Effect of β-Glucan on cold-stress resistance of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878)

S. Soltanian[1], M.N. Adloo[2], M. Hafeziyeh[3], N. Ghadimi[2]

[1]School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[2]Islamic Azad University, Bandar Abbas, Iran
[3]Iranian Fisheries Research Organization, Tehran, Iran

ABSTRACT: These experiments were performed to determine the effects of dietary β-glucan on stress responses of striped catfish (*Pangasianodon hypophthalmus*). Fish were fed for nine weeks with a diet containing 0 (control), 0.5% (G1), 1% (G2) and 2% (G3 group) β-glucan. Subsequently, stress responses were studied by evaluating serum cortisol and glucose levels following a constant 24 h cold shock (from 28 °C to 15 °C). Serum cortisol and glucose concentrations were measured after cold treatments of varying durations (prior to, and after one, 12 and 24 h of cold shock stress, respectively). No differences in serum cortisol and glucose levels were found between control and β-glucan-treated fish. However, the mortality rate was significantly lowered in cold challenged fish fed appropriate doses of β-glucan (in G1 and G2 vs. G3 and control group). The results of the present study demonstrate that a proper administration of β-glucan in the diet could ameliorate the detrimental effects of a severe stress resulting in a reduction in fish mortality.

Keywords: β-glucan; *Pangasianodon hypophthalmus*; physiological response

Physiological stress is the primary contributing factor to fish disease and mortality in aquaculture. Stress is defined as any biological, physical or chemical factor that causes bodily reactions that may contribute to disease and death.

Among the natural stressors that fish can experience throughout their life cycle are thermal changes. Fluctuations in water temperature either resulting from a transient (daily change) or seasonal change are associated with disease and mortality in catfish (Ju et al. 2002). Cold-shock stress occurs when a fish has been acclimated to a specific water temperature or range of temperatures and is subsequently exposed to a rapid decrease in temperature, resulting in a cascade of physiological and behavioural responses and, in some cases, death.

To deal with environmental change, fish respond by altering physiological functions including those associated with the stress response (Barton and Iwama 1991; Barton 2002). The physiological stress response in fish is mediated by the neuroendocrine system and includes the release of hormones such as cortisol and adrenaline (Barton and Iwama 1991). In response to most stressors fish will exhibit an increase in plasma cortisol concentrations, which is generally followed by an elevation in plasma glucose levels.

Temperature shock can have negative effects for fish by reducing metabolic rates (Galloway and Kieffer 2003), affecting swimming performance (Hocutt 1973), and by impairing immune functions (Hurst 2007), thus leading to a reduced ability to capture prey, increased susceptibility to disease and enhanced mortality (Donaldson et al. 2008). Temperature shock can also impede predator avoidance (Ward and Bonar 2003), alter rates of recovery from exercise (Hyvarinen et al. 2004; Suski et al. 2006), and disrupt homeostasis (Galloway and Kieffer 2003; Suski et al. 2006; Vanlandeghem et al. 2010).

It is, therefore, important to enhance the tolerance against various stressors (e.g. thermal stress) which target cultured fish (Yokoyama et al. 2005). Recent studies have indicated that immunostimulants, isolated from plants, animals and microorganisms (Sakai 1999) can, when administered in stressful situations, ameliorate the deleterious effects mediated by stressors (Ortuno et al. 2003; Sarma et al. 2009). In fact, as commercially important fish are inevitably grown in stressful conditions, it is important to boost immune function through immunostimulants and to so enhance their resistance and tolerance to diseases and unfavourable environmental conditions (Yokoyama et al. 2005).

β-Glucans are the most commonly applied immunostimulants in aquaculture (Soltanian et al. 2009; Kiron 2012). Under intensive farming, their anti-stress characteristics are of immense use without posing any environmental hazard (Maqsood et al. 2011). Therefore, they have been extensively used to reduce the negative effects of stress, increase disease resistance, and improve various physiological parameters (e.g. growth and feed conversion rate) (Cain et al. 2003; Shelby et al. 2007; Welker et al. 2007).

Pangasius catfishes play an important role in Asian aquaculture and commercial fishing (Ling 1977). *Pangasianodon hypophthalmus* formerly referred to as *Pangasius sutchi* and/or *Pangasius hypophthalmus* is native to the Chao Phraya River in Thailand and the Mekong in Vietnam. It is found abundantly in the Amazon River, in parts of Russia and in other places of the world under different names (Abbas et al. 2006). Moreover, fingerlings of the species are often collected and transported to pet fish shops in several countries (Baska et al. 2009).

This species is emerging as a promising candidate for aquaculture purposes particularly outside of tropical regions of South East Asia, as it can be successfully cultured in the western tropics. However, development of this catfish culture industry has faced difficulties partly related to the limited knowledge of the biology, ecology, and physiology of these fish (Hung et al. 2003).

In the present study, the effect of dietary β-glucan on the stress response in tropical fish during a 24 h exposure to a severe cold stress (13 °C decrease in water temperature) was investigated. The levels of cortisol and glucose and mortality rate prior to and after different intervals of cold stress (one, 12 and 24 h cold shock) were studied in fish either treated or untreated (control) with β-glucan. No recovery was appointed in this study.

MATERIAL AND METHODS

Experimental diets. A practical diet (obtained from CP commercial diet Malaysia with composition details in Table 1 was supplemented with β-glucan (Macrogard Biotec-Mackzymal, Norway) at the rates of 0% (Control), 0.5% (G1), 1% (G2) and 2% (G3 group), respectively. Determined dosages of β-glucan were mixed with feed for 20 min, and dried and stored at 4 °C in a glass jar until used (Jeney et al. 1997; Sahan and Duman 2010).

Table 1. Proximate chemical composition of basic experimental diet

Feed proximate composition	%
Dry mater	91.6
Protein	29.27
Fat	6.4
Ash	10.66
Carbohydrate	45.27

Experimental design. Juvenile pangasius catfish (average initial weight 1.27 ± 0.24 g and initial length 5.55 ± 0.45 cm) were purchased from a local commercial pet fish shop and held in a 1000 l glass tank for three weeks to allow acclimation to the experimental conditions. At the beginning of the experiment, the fish were fasted for 24 h and then weighed. Fish of similar sizes were randomly distributed into 12 glass tanks (150 l), and each tank was stocked with 35 fish (three replicates per each treatment (cold shock and control)).

Fish were handfed with experimental diets at 2–3% of body weight to apparent satiation twice daily for nine weeks. Water temperature (28.42 ± 0.67 °C), pH (7.88 ± 0.07) and dissolved oxygen (4.80 ± 0.29) were constant throughout this period.

Stress tests and sampling. At the end of the experiment (Day 63), 25 fish from each rearing tank were randomly removed and directly transferred to 150 l tanks in which the water temperature decreased by 15 °C by adding ice to the tanks. An YSI model 55 probe was used during the cold shock to monitor water temperature and dissolved oxygen concentration. To account for handling procedures, fish from all treatments (β-glucan-supplemented and control groups) were transferred to tanks with the same initial water temperature (28.42 ± 0.67 °C). Food was withheld 24 h before the onset of the cold shock.

Prior to, and after one, 12 and 24 h of cold stress, fish were sampled from each group and anesthetised with clove oil (50 mg/l). Blood samples were collected immediately after caudal vein amputation and transferred into sterile tubes and allowed to clot at room temperature for 1 h and then kept at 4 °C for 5 h. Then, serum was separated by centrifugation at 3000g for 10 min and stored at –20 °C until required.

Assays for determination of stress. Serum cortisol levels were measured by radioimmunoassay (RIA) according to Rottlant et al (2001) and expressed as ng/ml.

The quantitative determination of glucose was carried out using commercially available diagnostic experimental protocol kits (Pars Azmun, Iran, 1 00 0178) (Hoseini and Hoseini 2010), at 546 nm and 37 °C according to the glucose oxidase method suggested by Trinder (1969). All measurements were made in triplicate.

Statistical analysis. Data were analysed by one-way analysis of variance (ANOVA) using the statistical software SPSS, version 16.0. Significant differences between means were delineated using the Duncan test. $P < 0.05$ was considered significant.

RESULTS

No differences in serum cortisol or glucose levels were found between fish from control and β-glucan-treated groups at the different time points of sampling (Figures 1 and 2).

Neither 1 h cold shock treatment nor handling stress alone caused any mortality in any of the experimental groups (Figure 3).

In all treatments, irrespective of beta-glucan supplementation, the highest mortality was observed over the first 12 h of cold shock. In addition, except for G3, β-glucan-treated fish exhibited a lower mortality rate compared to untreated fish. The lowest mortality rates were observed equally in both the G1 and G2 treatment groups, which were significantly different from G3 and from the control group ($P < 0.05$)(Figure 3).

DISCUSSION

Despite no significant changes in physiological parameters (cortisol and glucose values) observed in any of the experimental groups, appropriate dosages of β-glucan could significantly decrease the mortality rates in cold-challenged fish especially after the second half of a 24 h cold shock (Figure 3).

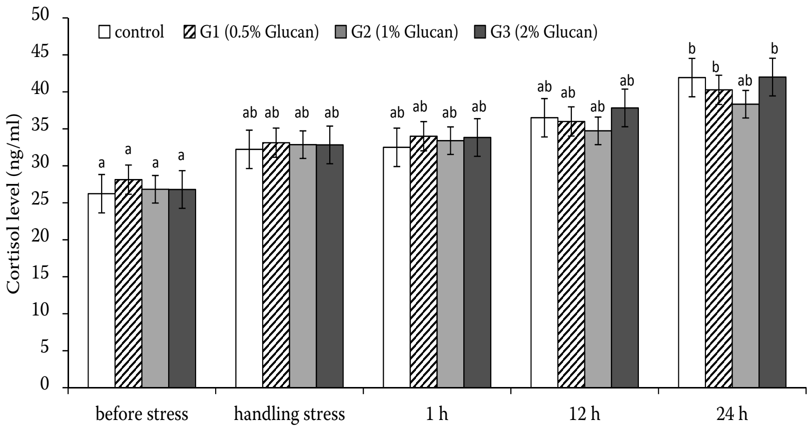

Figure 1. Serum cortisol levels in Pangasius catfish fed different dosages of β-glucan prior to, and after one, 12 and 24 h of cold stress. Data are expressed as mean ± SE. Significant differences between values are indicated by different letters

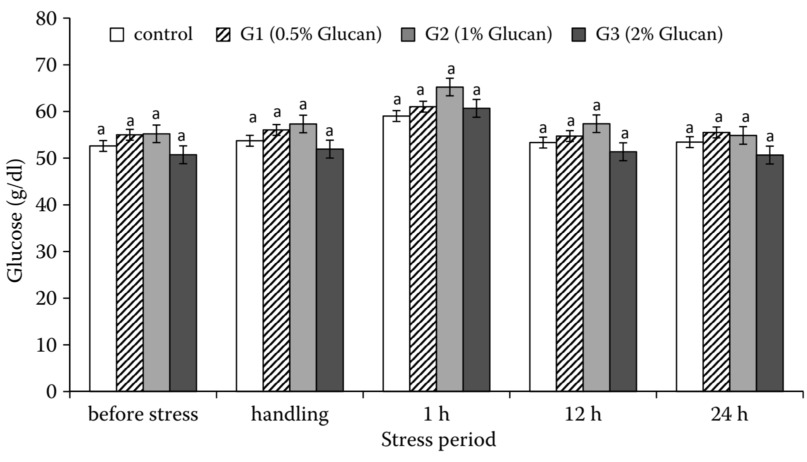

Figure 2. Serum glucose concentrations in Pangasius catfish fed different doses of β-glucan and sampled prior to, and after one, 12 and 24 h of cold stress. Data are expressed as mean ± SE. Significant differences between values are indicated by different letters

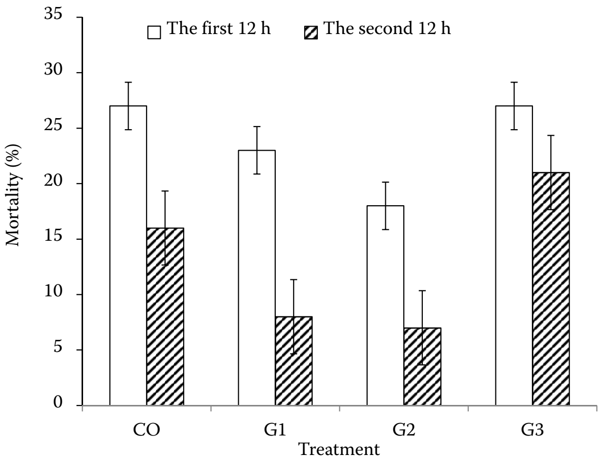

Figure 3. Mortality rates over the first and second half of a 24h cold shock stress imposed on striped catfish fed different dosages of β-glucan. Data are expressed as mean ± SE

In spite of the extensive use of cortisol and glucose as two of the main fish stress indicators (Tanck et al. 2000; Hsieh et al. 2003), the effectiveness and reliability of these parameters is now questioned. In fact, some inconsistencies have been reported in the results of several experimental studies, much of them associated with undefined and uncontrolled variables, which may alter the secretion of cortisol and glucose into the bloodstream. Most of these factors are not considered as direct stressors but have an effect on the intensity of the response which makes them a source of error (Kawauchi et al. 1984; Davis and Parker 1990; Vijayan and Leatherland 1990; Lamers et al. 1991; Sun et al. 1992; Sun et al. 1995; Reid et al. 1998; Wilson et al. 1998; Arends et al. 1999; Fevolden et al. 1999; Mommsen et al. 1999; Pottinger et al. 1999; Arends et al. 2000; Grutter and Pankhurst 2000; Tanck et al. 2000; Iwama et al. 2004; Koldkjær et al. 2004; Davis and Peterson 2006; Iwama 2007; Inoue et al. 2008; Martinez-Porchas et al. 2009; Gholipour Kanani et al. 2011).

Mass mortality of fish was observed following a sudden cold shock (15 °C decreases in water temperature from 27 °C to 12 °C over 24 h) in matrinxã (*Brycon cephalus*). In addition, a sudden 12 °C decrease in water temperature (from 27 °C to 15 °C over 24 h) caused 20% mortality in this fish species. Comparable to matrinxã, striped catfish are warm-water fish typically living within the temperature range of 22–28 °C. In the present study, the highest mortality occurred over the first half of a 24 h cold shock stress in all treatment groups. However, the rate of mortality significantly decreased by the end of the second half, likely due to a long-term acclimation to lower temperature mediated by a stress response which could, however, not be detected at the endocrine level. In fact, a lack of response would evidence the inability to adapt to cold, which could eventually lead to fish death. Indeed, mass mortality of matrinxã due to a sudden decrease of water temperature has been previously reported in aquaculture facilities close to subtropical areas (Inoue et al. 2008). In contrast, the partial mortality observed in the control group indicated that fish were indeed stressed despite the lack of an endocrine response.

As mentioned above, in aquaculture immunostimulants are usually administered to enhance stress resistance thus reducing mortalities during stressful situations.

In the current study, β-glucan supplementation at concentrations of 0.5% or 1% (G1 and G2, respectively), resulted in significantly lower mortality over the second half of the cold shock period. However, at a higher dosage (2%), fish mortality was not reduced. The immunomodulatory effects of glucans are not unequivocal and have been shown to vary depending on fish species, type and doses, route of administration, and the association with other immunostimulants (Couso et al. 2003; Bridle et al. 2005; Del Rio-Zaragoza et al. 2011; Jaafar et al. 2011).

It was suggested that the effect of β-glucan on stress resistance is markedly affected by dose and duration of the experiment (Jeney et al. 1997; Volpatti et al. 1998; Bagni et al. 2005; Selvaraj et al. 2005). For instance, various studies demonstrated that overdoses of β-glucan could even induce immunosuppression (Raa 1996; Jeney et al. 1997; Sakai 1999; Cook et al. 2001). This might be the case for the G3 treatment group where incorporation of 2% β-glucan into the diet increased mortality compared to control.

In conclusion, despite an apparent low-level endocrine response to cold stress in striped catfish, which may be related to their evolutionary history, neuroendocrine mechanisms involved in corticosteroid responses, or the anatomy of their interrenal tissue, an appropriate administration of β-glucan in the diet can ameliorate the detrimental effects of a severe stress, thus resulting in lower fish mortality.

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Corresponding Author:

Siyavash Soltanian, Shiraz University, School of Veterinary Medicine, Aquatic Animal Health and Diseases Department, Shiraz, Iran

Tel. +989173364632, Fax +987112286940, E-mail: siyavashsoltanian@yahoo.com