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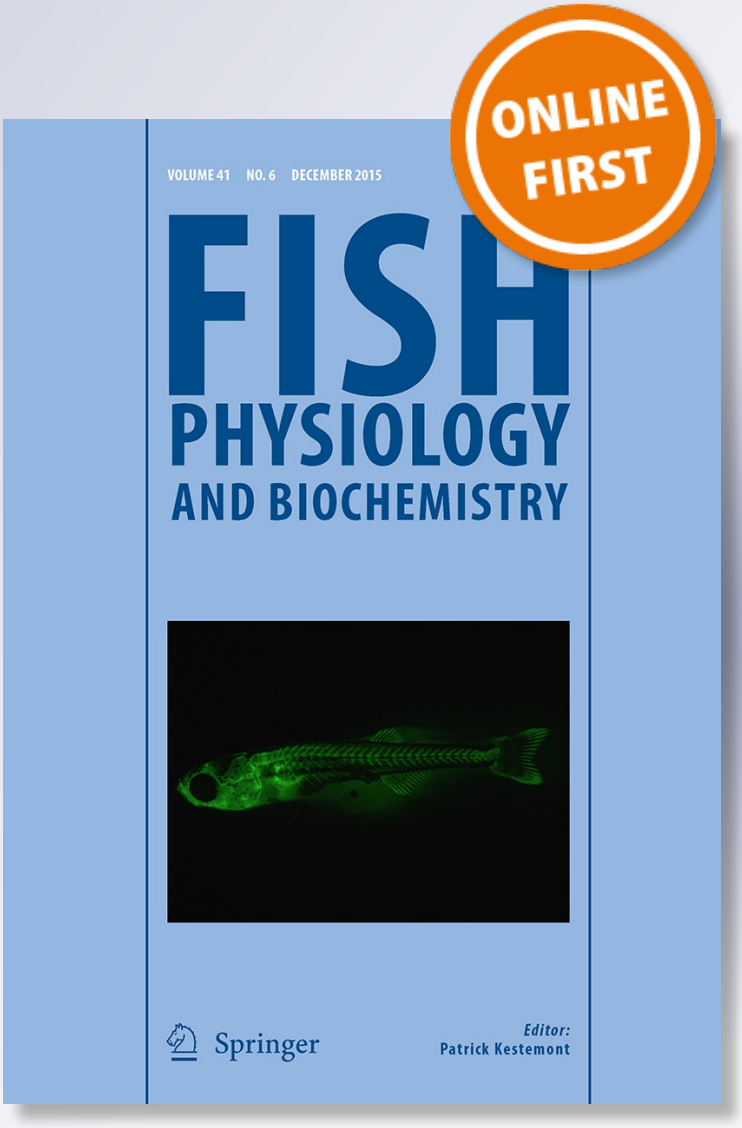
Fish Physiology and Biochemistry

ISSN 0920-1742

Fish Physiol Biochem
DOI 10.1007/s10695-015-0188-9



Springer

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Astragalus membranaceus (AM) enhances growth performance and antioxidant stress profiles in bluegill sunfish (*Lepomis macrochirus*)

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Received: 7 August 2015 / Accepted: 22 December 2015

Abstract This study was designed to assess the potential effects of *Astragalus membranaceus* (AM) on the growth performance and antioxidative stress response in bluegill sunfish (*Lepomis macrochirus*) exposed to challenging cold water temperature conditions. In this regard, fish with an average weight of 43 ± 1 g were divided into four groups and fed daily with an AM-free diet (control), and 1.5, 3, and 4.5 % (w/w) AM-incorporated diets for an 8-week period. Oxidative stress response, biochemical, and growth parameters were measured, and subgroups of fish were exposed to the outside challenging cold pond water temperature (1.6–9.9 °C) with an average of 7.0 ± 0.1 °C beyond the optimal temperature. The results showed that incorporating AM in the diet significantly improved growth performance parameters (body mass gain, specific growth rate, length, condition factor, and feed conversion ratio) and biochemicals (aspartate aminotransferase and alanine transaminase activities, and glucose and cortisol concentrations). In addition, markedly up-regulated superoxide dismutase, glutathione peroxidase, and catalase activities were observed in AM-treated fish groups over the control. Conclusively, feeding AM diets significantly increased ($P < 0.05$) growth performance and antioxidative stress profiles throughout the entire experiment, and this increase was much more pronounced at 8 weeks after the water temperature began to rise, which can be related to the nature of Bluegill fish as it is known to be a warm water fish. These findings are considered to be of great importance for sustainable aquaculture.

Keywords Growth performance · Antioxidative stress biomarkers · Biochemical biomarkers · Temperature · Bluegill

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Introduction

In aquaculture and natural environment, it is commonly known that fish stressed with chemical, biological, and physical disturbances are more susceptible to infection, and their immune system can also be greatly influenced, which may result in

Published online: 04 January 2016

mortality and substantial economic losses. Fish health status depends on the interrelationship of the fish and the environment in which they live. The environment may be the most critical component of the fish health matrix as it influences the fish's physiological well-being, feeding regimes, growth rate, and ability to maintain immune response (Magnadottir 2010; Plumb et al. 2011). The overall effect of stress can be considered to be a deviation in biological condition beyond the norm that challenges homeostasis and thus presents a threat to fish health (Villa-Cruz et al. 2009). Deviation in the ambient temperature is considered as a stressor that can affect all kinds of biological processes (Bly and Clem 1992; Bowden 2008). Stress induced by changes in temperature has been associated with enhanced reactive oxygen species (ROS) generation, which may seriously affect immune function and lead to oxidative stress because fish are unable to detoxify the ROS or repair injury (Halliwell 1994; An et al. 2010).

Most phytochemicals are considered as natural antioxidants and redox-active molecules similar to that of superoxide dismutase (SOD), which is a metal-ion chelator and xanthine oxidase inhibitor. Their activity can be attributed to many types of active components that they contain (Citarasu 2010; Chakraborty and Hancz 2011). *Astragalus membranaceus*, which belongs to *Leguminosae* family, is widely distributed throughout the temperate regions of the world. It locates principally in Northern China, and its dried root is a traditional Chinese medicinal herb (Li et al. 2010) that contains over 126 different components (Wu and Chen 2004) such as Astragalus polysaccharides (APS). APS have been shown to possess immunostimulating and hepatoprotective effects (Yan et al. 2009). It also contains organic acids, alkaloids, glucosides, and volatile oil as major components that have been found to enhance fish immune function (Galina et al. 2009). In common carp (*Cyprinus carpio*) and large yellow croaker (*Pseudosciena crocea*), immune response parameters were significantly increased after feeding with a ration containing a mixture of *A. membranaceus* and *Angelica sinensis* (Jian and Wu 2003, 2004). Similar results have been reported in several experiments on various fish species, e.g., rainbow trout (*Oncorhynchus mykiss*), Indian major carp (*Catla catla*), and Mozambican tilapia (*Oreochromis mossambicus*) (Dey and Chandra 1995; Logambal and Michael 2000; Dügenci et al. 2003).

Bluegill (*Lepomis macrochirus*) is an important freshwater sport fish as well as food fish species in North America. This species has been introduced presently in most parts of North America and also have been transported into Europe, South Africa, Asia, South America, and Oceania (Schultz 2004).

The purpose of the present study was to examine the effects of dietary administration of *A. membranaceus* on growth, body indices, and some biomarkers for liver functions and oxidative stress on bluegill (*L. macrochirus*) exposed to both simulating cold winter conditions and natural water temperature conditions.

Materials and methods

Fish

Lepomis macrochirus with average weight of 43 ± 1.0 g were procured from Jones Fish Hatcheries, Ohio, USA, were transferred to the wet laboratory of the Ohio State University South Centers, Ohio, USA, and kept in a 2200-L fiberglass aerated tank filled with disinfected dechlorinated well water. The health status was examined for evidence of diseases according to the methods of (Austin and Austin 1989), and fish were acclimated for 10 days feeding basal diet. Water quality parameters were monitored daily throughout the experiment. Temperature was maintained at 15 ± 0.4 °C and dissolved oxygen concentration at 7.0 mg/L.

All the experimental procedures involving animals were performed according to the protocol approved by the Ohio State University Institutional Animal Care and Use Committee. The study was carried out from the period of January to April 2014.

Astragalus membranaceus

Astragalus membranaceus was a commercial herbal product provided by Oregon's wild harvest (Sandy, Oregon, USA). It consists of astragalosides I through VII (astragaloside IV is around 1083.14 mcg/g dry weight of the root), isoastragalosides I–III, Astragalus polysaccharides (APS-I and APS-II), astragalin (0.073 mg/g), formononetin, dimethylhomoptercarpin, glycosides (methylinissolin and licoagroside

D), flavonoids (kaempferol, quercetin, isorhamnetin, and rhamnocitrin), vesticarpan, pendulone, cyclocephaloside II, astrapterocarpan, gluconic acid, choline and betaine (trace amounts), and nicotinic acid.

Experimental diets

Four experimental diets were prepared. Briefly, fine commercial powdered basal diet *Aquamax® Fingerling starter 300* (*PMI Nutritional International, LLC., Brentwood, MO, USA; Lot # 5D03*), crude protein minimum 50.0 %, crude fat minimum 16.0 %, crude fiber maximum 3.0 %, calcium minimum 2.0 %, phosphorous (P) minimum 1.3 %, sodium (Na) minimum 0.1 %, and ash maximum 12.0 % were divided into four portions; first three portions were incorporated with 15, 30, and 45 g *A. membranaceus*/kg feed to obtain the desired concentrations of 1.5, 3, and 4.5 %/kg, and the last portion was kept free of any additives (control). Each component was then mixed for 15 min. After that, each blend was adjusted to a desired pre-extrusion moisture content of ~45 % by adding adequate amounts of water and then mixed again for 15 min. After extrusion, the pelletized feed blends were dried in a laboratory oven (Thelco Precision, Jovan, Winchester, Virginia, USA) at 50 °C for 24 h. After drying, the diets were broken up and sieved into proper pellet size (1.5 × 3.0 and 2.5 × 5.0 mm), then packed in clean dry plastic containers, and stored at −15 °C until use.

Experimental design

The experiment was designed to include three phases (I, II, and III). Total experimental period was 8 weeks after the initial acclimation.

Phase I

Fish were assigned to four experimental groups, and each group was randomly distributed into flow-through rectangle fiberglass tanks (240 × 60 × 30 cm) with three-chamber replicates (30 fish per replicate) and fed with 0 (controls), 1.5, 3, or 4.5 % (w/w) *A. membranaceus*-added diet (AM diet). Fish were fed to satiation at 9:00 a.m. and 4:00 p.m. daily for 4 weeks. Water quality was monitored throughout the experiment and adjusted at 15 ± 0.4 °C and dissolved oxygen (DO) at 7.0 mg/L. Temperature and DO were measured in the morning and afternoon daily and recorded for each tank. All chambers were siphoned daily to remove excess feed and fecal matter to maintain good water quality. Water flow was adjusted across all tanks, and number of dead fish was recorded daily in all groups.

Phase II

After 4 weeks, fish were moved to 12 347-L floating cages that were held in a large outside 10,000-L fiberglass tank (supplied with pond water to mimic the natural cold water condition in the pond), maintaining the same experimental groups and replicates with same feeding regime as used in phase I in each cage for 2 weeks. Water temperature ranged from 1.6 to 9.9 °C with an average of 7.0 ± 0.1 °C, and DO was 8.0 ± 0.1 mg/L. This temperature range is considered a challenging range beyond its 30 °C optimal level (Lemke 1977; Wang et al. 2014).

Phase III

When the water temperature began to rise, experimental feed was withheld, and all the fish in all cages received the basal diet following the previous feeding regime for an additional 2 weeks with the same described management regime in phases I and II. Water temperature ranged from 5.6 to 14.3 °C with an average of 11 ± 0.7 °C, and DO was 7.0 ± 0.7 mg/L.

Growth performance parameters

Body mass gain (BMG, %), specific growth rate (SGR, %/day), condition factor (*K*), feed conversion ratio (FCR), length gain rate (LGR, %), and survival rate were calculated as follows:

$$\text{BMG}\,(\%) = 100 \times \left[\frac{\text{final body mass}\,(\text{g}) - \text{initial body mass}\,(\text{g})}{\text{initial body mass}\,(\text{g})}\right]$$

$$\text{SGR}\,(\%/\text{day}) = \left[\frac{\ln\ \text{final body mass in}\,(\text{g}) - \ln\ \text{initial body mass in}\,(\text{g})}{\text{number of trial days}}\right] \times 100$$

$$\text{Length gain rate}\,(\%) = 100 \times \left[\frac{\text{Average terminal body length}\,(\text{cm}) - \text{Average initial body length}\,(\text{cm})}{\text{Average initial body length}\,(\text{cm})}\right]$$

$$K\ \text{factor} = 100 \times \left[\frac{(\text{Weight, g})}{(\text{Length, cm})^3}\right]$$

$$\text{FCR} = \frac{F}{(W_f - W_i)};$$

where F is the weight of feed offered to fish, W_f is the final weight of fish, and W_i is the weight of fish at stocking.

$$\text{Survival rate}(\%) = 100 \times \left(\frac{\text{Final number of juveniles}}{\text{Number of juveniles at end of experiment}}\right).$$

Blood and tissue sampling

Sampling included two sampling points: (1) at 6 weeks (at the end of phase II) and (2) at 8 weeks (at the end of phase III). Three fish per replicate (nine fish per treatment) were carefully netted and were euthanized using tricaine methanesulfonate (MS222) at 250–350 mg/L in water for blood and tissue sampling. Blood samples were drawn near the caudal peduncle using 1-cc U-100 syringes without anticoagulant (Becton–Dickinson, Franklin Lakes, NJ, USA) and transferred into Eppendorf tubes and allowed to clot at room temperature in a slanting position. The blood samples were subsequently used to obtain serum (by centrifugation at 10,000*g* for 5 min at 4 °C) and stored in clean centrifuge tubes at −80 °C until used for assaying glucose, cortisol, AST, ALT, and SOD values. Then, fish were carefully dissected, and liver samples were taken and rinsed with phosphate-buffered saline (PBS, pH 7.4) to remove any red blood cells and clots. The samples were then transported on ice, and each sample was divided into two portions, one portion was homogenized in the AST kit assay buffer for assaying AST, and the second portion was homogenized in 50 mM PBS containing 1 Mm EDTA (pH 7.4) for assaying superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx),and then centrifuged at 15,000×*g* for 10 min at 4 °C to remove insoluble materials. Supernatant was separated and stored in clean centrifuge tubes at −80 °C until assayed.

Biochemical assays

Cortisol and Glucose concentrations

Serum glucose and cortisol concentrations were assayed spectrophotometerically in triplicates at 450 nm (BioTek's Epoch™, USA) using a commercial kit (Abnova, USA) and spectrophotometer's built-in Gen5™ data analysis software for each sample for cortisol. Glucose concentration was measured using a colorimetric assay kit (Biovision, USA) according to the following formula:

$$\text{Glucose concentration} = \frac{s_\text{a}}{s_\text{v}}\,(\text{nmol}/\mu\text{L}),$$

where S_a is sample amount (in nmol) calculated from standard curve, and S_v is sample volume (in μL) in each well. The background was corrected by subtracting the 0 glucose control value from all the readings.

Aspartate aminotransferase (AST) and alanine transaminase (ALT) activities

AST and ALT activities were assayed spectrophotometerically (Biotex EIx800, USA) using commercial kits (Sigma-Aldrich, USA) at 450 nm (AST) and 340 nm (ALT) according to the following equations:

$$\text{AST activity (milliunit/mL)} = \frac{\text{B} \times \text{Sample dilution factor}}{(\text{Reaction time}) \times V},$$

where $B =$ amount (nmol) of glutamate generated between T_{initial} and T_{final}, reaction time $= T_{\text{final}} - T_{\text{initial}}$ (minutes) and $V =$ sample vol. (mL) added to well. One unit of AST is the amount of enzyme that will generate 1.0 mol of glutamate per minute at pH 8.0 at 37 °C.

$$\text{ALT activity (U/μL)} = \left[\frac{(\Delta A_{340}/\text{min} \times 0.21\,\text{mL})}{(4.11\,\text{mM}^{-1} \times 0.02\,\text{mL})}\right] \times \text{Sample dilution},$$

where ΔA_{340} is the change in absorbance per minute. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 μmol of NADH to NAD^+ per minute at 37 °C.

Antioxidant enzyme assays

SOD, CAT, and GPx activities were measured spectrophotometerically (BioTek's Epoch™, USA) at 450 nm (SOD), 540 nm (CAT), and 340 nm (GPx), using colorimetric kits (Cayman Chemical, USA) according to the following formulas:

$$\text{SOD (U/}\mu\text{L)} = \{[(\text{sample LR} - y\text{-intercept})/\text{slope}] \times (0.23\,\text{mL})/(0.01\,\text{mL})\} \times \text{sample dilution}$$

$$\text{CAT activity(nmol/min/mL)} = [(\mu\text{M of sample})/(20\,\text{min})] \times \text{sample dilution}$$

$$\text{GPx activity (nmol/min/mL)} = \left[(\Delta A_{340}/\text{min})/(0.00373\,\mu\text{M}^{-1})\right] \times 9.5\,\text{mL} \times \text{sample dilution}$$

where ΔA_{340} is the change in absorbance per minute.

One unit is defined as the amount of enzyme that is needed to exhibit 50 % dismutation of the superoxide radical for SOD, that will cause the formation of 1.0 nmol of formaldehyde per minute at 25 °C for CAT, and that will cause the oxidation of 1.0 nmol of NADPH to NAD^{P+} per minute at 25 °C for GPx.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA), and Duncan's multiple range tests were used to determine significant difference between groups using the Statistical Package for the Social Sciences (SPSS) software (version 22.0). Values are expressed as mean ± standard error, and a value of $P < 0.05$ was considered significant.

Results

Growth performance

Growth performance parameters of Bluegill sunfish are presented in Table 1. Bluegill appeared to be healthy without any recorded mortalities throughout the duration of the experiment. Incorporating Bluegill diets with *A. membranaceus* (AM diet) significantly enhanced the growth performance parameters (SGR, BMG, LGR, FCR, and condition factor) throughout the experiment, with the highest significant increase ($P < 0.05$) for group fed with 4.5 % (w/w) AM diet followed by those fed with 3 and 1.5 % (w/w) AM diet, respectively, at 6 weeks after exposure to cold pond water temperature, and this increase continued at 8 weeks after withholding experimental diets, except for the group fed with 1.5 % (w/w) AM diet that did not show a significant increase in LGR (Table 1). The feed conversion ratio (FCR) showed the same significant decrease in all AM-diet-fed fish compared with the control groups throughout the experimental period (Table 1). There was no significant difference in the condition factor among all the groups at 6 weeks after exposure to cold pond water temperature. While at 8 weeks after withholding the experimental diets, the highest significant increase ($P < 0.05$) in condition factor was for the group fed with 1.5 % (w/w) AM diet followed by those fed with 3 and 4.5 % (w/w) AM diet, respectively (Table 1).

Biochemical assays

Adding 4.5 % (w/w) AM to bluegill diets gave the highest significant decrease ($P < 0.05$) in cortisol levels (Fig. 1a). The most significant decrease ($P < 0.05$) in glucose levels was for 3 % (w/w) AM group throughout the experimental period and for 4.5 % group at 6 weeks after exposure to cold pond water temperature compared to the control groups, while group fed with 1.5 % (w/w) AM showed a significant increase in glucose level at 6 weeks after

Table 1 Growth performance parameters of *L. macrochirus* at 6 weeks after feeding with different levels of AM-incorporated diets and at 8 weeks after withholding experimental diets and feeding on control diet

AM %/kg feed	SGR (%)	BMG (%)	LGR (%)	*K*	FCR
Incorporated diets (6 *weeks*)					
0	0.03 ± 0.01^{d}	1.20 ± 0.29^{d}	2.57 ± 0.04^{c}	2.06 ± 0.01^{a}	3.15 ± 0.07^{b}
1.5	0.26 ± 0.00^{c}	12.50 ± 0.22^{c}	4.17 ± 0.00^{c}	2.19 ± 0.01^{a}	2.92 ± 0.05^{a}
3	0.92 ± 0.01^{b}	52.87 ± 0.38^{b}	15.85 ± 0.55^{b}	2.16 ± 0.03^{a}	0.69 ± 0.01^{a}
4.5	1.26 ± 0.03^{a}	76.86 ± 0.54^{a}	21.39 ± 1.60^{a}	2.21 ± 0.12^{a}	0.46 ± 0.02^{a}
Control diets (8 *weeks*)					
0	0.54 ± 0.05^{c}	28.2 ± 2.83^{c}	15.28 ± 3.68^{b}	1.86 ± 0.18^{c}	1.32 ± 0.13^{b}
1.5	1.54 ± 0.03^{b}	103.5 ± 3.16^{b}	12.50 ± 2.41^{c}	3.16 ± 0.20^{a}	0.35 ± 0.01^{a}
3	1.52 ± 0.02^{b}	101.2 ± 2.38^{b}	18.06 ± 1.39^{ab}	2.70 ± 0.12^{ab}	0.36 ± 0.01^{a}
4.5	1.78 ± 0.04^{a}	126.5 ± 4.14^{a}	25.00 ± 0.00^{a}	2.55 ± 0.05^{b}	0.29 ± 0.01^{a}

Values [means ($n = 30$) ± SEM] within the same column with different superscript letters were significantly different ($P < 0.05$) from the control

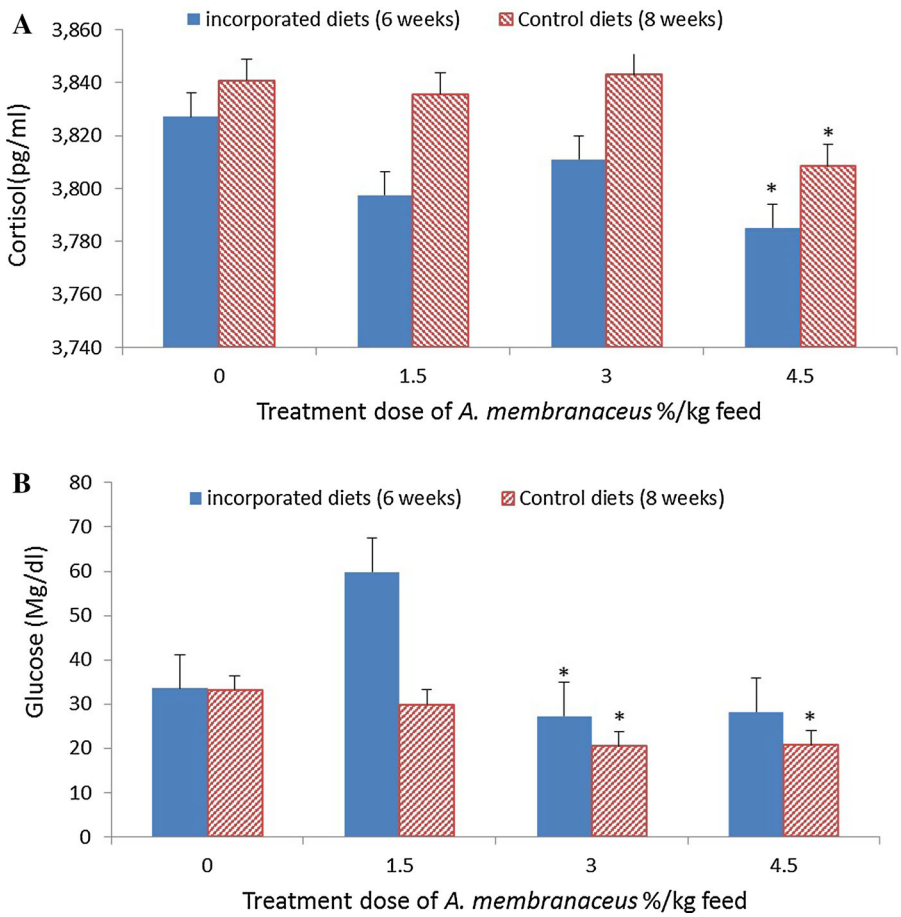

Fig. 1 Effects of AM-incorporated diet on cortisol (**a**) and glucose (**b**) levels of *L. macrochirus* at 6 weeks (after feeding on incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

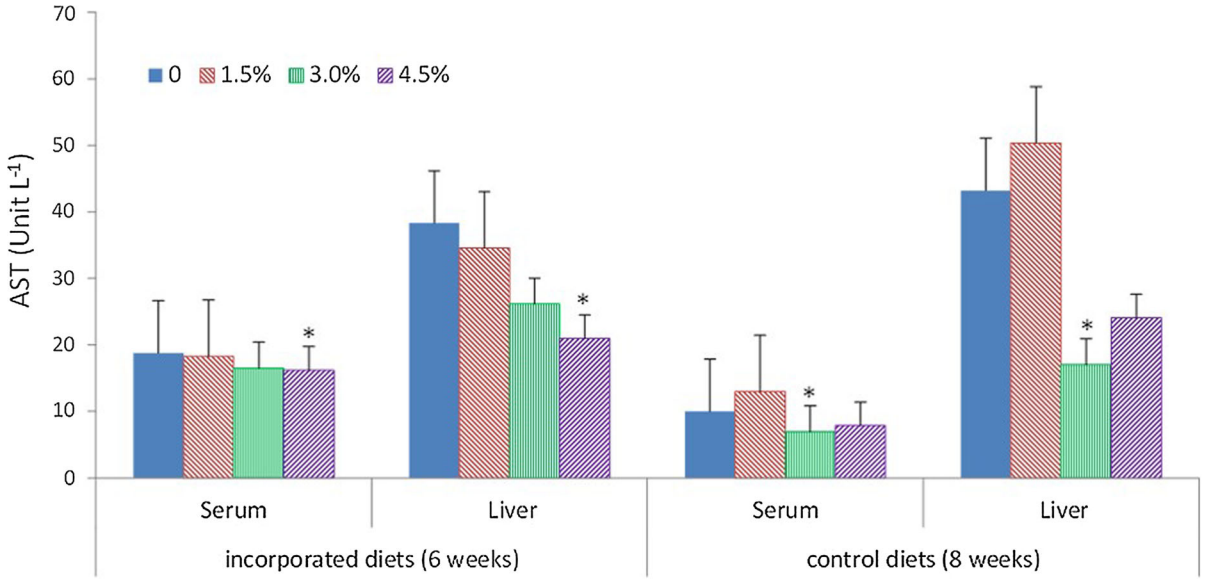

Fig. 2 AST in serum and liver of *L. macrochirus* at 6 weeks (after feeding on AM-incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

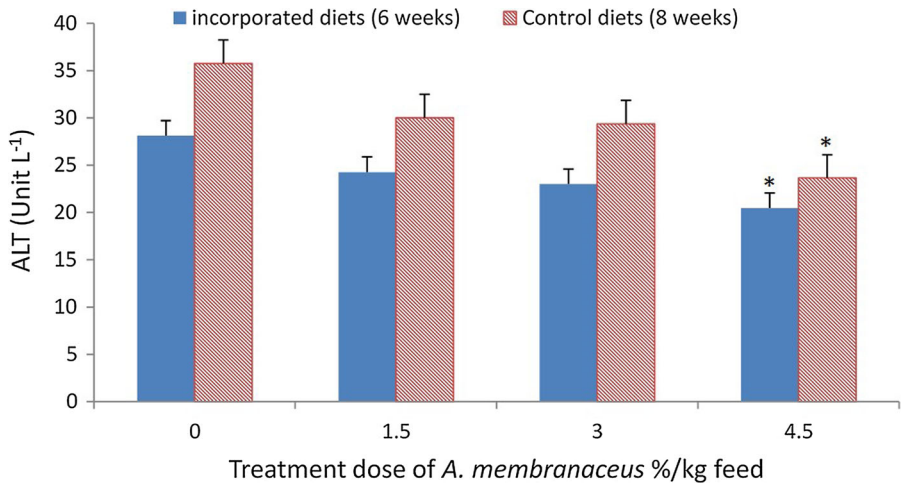

Fig. 3 ALT in serum of *L. macrochirus* at 6 weeks (after feeding on AM-incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

exposure to cold pond water temperature compared to the control group (Fig. 1b).

ALT and AST activities showed the maximum significant decrease ($P < 0.05$) in 4.5 % (w/w) AM-treated groups at 6 weeks after exposure to cold pond water temperature compared to the control groups (Figs. 2, 3). At 8 weeks from the start of the experiment, the 4.5 % (w/w) AM diet group continued showing the most significant decrease ($P < 0.05$) in ALT activity (Fig. 3), while the highest decrease ($P < 0.05$) in AST activity was for 3 % (w/w) AM diet group (Fig. 2).

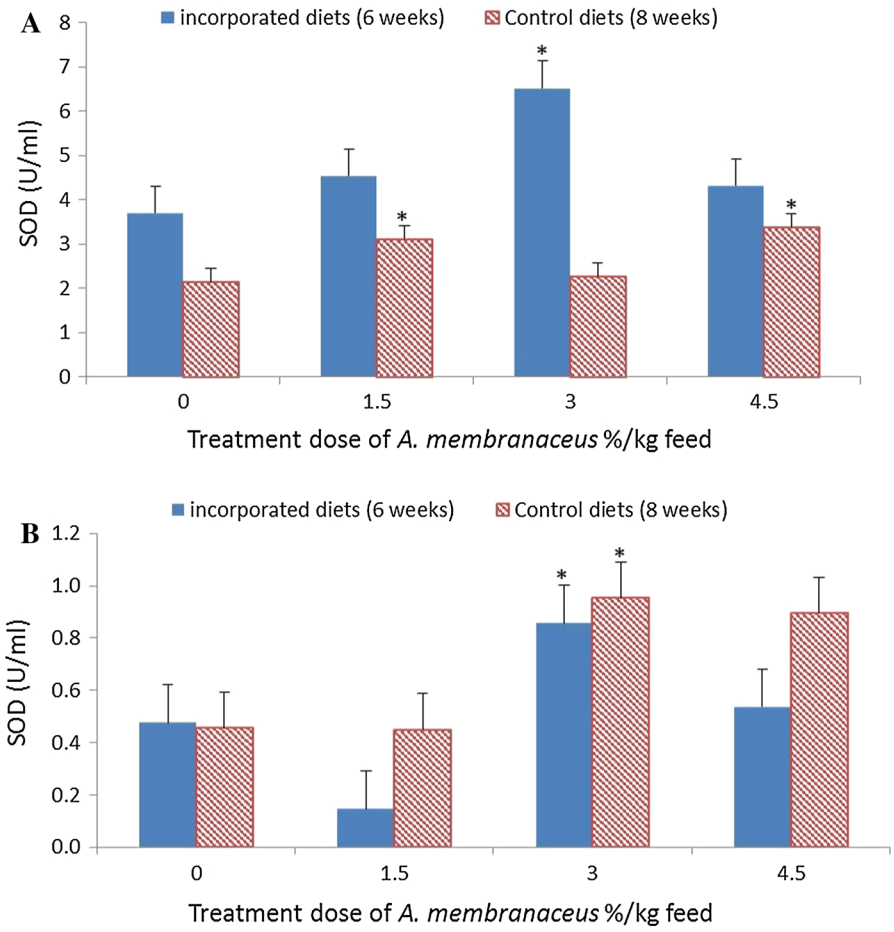

Fig. 4 SOD activity in serum (**a**) and liver (**b**) of *L. macrochirus* at 6 weeks (after feeding on AM-incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

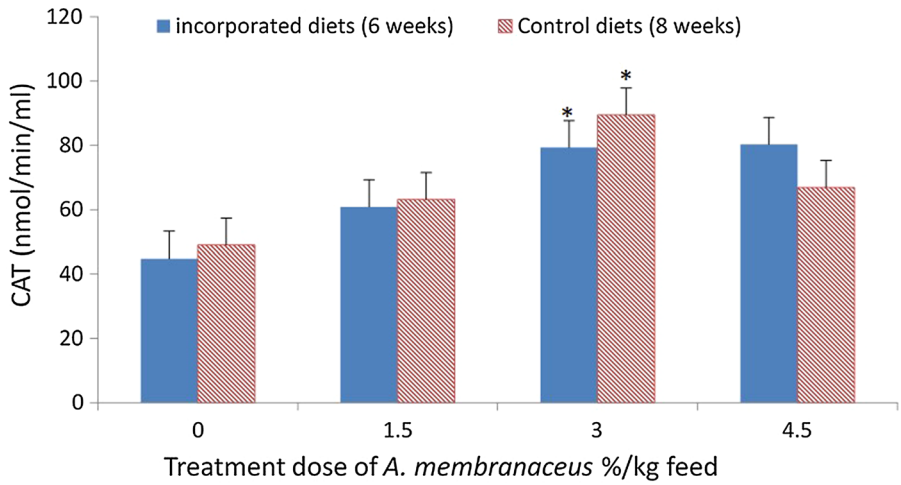

Fig. 5 Catalase (CAT) activity in liver of *L. macrochirus* at 6 weeks (after feeding on AM-incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

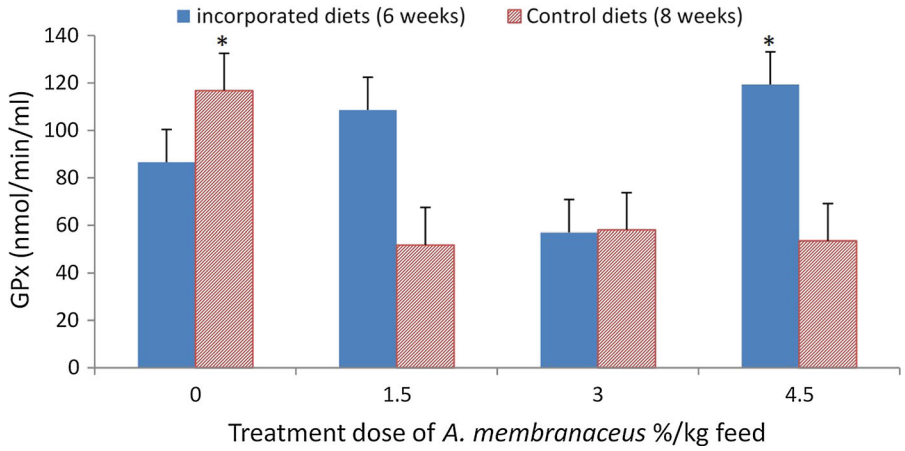

Fig. 6 GPx activity in liver of *L. macrochirus* at 6 weeks (after feeding on AM-incorporated diets with exposure to cold water temperature challenge) and at 8 weeks (after withdrawing AM diets and feeding on control diets). Values are mean ($n = 9$) ± SEM. Mean values with *asterisk* are different significantly ($P < 0.05$)

Antioxidant enzyme assays

SOD and CAT activities were significantly higher ($P < 0.05$) in most AM-incorporated diet groups throughout the experiment, with the most significant increase for 3 % (w/w) AM-incorporated diet groups compared with the control (Figs. 4, 5), except for 1.5 % AM group that did not show a significant difference in SOD activity in liver than control group (Fig. 4b) and 4.5 % AM group at 8 weeks that did not show a significant difference in CAT activity than control group (Fig. 5).

GPx level showed the highest significant increase ($P < 0.05$) in 4.5 % (w/w) AM-incorporated diet groups at 6 weeks after exposure to cold pond water temperature compared with the control. While at 8 weeks after withholding the incorporated experimental diets, all AM-incorporated diet groups showed a significant decrease in GPx level compared with the control (Fig. 6).

Discussion

The current findings that AM diets significantly increased growth performance and antioxidative stress profiles are supported by those of Zahran et al. (2014) as they found that adding APS (1500 mg/kg of diet) to *Oreochromis niloticus* basal diets showed a significant increase in growth parameters compared to control. Also, El-Desouky et al. (2012a, b) found that incorporating *Macrobrachium rosenbergii* basal diets with different concentrations of both *Zingiber officinalis* and *Cyanodon dactylon* showed significant increase in growth performance parameters compared to control along the whole experimental period. In addition, Nya and Austin (2009a, b), Talpur and Ikhwanuddin (2012), and Talpur et al. (2013) who fed garlic and ginger diets to rainbow trout and *L. calcarifer*, respectively, observed significantly higher growth rates in treated fish over the control. Moreover, Talpur (2014) reported that incorporating *Mentha piperita* in Asian sea bass feed led to reduced mortalities and significantly improved growth performance. Those results may be attributed to the active ingredients that *A. membranaceus* contain as APS, which have been shown to possess immunostimulating and hepatoprotective effects (Galina et al. 2009). It also contains monosaccharides, organic acids, alkaloids, glucosides, and volatile oil as major active components that have been found to enhance immune function in fish together with choline, betaine, and folic acid (Galina et al. 2009).

Plasma cortisol is considered one of the primary responses of fish to different kinds of stressors (Wendelaar Bonga 1997). In the present study, dietary AM supplementation significantly decreased cortisol level compared to the control group. Similar results have been reported by Barros et al. (2014) who showed that all Nile Tilapia supplemented with 0.1 % of β-

glucan and 600 mg of Vit C/kg and fed for at least 15 days returned to the baseline cortisol values after transportation stress. Additionally, Abasali and Mohamad (2010) found that cortisol levels in a swordtail treated with *Valeriana officinalis* root extract were significantly lower than the control group. This significant decrease might be attributed to active components of AM that have been found to possess antistress and immunostimulating properties (Galina et al. 2009).

The significant decrease in serum glucose level in this study can be possibly explained that AM might have stimulated the insulin activity, consequently reducing the glucose level. Serum insulin level is usually found to increase by decreasing the blood glucose level when an immunostimulant diet is administered (Ahmed and Sharma 1997). These results came in concurrence with Talpur (2014) who demonstrated a significant reduction in serum glucose levels for MP-diet-treated fish compared to the control. Similar results were observed in freshwater catfish *Clarias gariepinus* exposed to deltamethrin pesticide toxicity and fed high doses of ascorbic acid (100 mg/100 g diet) (Datta and Kaviraj 2003).

The finding that AM-incorporated diets significantly lowered ALT and AST activities throughout the experiment can be attributed to the immunostimulating and hepatoprotective effects of APS, which is one of the active ingredients (Jia et al. 2012). Our results agree with Zaki et al. (2012) who reported that lowest AST and ALT activities were obtained in *O. niloticus* group fed with 1 % fenugreek sprouts meal.

Oxidative stress occurs when there is an imbalance between the production of ROS (superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and the activity of antioxidants (Halliwell 1994; Kammer et al. 2011). Therefore, antioxidant systems are important to improve this imbalance in biological systems (Parihar et al. 1997; Madeira et al. 2013). SOD, GPx, and CAT are important antioxidant enzymes (Livingstone 2001; Somogyi et al. 2007; Madeira et al. 2013). Throughout the experiment, most AM supplementation markedly increased SOD and CAT activities. At 8 weeks, all incorporated groups showed a significant decrease in GPx level that may indicate inefficiency of to neutralize oxidative impacts after withdrawing experimental diets. Other studies are supportive to our results. Zahran et al. (2014) showed that dietary supplementation with *O. niloticus* and APS up-regulated SOD and GPx activities compared with the control. Jia et al. (2012) showed that in vitro APS supplementation in carp primary hepatocytes significantly improved cell viability and increased the reduced level of SOD. Also, in vivo administering 1.5 and 3 g/kg APS in the diet for 60 days prior exposure to carbon tetrachloride (CCl_4), intoxication markedly increased the reduced levels of SOD, glutathione, and total antioxidant. The significant increase in antioxidant enzyme activities may be attributed to the antioxidant and hepatoprotective activity effects of APS (Li et al. 2010; Yan et al. 2010; Jia et al. 2012; Zahran et al. 2014). In addition to the ASP that were shown to be related to its ability to scavenge free radicals (Hattori et al. 1991; Jia et al. 2012), other studies suggested the ability of probiotics to protect Yellow Perch (*P. flavescens*) against oxidative stress (Shaheen et al. 2014). The significant increase in growth performance and antioxidative stress profiles continued after switching to control diets and after water temperature began to rise, suggesting the ability of AM diets to up-regulate immune system using pulse feeding strategies that offers flexibility as the immunostimulant can be fed during periods of increased risk, such as winter conditions and can be also related to the nature of bluegill that is known to be a warm water fish, as described by Lemke (1977) and Wang et al. (2014). Similar result was explained by Bricknell and Dalmo (2005). Conclusively, the present study revealed that *A. membranaceus* dietary supplementation can be used as a natural growth promotor, hepatoprotective, anti-cold water stress, and redox-active agent in bluegill and some other fish species. Thus, it can contribute to the enhancement of fish welfare and economic growth for sustainable aquaculture and fisheries.

Acknowledgments This study was supported by the National Institute of Food and Agriculture (NIFA), US Department of Agriculture, under Agreement No. 2010-38879-20946, and Cultural Affairs and Missions sector, Ministry of Higher Education and Scientific Research, Egypt. Salaries and research support were provided by state and federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center. We thank Kun-Qian Zhu, Dean Rapp, and Paul O'Bryant for their assistance throughout the experiment, and Joy Bauman for her comments on the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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