOD) is one of the main anti-oxidant defense enzymes generated in response to oxidative stress that converts the highly toxic superoxide anions into hydrogen peroxide (Fridovich, 1995). SOD decreases in WSSV-infected *P. monodon* (Chang *et al*., 2003). The activity of SOD was significantly lowered in WSSV-infected *F. indicus* (Sarathi *et al*., 2008). The activity of superoxide dismutase responsible for the scavenging of reactive oxygen species (ROS) decreased leading to the increases of superoxide anion (Liu and Chen, 2004). An increase in the superoxide anion production against pathogens is considered to be beneficial after exposing shrimp to immunostimulants (Downs *et al*., 2001). In the present study, the activity of SOD was highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus*, which is in line with blood serum lysozyme activity. The highest level of SOD activity was considered to be beneficial after exposing *S. meridionalis* to immunostimulants compared to *S. asotus* and *C. fuscus.*

Catalase activity (CAT) is one of the primary antioxidant enzymes involved in ROS removal (Sun *et al*., 2012). Superoxide anion ($O_2\bullet-$) was reduced to H_2O_2 by SOD, and H_2O_2 was converted to water and oxygen by CAT (Pandey *et al*., 2003). The production of O_2^- has been reported to be an accurate method to measure the effectiveness of potential immunostimulants (Song and Hsieh, 1994). An increase in H_2O_2 and superoxide anion is considered beneficial in protecting against disease and increasing immunity (Song and Hsieh, 1994). A similar tendency as that observed for SOD and lysozyme activity was found for CAT; CAT activity was highest in *S. meridionalis*, followed by *C. fuscus*, and was lowest in *S. asotus,* indicating that *S. meridionalis* had the potentially strongest resistance to harmful invaders.

Increased phosphatase activity indicates higher breakdown of the energy reserve, which is utilized for growth and survival of fish (Sahu *et al*., 2008). Alkaline phosphatase plays an important role in metabolic regulation, which is directly involved in the transfer of phosphate groups and calcium phosphorus metabolism. Alkaline phosphatase also changes the surface structure of pathogens and strengthens the recognition and phagocytosis of pathogens (Hu *et al*., 2008). Alkaline phosphatase activity was increased in the group of fish fed turmeric over a number of days. An increase in the alkaline phosphatase activity is considered beneficial for fish disease resistance (Hu *et al*., 2008). In another study, acid phosphatase activity was increased post-challenge with *Aeromonas hydrophila* in all groups (Das *et al*., 2009). In the present study, blood serum alkaline phosphatase activity was highest in *S. meridionalis*, followed by *S. asotus* , and was lowest in *C. fuscus.* Blood serum acid phosphatase activity was also highest in *S. meridionalis*, while blood serum acid phosphatase activity of *C. fuscus* was higher compared to *S. asotus.* There are interspecific and intraspecific differences in non-specific immunity in different fish. Both blood serum acid phosphatase activity and alkaline phosphatase activity were highest in *S. meridionalis*, which is in line with lysozyme activity, superoxide dismutase (SOD) activity, catalase activity, and head kidney and spleen body ratios. The immune function of *S. meridionalis* was found to be strongest,

and it had strongest resistance to harmful invaders like parasites, bacteria, and viruses compared to *S. asotus* and *C. fuscus.*

The present study compared, for the first time, the non-specific immune indices of *C. fuscus*, *S. asotus* and *S. meridionalis.* In the present study, the head kidney index, spleen index, blood serum lysozyme activity, superoxide dismutase (SOD) activity, and alkaline phosphatase activity were highest in *S. meridionalis*, followed by *S. asotus*, and was lowest in *C. fuscus*. Meanwhile, both acid phosphatase and catalase activities were highest in *S. meridionalis*. These results suggested that the immune function of *S. meridionalis* was strongest, and it had strongest resistance to harmful invaders like parasites, bacteria, and viruses compared to *S. asotus* and *C. fuscus*. These results provide a theoretical basis for selection of varieties and optimization of germplasm in fish culture production, and are valuable for future research and application.

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The authors declare no conflict of interests.

References

Basha K A, Raman R P, Prasad K P, 2013. Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton). *Fish Shellfish Immunol*, 35(5), 1433-1441.

Chang C F, Su M S, Chen H Y, 2003. Dietary-1,3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish Shellfish Immunol*, 15, 297-310.

Das B K, Debnath C, Patnaik P, 2009. Effect of β-glucan on immunity and survival of early stage of *Anabas testudineus* (Bloch). *Fish Shellfish Immunol*, 27(6), 678-683.

Downs C, Fauth J E, Woodley C M, 2001. Assessing the health of grass shrimp (*Palaemonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. *Mar Biotechnol*, 3, 380-397.

Ellis A E, 2001. Innate host defense mechanisms of fish against viruses and bacteria. *Dev Comp Immunol*, 25, 827-839.

Ellis A E, 1990. Immunity to bacteria in fish. *Fish Shellfish Immunol*, 9, 291-308.

Fu S J, Pang X, Cao Z D, 2011. The effects of fasting on the metabolic interaction between digestion and locomotion in juvenile southern catfish (*Silurus meridionalis*). *Comp Biochem Physiol A*, 158, 498–505

Fridovich I, 1995. Superoxide radical and superoxide dismutases. *Annu Rev Biochem*, 64, 97-112.

Hu B, Li X Q, Leng X J, 2008. Effects of dietary vitamin C on growth, meat quality and non-specific immunity indices of grass carp, *Ctenopharyngodon idellus*. *J Fishery Sci China*, 15(5), 794-800.

Ingram G A, 1980. Substances involved in the natural resistance of fish to infection - a review. *J Fish Biol*, 16, 23-60.

Kumar S, Raman R P, Pandey P K, 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. *Fish Shellfish Immunol*, 34(2), 564-573.

Low K W, Sin Y M, 1996. In vivo and in vitro effects of mercuric chloride and sodium selenite on some non-specific immune responses of blue gourami, *Trichogaster trichopterus* (Pallus). *Fish Shellfish Immunol*, 6, 351–362.

Liu C H, Chen J C, 2004. Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish Shellfish Immunol*, 16(3), 321-334.
Manning M J, Turner R J, 1994. Immunology: a comparative approach. In: Turner, R.J. (Ed.), Fishes. John Wiley & Sons, UK, 66 – 99 pp.
Park I S, Im J H, Hur J W, 2004. Morphometric characteristics of catfish (*Silluridae*) in Korea. *Kor J Ichthyol*, 16, 223-228.
Pandey S, Parvez S, Sayeed I, 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). *Sci Total Environ*, 309, 105–115.
Qiao Q, Liang H L, Zhang X Z, 2013. Effect of cyanobacteria on immune function of crucian carp (*Carassius auratus*) via chronic exposure in diet. *Chemosphere*, 90, 1167-1176.
Rijkers G T, Frederix Wolters E M H , Van Muiswinkel W B, 1980. The haemolytic plaque assay in carp (*Cyprinus carpio*). *J Immunol Methods*, 33, 79 – 86.
Sarathi M, Nazeer Basha A, Ravi M, 2008. Clearance of white spot syndrome virus (WSSV) and immunological changes in experimentally WSSV-injected *Macrobrachium rosenbergii*. *Fish Shellfish Immunol*, 25, 222-230.
Sahu S, Das B K, Mishra B K, 2008. Effect of dietary Curcuma longa on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*. *Aquacult Res*, 39, 1720-1730
Sun H, Lü K, Minter E J, 2012. Combined effects of ammonia and microcystin on survival, growth, antioxidant responses and lipid peroxidation of bighead carp *Hypophthalmythys nobilis* larvae. *J Hazard Mater*, 30, 213-219.
Song Y L, Hsieh Y T, 1994. Immunostimulation of tiger shrimp *Penaeus monodon* haemocytes for generation of microbicidal substances: analysis of reactive oxygen species. *Dev Comp Immunol*, 18, 201-209.
Tort L, Balasch J C, Mackenzie S, 2003. Fish immune system. A cross roads between innate and adaptive responses. *Imunología*, 3, 277-286.
Wang W B, Wang Y P, Hu W, 2006. Effects of the "all-fish" growth hormone transgene expression on non-specific immune functions of common carp, *Cyprinus carpio L*. *Aquaculture*, 259, 81–87.
Xia H, Tang Y, Lu F H, 2017. The effect of *Aeromonas hydrophila* infection on the non-specific immunity of blunt snout bream (*Megalobrama amblycephala*). *Central European J Immunol*, 42(3), 239-243
Xia H, Liu W J, Wu K, 2015. Spatio-temporal expression of blunt snout bream (*Megalobrama amblycephala*) mIgD and its immune response to *Aeromonas hydrophila*. *Central European J Immunol,* 40 (2): 132-141.
Yang W S, Gil H W, Yoo G Y, 2015. Identification of Skeletal Deformities in Far Eastern Catfish, *Silurus asotus* under Indoor Aquaculture Condition. *Dev Reprod*, 19(3), 153-161.
Zhou C, Wang X, Guan L, 2015. The complete mitochondrial genome of *Clarias fuscus* (Teleostei, Siluriformes: Clariidae). *Mitochondrial DNA*, 26(2), 270-271.
Zhang B, Xie X J, 2000. Starvation metabolism in the southern catfish (*Silurus meridionalis* Chen). *Oceanol Limnol Sin*, 31, 480–484
Zeng L Q, Fu S J, Li X M, 2014. Physiological and morphological responses to the first bout of refeeding in southern catfish (*Silurus meridionalis*). *J Comp Physiol B*, 184(3), 329-46.