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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

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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).

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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	