ORIGINAL ARTICLE

Effects of dietary *Allium mongolicum Regel* polysaccharide on growth, lipopolysaccharide-induced antioxidant responses and immune responses in *Channa argus*

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Abstract

The present study evaluated the effects of dietary *Allium mongolicum Regel* polysaccharide (AMRP) on growth, lipopolysaccharide-induced antioxidant responses and immune responses in *Channa argus*. A basal diet was supplemented with AMRP at 0, 1, 1.5 or 2 g/kg feed for 56 days. After the 56 days feeding period, weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) were significantly increased or decreased ($P < 0.05$) by dietary AMRP, with the highest WG, SGR and the minimum FCR occurring in 1.5 g/kg AMRP group. Furthermore, AMRP supplementation conferred significant protective effects against LPS challenge by preventing alterations in the levels of complements 3 (C3) and complements 4 (C4), lysozyme, superoxide dismutase (SOD), glutathione-S-transferase (GST), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) while regulating the expression of immune-related genes including heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), SOD, GST, IL-1 and TNF-α. Finally, AMRP supplementation significantly increased serum total protein, albumin and globulin concentrations and reduced mortality after LPS challenge. Taken together, our results suggest that the administration of AMRP could attenuate LPS-induced negative effects in *C. argus*, with 1.5 g/kg considered a suitable dose.

Keywords *Allium mongolicum Regel* · Growth · Immune response · Antioxidant response · *Channa argus*

Introduction

In recent decades, *Channa argus* consumption and aquaculture activity have increased [1]. Intensive aquaculture of *C. argus* have produced various sources of environmental stress and accelerated susceptibility of fish to pathogenic microorganisms. Therefore, the disease of fish in the water environment is frequent, causing huge economic losses for the farm. Antibiotics, vaccines, and immunostimulants have been used to prevent and/or control diseases to reduce mortality [2]. However, the use of antibiotics will not only affect the safety of fish products, but also cause environmental pollution and induce drug-resistant bacterial populations [3–5]. Vaccines are only effective against specific pathogens, but they are costly [6]. Immunostimulants could promote the immune defense function of fish, not only can improve the innate immune response, but also can enhance the adaptive immune response [7]. Therefore, it is necessary to develop green, economic and effective immunopotentiator to promote the healthy development of commercial aquaculture. Various immunostimulants, such as vitamin E [8], emodin [9], chitosan [10], astaxanthin [11, 12], medicinal plant polysaccharide [13], and plant flavonoids [14], have been used as feed additives. All promote growth or immune function and attenuate oxidative stress or inflammatory responses. Some plant extracts are attractive for the manufacturing of these immunostimulants because they have wide sources, are generally cost-effective and environmentally friendly [2].

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Bioactive ingredients in plants and their extracts are very complex. The most common ingredients are polysaccharides, flavonoids, glycosides and essential oils [13]. The plant *Allium mongolicum Regel* is a member of the *Liliaceae* family and the *Allium* genus [14]. It grows in high-altitude deserts, has a unique flavour, and is rich in protein, flavonoids, polysaccharide, and other components [15]. There are some reports have indicated that *A. mongolicum Regel* possesses several biological effects, including growth-promoting, antioxidant, and anti-inflammatory effects [14, 15]. Polysaccharide is an important active component in *A. mongolicum Regel*. Related research has found that plant polysaccharide can be used as immunostimulants to enhance the immunologic function in fish [13]. To the best of our knowledge, the effects of *A. mongolicum Regel* polysaccharide as an immunopotentiator in fish have not been reported. Therefore, the purpose of this study was to investigate the protective effect of dietary supplementation with *A. mongolicum Regel* polysaccharide (AMRP) on growth and LPS-induced immune and antioxidant responses in *C. argus*.

Materials and methods

Allium mongolicum Regel polysaccharide preparation and diet

Fresh *A. mongolicum Regel* were purchased from a commercial pasture (Tongliao, China). The *A. mongolicum Regel* were cleaned with distilled water, and dried at room temperature. The dried *A. mongolicum Regel* (4.0 kg) were ground into powders and pretreated with 95% alcohol under reflux for 2 h to remove lipids and small molecular materials, then dried at 65 °C to constant weight. Then, the extractive was extracted with 20 L distilled water three times (2 h for each) at 100 °C, the supernatants were collected. The combined aqueous parts were filtered and concentrated to one-fifth of initial volume with a vacuum rotary evaporator (RE-52C; YaoYu Equipment Company, Shanghai, China). The supernatant was mixed with four-fold volume dehydrated ethanol. After standing at 4 °C for 24 h, the mixture was centrifuged to precipitate the crude polysaccharide, and then collected, lyophilized and stored at 4 °C. Referring to the anthrone-sulfuric method [13], a glucose standard curve was used to determine *A. mongolicum Regel* polysaccharides content (83.4%).

The basal diet was the same as those used in our previous study (dry matter: crude protein 48.1%, crude lipid 11.3%, ash 12.3% carbohydrate 20.1%, gross energy 19.3 kJ/g and protein/energy ratio 26.0 mg/kJ) [16]. The AMRP (0, 1, 1.5 and 2 g/kg) was sprayed slowly into the basal diet, when selecting the concentration of AMRP, we combined the concentration of polysaccharides from various plant [2, 17]. The diet were evenly mixed, dried and pelleted at room temperature, and stored at −4 °C until used.

Fish and rearing management

Channa argus (30 ± 0.12 g) was purchased from a commercial farms (Huzhou, China). *C. argus* was acclimated in 300 L glass aquaria for 14 days. During acclimation, all fish was fed with basal diet. After acclimation, 400 *C. argus* were randomly divided into four groups (5 tanks per group, 20 fish per tank). The glass aquaria was kept under experimental conditions (temperature: 26 ± 2 °C; pH 7.1 ± 0.1; ammonia: less than 0.5 mg/L; nitrites: less than 0.05 mg/L and dissolved oxygen: 6.21 ± 0.41 mg/L) and a regular light cycle (12 h light/12 h dark photoperiod). The fish were fed two times (08:00 and 16:00) a day for 56 days at a rate of 3–4% of the wet body weight. All the experimental animals used in the study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Jilin Agricultural College.

LPS challenge experiment and sample collection

After the 56 days feeding trial, six fish from each tank (5 tanks per group) was weighed and used for the LPS challenge. Fish from each group (30 fish for each group) were injected intraperitoneally with LPS (4 mg/kg of fish). Before challenge, LPS (*Escherichia coli 055:B5*, Sigma–Aldrich, USA) were diluted into different concentrations (1, 2, 4, 8, 16, 32 and 64 mg/mL) with a 0.9% NaCl solution for Lethal dose 50 (LD 50) determination for 14 days, 4 mg LPS/kg fish was used for a challenge test. Pre-treatment/challenge groups: Ctrl/PBS, Ctrl/LPS, and AMRP/LPS. Daily mortality was recorded from days 1 to 14. Fish continued to receive their assigned diets after the injection. Fish was fasted before sample collection for 24 h. After the challenge trial, five fish were randomly collected from each treatment groups and anaesthetized using 300 mg/L of buffered MS-222 (Sigma–Aldrich, USA) for blood sampling. Later, liver and spleen of *C. argus* were sampled. Blood samples were centrifuged at 3000 rpm/min for 10 min to obtain serum. All tissues were flash-frozen in liquid nitrogen. All samples were stored at −80 °C for further analysis.

Immunological parameters

Serum complements 3 and complements 4 and lysozyme activity

Serum lysozyme activity (LZM) and complement 3 (C3) and complement 4 (C4) concentrations were determined using a commercial Kit (Nanjing Jiancheng Bioengineering

Institute, China). Lysozyme activity was determined using the turbidimetric method described as Gou et al. [2]. The concentrations of C3 and C4 were determined using the turbidimetric method based on the increase in turbidity after the response of C3 and C4 [18]. The results for LZM, C3 and C4 are expressed as units/mL, mg/L, and mg/L, respectively.

Serum total protein, globulin and albumin levels

Serum total protein, globulin and albumin were assayed enzymatically with an automatic biochemical analyser (Beckman Coulter AU680, California, USA) [2]. The standard product of total protein and albumin reagent was added to the colorimetric cup, and then the absorbance at 540 nm was measured to establish the standard curve. The absorbance of serum was measured and the concentration of total protein and albumin was calculated.

Liver and spleen TNF-α and IL-1β

The protein concentrations in the liver and spleen were determined by spectrophotometry according to the methods of Jiang et al. [19]. IL-1β and TNF-α content were determined using a commercially available ELISA kit (Nanjing Jiancheng Bioengineering Institute, China) [10]. The minimum detectability of IL-1β and TNF-α was 1.0 ng/g protein. The results for IL-1β and TNF-α are expressed as ng/g protein.

Antioxidant parameters

Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity in liver and spleen were determined using an assay kit according to Jiang et al.

[19]. MDA content was using the thiobarbituric acid reaction [19]. SOD and GST activity was monitored at 450 nm and 340 nm, respectively [19]. The results for MDA content is expressed as nmol/mg protein. The results for SOD and GST activity are expressed as units/mg protein.

Real-time PCR analysis

Total RNA were extracted from liver and spleen using commercial kits (Takara, Dalian, China). The RNA purity were analysed by using spectrophotometry. Then, cDNA was synthesized with a reverse transcriptase cDNA synthesis kit (Takara, Dalian, China). SOD, GST, TNF-α, IL-1β, HSP70 and HSP90 gene expression levels were analysed by real-time PCR with SYBR with the Premix Ex Taq™ II kit (Takara, Dalian, China). The primer sequence is shown in Table 1. The real-time PCR reactants are as follows: SYBR qPCR Mix (10 μL), forward and reverse primer (10 mM, 1 μL), cDNA (1 μL), and DEPC-treated water (7 μL). The reaction conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, annealing for 30 s and 72 °C for 30 s. The levels of gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized using β-actin expression [2].

Statistical analysis

Date are presented as the mean ± SD. The results are subjected to one-way analysis of variance (ANOVA) to determine the significant differences. Tukey's multiple range test was used to compare the mean values to indicate significant differences ($P < 0.05$). Analysis was conducted using SPSS statistics 19.0 software (IBM, USA).

Table 1 Primer sequences and annealing temperatures for polymerase chain reactions

Target gene	Sequence (5′–3′)	Annealing temp. (°C)	GenBank ID
SOD-F	GCAGGACCCCACTACAAT	59	HF585624
SOD-R	CTGAGCGATGCCTATGAC		
HSP90-F	TGTATGTCAGGAGGGTGTTT	55	KU946993.1
HSP90-R	TAGATTGATTTCTGGTTTTC		
HSP70-F	ATTTTGAATGTGTCTGCGGT	56	KU883613.1
HSP70-R	ACTTGCTGATGATGGGGTTA		
IL-1-F	GTTTACCTGAACATGTCGGCTTACG	59	JN085956.1
IL-1-R	AGGGTGCTGATGTTCAGCCCA		
TNF-α-F	ACAATACCACCCCAGGTCCCA	61	KC843460.1
TNF-α-R	ACGCAGCATCCTCTCATCCAT		
GST-F	GAGGGAGAGCGACACGAC	62	JN104637
GST-R	TCACATAACCCAAAACGG		
β-actin-F	CACTGTGCCCATCTACGAG	57	EF452499
β-actin-R	CCATCTCCTGCTCGAAGTC		

Results

Growth

The weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) of *C. argus* fed AMRP are presented in Table 2. Dietary AMRP significantly increased ($P < 0.05$) WG and SGR of *C. argus*, with the highest WG and SGR occurring in fish fed diet with 1.5 g/kg AMRP after the 56 days feeding period. Dietary AMRP remarkably decreased ($P < 0.05$) FCR of *C. argus*, with the lowest FCR occurring in fish fed diet with 1.5 g/kg AMRP.

Immunological parameters

The serum LZM and C3 and C4 concentrations fed AMRP are showed in Fig. 1. Compared with the Ctrl/PBS group, the Ctrl/LPS group showed a reduction in serum LZM and C3 and C4 levels ($P < 0.05$). However, AMRP pre-treatment (AMRP/LPS group) significantly increased the levels of serum LZM, C3 and C4 compared with those in the Ctrl/LPS group ($P < 0.05$).

Serum total protein, globulin and albumin concentrations in *C. argus* fed AMRP are presented in Fig. 2. LPS challenge significantly increased serum total protein, globulin and albumin concentrations than Ctrl/PBS groups ($P < 0.05$), while AMRP pre-treatment increased serum total protein, globulin and albumin concentrations compared with those in the Ctrl/LPS group ($P < 0.05$).

The TNF-α and IL-1β concentrations in the liver and spleen of *C. argus* fed AMRP are presented in Fig. 3. LPS challenge resulted in a significant increase in TNF-α and IL-1β concentrations in the liver and spleen ($P < 0.05$). However, when fish were supplemented with AMRP, the increases in TNF-α and IL-1β concentrations were attenuated ($P < 0.05$).

Antioxidant parameters

The levels of MDA, SOD and GST in the liver and spleen fed AMRP are showed in Fig. 4. LPS challenge resulted in a reduction in SOD and GST activity in the liver and spleen ($P < 0.05$). In contrast, LPS challenge resulted in a significant increase in MDA concentrations in the liver and spleen ($P < 0.05$). However, AMRP pre-treatment elevated SOD and GST activity and decreased MDA concentrations ($P < 0.05$) compared with that in the Ctrl/LPS group.

Real-time PCR analysis

The HSP70, HSP90, SOD, GST TNF-α and IL-1β gene expression in the liver and spleen are displayed in Fig. 5. The gene expression levels and the concentrations of SOD, GST, TNF-α and IL-1β in the liver and spleen showed similar trends. LPS challenge resulted in a reduction in SOD and GST and a rise in TNF-α and IL-1β gene expression levels in the liver and spleen ($P < 0.05$). However, AMRP pre-treatment attenuated these changes ($P < 0.05$). LPS challenge significantly increased HSP70 and HSP90 gene expression ($P < 0.05$), and AMRP pre-treatment increased HSP70 and HSP90 gene expression compared with that in the Ctrl/LPS group ($P < 0.05$).

Survival rate

The survival rate is shown in Fig. 6. LPS challenge in the Ctrl/LPS group significantly decreased the survival rate of fish compared with that in the Ctrl/PBS group ($P < 0.05$). However, AMRP pre-treatment significantly reduced mortality compared with that in the Ctrl/LPS group ($P < 0.05$). No significant difference in the survival rate was observed among fish in the 1.5 and 2 g/kg AMRP/LPS groups and the Ctrl/PBS group ($P > 0.05$).

Table 2 Growth performance of *Channa argus* fed with AMRP

AMRP (g kg diet^{-1})	IW (g)	FW (g)	WG[a] (%)	SGR[b] (% day^{-1})	FCR[c]
Control (0.0)	30.07 ± 0.09a	116.73 ± 2.52a	288.69 ± 8.14a	2.42 ± 0.04a	1.30 ± 0.04c
1.0	30.04 ± 0.05a	126.53 ± 2.29b	321.20 ± 7.94b	2.57 ± 0.03b	1.22 ± 0.06b
1.5	30.06 ± 0.05a	136.36 ± 2.87c	353.58 ± 9.52c	2.70 ± 0.04c	1.13 ± 0.03a
2.0	30.03 ± 0.07a	127.22 ± 1.80b	323.70 ± 6.80b	2.58 ± 0.03b	1.21 ± 0.03b

Data are expressed as the mean ± S.D (n = 5). Values with different superscripts are significantly ($P < 0.05$) as determined by the Tukey's test

IW initial body weight, *FW* final body weight

[a]Weight gain (WG) = (FW − IW) × 100/IW

[b]Specific growth rate (SGR) = (Ln FW – Ln IW) × 100/T, T is the culture period in days

[c]Feed conversion ratio (FCR) = total diet fed (kg)/total wet weight gain (kg)
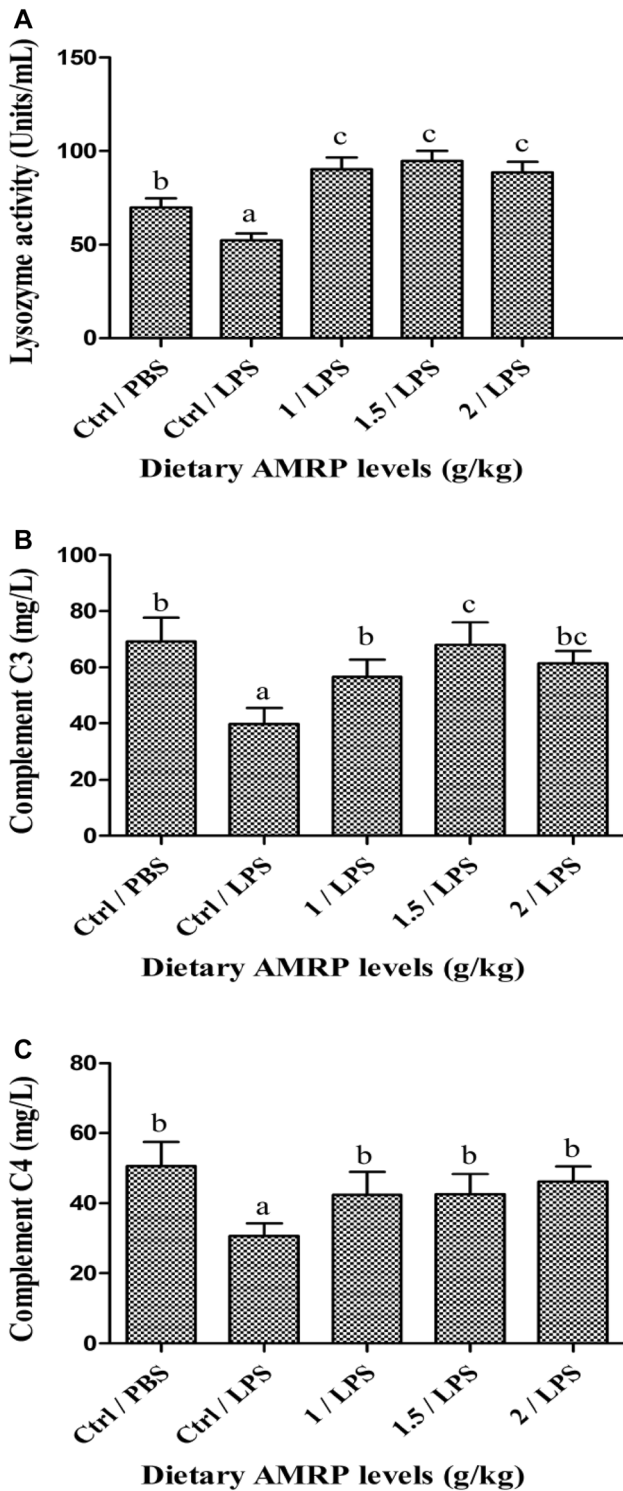
Fig. 2 Effects of AMRP on serum total protein, globulin and albumin levels in *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference (P < 0.05) as determined by the Tukey's test
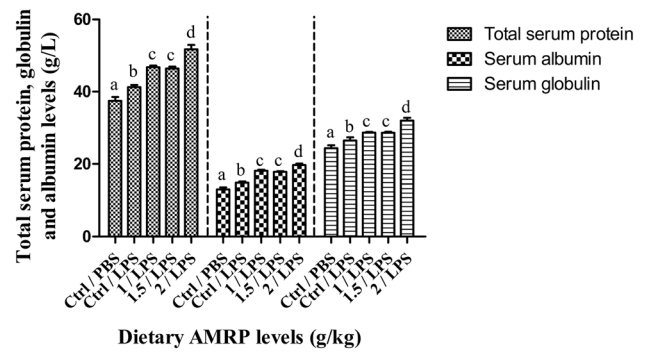

Fig. 3 Effects of AMRP on IL-1β (**a**) and TNF-α (**b**) concentrations in the liver and spleen of *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference (P < 0.05) as determined by the Tukey's test
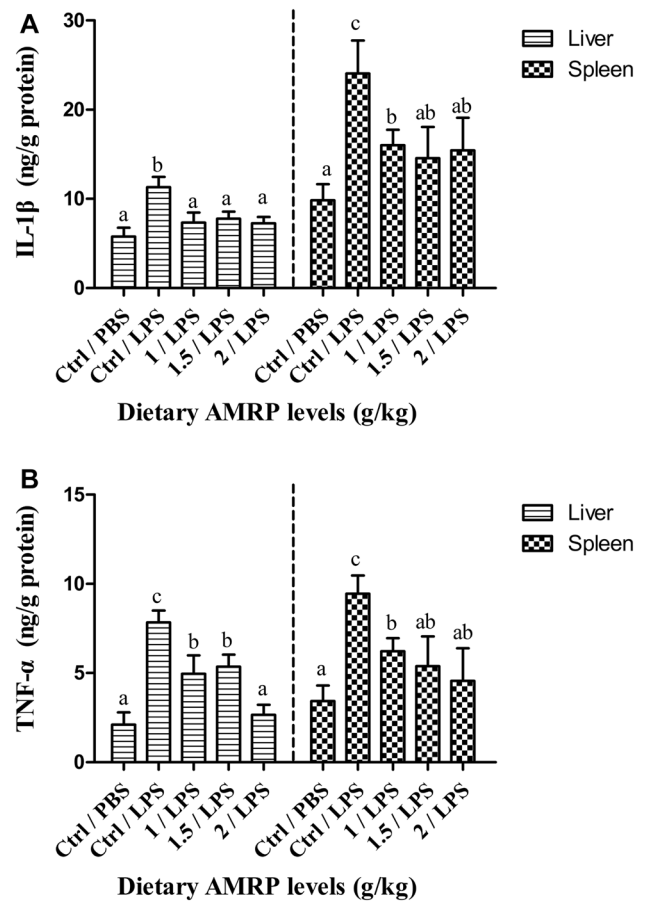

Fig. 1 Effects of AMRP on serum lysozyme (**a**), C3 (**b**) and C4 (**c**) levels in *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference (P < 0.05) as determined by the Tukey's test
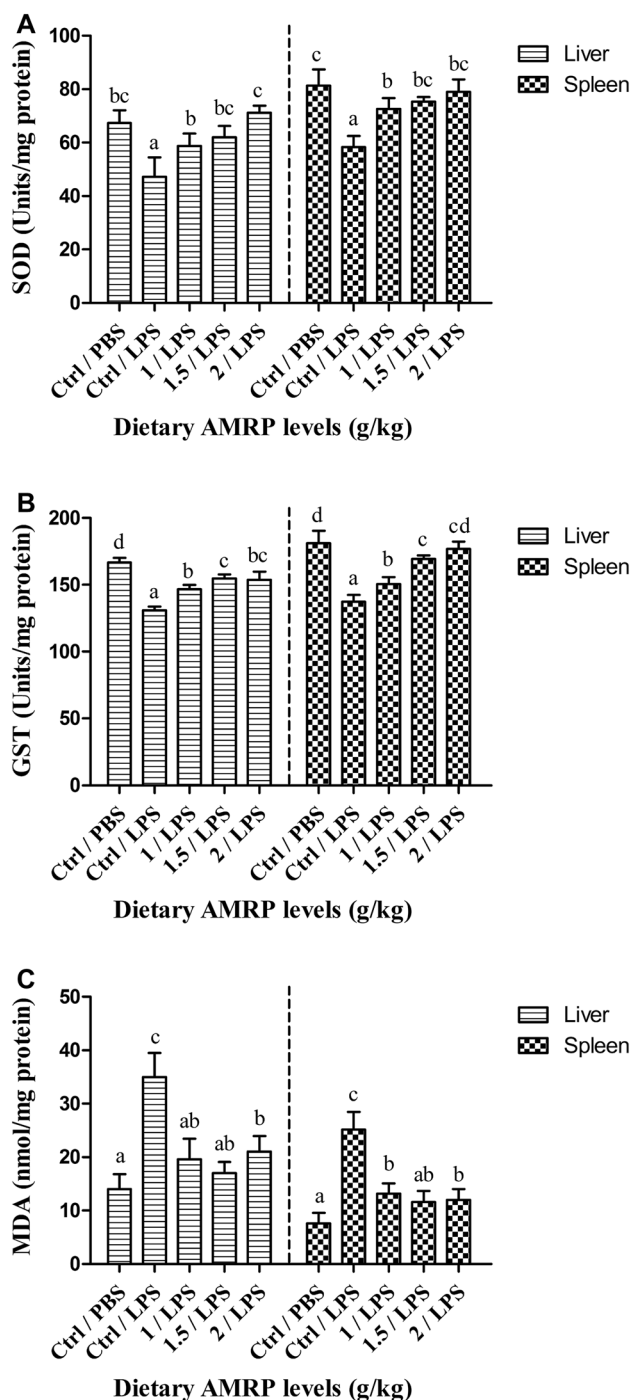
Fig. 4 Effects of AMRP on SOD (a), GST (b) and MDA (c) levels in the liver and spleen of *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference ($P < 0.05$) as determined by the Tukey's test

Discussion

Many plants and their extracts have attracted great attention as safe immunostimulants for animals [13, 20, 21]. Polysaccharide, the main active components of plants, are

considered as the best candidate for the development of immunomodulators in aquaculture. The effects of plant polysaccharide on growth performance, antioxidant responses and immune responses are somewhat variable, but supplemental *Astragalus* polysaccharide have been found to improve the growth performance and immune response in *Oreochromis niloticus* [17]. Wang et al. [22] and Giri et al. [23] suggested that dietary supplementation with Chinese herbal polysaccharide can enhance disease resistance in fish. Yang et al. [24] and Gou et al. [2] found that dietary supplementation with *Ficus carica* polysaccharide and *Hericium* polysaccharide provoked immune responses in *Ctenopharyngodon idella* against *Flavobacterium columnare* and *Aeromonas hydrophila*. In this study, fish fed with AMRP showed notably promoted WG and SGR. In line with our results, dietary supplementation with *Astragalus* polysaccharide can promote growth as determined by different parameters in fish (WG, SGR and feed conversion ratio), indicating that *Astragalus* polysaccharide may contribute to the efficient utilization of feed [17]. A previous study showed that meat sheep fed *A. mongolicum Regel* flavonoids had significantly increased growth performance and serum growth hormone levels, suggesting that increased growth performance may be related to physiological mechanisms [14].

LPS, a bacterial endotoxin, is able to trigger the immune response in animals [19]. Lysozyme plays an important role in dissolving bacteria and activating complements in fish [2, 25]. In this study, AMRP pre-treatment significantly enhanced the serum lysozyme levels of fish after LPS challenge. Many authors have reported the enhancement of lysozyme activity in response to the administration of *Hericium caput-medusae* polysaccharide [2] and *Coriolus versicolor* polysaccharide [26] in grass carp and crucian carp, respectively. As far as we know, the protective effect of plant polysaccharides on LPS-induced immune response has not been studied. The higher lysozyme activity in the AMRP treatments suggested that lysozyme levels were enhanced to protect the fish from bacteria infection.

Complements are crucial components of humoural immune responses and play an essential role in promoting inflammatory responses and clearing pathogens [18, 27, 28]. A previous study showed that oral administration of *Coriolus versicolor* polysaccharide significantly enhanced serum C3 and C4 levels in crucian carp [26]. The present results showed that AMRP pre-treatment in fish significantly enhanced C3 and C4 levels compared with those of fish fed the basal diet after LPS challenge. A previous study demonstrated that dietary administration of *Hericium caput-medusae* polysaccharide [2], *Ficus carica* polysaccharide [24] and soybean isoflavones [18] promoted complement levels during the late phase of feeding trials. Short-term improvements in complement levels are usually beneficial to fish. Thus, the
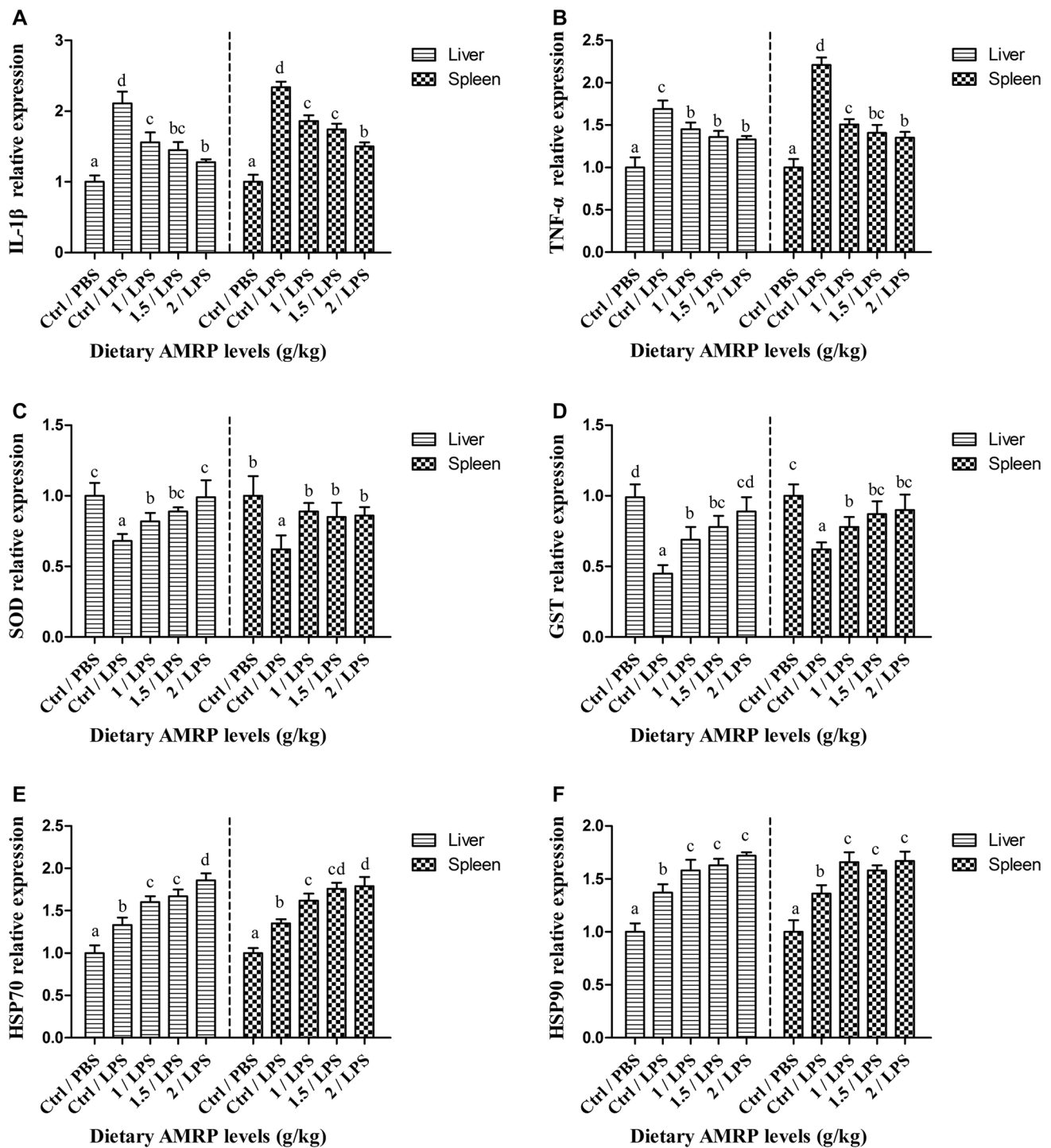
Fig. 5 Effects of AMRP on IL-1β (**a**), TNF-α (**b**), SOD (**c**), GST (**d**), HSP70 (**e**) and HSP90 (**f**) gene expression in *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference ($P < 0.05$) as determined by the Tukey's test

enhance of serum complement levels in AMRP groups may help fish cope with LPS challenge.

Serum proteins, including albumin and globulin, are important compounds in the serum. LPS is considered as general immunostimulatory components to triggers host

defense responses by promoting inflammatory cytokines, antibodies and the function of lymphocytes and macrophages. Increases in serum protein concentrations are thought to be related to an intense innate immune response in fish [13]. Previous study showed that serum total protein,
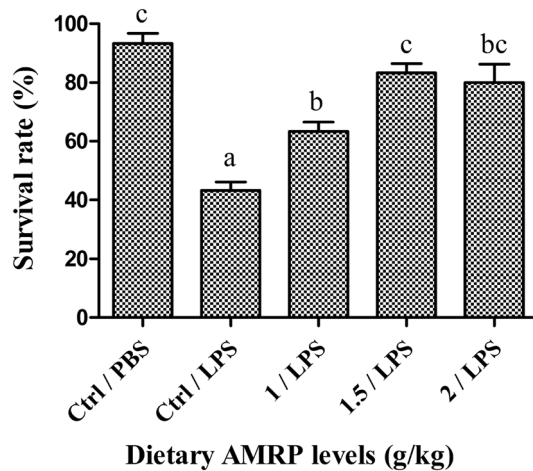
Fig. 6 Effects of AMRP on the survival rate in *Channa argus*. Data are expressed as the mean ± SD (n = 5). Values with different superscripts are significantly difference (*P* < 0.05) as determined by the Tukey's test

albumin and globulin levels were significantly enhanced after dietary administration of various herbal extracts [2, 13, 24]. In this study, a significant enhancement of total serum protein, albumin and globulin levels occurred in the AMRP pre-treatment groups after LPS challenge. AMRP pre-treatment groups may be beneficial for the synthesis of more proteins for repairing immune damage caused by LPS.

MDA, a product of lipid peroxidation, is a reliable index of oxidative stress and cell damage [19]. A previous study showed that LPS induced profound elevations in ROS and MDA concentrations in Jian carp [19]. Administration of plant extracts has been reported to reduce hepatic MDA concentrations in fish [18]. AMRP pre-treatment was found to attenuate LPS-induced increases in MDA concentrations in the liver and spleen after LPS challenge in the present study. SOD and GST, endogenous antioxidative molecules, play critical roles in removing damaging ROS and preventing oxidative stress [29]. LPS-induced liver and spleen oxidative stress associated with chaos of the antioxidant system. The findings of the present study demonstrated that AMRP remarkably enhanced the SOD and GST activity in the liver and spleen after LPS challenge, which was a pattern opposite to that of the MDA content. Increased antioxidant levels are beneficial for inhibiting ROS and decreasing MDA.

Inflammatory factors are a series of proteins secreted by endothelial cells, lymphocytes, monocytes and fibroblasts that play an important role in regulating inflammatory processes [19, 30, 31]. It was well known that IL-1β and TNF-α were two effective pro-inflammatory cytokines that can accelerate the process of inflammation [32, 33]. The liver and spleen are considered as the main immune tissues of fish that responds to pathogen invasion [34]. LPS could induce various host immune cells

secretion of pro-inflammatory cytokines [19, 35]. In this study, LPS challenge prominently increased the concentrations and mRNA of TNF-α and IL-1β in *C. argus*. To our knowledge, LPS challenge has been reported to exert a pro-inflammatory response in fish [19]. To date, no information is available about the positive roles of AMRP against the LPS-induced inflammatory reaction. AMRP pre-treatment was found to attenuate LPS-induced increase of IL-1β and TNF-α levels after LPS challenge. A previous study showed that LPS-induced increases in IL-1β and TNF-α mRNA in fish may be related to activated NF-κB and MAPK signalling pathways [19].

Heat shock proteins (HSPs), which act as chaperone proteins, are synthesized in large quantities in all cells when they are exposed to various stresses [18, 36–38]. HSP70 and HSP90 play an important in the formation of glucocorticoid receptors (GR) protein complexes. In addition, studies have shown that the molecular mechanism of NF-κB signaling pathway inhibition is closely related to the interaction of GR and NF-κB. Dong et al. reported that thermal stress and osmotic stresses could increase the levels of HSP70 in sea cucumber [39]. Zhou et al. reported higher mRNA levels of hepatic HSP70 and HSP90 after pH stress in golden pompano [40]. Furthermore, Liu et al. reported that Chinese herbs can increase the levels of HSP70 in *Megalobrama amblycephala* [41]. AMRP pre-treatment was found to upregulate HSP70 and HSP90 gene expression after LPS challenge in the present study. HSP70 and HSP90 can enhance cell tolerance in stress environment. The higher HSP70 and HSP90 levels may be beneficial for raising the anti-inflammatory response of cells and tissues.

Relative survival rate has often been used as a final visual index of fish health status after bacterial challenge tests [18]. A previous study showed that dietary supplementation with many plant polysaccharide reduced mortality in fish after challenge with *Aeromonas hydrophila* [2, 13]. In the present study, the group receiving AMRP pre-treatment had a higher survival rate than did the control group after LPS challenge. This finding suggested that AMRP enhanced the survival rate by regulating antioxidant responses and immune responses to protect *C. argus* against LPS damage.

Conclusion

The present study is, to our knowledge, the first to report that dietary supplementation with AMRP (1–2 g/kg) improved immune and antioxidant responses and regulated gene expression, thus enhancing the growth of *C. argus*. These results suggested that AMRP have the potential to act as an immunopotentiator to promote the immune status and production of fish, with 1.5 g/kg considered a suitable dose.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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