Effects of long term dietary administration of β-Glucan on the growth, survival and some blood parameters of striped catfish, *Pangasianodon hypophthalmus* (Siluriformes: Pangasiidae)

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Abstract: The present experiment was undertaken to study the effect of different dosages of β-glucan on the growth, survival and physiological responses in juvenile striped catfish (*Pangasianodon hypophthalmus*). Over nine weeks of feeding fish with a diet containing 0, 0.5, 1 and 2% β-Glucan (as control, G1, G2 and G3 group, respectively), some blood parameters such as serum lysozyme activity, total protein and glucose values as well as fish survival rate and growth performance were determined. No significant differences were observed either in the growth and survival indexes or in the glucose value in fish fed with and/or without different ratios of β-glucan. However, highest serum lysozyme activity was detected in fish fed on a diet containing 0.5% of β-glucan (10.8 µg/ml serum vs 8.00 µg/ml serum, in control group). In addition, the protein content was significantly increased in all β-glucan treatments compared to control. Thus, the administration of β-glucan might be beneficial for health enhancement in striped catfish juveniles.

Keywords: Immunostimulants, Lysozyme, Physiological responses.

Introduction

Pangasius catfishes play an important role in Asian aquaculture and commercial fishing (Ling 1977). *Pangasianodon hypophthalmus* (Sauvage, 1878) formerly referred to as *Pangasius sutchi* Fowler, 1937 and *Pangasius hypophthalmus* (Sauvage, 1878) is limited to the Mekong River, the Chaopraya River and possibly the Mekong basins in Cambodia, Lao People's Democratic Republic, Thailand and Vietnam, together with the Ayeyawady basin of Myanmar. The species has a variety of common English names including Sutchi catfish, iridescent shark-catfish, and striped catfish. It is abundantly available in the Amazon River, in parts of Russia and in other places of the world under different names (Abbas et al. 2006). The fish is extensively cultured by commercial fish farms in Vietnam, Thailand, Malaysia, China, Indonesia, India, Bangladesh and Myanmar (Roberts et al. 1990; Islam et al. 2008; Dung et al. 2008; Singh et al. 2011; Abbas et al. 2006; Islam et al. 2006; Rahman et al. 2006)

Moreover, fingerlings of the species are often collected and transported to pet fish shops of several countries (Baska et al. 2009). Nowadays, this species emerged as a promising potential for aquaculture purposes particularly outside of tropical regions of south-east Asia, which 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 biology, ecology, and physiology reported in some cultivated stocks (Hung & Storebakken 1994).

Recent studies have indicated that immunostimulants, isolated from plants, animals and
Microorganisms (Sakai 1999) even applied in stressful situations, can reverse the deleterious effects mediated by stress (Ortuno et al. 2003; Sarma et al. 2009). Beta-glucans are the most commonly applied immunostimulants in aquaculture (Soltanian et al. 2009; Kiron 2012). Under intensive farming, the anti-stress characteristics of beta-glucans could be 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 diseases resistance, and improve various physiological performances (e.g. growth and feed conversion rate) (Cook et al. 2003; Cain et al. 2003; Shelby et al. 2007; Welker et al. 2007). However, little is known about how long the non-specific defense mechanisms of fish can be immunostimulated without posing harmful effects including immunosuppression and increased diseases susceptibility (Siwicki et al. 1990). More specifically, the effect of long-term administration of beta-glucans is not fully understood. Hence, the current study was undertaken to investigate the effects of prolonged application of beta-glucan on the immunity, growth performance and survival of striped catfish juveniles.

**Materials and Methods**

**Experimental diets:** A practical diet (obtained from CP commercial diet, Malaysia with proximate chemical composition details in Table 1) was supplemented with beta-Glucan (Macrogard Biotec-Mackzynal, Norway) at the rates of 0% (Control), 0.5% (G1), 1% (G2) and 2% (G3 group). Determined doses of glucan were mixed with feed for 20 min, pelleted, dried and stored at 4°C in a glass jar until used (Sahan & Duman 2010).

**Experimental design:** Juvenile pangasius catfish (with average initial weight of 1.27±0.24g and initial length of 5.55±0.45cm) were purchased from a local commercial pet fish shop and held in 1000 L glass tank for three weeks to be acclimated to the experimental conditions. In the beginning of the experiment, the fish were fasted for 24h and then weighed. Fish of similar sizes were randomly distributed into 12 glass tanks (150L), and each tank was stocked with 35 fish (3 replicates per each treatment). For a period of 9 weeks, fish were handfed twice daily with experimental diets at 2-3% of body weight. Water temperature (28.42±0.67°C), pH (7.88±0.07) and dissolved oxygen (4.80±0.29 mg/l) were constant throughout this period. Growth performance of catfish fed with different level of beta-Glucan incorporated diets was considered by calculating weight gain (WG), specific growth rate (SGR), daily growth index (DGI) and feed efficiency ratio (FER).

\[
\text{WG} (%) = \left(\frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}}\right) \times 100
\]

\[
\text{SGR} (%) = \left(\frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{days}}\right) \times 100
\]

\[
\text{DGI} = 100 \times \left(\frac{\text{Final body weight}^{\frac{1}{3}} - \text{Initial body weight}^{\frac{1}{3}}}{\text{Time days}}\right)
\]

\[
\text{FER} = \frac{\text{Weight gain (g)}}{\text{dry feed fed (g)}}
\]

**Physiological assays:** Immediately, one day before starting the experiment and at the end of feeding period (day 63), 3 fish from each tank were sampled and anesthetized 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 1h and then kept at 4°C for 5h. Afterwards, serum was separated by centrifugation at 3000g at 4°C for 10min and stored at -20°C until required.

Serum lysozyme activity was determined according to the method of Demers & Bayne (1997),
based on the lysis of the lysozyme sensitive gram positive bacterium, *Micrococcus lysodeikticus* (Sigma, MO, USA). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20µg/ml (in 0.1M phosphate citrate buffer, pH; 5.8) were considered as the standard. This along with the undiluted serum sample (25µl) were placed into wells of a 96-well plate in triplicate. One hundred and seventy-five µl of M. *lysodeikticus* suspension (75 µg/ml) prepared in the same buffer, was then added to each well. After rapid mixing, changes in turbidity were measured every 30s for 5min at 450nm at approximately 20°C using a microplate reader (Biorad, USA). The equivalent unit of activity of the sample as compared to the standard were determined and expressed as µg/ml serum.

The quantitative determination of glucose was carried out according to the glucose oxidase method suggested by Trinder (1969) using commercially available diagnostic Experimental Protocols kits (Pars Azmun, Iran, 1 500 0178) (Hoseini et al. 2011). The plasma total protein level was determined by Bradford method (Kruger 1996).

**Statistical analysis:** Data were analyzed by One-Way analysis of variance (ANOVA) followed by least significant differences (Duncan) test using SPSS, version 11.0. All the measurements were made in triplicate. Correlation coefficients were significant with *P*<0.05.

**Results**

Average weight gain (WG), specific growth rate (SGR), daily growth index (DGI), feed efficiency

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WG a</th>
<th>SGR b</th>
<th>DGI c</th>
<th>FER d</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>12.08±2.30 a</td>
<td>3.25±0.27 a</td>
<td>8.49±1.68 a</td>
<td>88.88±2.14 a</td>
<td>98.33 a</td>
</tr>
<tr>
<td>G2</td>
<td>13.34±0.25 a</td>
<td>3.37±0.05 a</td>
<td>9.22±0.32 a</td>
<td>89.90±0.32 a</td>
<td>97.67 a</td>
</tr>
<tr>
<td>G3</td>
<td>13.92±2.49 a</td>
<td>3.48±0.27 a</td>
<td>10.15±1.95 a</td>
<td>90.53±1.83 a</td>
<td>98.67 a</td>
</tr>
<tr>
<td>CO</td>
<td>12.71±3.23 a</td>
<td>3.21±0.44 a</td>
<td>8.40±2.56 a</td>
<td>88.35±3.72 a</td>
<td>92.33 a</td>
</tr>
</tbody>
</table>

**Table 1.** Growth performance and survival rate of Sutchi catfish over a 63 days feeding on diets containing various levels of β-glucan. Weight Gain (WG); Specific Growth Rate (SGR), Daily Growth Index (DGI), Feed Efficiency Ratio (FER). G1; fish fed with 0.5% β-glucan; G2; fish fed with 1% β-glucan; G3; fish fed with 2% β-glucan; CO; fish fed with diet without β-glucan. Different letters over bars represent significant difference in each column (*p*Tukey<0.05).

![Fig.1](image1.png)

**Fig.1.** Glucose content (g/dl) in fish fed with and/or without (control) various dosages of β-glucan over a 9 week feeding trial (Mean±S.D., n=6). Bars bearing the same superscript letters are not significantly different (*p*Tukey>0.05).

![Fig.2](image2.png)

**Fig.2.** Total protein content (g/dl) in fish fed with and/or without (control) with various dosages of β-glucan over a 9 week feeding trial. (Mean±S.D., n=6). Bars bearing the same superscript letters are not significantly different (*p*Tukey>0.05).
The current data showed that growth and survival parameters were not affected by any dietary dosage of β-gluca.

Conversely, Misra et al. (2006) reported an enhancing effect of β-gluca on immunity, growth and survival of *Labeo rohita* fingerlings. Moreover, significant improvements in weight gain, specific growth rate and feed conversion ratio was noted in mirror carp fed diets containing 1% and 2% MacroGard in comparison to fish fed both the control and the 0.1% MacroGard containing diet (Kühlwein et al. 2013).

Data showing that serum glucose concentration was not affected by any β-gluca supplementation ratio. These findings are broadly in accordance with the study of Misra et al. (2006), who found no effect of feeding different β-gluca ratios on Rohu carp serum glucose concentration. On the other hand, a reduction in serum glucose following β-gluca administration has also been reported (Ahmad et al. 2009). It is speculated that decreases or no changes in serum glucose is probably due to increased intestinal viscosity that is an outcome of ingestion of β-gluca containing diets, which resulted in slow absorption of glucose in the blood stream (Ahmad et al. 2009).

In agreement with a number of studies (Siwicki et al. 1994; Misra et al. 2006; Sych et al. 2013), serum total protein concentration was significantly elevated using various rates of β-gluca supplementation in diet. Nevertheless, in mirror carp no elevation in total protein content was observed after 8 week feeding with different dosages of β-gluca.

In the present research, a significant increase in serum lysozyme activity was only observed in fish...
treated with 0.5% of β-glucan in diet (10.95 μg/ml serum in G1 group vs 8.66 μg/ml serum in control group). Accordingly, several authors have also reported enhancement of lysozyme activity following administration of different levels of β-glucan (Ortuno et al. 2002; Bagni et al. 2005; Misra et al. 2006; Siwicky et al. 2009; Lauridsen & Buchmann 2010).

In large yellow croaker, *Pseudosciaena crocea*, the results of 8 weeks feeding trial showed that high glucan supplementation (0.18%) significantly enhanced the serum lysozyme activity, whereas low (0.09%) supplementation did not (Ai et al. 2007). Furthermore, β-glucan supplementation at a rate of 0.1% in the feed significantly enhanced lysozyme activity following 8 week feeding to Asian catfish, *Clarias batrachus* (L.) (Kumari & Sahoo 2006).

In spotted rose snapper, *Lutjanus guttatus*, during 5 weeks feeding with three levels of β-glucans (0.05%, 0.1% and 0.5%/ kg feed), the highest lysozyme activity was observed at week 3 in the 0.5% group and thereafter with lysozyme increment in week 4 in both the 0.05% and 0.5% treatments (Del Rio-Zaragoza et al. 2011).

Some studies indicated that any alteration either in immune response or in growth performance is highly linked with different doses and different duration of β-glucan administration, that may not coincide exactly in magnitude or in time with the changes of other responses, and such effects on immune and different blood parameters are common in this kind of studies (Ortuno et al. 1999; Fletcher 1986). In fact, several parameters such as time and duration of administration, type of glucan, dose and different manufacturing processes of glucan, together with environmental parameters, i.e. temperature, can affect results among different experiments, and this could at least partly explain the differences in experimental results (Couso et al. 2003; Bridle et al. 2005; Mohammad et al. 2011; Del Rio-Zaragoza et al. 2011).

To conclude, the present finding provides evidences that a proper administration of β-glucan may have some beneficial effects on health condition in striped catfish.

**Acknowledgement**

This study was supported by the Aquatic Animal Health and Diseases Department, School of Veterinary Medicine, Shiraz University, through a research grant to the second author. We express our sincere thanks to Mr. Fereidouni for his kind cooperation and technical assistance.

**References**


