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Protective effects of *Sargassum horneri* against ammonia stress in juvenile black sea bream, *Acanthopagrus schlegelii*

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Abstract
Ammonia is an important environmental toxic pollutant that represents a biochemical and physiological hazard to living organisms, especially in intensive culture systems with high stocking density. This study aimed at evaluating the protective effects of *Sargassum horneri* (SH) on ammonia stress in black sea bream. Four hundred and eighty fingerlings with approximate mean body weight 12.0 ± 0.1 g were randomly distributed into four experimental groups in triplicate. Each group was stocked with 40 fish and fed with isonitrogenous (42% crude protein) and isolipid (12% crude lipid) experimental diets containing either 0% SH (control), 3% SH, 6% SH, or 9% SH in the feed. At the end of 8 weeks of experimental feeding, fish were exposed to ammonia for 24 h. The results indicated that ammonia stress led to an increase in oxidative stress as shown by elevation in the levels of cortisol and liver lipid peroxidative damage markers as well as decrease in antioxidant biomarkers. Furthermore, humoral immunity was suppressed during the stress period. However, fish supplied with *S. horneri* had significant improvement in immune response, antioxidant capacity, and resistance against ammonia stress, particularly in the 6% SH group. Therefore, dietary *S. horneri* supplementation at an optimum level of 6% could increase the resistance of juvenile black sea bream to ammonia-induced stress.

Keywords *Sargassum horneri* · Phaeophyta · Immunosuppression · Oxidative stress · *Acanthopagrus schlegelii*

Introduction

Several factors including dissolved oxygen, ammonia, pH, nitrites etc., can affect water quality, with these water quality parameters impacting on fish health. Ammonia is one of the most detrimental water quality parameters besides oxygen depletion, which affects fish, as it can generate severe stress, especially in intensive culture systems with high stocking density (Shrivastava et al. 2016). An elevation in water ammonia concentration in intensive culture systems is not just due to insufficient water exchange and filtration, but is due to many factors such as bacterial decomposition products of leftover food, chemical fertilizer from agriculture fields, a wide variety of other anthropogenic processes, as well as fish metabolism and excretion by gills (Yang et al. 2010; Ankley et al. 2011). Previous studies have indicated that the excretory mechanism of nitrogenous products (such as by gills and kidneys) in most teleosts could be damaged, reduced, or the concentration gradient even reversed, eventually leading to ammonia poisoning when the ammonia concentrations become very high (Claiborne and Evans 1988; Meade 1989). Similarly, when fish are exposed to low ammonia environment for a long time, it results in poor growth, low resistance to bacterial infections, as well as become more sensitive to routine handling (Li et al. 2014; Bucking 2017). Some signs and symptoms of ammonia poisoning in fish include lethargy, inappetence, hovering at bottom of the tank, gasping at the surface (Rama and Manjabhat 2014), and sometimes, fast breathing, and convulsions till death in cases of acute ammonia toxicity.

Ammonia toxicity is physiologically harmful to fish and can rapidly elevate serum glucose and lactate levels (Zhang et al. 2015a), increase lysozyme (LZM) activity in fish serum as well as other immune factors such as complement 3 (C3), complement 4 (C4) and immunoglobulin M (IgM) at the onset of water stress (Jia et al. 2017). Thus, ammonia exposure impacts the innate immune system of fish. Elevated levels of ammonia may also result in oxidative stress in fish, as it has been shown that production of reactive oxygen species (ROS) can be induced in the liver of Nile tilapia upon exposure to chronic ammonia stress (Hegazi et al. 2010). Similarly,

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ammonia attenuates the activity of catalase (CAT), superoxide dismutase (SOD), and other antioxidant enzymes such as glutathione peroxidase (GPx) and glutamine synthetase (GSH) when fish are exposed to high environmental ammonia (Ching et al. 2009; Sinha et al. 2015). Fish have several different strategies in detoxifying ammonia including reducing ammonia production, converting ammonia to less toxic compounds, or increasing ammonia excretion through the gills (Randall and Tsui 2002). However, an effective way to ameliorate ammonia toxicity by varying nutritional factors has attracted interest, with a number of studies exploring the use of diverse dietary supplements as immunostimulants, with the hope that this could be an appropriate strategy to relieve ammonia toxicity (Chen et al. 2012; Rama and Manjabhat 2014; Suwaree et al. 2015).

Seaweeds are considered suitable sources of natural immunostimulants, as they possess valuable biologically active compounds, especially polysaccharides (Wan et al. 2016), and which have anti-inflammatory, antimicrobial, anti-viral, immunity enhancing, as well as efficient antioxidant properties (Araújo et al. 2016). The brown macroalgae *Sargassum horneri* (SH) (Sargassaceae, Phaeophyta) is widely distributed along the southeastern coast of China to the Sea of Japan, from mid-littoral to sublittoral zones (Hu et al. 2011). Some in vitro studies have shown that the polysaccharide, fucoidan, derived from different species of *Sargassum*, has important biological properties, including anti-oxidant, anti-bacterial, anti-viral, anti-tumor, and immune enhancement activities (Shao et al. 2015; Kang et al. 2016; Telles et al. 2018). Moreover, in teleosts, in vivo studies have revealed that polysaccharides are immune system stimulators, as they are capable of boosting immune responses and resistance against infection. For example, extracts of *Sargassum* sp. have been shown to have an effect on Asian sea bass (*Lates calcarifer*) (Yangthong et al. 2016), with *Sargassum angustifolium* extracts having an effect on rainbow trout (*Oncohrynchus mykiss*) (Zeraatpisheh et al. 2018), while extracts of *Sargassum horneri* have an effect on yellow catfish (*Pelteobagrus fulvidraco*) (Yang et al. 2014). In fact, Wang et al. (2018) have reported that the dry powder of *S. horneri* enhances immunity of turbot (*Scophthalmus maximus*) and resistance to pathogenic bacteria. It is clear from the foregoing that *S. horneri* could serve as promising supplement in aquatic diet as well as for therapeutic application for immune inhibition and resistance against stress.

The black sea bream (*Acanthopagrus schlegelii*), an omnivorous fish that consumes seaweed as part of its natural food, is considered an important marine species in China and Japan due to its high economic value and excellent meat quality (Nip et al. 2003). Moreover, *A. schlegelii* can be used as an animal model in environment pollution monitoring. However, in commercial farming, chopped or minced trash fish are commonly used as feed inputs, which result in serious water eutrophication problems, particularly ammonia (Alongi et al. 2003). High levels of ammonia cause severe damage to the anti-oxidation and immune system of fish, thus resulting in heavy losses in intensive aquaculture.

The current study sought to evaluate the effects of *S. horneri* dietary supplementation on anti-oxidation and immunological parameters in black sea bream, and to also investigate whether this seaweed could protect against damages caused by exposure to ammonia stress.

Materials and methods

Experimental diets

Fresh *Sargassum horneri* were collected from Shenao Bay, Nan'ao Island, Guangdong province, China. After being thoroughly washed in seawater, the algae were air-dried and further dried at 65 °C for 4 h, followed by grinding into powder with a small laboratory feed grinder. The other ingredients were purchased from commercial suppliers. Proximate composition of the major ingredients in the experimental diets is shown in Table 1. Four isonitrogenous (42% crude protein) and isolipid (12% crude lipid) experimental diets were formulated to contain 3%, 6%, and 9% *S. horneri* meal by replacing with soybean meal in order to attain an equal weight basis. An experimental diet without seaweed supplementation was used as control (Table 2).

All dry ingredients were finely ground into powder, passed through a 90-mesh sieve, and then thoroughly mixed with soybean oil and water to form a stiff paste. Next, pellets were made by a laboratory pelletizer equipped with a 2-mm die, and the extrusion temperature was set mainly at 100 °C during the process. After natural air-drying, the pellets were packed and stored at − 20 °C until used. About 50 g of diet in each triplicate formulated diet was sampled for biochemical composition analysis.

Experimental fish and feeding trial

Juvenile black sea bream *Acanthopagrus schlegelii* were obtained from a local marine fish hatchery, and then cultured in a floating sea cage (2.5 × 2.5 × 2.5 m, L/W/H) for 2 weeks to acclimatize to the experimental culture conditions. Fish were fed a commercial diet during acclimation. Next, fish were starved for 24 h, after which fish with approximate mean body weight of 12.0 ± 0.1 g were selected and randomly allocated into 12 sea cages (1.0 × 1.0 × 1.2 m, L/W/H). Each cage was stocked with 40 fish, while each diet was randomly assigned to triplicate cages.

Fish were fed twice a day (7:00 and 17:00) for each treatment group at 3–5% biomass for 8 weeks. During this period, water temperature ranged from 24 to 33 °C, oxygen level was

Table 1 Proximate composition of the major ingredients (%, dry matter)

Ingredient	Dry matter	Crude protein	Crude lipid	Crude ash	NFE
S. horneri meal[a]	88.21	17.21	0.45	19.36	62.98
Fish meal[b]	92.48	69.04	9.13	12.85	8.89
Soybean meal[b]	90.76	43.62	1.46	5.68	49.24
Tapioca starch[b]	89.55	3.52	2.37	1.85	92.26
Casein[c]	93.45	91.08	–	1.24	–

NFE nitrogen free extract = 100 − (crude protein + crude lipid + ash)

[a] Shenao Bay, Guangdong Province, China

[b] Yuequn feed company, Jieyang, Guangdong Province, China

[c] Hualing casein company, Gannan, Gansu Province, China

approximately 7.2 mg L^{-1}, and the ammonia concentration did not exceed 0.3 mg L^{-1}.

At the end of the feeding trial, fish blood was withdrawn and liver tissues removed from randomly selected fish per group in each cage ($n = 9$), snap frozen in liquid nitrogen, and stored at − 80 °C for blood biochemical assays and liver tissue antioxidant parameter analysis.

Table 2 Formulation and chemical composition of experimental diets (%, dry matter)

Ingredient	Control	3% SH	6% SH	9% SH
Fish meal	45	45	45	45
S. horneri meal	0	3	6	9
Soybean meal	9	8	7	6
Casein	10	10	10	10
Tapioca starch	22.5	20.5	18.5	16.5
Soybean oil	8.5	8.5	8.5	8.5
MCC[a]	1	1	1	1
CaH_2PO_4	1.8	1.8	1.8	1.8
Choline chloride	0.2	0.2	0.2	0.2
Vitamin premix[b]	1	1	1	1
Mineral premix[c]	1	1	1	1
Proximate composition				
Dry matter	90.25	90.07	89.93	90.34
Crude protein	42.11	42.06	42.40	42.50
Crude lipid	12.07	12.42	12.45	12.40
Crude ash	10.87	10.61	10.50	11.27

[a] MCC, microcrystalline cellulose, Sunhere Pharmaceutical Excipients Co., Ltd., Hefei, Anhui Province, China

[b] Per kilogram of mineral premix: NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; NaCl, 100 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; zoelite, 15.45 g. Formulated with cellulose as filling, Sintun Aqua-Tech Co., Ltd., Guangzhou, Guangdong Province, China

[c] Per kilogram of vitamins premix: A, 4×10^6 IU; D_3, 2×10^6 IU; E, 60 g; K_3, 6 g; B_1, 7.5 g; B_2, 16 g; B_6, 12 g; B_{12}, 100 mg; nicotinic acid, 88 g; pantothenic acid, 36 g; folic acid, 2 g; biotin, 100 mg; inositol, 100 g; C monophospholipid compound, 200 g, Sintun Aqua-Tech Co., Ltd., Guangzhou, Guangdong Province, China

Ammonia stress test

Ammonia stress test was performed for 24 h at the end of the feeding experiment. Briefly, the remaining fish ($n = 20$) from each experimental group were collected and transferred to the corresponding tank, then exposed to ammonia (35 mg L^{-1}), which is NH_4Cl (10 g L^{-1}) added in advance. The LC_{50} at 24 h was 42.35 mg ammonia L^{-1} under the present experimental conditions (salinity 29‰,water temperature 27 ± 1.0 °C, pH 7.2, dissolved oxygen 6.8–7.5 mg L^{-1}). The ammonia content of the tank was monitored every 4 h with a water quality indicator (YSI, Professional Plus). At 24 h post the ammonia induction, blood and liver tissues were sampled from randomly selected fish from each tank, snap frozen in liquid nitrogen, and stored at − 80 °C for analysis of related indices.

Sample collection

All fish were starved for 24 h after which blood samples were collected with needle and syringe through the caudal vein into tubes after anesthetizing with tricane methane sulfonate MS-222 (80 ml L^{-1}). Blood was separated in a 4 °C centrifuge (3500 rpm, 10 min) and the serum stored at − 80 °C for biochemical assays. Fish were dissected, liver tissue removed onto ice, homogenized at tissue: solution of 1: 9 using physiological saline solution, and then centrifuged at 3500 rpm for 10 min at 4 °C. Finally, the supernatant was collected and stored at − 80 °C for antioxidant enzyme assay.

Sample analyses

Stress response

Analysis of serum glucose content was carried out by the glucose oxidase method as described by Asadi et al. (2009), while serum lactate concentration was measured by the lactate oxidase method, using a commercial kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Serum cortisol level was determined using an

enzyme-linked immunosorbent assay (ELISA) kit for fish obtained from Shanghai Chuangxiang Biotechnology company (Shanghai, China), following the method previously described by Rodriguez et al. (1989).

Immune parameters

Serum acid phosphatase (ACP) and alkaline phosphatase (AKP) activities were assessed based on a disodium phenyl phosphatase method as described by Xie et al. (2008). LZM activity was measured using the turbid metric method described by Yue et al. (2014) with slight modification. C3, C4, and IgM contents were determined by the same method as that for cortisol, using ELISA kits for fish purchased from the same company.

Liver antioxidant capacity

Liver antioxidant indices including superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) were measured using commercial assay kits supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The SOD and CAT activities were expressed as U (units) per microgram of soluble protein in the liver. T-AOC was assayed based on the method described by Miller et al. (1993). The method used to measure MDA level is as described by Livingstone et al. (1990).

Statistical analysis

The data for each treatment group are presented as the mean ± standard error (SE). The stress test parameters were compared by *t* test. A one-way ANOVA followed by Duncan's multiple range test was used to assess the statistically significant differences between the means among experimental groups using SPSS 19.0 software. The level of significance was set at $P < 0.05$.

Results

Stress response

The effects of *S. horneri* on serum glucose, lactate, and cortisol contents of black sea bream before and after ammonia stress are shown in Table 3. It was observed that before the ammonia stress, there were generally no significant differences in the serum levels of glucose, lactate, and cortisol between the control and SH treatment groups ($P > 0.05$). However, a significant change was observed in only serum glucose level of the 6% SH group compared to control ($P < 0.05$). On the other hand, after the ammonia stress, the level of cortisol significantly increased in all groups ($P < 0.05$), with similar changes also found in serum glucose levels of 6% and 9% SH groups, as well as the lactate levels of control and 9% SH groups ($P < 0.05$). The lowest serum lactate and cortisol levels were found in the 6% SH group compared to control ($P < 0.05$). However, there was no significant change in serum glucose level among the various treatment groups post ammonia stress ($P > 0.05$).

Immune response

In terms of the immune response parameters before and after the ammonia stress test in Table 4, it was observed that the levels of serum LZM, AKP (except for 9% SH group), C3, and C4 were significantly increased among the various *S. horneri* groups compared to control ($P < 0.05$), with the highest AKP and C3 levels found in the 6% SH diet group. The activity of ACP was significantly elevated in the 6% and 9% SH groups ($P < 0.05$), while no significant change in the IgM content was observed among all treatment groups ($P > 0.05$). There was generally some decrease in the immune parameters after stress exposure, except for ACP and IgM, while a significant reduction in the levels of AKP and C3 was observed ($P < 0.05$). Similarly, before the stress exposure, the levels of LZM and C4 were significantly increased with *S. horneri* treatment groups compared to the control ($P < 0.05$), with the highest found in the 6% SH group. The IgM content was significantly elevated in the 3% and 6% SH groups ($P < 0.05$), while C3 content significantly increased in the 6% and 9% SH groups compared to control ($P < 0.05$). No significant change in the activity of AKP was observed ($P > 0.05$), although there was some increase between the various groups.

Liver antioxidant capacity

As was shown in Table 5, apart from MDA and CAT, the activities of T-AOC and SOD in fish fed with *S. horneri* were significantly higher compared with fish fed with the control diets before stress ($P < 0.05$), with the highest observed in the 6% SH group. All parameters, except MDA, were significantly reduced following exposure to ammonia stress for 24 h ($P < 0.05$). Supplementation with 6% SH significantly enhanced the T-AOC, SOD, and CAT activities ($P < 0.05$), but had little effect on MDA content in all groups ($P > 0.05$).

Discussion

Determination of serum glucose, lactate, and cortisol levels generally serves as reliable parameters in evaluating stress caused by a variety of stressors (Costas et al. 2011). Several reports have indicated that one or all of these parameters (serum glucose, lactate, and cortisol) are elevated when exposed to different stressors, such as harsh handling (Wedemeyer

Table 3 Effects of *S. horneri* on stress response of black sea bream

Treatments	Control	3% SH	6% SH	9% SH
Before stress				
Glucose (mmol L^{-1})	3.17 ± 0.22Ab	3.09 ± 0.12Ab	2.53 ± 0.02Aa	2.95 ± 0.08Ab
Lactate (mmol L^{-1})	4.16 ± 0.10A	4.07 ± 0.14A	4.03 ± 0.14A	4.12 ± 0.10A
Cortisol (pg mL^{-1})	252.23 ± 13.12A	236.41 ± 10.99A	238.27 ± 15.54A	241.56 ± 4.66A
After stress				
Glucose (mmol L^{-1})	3.38 ± 0.24A	3.35 ± 0.09A	3.24 ± 0.05B	3.36 ± 0.13B
Lactate (mmol L^{-1})	4.87 ± 0.32Bb	4.13 ± 0.29Aa	3.95 ± 0.45Aa	5.02 ± 0.45Bb
Cortisol (pg mL^{-1})	321.37 ± 11.74Bb	331.65 ± 16.36Bb	292.43 ± 17.77Ba	315.14 ± 15.42Bb

Data represent mean ± SD for three replicates

Data in the same column with different letters (A, B) for before and after ammonia stress show significant difference ($P < 0.05$)

Data in the same row with different letters (a, b, c) show significant difference ($P < 0.05$)

1972), careless transportation (Barton and Schreck 1987), inappropriate stocking density (De las Heras et al. 2015), low dissolved oxygen (Ni et al. 2014), and pathogen infection (Prabu et al. 2016). In the present work, ammonia stress resulted in significant increase in serum cortisol level of black sea bream, coupled with an increasing trend in the levels of serum glucose and lactate. This observation thus indicated that high ammonia could cause stress response in black sea bream, which is in agreement with previous reports on blunt snout bream (*Megalobrama amblycephala*) (Zhang et al. 2015a), rohu (*Labeo rohita*) (Acharya et al. 2005), and sablefish (*Anoplopoma fimbria*) (Kim et al. 2017). It is believed that an elevation in serum cortisol level might be regulated through the hypothalamic-pituitary-interrenal axis, with an increased release of corticosteroid hormones when animals are subjected to stress (Zhang et al. 2015b). In fact, Laiz-Carrión et al. (2002) hypothesized that cortisol could facilitate liver glucogenesis and gluconeogenesis in fish that are in suboptimum or stressful situations, thereby raising serum glucose level. As a substrate for gluconeogenesis, lactate is closely related to muscle glycolysis (Stallknecht et al. 1998). Thus, once fish are subjected to acute stress, anaerobic metabolism occurs in order to meet the short-term energy needs, therefore resulting in the abnormal accumulation of lactate (Hur et al. 2007). In this study, it was observed that after the ammonia stress, the serum cortisol and lactate levels in fish fed 6% SH were significantly lower than fish fed with the control diet, especially the lactate level, which was lower than the pre-stress level. This indicated that 6% SH could effectively relieve the adverse effects of high ammonia stress. A previous

Table 4 Effect of *S. horneri* on the immune parameters of black sea bream before and after stress

Treatments	Control	3% SH	6% SH	9% SH
Before stress				
LZM (U mL^{-1})	108.98 ± 11.38Aa	136.29 ± 8.64Ab	149.81 ± 8.82Ab	134.17 ± 7.82Ab
ACP (U 100 mL^{-1})	5.96 ± 0.30Aa	5.77 ± 0.23Aa	7.01 ± 0.29Ab	6.84 ± 0.33Bb
AKP (U 100 mL^{-1})	12.33 ± 0.80Ba	16.25 ± 1.42Bb	20.28 ± 1.22Bc	12.75 ± 1.31Ba
IgM (g L^{-1})	1.07 ± 0.12A	1.28 ± 0.11A	1.20 ± 0.14A	1.02 ± 0.14A
C3 (μg mL^{-1})	113.73 ± 3.68Ba	130.92 ± 4.05Bb	146.58 ± 8.05Bc	130.53 ± 8.91Bb
C4 (μg mL^{-1})	85.60 ± 3.75Ba	101.56 ± 7.52Ab	107.28 ± 5.21Ab	99.24 ± 6.56Ab
After stress				
LZM (U mL^{-1})	103.61 ± 7.18Aa	132.60 ± 11.54Abc	145.78 ± 6.89Ac	130.48 ± 5.00Ab
ACP (U 100 mL^{-1})	6.01 ± 0.08Ab	6.33 ± 0.38Ab	6.67 ± 0.35Ab	5.76 ± 0.06Aa
AKP (U 100 mL^{-1})	8.46 ± 0.98A	9.34 ± 0.87A	9.09 ± 1.07A	8.67 ± 0.76A
IgM (g L^{-1})	1.16 ± 0.02Aa	1.26 ± 0.04Ab	1.36 ± 0.03Ac	1.18 ± 0.02Aa
C3 (μg mL^{-1})	103.04 ± 4.77Aa	112.20 ± 5.88Aab	132.83 ± 8.45Ac	117.93 ± 4.77Ab
C4 (μg mL^{-1})	74.97 ± 4.63Aa	95.83 ± 6.36Ab	102.78 ± 11.57Ab	104.42 ± 3.47Ab

Data represent mean ± SD for three replicates

Data in the same column with different letters (A, B) for before and after ammonia stress show significant difference ($P < 0.05$)

Data in the same row with different letters (a, b, c) show significant difference ($P < 0.05$)

study showed that the olive flounder (*Paralichthys olivaceus*) when fed 5% *Undaria pinnatifida* could increase its resistance to temperature stress, as well as significantly lower its plasma glucose and cortisol level compared to control (Kwon et al. 2003). Similarly, Magnoni et al. (2017) reported that feeding gilthead seabream (*Sparus aurata*) with heat-treated *Gracilaria* could mitigate stress by hypoxia. Moreover, they revealed that the serum cortisol and lactate levels were significantly lower in fish fed 5% heat-treated *Gracilaria* compared with control, while subsequent recovery to normoxia also resulted in a marked decrease in serum lactate level in the 5% heat-treated *Gracilaria* group. However, the exact mechanisms of the high ammonia stress relieving effects by *S. horneri* are not clear, since there are limited reports on it, which therefore requires further studies.

The humoral immunity of fish, like that of higher vertebrates, is composed of non-specific and specific immune defense responses, with the non-specific immune response considered the most crucial defense mechanism in fish. Fish immunity is evaluated using some parameters such as LZM, ACP, AKP, complement system, IgM, etc., which are factors involved in the first line of defense against invading pathogens or xenobiotic stress (Uribe et al. 2011). In the present study, all the indices measured (except IgM) in the serum of black sea bream were remarkably elevated in fish fed *S. horneri*, especially in the 6% group. These results are similar to that recently reported by Wang et al. (2018), where dietary 7.5% SH power increased the activity of serum ACP. Furthermore, given that it has previously been reported that seaweeds may act as promising prebiotic supplements when used in aquatic feeds on account of their rich immunostimulant compounds (mainly polysaccharides, such as fucoidan) (Kim and Lee 2008; Lee et al. 2016), this might account for the enhanced non-specific immunity of fish observed in this study. Several similar results on the non-specific immune enhancement by seaweeds and/or seaweed products have been reported. For instance, *S. horneri*-derived fucoidan was shown to produce an increase in serum lysozyme and respiratory burst activity of yellow catfish phagocytes (Yang et al. 2014). Similarly, fucoidan extracted from *U. pinnatifida* significantly enhanced total hemocyte counts, phenol oxidase activity, and serum antibacterial activity of kuruma shrimp (*Marsupenaeus japonicas*) (Traifalgar et al. 2010), while Asian sea bass administrated with a rich fucoidan aqueous extract of *Sargassum* sp. had an increased immune response (Yangthong et al. 2016). However, there has not been an exact explanation of the mechanism or how fucoidan affects non-specific immunity in fish. It is believed that fucoidan might be involved in the activation of a signaling pathway that enhances interleukin (IL)-12 production in macrophages (Kawashima et al. 2012). In some mammalian studies, fucoidan was found to significantly limit plasma production of proinflammatory cytokines (TNF-α and IFN-γ), thereby promoting the formation of anti-inflammatory cytokine (IL-10) (Saito et al. 2006; Hong et al. 2012; Omar et al. 2013).

There was a general decrease in all the immune-related parameters in all the treatment groups following ammonia challenge, except ACP and IgM. Similar results have previously been reported in turbot, where a dramatic decrease in C3, C4, and IgM levels was observed when fish were subjected to high ammonia (20 or 40 mg L^{-1}) for 48 and 96 h (Jia et al. 2017). It has also been reported that, when teleosts were subjected to high concentrations of ammonia, there was a net influx of free ammonia from the medium into tissues, therefore leading to large accumulation of ammonia in immune organs (kidney and spleen) until they became dysfunctional (Ching et al. 2009; Sinha et al. 2013). Besides, elevated serum ammonia levels induce the overproduction of ROS, which is one of the main culprits of cell apoptosis and inflammation (Kim et al. 2011). Given that fish fed *S. horneri* and subjected to ammonia stress had elevated serum levels of LZM, C3, C4, and IgM compared to control, it seems to suggest that *S. horneri* has a protective effect on stress response. This finding is in agreement with a previous report on shrimp that received fucoidan and were found to have a higher capability to recover early to ambient stress and to maintain homeostasis in their immune parameters (Suwaree et al. 2015).

Unlike mammals, fish are susceptive to ambient water conditions, especially in intensive culture systems with high stocking density, where there could be severe oxidative stress due to the generation of ROS. The antioxidant system of organisms is divided into two major parts, i.e., enzymatic and non-enzymatic (Akbary and Aminikhoei 2018). SOD and CAT are the main antioxidant enzymes that protect organisms or tissues from ROS (Seifried et al. 2007). As an important antioxidant enzyme, SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), and therefore plays a vital role in ameliorating stress-mediated immunosuppressive responses (Fridovich 1995). On the other hand, CAT serves as a biomarker of the antioxidant defense status and converts H_2O_2 to O_2, thereby protecting the organism against superoxide radical toxicity (Pandey et al. 2003). Similarly, T-AOC can prevent the deleterious effects of ROS and directly reflect total intracellular antioxidant capacity of fish (Wu et al. 2014), while MDA is the final product of lipid peroxidation, and therefore indirectly reflects the severity of free radical attack (Doyotte et al. 1997). Previous studies have indicated that *S. horneri* extracts contain a variety of antioxidant compounds, with stronger in vitro and in vivo antioxidant activity. For instance, Shao et al. (2014) showed that polysaccharides from *S. horneri* had strong scavenging activity towards superoxide radicals, hydroxyl radical, ABTS radicals, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Similarly, Wen et al. (2014) demonstrated that high sulfated polysaccharides from *S. horneri* protected RAW264.7 cells from oxidative stress, suggesting

Table 5 Effect of *S. horneri* on the liver antioxidant capacity of black sea bream before and after ammonia stress

Treatments	Control	3% SH	6% SH	9% SH
Before stress				
SOD (U mg^{-1} prot)	166.86 ± 12.88Ba	205.43 ± 11.32Bb	216.15 ± 20.67Bb	199.74 ± 17.90Bb
CAT (U mg^{-1} prot)	3.71 ± 0.22B	3.70 ± 0.09B	3.68 ± 0.43B	3.98 ± 0.06B
T-AOC (U mg^{-1} prot)	9.95 ± 0.84Aa	13.16 ± 0.35Bb	16.03 ± 0.76 Bd	14.86 ± 0.32Bc
MDA (nmol mg^{-1} prot)	3.21 ± 0.14Ab	2.36 ± 0.08Aa	2.36 ± 0.19Aa	2.34 ± 0.09Aa
After stress				
SOD (U mg^{-1} prot)	143.23 ± 3.36Aa	166.77 ± 3.72Ab	190.34 ± 3.49Ac	169.14 ± 2.34Ab
CAT (U mg^{-1} prot)	1.55 ± 0.09Aa	1.95 ± 0.11Ab	2.27 ± 0.11Ac	1.84 ± 0.10Ab
T-AOC (U mg^{-1} prot)	9.30 ± 1.70Aa	9.77 ± 0.45Aa	13.28 ± 1.02Ab	12.43 ± 0.93Ab
MDA (nmol mg^{-1} prot)	6.86 ± 0.22B	6.54 ± 0.14B	7.18 ± 0.32B	6.89 ± 0.56B

Data represent mean ± SD for three replicates

Data in the same column with different letters (A, B) for before and after ammonia stress show significant difference ($P < 0.05$)

Data in the same row with different letters (a, b, c) show significant difference ($P < 0.05$)

that fucose might play a vital role in the antioxidant function of the polysaccharides. Studies by Terasawa et al. (2011) revealed that *S. horneri* was an excellent source of natural antioxidants and could serve as food for people on account of its potential to mitigate the onset of metabolic diseases due to ROS. In a related study, Yang et al. (2014) reported that polysaccharides from *S. horneri* could elevate the activities of serum SOD and CAT of yellow catfish, thereby significantly decreasing levels of MDA, hence, improving antioxidant status. Consistent with these previous reports, we observed in the present study that dietary *S. horneri* supplementation significantly elevated the activities of liver antioxidant enzymes (SOD, T-AOC), while decreasing MDA content. These results revealed that *S. horneri* improved the liver anti-oxidative status of black sea bream. Following 24 h post-ammonia stress, the activities of the antioxidant enzymes decreased, while the MDA level increased. This could probably be due to excess generation of ROS that exceeded the scavenging capacities of SOD and CAT, with the excess ROS in turn inactivating the activities of SOD and CAT (Escobar et al. 1996; Bagnyukova et al. 2006). In addition, the excess ROS might have caused lipid peroxidative damage (evidence by an elevation in MDA content), therefore resulting in a change in the antioxidant status (represented by a reduction in T-AOC level) (Geret et al. 2002). However, when fish were fed *S. horneri*, the activities of SOD, CAT, and T-AOC were significantly increased. From this observation, we therefore conclude that *S. horneri* has a protective effect on ammonia-induced oxidative stress, which is likely to be exerted indirectly through the enhancement of the activities of SOD and CAT. As noted above, this might be due to higher contents of antioxidant compounds particularly polysaccharides, polyphenols (Luo et al. 2010; Shipeng et al. 2015), and fucoxanthin present in *S. horneri* (Zhang et al. 2007). All these compounds have efficient superoxide anions and hydroxyl free-radicals scavenging properties. Unlike in the pre-ammonia stress exposure, there were no significant changes in the MDA level with dietary *S. horneri* supplementation after the ammonia stress. One possible reason could be that the excess ROS generated might have caused lipid peroxidative damage to fish exposed to the ammonia environment, with the protective effects of *S. horneri* not sufficient to eliminate the damage caused by the ammonia. This observation is supported by the finding of Zhang et al. (2015a), who reported that the MDA level of blunt snout bream fed 0.4% fructooligosaccharides was significantly decreased before ammonia stress, and did not change after 24-h ammonia stress.

In conclusion, dietary *S. horneri* supplementation could enhance immunity and antioxidant capacity of juvenile black sea bream, with the optimum level of *S. horneri* being 6% under our present experimental conditions. Moreover, ammonia stress could decrease immunity and cause liver oxidative stress, while the impact of the ammonia was alleviated with dietary *S. horneri* supplementation. This study provides important information regarding the potential application of *S. horneri* as a feed additive for black sea bream aquaculture.

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