Aquaculture Reports 8 (2017) 21–30

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Anti-oxidative effects of some dietary supplements on Yellow perch (*Perca flavescens*) exposed to different physical stressors

Hiam Elabd[a,b], Han-Ping Wang[a,*], Adel Shaheen[b], Hong Yao[a], Amany Abbass[b]

[a] Aquaculture Genetics and Breeding Laboratory, The Ohio State University South Centers, Piketon, Ohio, 45661, USA
[b] Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, 13736, Egypt

ARTICLE INFO

Keywords:
Antioxidative stress biomarkers
Biochemical biomarkers
Gene expression
Histopathology
Stress
Yellow perch

ABSTRACT

In current study, Yellow perch (*P. flavescens*) was exposed to common forms of physical stressors and anti-oxidative effects of dietary incorporated *Astragalus membranaceus* (AM) and *Glycyrrhiza glabra* (liquorice) were assessed. To address this, for a four-week five groups of fish (31 ± 1.0 g, average weight) received 1, 2, and 3% (w/w) *Glycyrrhiza glabra*; and 1% *G. glabra- A. membranaceus* mixture daily. Control group fed an additive-free basal diet. Immunological, biochemical and histopathological profiles were evaluated; and fish were redistributed to be exposed to heat, cold, hypoxia and capture stressors. The current findings demonstrated that *A. membranaceus* and *G. glabra* dietary incorporation remarkably enhanced antioxidative and biochemical parameters. Also, the study showed markedly up-regulation of related genes expression; and revealed better liver histology in supplemented groups over the control. In conclusion, *A. membranaceus* and *G. glabra* dietary supplementation markedly enhanced antioxidantive responses throughout the experimental period, indicating the ability of both herbal plants to confer protection against different physical stressors.

1. Introduction

In aquaculture, physiological stress response may be caused by various husbandry practices such as elevated rearing densities, thermal stress, handling, low dissolved oxygen and transportation stressful conditions (Ackerman et al., 2000; Palmisano et al., 2000; Tort, 2011).

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 of injury (An et al., 2010; Halliwell, 1994). Oxygen levels can modulate the immune response; hypoxia may weaken the fish immune system resulting in increased susceptibility to disease (Bowden, 2008).

In the current study, fish were exposed to heat, cold, hypoxia and capture stressors as the most common experienced stress conditions. Diets were supplemented with *Glycyrrhiza glabra* and *Astragalus membranaceus*. *G. glabra* is one of the common used herbal medicinal plants (Wang and Nixon, 2001). Glycyrrhizic acid is considered the main active component of *G. glabra* (Kamei et al., 2003; Kim et al., 2004). It is mostly known for possessing antioxidative properties (Guojun Yin, 2011). *A. membranaceus* is also an important Chinese herbal plant (Li et al., 2010) that contains Astragalus polysaccharides (APS), alkaloids and glucosides and volatile oil as main active ingredients that are known for its immunostimulantry and hepatoprotective effects (Galina et al., 2009; Yan et al., 2009).

The role of the currently investigated dietary supplements on mitigating physical stressors in yellow perch has not been previously addressed. Yellow perch is one of North America most important fish species which has been introduced in most of the parts of North America, and also been transported into Europe, South Africa, Asia, South America, and Oceania (Brown et al., 2009). The aim of current work was to evaluate the action of incorporated *G. glabra* and *G. glabra* − *A. membranaceus* mixture on immune response of yellow perch exposed to some physical stressors.

2. Material and methods

2.1. Fish and experimental conditions

Yellow perch, *P. flavescens* (average weight 31 ± 1.0 g) were obtained from South Centers, The Ohio State University, United States and stocked into an aerated 2200-L fiberglass tank. Fish health examination was performed according to the methods of (Austin, 1951). Fish acclimatization and all the environmental conditions were maintained as addressed by Elabd et al. (2016b).

Our current study including all procedures involving animals were

* Corresponding author.
E-mail address: wang.900@osu.edu (H.-P. Wang).

http://dx.doi.org/10.1016/j.aqrep.2017.09.002
Received 5 May 2017; Received in revised form 31 August 2017; Accepted 2 September 2017

carried out according to the Ohio State University approved protocol for institutional animal care and use.

2.2. Diets preparation and experimental design

G. glabra and *A. membranaceus* were commercial products obtained from Oregon's wild harvest company, Oregon, United States in powder form and mixed with commercial fine powdered basal diet *Aquamax*® (Elabd et al., 2016a,b) to achieve five experimental diets at concentrations of 1, 2, 3% *G. glabra*/kg and 1% *G. glabra:* A. *membranaceus*/kg mixture. Control group was kept free without any additives. Diets were prepared and stored following same methods of (Elabd et al., 2016a,b).

The experiment was carried out on two phases as following:

2.2.1. Phase I (Pre-exposure)

Yellow perch were assigned for five tanks (240 × 60 × 30 cm). Each tank (90 fish/tank) divided into three replicates and fed twice daily for four weeks with the five prepared experimental diets in two equal parts at 9:00 a.m. and 4:00 p.m. All the conditions and daily routine work were performed according to Elabd et al. (2016a,b).

2.2.2. Phase II (Stressors exposure)

Nine fish were randomly transferred from each experimental tank (three per replicate) to a 50 L tanks set (each tank represent one experimental group) for application of different stressors that might be experienced in the environment. After exposure to stressors, 3 fish from each group were sampled. Each stressful condition was introduced as following:

2.2.2.1. Heat. This stressor lasted for 15 min. Fish were netted from (17 ± 1.2 °C, ambient temperature) and transferred to the 50 l tanks set each containing aerated water heated to 29 ± 0.50 °C.

2.2.2.2. Cold. Fish were carried from (17 ± 1.2 °C, ambient temperature) to the 50 l tanks set each containing aerated water cooled to 8 °C for 2 h.

2.2.2.3. Capture. From each group, nine fish were netted, held in net outside water and left to struggle for 20 s. Then, while in the net, returned in water. This method was repeated three times before sampling.

2.2.2.4. Hypoxia. Nine fish were transferred from each experimental group to static 50-L tanks set after shutting off the supplemental aeration and decreasing the water column. Fish kept hypoxia signs appearance (surfacing, rapid opercula movement, gasping and lethargy).

2.3. Tissue sampling

Two sampling points: (1st) after four weeks receiving incorporated diets and pre-exposure to different stressors and (2nd) after exposure to different stressors (post-exposure). Nine fish per each group were euthanized using 250 ppm tricaine methanesulfonate (Vancouver, British Columbia). Liver samples were carefully isolated from dissected fish and later divided into two parts according to same methods and procedures previously mentioned by Elabd et al. (2016a,b).

2.4. Antioxidative stress parameters

Antioxidant enzymes; Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Lipid Peroxidase (LPx) activities were measured following Elabd et al. (2016a,b).

2.5. Biochemical parameters

Measuring both Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) activities was done according to Elabd et al. (2016a,b).

2.6. Liver histopathology

Liver samples were collected from three fish per tank at (pre-exposure), (post-heat stress) and (post-cold stress) exposure, preserved in 20% formaldehyde (in PBS) and processed for hematoxylin and eosin (H & E) staining. Preparation and evaluation of H & E sections was carried out by and according to Department of Veterinary Biosciences, Comprehensive Cancer Center, Ohio State University, Ohio, USA.

2.7. Gene expression

2.7.1. RNA extraction and cDNA synthesis

Trizol method (Invitrogen, Carlsbad, USA) was used for total RNA extraction according to same procedures described by Elabd et al. (2016a,b). Invitrogen® high capacity cDNA reverse transcription kit was used for total RNA reverse transcription to cDNA using protocol previously mentioned by Elabd et al. (2016a,b).

2.7.2. Primer design and Real-time PCR

Primer-BLAST (NCBI) was used for primer design. Table 1 describes forward and reverse primer sequences for β-actin, SOD, GPx, HSP70, Serum amyloid A (SAA), Complement Component C3 (CCC3), Alpha 2 Macroglobulin (A2M) genes. Two pairs for each primer at least were tested and best performance pairs were selected. Primers were obtained from IDT (Coralville, IA, USA).

PCR amplification reactions were carried out using Applied Biosystems® Real-Time PCR System (United States) Following Elabd et al. (2016a,b).

2.8. Statistical analysis

One-Way ANOVA was used for results statistical analysis. Results are expressed as mean ± standard error. Significant difference among groups based on the different dietary supplements concentrations as main factor, was determined by Duncan's multiple range tests using the Statistical Package for the Social Sciences (SPSS, v.22.) software; and a probability of $P < 0.05$ was considered significant.

Table 1
Sequences of the primers used to evaluate gene expression in Yellow perch *P. flavescens*.

Gene	Primer sequence (5′-3′)	Function
β-actin	F: GCCTCTCTGTCCACCTTCCA R: GGGCCGGACTCATCGTACT	House keeping
SOD	F: GCATGTAGGAGACTTGGGCAAT R: CCGTGATTTCTATCTTGGCAACA	Oxidative stress
GPx	F: GTCTTGGGTAACCCCACCAG R: GACACTTGGATGCCACCTCA	
HSP-70	F: TGTTGGTCGGTGGCTCAA R: TTGAAGAAGTCCTGAAGCAGCTT	Protein folding and protection
A2M	F:TACAGGAGCACCAAGTGCAG R: GACTGACCACACGCTCTTCA	Immune related
SAA	F:ACCATGCTCGTTTGCCTTCT R:TGTGGCGAGCATACAGTGAT	
CCC3	F:GCACAGGAGAAGCAACAGTG R: AGGAGCTGCACTGACAAGTTA	

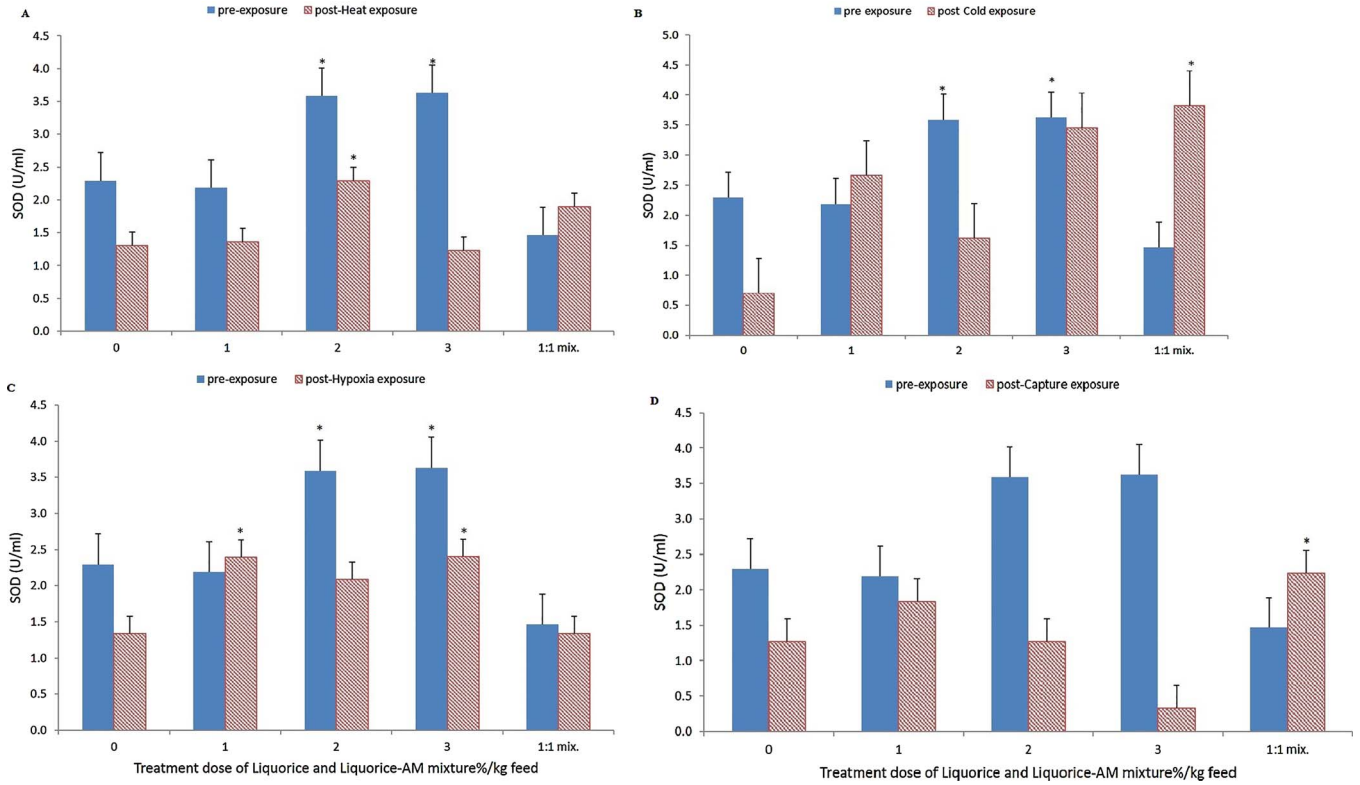


Fig. 1. SOD activity in yellow perch at post- exposure to Heat (A), Cold (B), Hypoxia (C) and Capture (D) stressors after feeding different concentrations of *G. glabra* and *G. glabra-A. membranaceus* supplemented diets. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Antioxidative stress parameters

3.1.1. Superoxide dismutase activity assay

2 and 3% (w/w) *G. glabra* groups showed significantly high SOD activity ($P < 0.05$) in compared with the control at pre-exposure phase (Fig. 1). Exposure to heat and cold stresses decreased SOD activity in control groups remarkably. 2% *G. glabra* at post-heat exposure and with 1% *G. glabra-A. membranaceus* combination at post-cold gave the highest increase ($P < 0.05$) in SOD activity (Fig. 1(A and B)). While SOD was significantly increased with exposure to hypoxia and capture stresses in control groups. Compared to the control, 1% Liquorice-AM combination at post-hypoxia and 3% *G. glabra* at post capture showed the most remarked decrease (Fig. 1(C and D)).

3.1.2. Glutathione Peroxidase (GPx) activity

1% *G. glabra* groups had the highest significant decrease in GPx before exposure to any stress (Fig. 2). In control groups, Exposure to all experimental stressors lead to marked increase in GPx activity, with the most remarked decrease for 1% (w/w) (Liquorice-AM mixture) treated groups post-heat and capture stresses (Fig. 2(A–D)); and for 1% (w/w) *G. glabra* incorporated groups post-cold and hypoxia (Fig. 2(B and C)).

3.1.3. Catalase activity assay

The highest significant increase in CAT activity was for 2% *G. glabra* groups compared with the control at pre-exposure phase (Fig. 3). Exposure to all experimental stressors, except for cold stress, lead to remarked decrease in catalase activity in control tanks. The most remarked increase was found in 1% Liquorice-AM combination groups post-heat and hypoxia stresses exposure (Fig. 3(A–C)); and for 3% (w/w) *G. glabra* post-capture exposure (Fig. 3(D)).

3.1.4. Lipid Peroxidase activity assay

Fish supplied with 1 and 2% *G. glabra* had the most remarked increase in LPx activity before stress exposure (Fig. 4). Subjection to all stressors caused remarked increase in LPx activity. 1% *G. glabra-A. membranaceus* mixture at post heat and hypoxia (Fig. 4(A–C)) and 1% *G. glabra* post cold and capture stress gave lowest LPx activity (Fig. 4(B–D)).

3.2. AST and ALT activities

Lowest ALT activity was revealed in 1% *G. glabra-A. membranaceus* groups throughout the experiment compared to the control groups (Fig. 5). AST activity The most remarked decrease was for 3% *G. glabra* group at pre-exposure and post-exposure to (Heat, Cold and Hypoxia) stresses (Fig. 6(A–C)) and for 2% (w/w) *G. glabra* post capture stress exposure (Fig. 6(D)).

3.3. Histopathological analysis (light microscopy)

Following exposure to heat stress, groups fed with 2% *G. glabra* and 1% *G. glabra-A. membranaceus* diets revealed less (moderate) hepatocellular vacuolization compared with the control group, that revealed marked vacuolization (Panels 1 and 2). While after exposure to cold stress, 1% *G. glabra-A. membranaceus* group showed a healthier liver with moderate hepatocellular vacuolization and without any signs of necrosis or degenerative changes compared with the control group, that revealed marked vacuolization with degenerative necrosis changes (Panels 1 and 2).

3.4. Gene expression

3.4.1. Gene expression after subjection to heat stress

GPx, Heat Shock protein 70 (HSP-70) and SAA genes were

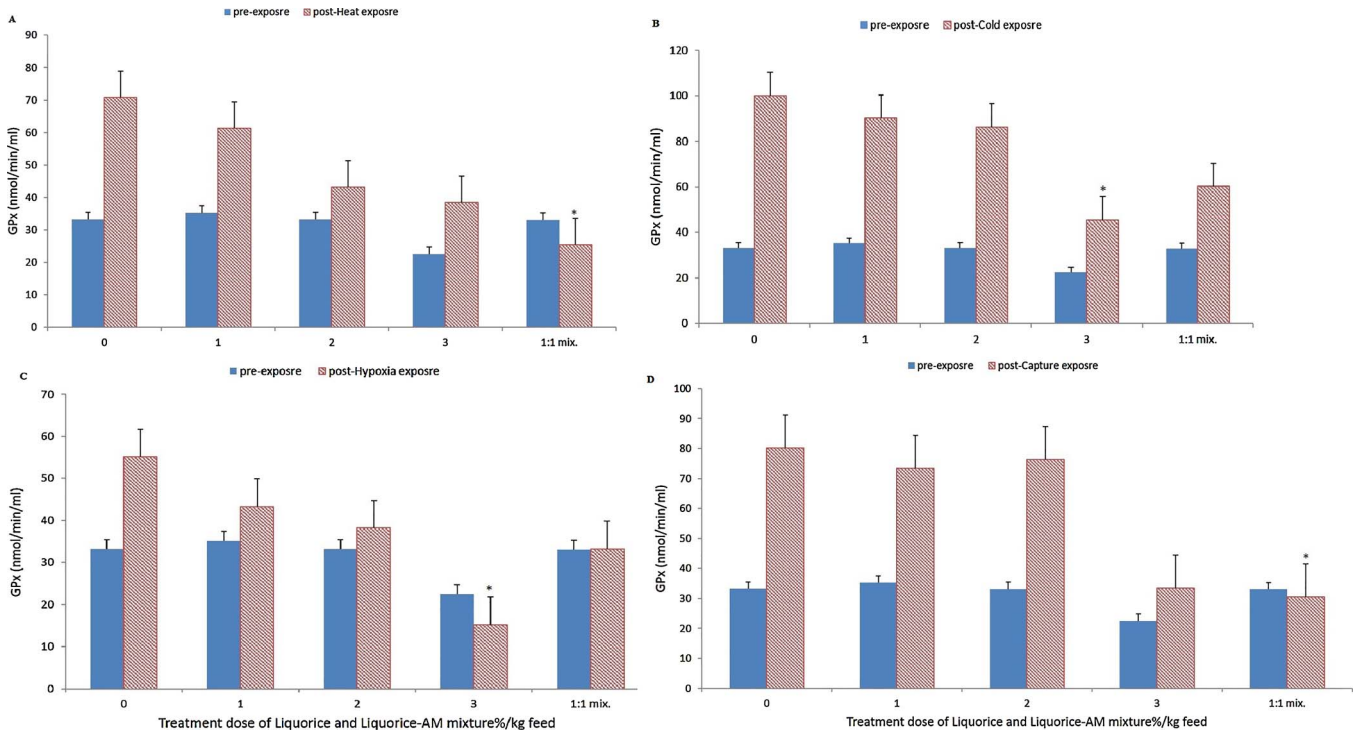


Fig. 2. GPx activity in yellow at post- exposure to Heat (A), Cold (B), Hypoxia (C) and Capture (D) stressors after feeding with different concentrations of *G. glabra* and *G. glabra-A. membranaceus* supplemented diets. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

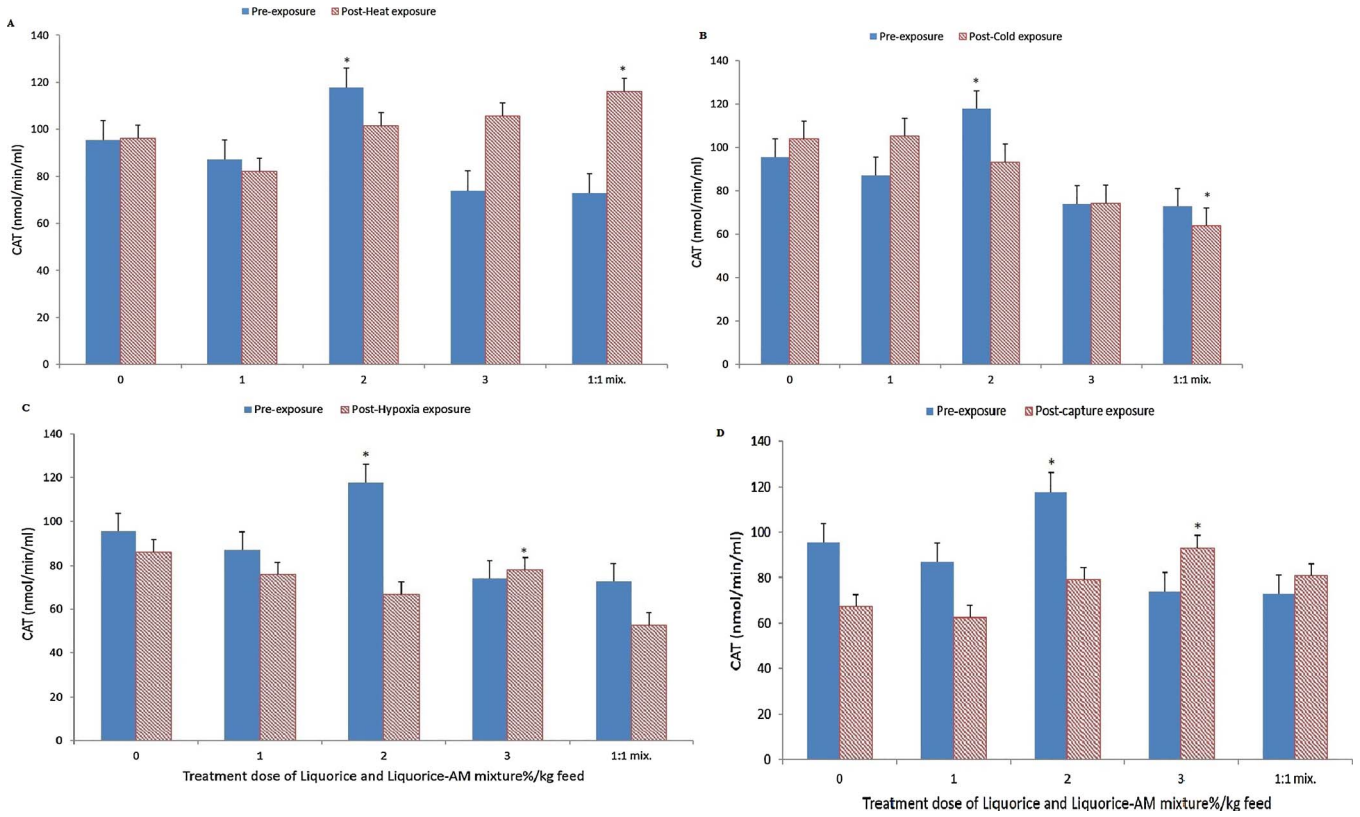


Fig. 3. CAT activity in yellow perch at post- exposure to Heat (A), Cold (B), Hypoxia (C) and Capture (D) stressors after feeding with different concentrations of *G. glabra* and *G. glabra-A. membranaceus* supplemented diets. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

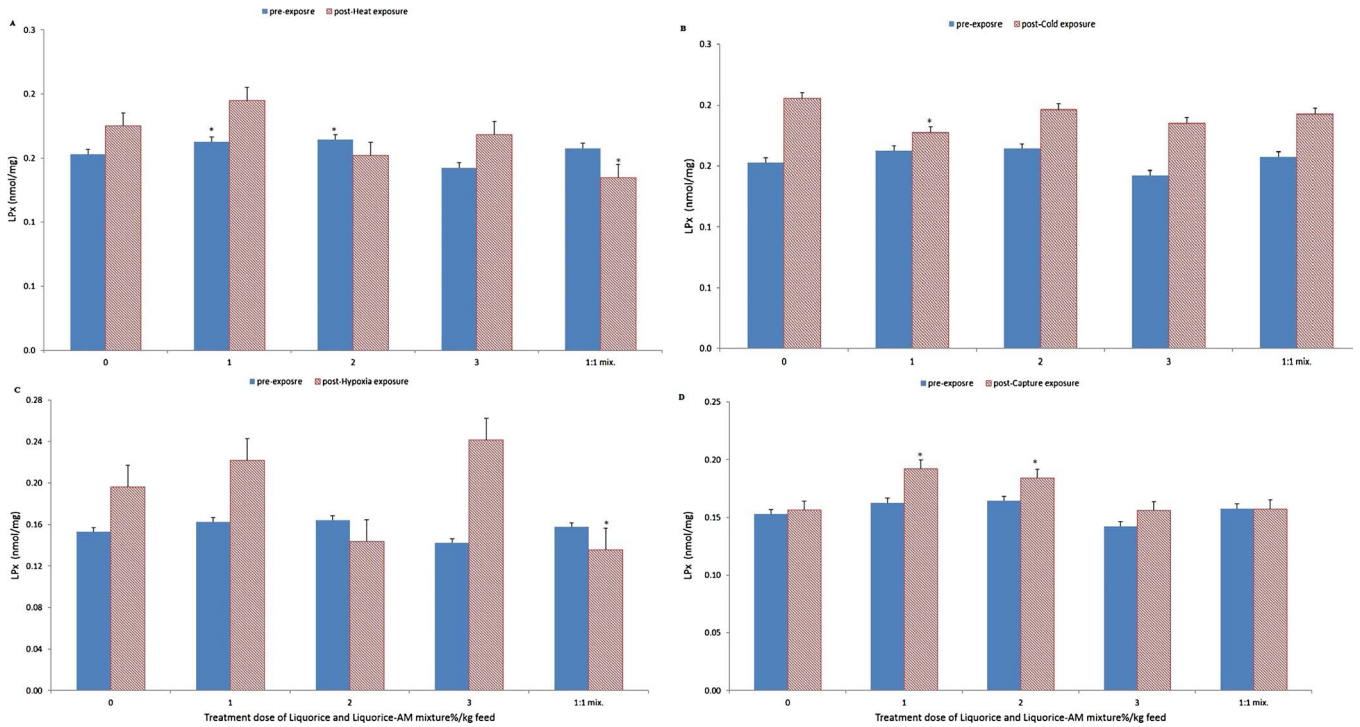


Fig. 4. LPx activity in yellow perch at post- exposure to Heat (A), Cold (B), Hypoxia (C) and Capture (D) stressors after feeding with *G. glabra* and *G. glabra-A. membranaceus* supplemented diets. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly unregulated 1% *G. glabra-A. membranaceus* mixture groups After exposure to heat (Fig. 7(B–E)). 1% *G. glabra* incorporated diet showed the most manifested up-regulation in SOD gene expression (Fig. 7(A)). Meanwhile, dietary incorporation did not show a significant up-regulation in of either A2M or CCC3 genes expression post- exposure to Heat (Fig. 7(C–F)).

3.4.2. Gene expression after subjection to cold stress

Feeding yellow perch with 1% *G. glabra-A. membranaceus* mixture remarkably unregulated both SOD and CCC3 genes expression than the control after exposure to cold challenge (Fig. 7(A–F)). 3% *G. glabra* incorporated diets group showed the most remarked up-regulation in HSP-70 gene expression (Fig. 7(E)). Meanwhile, at post- cold stress exposure incorporated diets did not significantly up-regulate any of GPx, A2M or SAA genes (Fig. 7(B–D)).

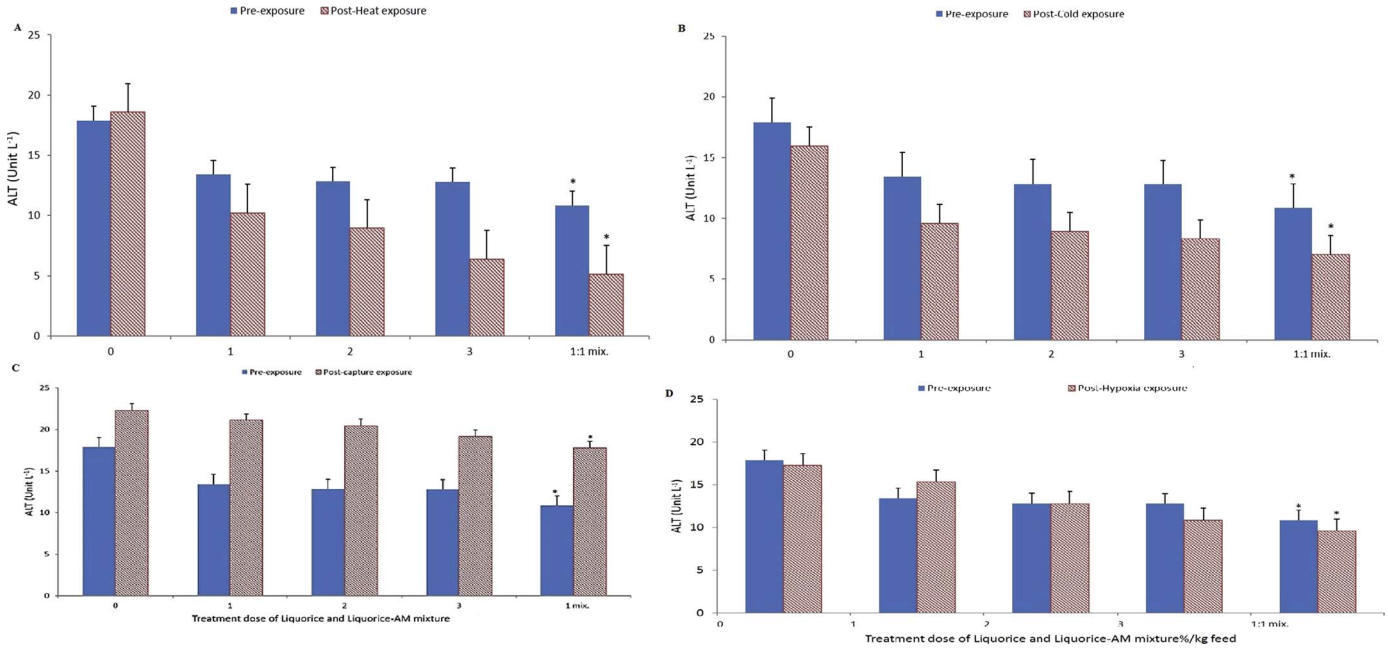


Fig. 5. *G. glabra* and *G. glabra-A. membranaceus* incorporated diets effect on yellow perch ALT activity after exposure to Heat (A), Cold (B), Capture (C) and Hypoxia (D) stressors. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

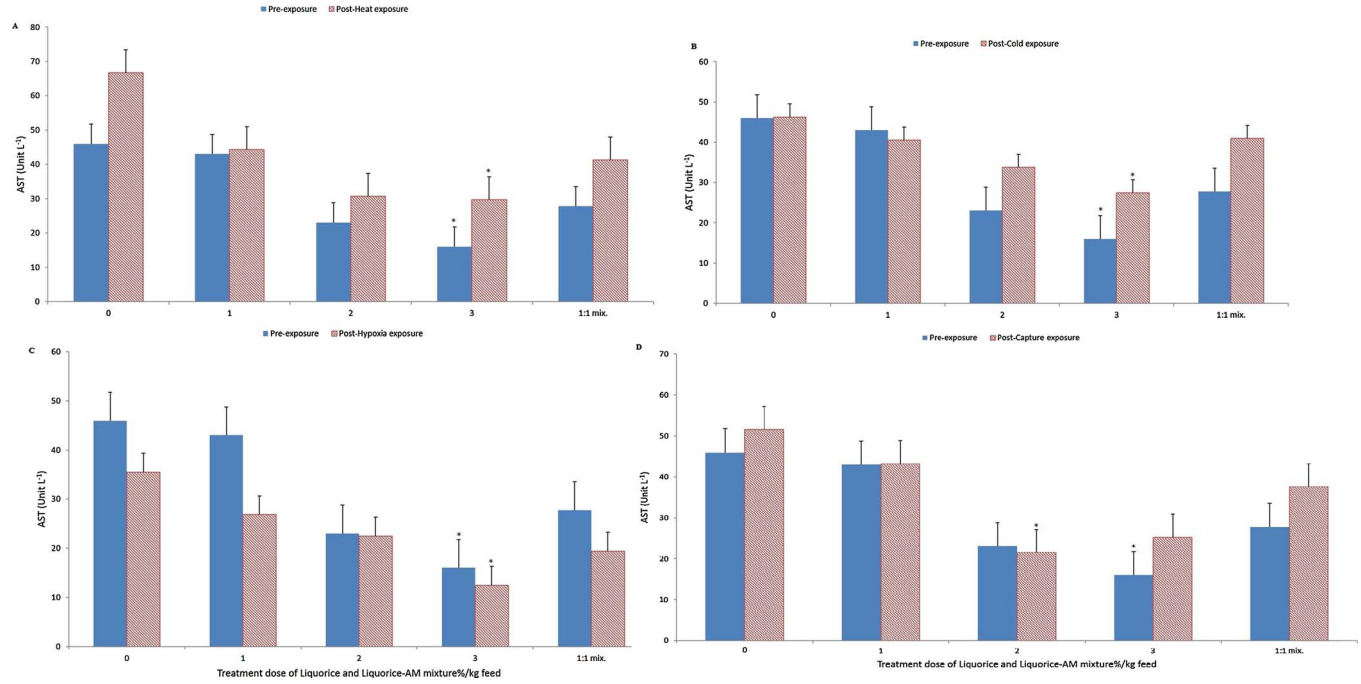


Fig. 6. *G. glabra* and *G. glabra-A. membranaceus* incorporated diets effect on yellow perch AST activity after exposure to Heat (A), Cold (B), Capture (C) and Hypoxia (D) stressors. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4.3. Gene expression after subjection to hypoxia stress

Following hypoxia exposure, 1% *G. glabra-A. membranaceus* mixture groups showed significantly higher ($P < 0.05$) expression of SOD, GPx, CCC3 and A2M genes (Fig. 8(A–C and F)). 2% *G. glabra* groups had the most significant up-regulation in HSP-70 gene expression (Fig. 8(E)). In addition, 3% *G. glabra* group gave the most remarkedd increase in expression of SAA gene after exposure to hypoxia stress (Fig. 8(D)).

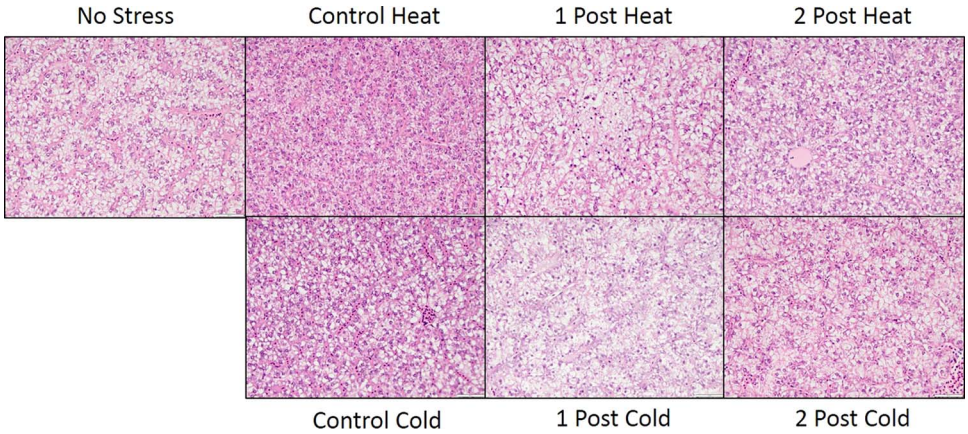
3.4.4. Gene expression after subjection to capture stress

Yellow perch supplied with 1% *G. glabra* had the most up-regulated HSP-70 expression and immune related genes (A2M, SAA, and CCC3) than control after exposure to capture stress (Fig. 8(C–F)). 1% *G. glabra-A. membranaceus* mixture showed the highest significant increase in GPx gene expression (Fig. 8(B)). Meanwhile, incorporated diets did not significantly increased the expression of SOD gene after exposure to capture stress (Fig. 8(A)).

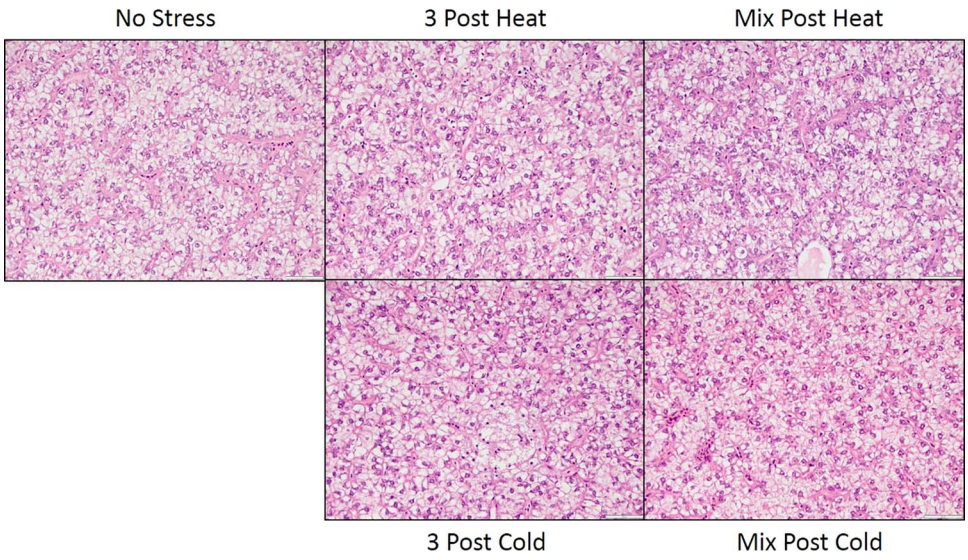
4. Discussion

Various husbandry practices such as elevated rearing densities, thermal stress, hypoxia, supersaturation of gases, handling, anesthesia and transportation stress conditions can subsequently cause physiological stress response (Ackerman et al., 2000; Palmisano et al., 2000; Tort, 2011). Many phytochemicals are classified as antioxidants by scavenging oxygen anions (Chakraborty and Hancz, 2011; Citarasu, 2010), (Shepherd et al., 2012; Shaheen et al., 2014a; Shaheen et al., 2014b; Eissa and Wang, 2013) and possess immunostimulating properties (El-Desouky et al., 2012a,b). Current study includes *Glycyrrhiza glabra* and *Astragalus membranaceus*, which are very important herbal plants with many beneficial active ingredients (Wang and Nixon, 2001; Li et al., 2010).

Antioxidant enzymes CAT, SOD, GPx, and LPx are reported to enhance biological systems imbalance (Livingstone, 2001; Madeira et al., 2013; Somogyi et al., 2007; Parihar et al., 1997; Kammer et al., 2011). Current work revealed that, although different stressors significantly decreased SOD activity, tested diets remarkably repaired this decrease and returned it to the control levels. Feeding 1% *G. glabra* the most up-



Panel 1. Liver of Yellow perch in control groups at pre-stress exposure (No stress), post-heat stress exposure (Control Heat) and post-cold stress exposure (Control Cold); and in groups incorporated with 1 and 2% *G. glabra* post-exposure to heat and cold stresses*. Group fed with 2% *G. glabra* incorporated diets showed less (moderate) hepatocellular vacuolization compared with the control group, that revealed marked vacuolization post-exposure to heat (H & E, Scale Bar 50 μm). * 1 and 2 Post Heat/Cold = 1% and 2% *G. glabra*, post- heat/cold stress exposure.



Panel 2. Liver of Yellow perch in control group at pre-stress exposure (No stress) and in groups incorporated with 3% *G. glabra* and 1% *G. glabra-A. membranaceus* post-exposure to heat and cold stresses*. 1% *G. glabra-A. membranaceus* diets revealed a healthier liver with moderate hepatocellular vacuolization and without any signs of necrosis or degenerative changes compared with the control group, that revealed marked vacuolization with degenerative necrosis changes post-exposure to cold (H & E, Scale Bar 50 μm). * 3 and Mix Post Heat/Cold = 3% *G. glabra* and 1% *G. glabra-A. membranaceus* mixture, post- heat/cold stress exposure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

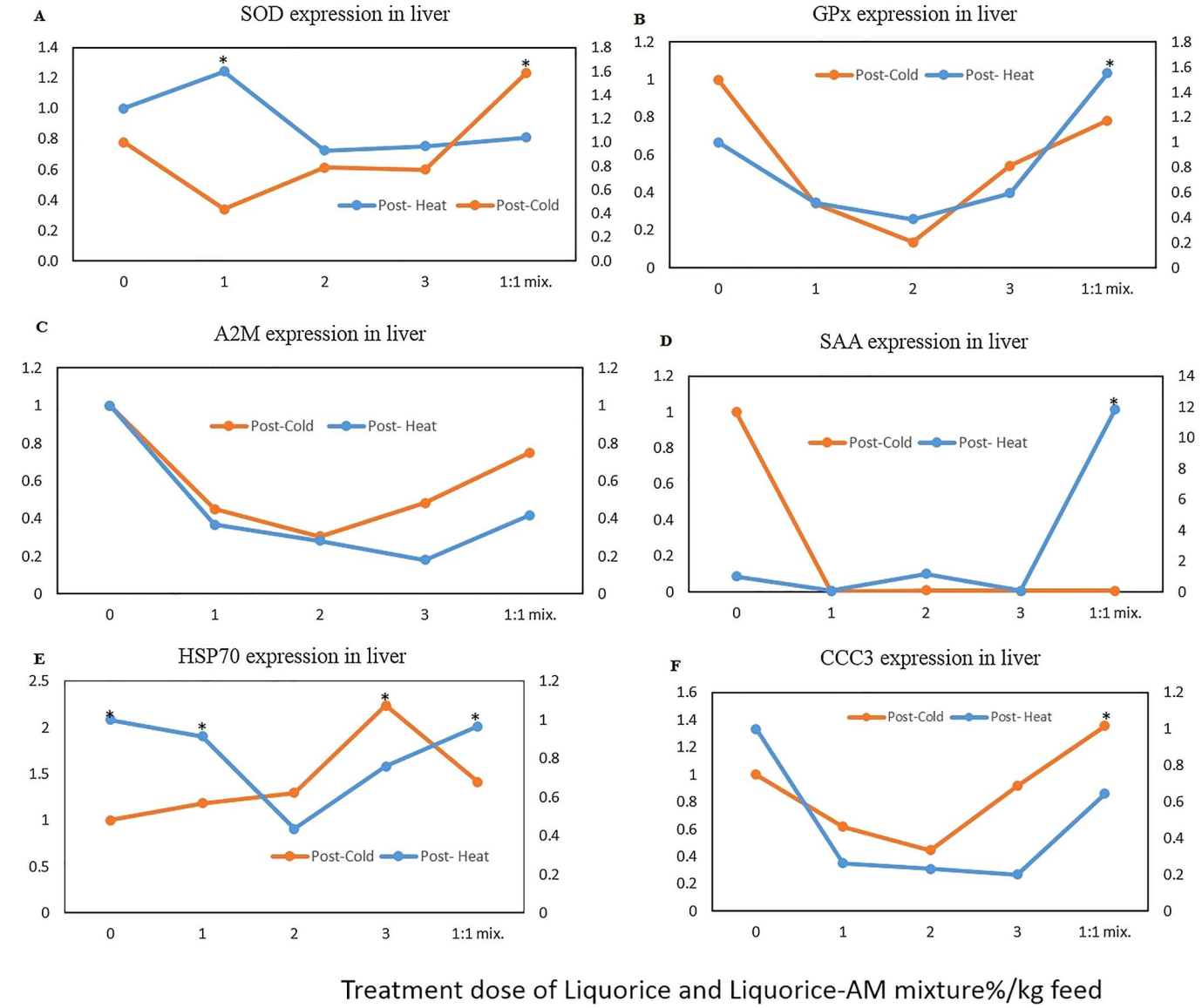


Fig. 7. Expression of SOD (A), GPx (B), A2 M (C), SAA (D), HSP70 (E) and CCC3 (F) genes in yellow perch after challenge with Heat and Cold stressors. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

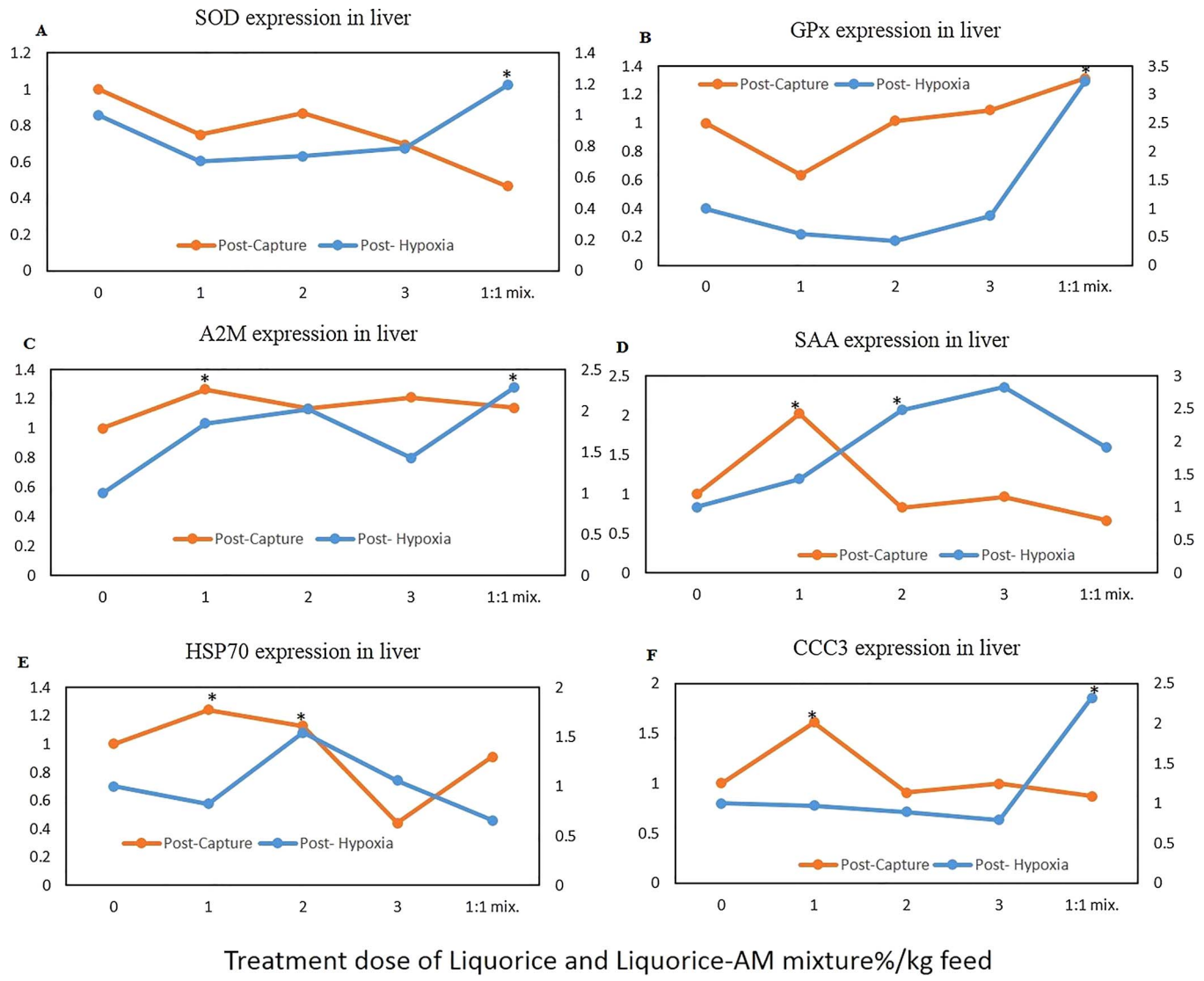


Fig. 8. Expression of SOD (A), GPx (B), A2 M (C), SAA (D), HSP70 (E) and CCC3 (F) genes in yellow perch after challenge with Hypoxia and Capture stressors. Data are mean (n = 9) ± SE. Values with asterisk (*) are significantly different. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulation in GPx activity before exposure to stress. Despite exposure to different stressors and significantly elevated GPx activity, incorporated diets significantly compensated this elevation and returned it to the control levels. In addition, 1% *G. glabra* diets had the most significant increase in CAT activity before exposure to stress. Most stressors significantly decreased CAT activity, and tested diets significantly compensated for this decrease. Furthermore, 1 and 2% *G. glabra* showed the most significant increase in GPx activity before exposure to different stressors, which lead to a significant decrease in LPx activity. Incorporated diets significantly mitigated this with the highest decrease for the 1% Liquorice-AM mixture group post-exposure to heat and hypoxia and for the 1% *G. glabra* group after cold and capture exposure. Our work is supported by previous studies, Yin et al. (2011) reported that (5 μg/ml) pre-treatment and (5 and 10 μg/ml) pre- and post-treatment of common carp hepatocytes with *G. glabra* extract significantly reduced the GOT, LDH, GPT and MDA elevated levels of, and increased the reduced SOD and GSH-Px levels of by carbon tetrachloride. In addition, Zahran et al. (2014) showed that dietary supplementation O. niloticus with APS (1500 mg/kg of diet) upregulated SOD, and GPx activities. Jia et al. (2012) revealed that in vitro supplementation with 200, 400, and 800 mu g/ml APS in carp primary hepatocytes significantly improved cell viability and increased the SOD reduced level of. Also, in vivo administration of 1.5 and 3 g/kg APS for 60 days prior to exposure to CCl4 intoxication markedly increased the SOD reduced levels and total antioxidants. The significant increase in antioxidant enzyme activities can be because of antioxidant effects of APS (Zahran et al., 2014; Jia et al., 2012). Li et al. (2010) and Yan et al. (2009) also have reported the same findings in rats. In addition, the hepatoprotective activities of ASP were found to be associated with its antioxidative activity (Jia et al., 2012; Hattori et al., 1991; Zhang et al., 1995).

Results demonstrated that 1% *G. glabra*-A. *membranaceus* mixture diets showed the highest decrease in ALT activity before and after exposure to all stressors. The lowest AST activity was for the 3% *G. glabra* diet before and after exposure to (Heat, Cold and Hypoxia) stresses and for 2% *G. glabra* group post-exposure to capture stress. These findings can be attributed to the antioxidative and hepatoprotective activities of A. *membranaceus* and *G. glabra* (Guojun Yin, 2011; Jia et al., 2012; Zhang et al., 1995). Our results are similar to Zaki et al. (2012) who reported that feeding *O. niloticus* with 1% fenugreek meal gave the lowest AST and ALT activities.

It is very important to use histological methods to assess the effects of feed on the digestive tract of fish (Rašković* Božidar et al., 2011). Light microscopy of H & E sections showed that after exposure to heat

stress, groups fed with 2% *G. glabra* and 1% *G. glabra*-A. *membranaceus* mixture diets showed less (moderate) hepatocellular vacuolization when compared with the control group. Meanwhile, after exposure to cold stress, the group fed with 1% *G. glabra*-A. *membranaceus* mixture diet revealed a healthier liver with moderate hepatocellular vacuolization, without any signs of necrosis or degenerative changes compared to the control group. Our results came in accordance with Zahran et al. (2014) who revealed that adding Astragalus polysaccharides (APS) at 0.15% to Oreochromis niloticus basal diets did not show any alteration in the number of microvilli between experimental groups, however their length increased in the APS-treated versus control group. In addition, Merrifield et al. (2011) who studied the effect of (5 g/kg^{-1} Ergosan) on intestinal histology of Nile tilapia and demonstrated normal morphology of the intestinal tract. In contrast, an increase in the length of intestinal villus, depth of intestinal crypts, and quantity of goblet cells was detected in APS-treated groups of tilapia (Quanxi et al., 2010). These findings can be attributed to the immunostimulating and hepatoprotective properties of A. membranaceus and *G. glabra* (Guojun Yin, 2011; Jia et al., 2012; Zhang et al., 1995). Similar results were recorded in sea bream (Dimitroglou et al., 2010).

On molecular level, at four weeks after feeding incorporated diets and before exposure to any stressor, our study revealed that the expression of immune-related genes was enhanced by *G. glabra* and *G. glabra*-A. *membranaceus* incorporation. After exposure to heat stress, oxidative and immune response-related gene expression were evaluated. Heat stress caused significant decrease in the expression of those genes. 1% *G. glabra*-A. *membranaceus* groups also showed the most remarked up-regulation in GPx, SOD, and SAA gene expression that was exposed to heat stress without any supplementation. Meanwhile, incorporated diets did not show significant increase in expression of A2M and CCC3 genes. GPx and SOD genes expression showed positive correlation with those enzymes biochemical assays. Following cold stress exposure, 1% *G. glabra*-A. *membranaceus* groups showed the most significant increase in SOD and CCC3 genes expression; and the 3% *G. glabra* group had the most significant increase in HSP-70 gene expression compared to the control. Hypoxia exposure also influenced targeted genes expression, with the 1% *G. glabra*-A. *membranaceus* groups showing the most significant increase in SOD, GPx, A2M and CCC3 genes expression; and the 3% G. *glabra* group had the most significant increase in HSP-70 and SAA gene expression. The 3% *G. glabra* group showed the highest significant increase in HSP-70, A2M, SAA, and CCC3 gene expression compared to the control. Other studies support our results, with hepatic Cd accumulation found to be linked to decreased SOD gene expression in yellow perch (Pierron et al., 2009). In addition, expression ratio of target genes (IL-8, IL-1β and TGF-β) of the head kidney of rainbow trout *Oncorhynchus mykiss* fed with lupin and mango revealed increased expression of genes of interest over control (Awad et al., 2011). In addition, head kidney leucocytes from trout cultured with lipopolysaccharide (LPS) showed an increase in IL-1β expression (Secombes et al., 1999). This may be related to the antioxidant effects of APS (Zahran et al., 2014; Jia et al., 2012). Li et al. (2010) and Yan et al. (2009) also have reported the same findings in rats, in addition to ASP hepatoprotective activities (Jia et al., 2012; Hattori et al., 1991; Zhang et al., 1995). In conclusion, the present study showed that dietary incorporation of *G. glabra* and *A. membranaceus* improved immune response, and the majority of most pronounced antioxidant profiles in Yellow perch revealing their possible usage as natural antioxidants.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by United States Department of Agriculture (Agreement No 2010-38879-20946), and Egyptian Missions sector, Ministry of Higher Education and Scientific Research. Salaries were provided by state and federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center. Authors wish to thank Dean Rapp, and Paul O'Bryant for maintaining experimental fish and conditions through the entire experiment, and Joy Bauman for her comments on the manuscript.

References

Ackerman, P.A., Forsyth, R.B., Mazur, C.F., Iwama, G.K., 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiol. Biochem. 23, 327–336.
An, K.W., Kim, N.N., Shin, H.S., Kil, G.S., Choi, C.Y., 2010.. Profiles of antioxidant gene expression and physiological changes by thermal and hypoosmotic stresses in black porgy (Acanthopagrus schlegeli). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 156, 262–268.
Austin, B., Austin, D.A., 1989. Methods for the Microbiological Examination of Fish and Shellfish. 1951.
Awad, E., Mitchell, W.J., Austin, B., 2011. Effect of dietary supplements on cytokine gene expression in rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish J. Fish Dis. 34, 629–634.
Bowden, T.J., 2008. Modulation of the immune system of fish by their environment. Fish Shellfish Immunol. 25, 373–383.
Brown, T.G., Runciman, B., Bradford, M.J., Pollard, S., 2009. A biological synopsis of yellow perch *Perca flavescens*. Can. Manuscr. Rep. Fish. Aquat. Sci. 2883 (i–v), 1–28.
Chakraborty, S.B., Hancz, C., 2011. 2011. Application of phytochemicals as immunostimulant: antipathogenic and antistress agents in finfish culture. Rev. Aquacult. 3, 103–119.
Citarasu, T., 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Ind. 18, 403–414.
Dimitroglou, A., Merrifield, D.L., Spring, P., Sweetman, J., Moate, R., Davies, S.J., 2010. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (Sparus aurata). Aquaculture 300, e182–e188.
Eissa, N., Wang, H., 2013. Physiological stress response of yellow perch subjected to repeated handlings and salt treatments at different temperatures. N. Am. J. Aquacult. 75, 449–454.
El-Desouky, H., Abbass, A., El-Asely, A., Gwad, E.A., Shaheen, A.A., 2012a. Effect of Zingiber officinalis and Cyanodon dactylon against pH stress in giant freshwater prawn Macrobrachium rosenbergii. In: Presented at the Proceedings of the 5th Global Fisheries and Aquaculture Research Conference. Faculty of Agriculture, Cairo University, Giza, Egypt, 1–3 Massive Conferences and Trade Fairs. pp. 286–298.
El-Desouky, H., El-Asely, A., Shaheen, A.A., Abbass, A., 2012b. Effects of Zingiber officinalis and Cyanodon dactylon on the Growth Performance and Immune Parameters of Macrobrachium rosenbergii. World J. Fish Mar. Sci. 4 (3), 301–307.
Elabd, H., Wang, H.-P., Shaheen, A., Yao, H., Abbass, A., 2016a. Astragalus membranaceus (AM) enhances growth performance and antioxidant stress profiles in bluegill sunfish (Lepomis macrochirus). Fish Physiol. Biochem. 1–12. http://dx.doi.org/10.1007/s10695-015-0188-9.
Elabd, H., Wang, H.-P., Shaheen, A., Yao, H., Abbass, A., 2016b. Feeding *Glycyrrhiza glabra* (liquorice) and *Astragalus membranaceus* (AM) alters innate immune and physiological responses in yellow perch (*Perca flavescens*). Fish Shellfish Immunol. 54, 374–384. http://dx.doi.org/10.1016/j.fsi.2016.04.024.
Galina, J., Yin, G., Ardó, L., Jeney, Z., 2009. The use of immunostimulating herbs in fish. An overview of researchh. Fish Physiol. Biochem. 35, 669–676.
Guojun Yin, L.C., 2011. Hepatoprotective and antioxidant effects of Glycyrrhiza glabra extract against carbon tetrachloride (CCl(4))-induced hepatocyte damage in common carp (Cyprinus carpio). Fish Physiol. Biochem. 37, 209–216. http://dx.doi.org/10.1007/s10695-010-9436-1.
Halliwell, B., 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 344, 721–724.
Hattori, T., Ito, M., Suzuki, Y., 1991. Studies on antinephritic effects of plant components in rats. (1). Effects of saikosaponins original-type anti-GBM nephritis in rats and its mechanisms. Folia Pharmacol. Jpn. 97, 13–21.
Jia, R., Cao, L., Xu, P., Jeney, G., Yin, G., 2012. In vitro and in vivo hepatoprotective and antioxidant effects of Astragalus polysaccharides against carbon tetrachloride-induced hepatocyte damage in common carp (Cyprinus carpio). Fish Physiol. Biochem. 38, 871–881. http://dx.doi.org/10.1007/s10695-011-9575-z.
Kamei, J., Nakamura, R., Ichiki, H., Kubo, M., 2003. Antitussive principles of Glycyrrhizae radix, a main component of the Kampo preparations Bakumondo-to (Mai-men-dongtang). Eur. J. Pharmacol. 469, 159–163.
Kammer, A.R., Orczewska, J.I., O'Brien, K.M., 2011. Oxidative stress is transient and tissue specific during cold acclimation of three spine stickle back. J. Exp. Biol. 214, 1248–1256. http://dx.doi.org/10.1242/jeb.053207.
Kim, S.C., Byun, S.H., Yang, C.H., Kim, C.Y., Kim, J.W., Kim, S.G., 2004. Cytoprotective effects of Glycyrrhizae radix extract and its active component liquiritigenin against cadmium-induced toxicity (effects on bad translocation and cytochrome c-mediated PARP cleavage). Toxicol 197, 239–251.
Li, R., Chen, W., Wang, W., Tian, W., Zhang, X., 2010. Antioxidant activity of Astragalus polysaccharides and antitumour activity of the polysaccharides and siRNA. Carbohydr. Polym. 82, 240–244. http://dx.doi.org/10.1016/j.carbpol.2010.02.048.
Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666. http://dx.doi.

org/10.1016/S0025-326X(01)00060-1.
Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2013. Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 166, 237–243. http://dx.doi.org/10.1016/j.cbpa.2013.06.008.
Merrifield, D.L., Harper, G.M., Mustafa, S., Carnevali, O., Picchietti, S., Davies, S.J., 2011. Effect of dietary alginic acid on juvenile tilapia (Oreochromis niloticus) intestinal microbial balance, intestinal histology and growth performance. Cell Tissue Res. 344, e135–e146.
Palmisano, A.N., Winton, J.R., Dickhoff, W.W., 2000. Tissue specific induction of hsp90 mRNA and plasma cortisol response in Chinook salmon following heat shock seawater challenge, and handling challenge. Mar. Biotechnol. 2, 329–338.
Parihar, M.S., Javeri, T., Hemnani, T., Dubey, A.K., Prakash, P., 1997. Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the freshwater catfish (Heteropneustes fossilis) to short-term elevated temperature. J. Therm. Biol. 22, 151–156. http://dx.doi.org/10.1016/S0306-4565(97)00006-5.
Pierron, F., Bourret, V., St-Cyr, J., Campbell, P.G.C., Bernatchez, L., Couture, P., 2009. Transcriptional responses to environmental metal exposure in wild yellow perch (Perca flavescens) collected in lakes with differing environmental metal concentrations (Cd, Cu, Ni). Ecotoxicol. Lond. Engl. 18, 620–631. http://dx.doi.org/10.1007/s10646-009-0320-7.
Quanxi, H.Y., Wang, L.X., Jianqiang, X., Jiaming, C., Jin, Z., Shugen, L.I.N., 2010. Effects of Astragalus polysaccharide on structure of intestinal villus and intestinal immunocyte of tilapia. Chin. J. Anim. Nutr. 1, 023.
Rašković* Božidar, S., Marko Stanković, B., Zoran Marković, Z., Vesna Poleksić, D., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agric. Sci. 56, 87–100.
Secombes, C.J., Bird, S., Cunningham, C., Zou, J., 1999. Interleukin −1 in fish. Fish Shellfish Immunol. 9, 335–343.
Shaheen, A.A., Eissa, N., Abou-Elgheit, E.N., Yao, H., Wang, H.-P., 2014a. Effect of probiotic on growth performance and growth-regulated genes in yellow perch (Perca flavescens). Glob. J. Fish. Aquacult. Res. 1, 01–15.
Shaheen, A.A., Eissa, N., Abou-Elgheit, E.N., Yao, H., Wang, H.-P., 2014b. Probiotic effect on molecular antioxidant profiles in yellow perch, Perca flavescens. Glob. J. Fish. Aquacult. Res. 1 (2), 16–29. http://dx.doi.org/10.13140/2.1.3260.3201.
Shepherd, B.S., Rees, C.B., Binkowski, F.P., Goetz, F.W., 2012. Characterization and evaluation of sex-specific expression of suppressors of cytokine signaling (SOCS)-1 and −3 in juvenile yellow perch (Perca flavescens) treated with lipopolysaccharide. Fish Shellfish Immunol. 33, 468–481.
Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z., Nagy, G., 2007. Antioxidant measurements. Physiol. Meas. 28, R41–R55. http://dx.doi.org/10.1088/0967-3334/28/4/R01.
Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375.
Wang, Z.Y., Nixon, D.W., 2001. Licorice and cancer. Nutr. Cancer 39, 1–11.
Yan, F., Zhang, Q.-Y., Jiao, L., Han, T., Zhang, H., Qin, L.-P., Khalid, R., 2009.. Synergistic hepatoprotective effect of Schisandrae lignans with Astragalus polysaccharides on chronic liver injury in rats. Phytomedicine 16, 805–813. http://dx.doi.org/10.1016/j.phymed.2009.02.004.
Yin, G., Cao, L., Xu, P., Jeney, G., Nakao, M., Lu, C., 2011. Hepatoprotective and antioxidant effects of Glycyrrhiza glabra extract against carbon tetrachloride (CCl4)-induced hepatocyte damage in common carp (Cyprinus carpio). Fish. Physiol. Biochem. 37, 209–216.
Zahran, E., Risha, E., AbdelHamid, F., Mahgoub, H.A., Ibrahim, T., 2014. Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol. 38, 149–157. http://dx.doi.org/10.1016/j.fsi.2014.03.002.
Zaki, M.A., Labib, E.M., Nour, A.M., Tonsy, H.D., Mahmoud, S.H., 2012. Effect some medicinal plants diets on mono sex nile tilapia (Oreochromis niloticus), growth performance, feed utilization and physiological parameters. APCBEE Procedia 4, 220–227. http://dx.doi.org/10.1016/j.apcbee.2012.11.037. 2nd International Conference on Asia Agriculture and Animal (ICAAA).
Zhang, Y., Ming, L., Li, W., Zhang, H., 1995. Studies on protective effects of Astragalus polysaccharide (APS) against experimental Liver injuries in mice? Acta Universitatis Medicinalis Anhui 30 (3), 182–184.