Effect of Prebiotic Supplementation on Growth Performance and Serum Biochemical Parameters of Kutum (Rutilus frisii kutum) Fries

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ABSTRACT
A study was conducted to investigate the effects of dietary GroBiotic-A (1-3%) on growth and blood profiles of kutum (Rutilus frisii kutum) fingerlings (0.78±0.19 g) compared to fish fed an unsupplemented prebiotic in diet. After 8 weeks of feeding on the experimental diets, total protein, albumin, creatinine, compleman 3, compleman 4 and IgM were significantly higher (p = 0.05), in fish fed GroBiotic-A with 3% than control group. Other biochemical parameter such as glucose, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase, cholesterol, blood urea nitrogen, amylase, calcium, iron, phosphorus and magnesium were measured and compared to the control group (0% GroBiotic-A), dietary GroBiotic-A had no effect on serum. There were no significant differences in growth parameters between experimental groups and control group (p = 0.05). These results indicate that fish blood profiles could be affected by probiotics which should be taken into account in preparing formula feed for artificial rearing fish.

Key words: Biochemical factor, GroBiotic-A, complement, Rutilus frisii kutum

INTRODUCTION
Good meat quality and good consumer acceptance has caused a great economical value for Caspian kutum, Rutilus frisii kutum Kamenskii, 1901. The kutum is the most important bony fish of North of Iran that more than 70% of caught fish by fisherman in Iran costal of Caspian sea consist of this fish (Yousefian and Mosavi, 2008).

But threatening Agents like water pollution, over fishing, decrease of input water from the rivers, destruction of natural spawning beds and etc. has caused a negative impact on its natural population. Now-a-days, Iranian fisheries organization produce and releases more than 100 million fingerlings into Caspian Sea Rivers for stock restoration, therefore the annual landing of kutum increased and reached 16118 mt in 2006 (Salehi, 2008).

In recent years there has been heightened research in developing dietary supplementation strategies in which various health promoting compounds have been evaluated in fish and other farmed animal (Radfar and Parhoormand, 2008). Also several treatments are suggested to prevent losses of growth of fish (Srinivasan et al., 2007; Yesillik et al., 2011). One group of health promoting compounds that has shown numerous beneficial effects in terrestrial animals as well as
in some aquatic animals is prebiotics (Burr et al., 2005). As the aquaculture industry expands, there is an increasing need for improved diagnostic methods (Hrubeck and Smith, 2000). The practical utility of this diagnostic technique is thus clear, as it permits the verification of possible errors in the farming practice so that, they can be dealt with before they show up clinically (Melotti et al., 2004). So, great attention has been recently paid to biochemical characterization of fish blood as to an index of the state of internal milieu (Svoboda et al., 2001). The analysis of blood can reveal the internal biochemical changes, physiological condition and living habitat situation of fish. Also it can point up for us the pollutants, nutrition, stress and ecological conditions and in general the status of fish.

Studies on kutum culture assist the aquaculture industry in meeting the ever increasing demand for kutum, by improving production and enhanced survival of progeny. The aim of the present study was to investigate the effect of dietary GroBiotic-A on some hematological and serum biochemical parameters of kutum fries.

MATERIALS AND METHODS

Kutum fingerlings used in this study acquired from the Shahid Rajaee Hatchery, Sari, Iran. Four hundred fish with average weight of 0.78±0.19 g were randomly distributed in 500 L fiberglass tanks and acclimatized to the experimental condition for 15 days feeding on a basal diet. During the study the fish were fed with experimental diet three times for 8 weeks. Water temperature, Oxygen and pH ranged from 21.6-24.8°C, 8.3-10.1 mg L⁻¹ and 7.9-8.3, respectively.

Diet preparation: Basal practical diet was formulated with estimated protein levels of 380 g kg⁻¹, respectively. Three levels (1-3%) of commercial prebiotic, GroBiotic-A were added to the basal diet (control).

Growth and feed efficiency parameters: The growth performance of fries such as, Body Weight Increase (BWI), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Condition Factor (CF) and survival rate were calculated based on the standard formulae:

\[
BWI = \left( \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \right) \times 100
\]

\[
SGR = \left( \frac{\text{In final weight} - \text{In initial weight}}{\text{Days}} \right) \times 100
\]

\[
FCR = \frac{\text{Feed consumption}}{\text{Body weight gain}}
\]

\[
PER = \frac{\text{Weight gain}}{\text{Protein intake}}
\]

$$CF = \frac{\text{Body weight}}{\text{Body length}} \times 100$$

and:

$$\text{Survival rate} = \frac{(\text{Final No. of fish})}{(\text{Initial No. of fish})} \times 100$$

according to Hung et al. (1993, 1997), Haghhighi et al. (2009) and Luo et al. (2010).

To avoid dietary effect on metabolic status, fish were not fed 12 h before sampling. It was important in this study to gain reliable health status on the fish so as to avoid any distortion of the results by the diseased fish in the database. Accordingly, the following conditions had to be met to ensure this: absence of clinical signs of disease and of pathological and anatomical changes in the post-mortem examination (Rehulkula et al., 2004).

Individual kutum was rapidly netted and carefully placed in a circular tank and were anesthetized with clove oil. Fish were bleed cutting the tail, collecting the blood from the caudal vein. Serum was separated by centrifugation at 3000 g for 15 min at 4°C. After separation all sera maintained at -20°C on shore until processed in the laboratory.

**Analysis methods:** Before analysis, the frozen samples were left to stand at room temperature to thaw and then inverted several times to mix. The serum samples for each specimen were analyzed together in one batch, to avoid run-to-run variability, for the following analyses: Total Protein (TP), Albumin (Alb), Glucose (Glu), Creatinine (CREA), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Creatine Phosphokinase (CPK), Cholesterol (CHOL), Complement C3, Complement C4, Blood Urea Nitrogen (BUN), Immunoglobulin (IgM), Amylase, Calcium (Ca), Iron (Fe), Phosphorus (P) and Magnesium (Mg). All analyses were performed using a blood chemistry AutoAnalyser (Model Eurolyser).

**Statistical analysis:** Blood biochemical values of the kutum were statistically evaluated using an analysis of variance procedure using one-way ANOVA. Differences in p<0.05 were considered to be significant and all results in the text were stated as Mean±Standard error (SE).

**RESULTS**

The effect of dietary GroBiotic-A supplementation on kutum (*Rutilus frisii kutum*) blood profiles is displayed in Table 1. Serum glucose, Alanine aminotransferase, Aspartate aminotransferase, Creatine phosphokinase, Blood urea nitrogen, Cholesterol, Amylase, Calcium, Iron, phosphorus and Magnesium were not significantly affected by the experimental diets (p>0.05). However, IgM and Albumin were significantly higher (p = 0.05), in fish fed GroBiotic-A than control group. Additionally C4 and creatinine were significantly higher (p = 0.05) in the 3% GroBiotic-A fed fish than in the control group. Serum C3 and total protein was significantly higher in the 3% oligofructose group than in the control group (p<0.05). The growth performance of kutum fries fed with the experimental diets for 8 weeks is shown in Table 2. At the end of trial, the final weight of the fish fed with the basal feed was not significantly different from that fish fed with the GroBiotic-A (p>0.05).

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Table 1: The blood parameters of kutum (Rutilus frisiis kutum) fed by GroBiotic-A supplementation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1 (%)</th>
<th>2 (%)</th>
<th>3 (%)</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g dL⁻¹)</td>
<td>3.30±0.10ᵃ</td>
<td>2.85±0.25ᵇ</td>
<td>4.40±0.50ᵇ</td>
<td>1.85±0.25ᵇ</td>
</tr>
<tr>
<td>Albumin (mg dL⁻¹)</td>
<td>2.20±0.50ᵇ</td>
<td>1.55±0.65ᵇ</td>
<td>2.15±0.05ᵇ</td>
<td>0.55±0.06ᵇ</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>4.10±1.67ᵇ</td>
<td>4.0±1.85ᵇ</td>
<td>5.4±1.50ᵇ</td>
<td>3.8±0.20ᵇ</td>
</tr>
<tr>
<td>Creatinine (mg dL⁻¹)</td>
<td>0.6±0.29ᵇ</td>
<td>0.57±0.15ᵇ</td>
<td>0.7±0.10ᵇ</td>
<td>0.4±0.10ᵇ</td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>52.0±12.65ᵃ</td>
<td>53.6±18.61ᵇ</td>
<td>52.0±9.54ᵇ</td>
<td>34.6±7.61ᵇ</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>75.0±11.14ᵇ</td>
<td>56.0±9.64ᵇ</td>
<td>75.0±18.19ᵇ</td>
<td>48.6±18.01ᵇ</td>
</tr>
<tr>
<td>C₃ (g dL⁻¹)</td>
<td>57.0±0.90ᵇ</td>
<td>59.9±8.25ᵇ</td>
<td>68.2±1.25ᵇ</td>
<td>52.6±3.06ᵇ</td>
</tr>
<tr>
<td>C₄ (g dL⁻¹)</td>
<td>57.35±9.45ᵃᵇ</td>
<td>59.15±13.95ᵇ</td>
<td>78.8±19.35ᵇ</td>
<td>34.0±6.96ᵇ</td>
</tr>
<tr>
<td>IgM (mg dL⁻¹)</td>
<td>144.7±16.15ᵇ</td>
<td>164.25±13.56ᵇ</td>
<td>138.8±7.15ᵇ</td>
<td>106.5±6.25ᵇ</td>
</tr>
<tr>
<td>CHOL (mg dL⁻¹)</td>
<td>61.0±13.75ᵇ</td>
<td>54.0±6.57ᵇ</td>
<td>66.0±13.75ᵇ</td>
<td>49.0±6.93ᵇ</td>
</tr>
<tr>
<td>CPK (IU L⁻¹)</td>
<td>39.67±9.29ᵇ</td>
<td>52.3±4.04ᵇ</td>
<td>59.6±13.01ᵇ</td>
<td>51.0±21.16ᵇ</td>
</tr>
<tr>
<td>BUN (mg dL⁻¹)</td>
<td>2.17±0.45ᵇ</td>
<td>3.17±0.35ᵇ</td>
<td>3.4±0.75ᵇ</td>
<td>2.83±0.90ᵇ</td>
</tr>
<tr>
<td>Amylase (IU L⁻¹)</td>
<td>149.0±22.87ᵇ</td>
<td>161.3±12.95ᵇ</td>
<td>162.0±22.14ᵇ</td>
<td>148.3±22.37ᵇ</td>
</tr>
<tr>
<td>Ca (mg dL⁻¹)</td>
<td>5.93±0.40ᵇ</td>
<td>7.47±0.71ᵇ</td>
<td>6.67±0.81ᵇ</td>
<td>5.73±2.41ᵇ</td>
</tr>
<tr>
<td>Fe (μg dL⁻¹)</td>
<td>35.67±5.52ᵇ</td>
<td>37.6±2.09ᵇ</td>
<td>38.5±8.69ᵇ</td>
<td>28.4±1.70ᵇ</td>
</tr>
<tr>
<td>P (mg dL⁻¹)</td>
<td>1.53±2.01ᵇ</td>
<td>1.63±2.35ᵇ</td>
<td>1.70±1.78ᵇ</td>
<td>1.47±0.38ᵇ</td>
</tr>
<tr>
<td>Mg (mg dL⁻¹)</td>
<td>3.37±0.87ᵇ</td>
<td>3.37±0.66ᵇ</td>
<td>3.27±0.49ᵇ</td>
<td>3.40±0.95ᵇ</td>
</tr>
</tbody>
</table>

Different alphabets on the mean values within rows represents a significant difference at p<0.05. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, C₃: C₄: Complement, IgM: Immunoglobulin, CHOL: Cholesterol, CPK: Creatine phosphokinase, BUN: Blood urea nitrogen, Ca: Calcium, Fe: Iron, P: Phosphorus and Mg: Magnesium.

Table 2: The growth performance of fries kutum (Rutilus frisiis kutum) fed with the experimental diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1% GroBiotic-A</th>
<th>2% GroBiotic-A</th>
<th>3% GroBiotic-A</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR</td>
<td>2.78</td>
<td>2.90</td>
<td>3.10</td>
<td>2.70</td>
</tr>
<tr>
<td>PER</td>
<td>1.03</td>
<td>0.97</td>
<td>0.99</td>
<td>1.10</td>
</tr>
<tr>
<td>SGR</td>
<td>0.96</td>
<td>1.02</td>
<td>1.18</td>
<td>0.70</td>
</tr>
<tr>
<td>CF</td>
<td>0.80</td>
<td>0.78</td>
<td>0.87</td>
<td>0.74</td>
</tr>
<tr>
<td>BWI</td>
<td>0.71</td>
<td>0.77</td>
<td>0.94</td>
<td>0.48</td>
</tr>
<tr>
<td>SR</td>
<td>96.00</td>
<td>97.00</td>
<td>95.00</td>
<td>95.00</td>
</tr>
</tbody>
</table>

FCR: Feed conversion ratio, PER: Protein efficiency ratio, SGR: Specific growth rate, CF: Condition factor, BWI: Body weight increase.

DISCUSSION

In the current study, we tested whether the addition of GroBiotic-A affect the growth performance and serum biochemical parameters of Kutum. There were no significant effects of supplementing the diet with GroBiotic-A on growth rate, FCR and PER of the Kutum. Reports about effect of probiotic on growth parameters in fish are inconclusive (Yousefian and Amini, 2009). The lack of growth response to the prebiotics is in agreement with results from studies on Red Drum (Burr and Gatlin, 2009), Gulf sturgeon (Pryor et al., 2003), turbot larvae (Mahious et al., 2006) and hybrid tilapia (Gene et al., 2007) and in contrast to studies with kutum (Ebrahimh, 2010), hybrid striped bass (Li and Gatlin, 2005), hybrid red tilapia, Jian carp, common carp, European catfish, preliminary reports reviewed by Stayklov et al. (2007), rainbow trout (Stayklov et al., 2007; Yilmaz et al., 2007) and European sea bass (Torrecillas et al., 2007).

Supplementation of Beluga’s (Huso huso) diet with 1, 2 and 3% inulin showed negative relationship between some performance indices including Weight Gain (WG), Specific Growth Rate
(SGR), Protein Efficiency Ratio (PER), Energy Retention (ER), Feed Efficiency (FE), Protein Retention (PR) and supplementation level of inulin. Also growth parameters in fish fed inulin was lower than control group (Reza et al., 2009). Like Reza et al. (2009), in this study we observed negative effect of higher level of prebiotic on growth parameters but not significantly (p>0.05). Olsen et al. (2001) observed that a diet supplemented with high level of prebiotic (15% inulin) caused harmful effects on enterocytes to Arctic charr, Salvelinus alpinus. The authors speculated that the reason for this effect may be linked to accumulation of lamellar structures and large vacuoles which may have been absorbed inulin. According to their theory inulin that cannot be degraded by the cells would accumulate to an extent at which cell function became impaired (Olsen et al., 2001).

Serum immunoglobulins are a major component of the vertebrate humoral immune system. Although, IgD has recently been described, IgM is the main immunoglobulin present in teleosts (Ellis, 1998; Wilson et al., 2001). Teleosts mainly produce a class of Ig which resembles mammalian IgM both in structure and physiological characteristics, being also present in soluble (secreted) and membrane-bound forms. The soluble form is secreted from B cells and is present in the blood and other fluids, where it plays a role as an immune effect or molecule (Ross et al., 1998). Among the immune parameters considered in fish immunity studies, changes in total seric IgM levels have been scarcely considered, in spite of the importance of such parameters for the potential prevention and/or control of fish diseases (Nikoskelainen et al., 2003). It is also worth noting that the observed changes in the biochemical factor levels are not concomitant with the effects produced by stimulants or stressors on other humoral immune responses, such as complement and lysozyme activities. Thus, this parameter might be considered one of the best candidates for determining the fish immune system status in certain fish-farm situations due to the non-invasive sampling protocol and because of the simplicity and reproducibility of the assay (Cuesta et al., 2004). All the GroBiotic-A doses administered in the diet to the kutum specimens increased total seric IgM levels and in comparison with control group were significantly higher (p<0.05). Cuesta et al. (2004), supplemented several immunomodulators (vitamin A, chitin, yeast cells or levamisole which act as immunostimulants in the diet of gilt-head seabream (Sparus aurata L.). Total serum IgM levels of fish fed with the assayed immunostimulant supplemented diets were statistically higher than those in fish fed a non-supplemented diet. Supplementation of great sturgeon’s (Huso huso) diet with a commercial prebiotic, Immunoster didn’t increase the serum total IgM (Taati et al., 2011).

Innate responses mediated by complement have been reported in several fish species (Tort et al., 1996; Rotllant et al., 1997). Popov and Popova (1997) also suggested that the alternative complement pathway is significantly enhanced by the use of polysaccharides to facilitate a better aggregation of immunoglobulins, cells and viruses which are subsequently removed by the phagocytic cells. Insoluble inulin (γ-inulin) has been suggested to possess adjuvant activity because it activates the alternative complement pathway (Silva et al., 2004). During complement activation, several complement fragments are released during the activation cascade. Some of the fragments have distinct effects (anaphylatoxin and chemotactic) on leucocytes harbouring specific receptors (Ringo et al., 2010). It is known that in the present study fish fed with 3% GroBiotic had significantly higher C3 levels than fish fed with 1, 2% and control group. In agreement with present results, in hybrid tilapia, fructooligosaccharide did not affect growth but increased survival and enhanced activity of alternative complement activity (Ringo et al., 2010). In the raceway reared trout, Mannan oligosaccharide supplementation significantly increased serum lysozyme activity, classical and alternative pathway complement activity (Merrifield et al., 2010).
The gamma globulin fraction is the source of almost all the immunological active protein of the blood. Globulins like gamma globulins are absolutely essential for maintaining a healthy immune system. Serum albumin and globulin values in the fish treated with different immunostimulants were always higher than control (Choudhury et al., 2005). Increase in the serum protein, albumin and globulin levels is thought to be associated with a stronger innate response in fish (Wiepertz et al., 1996). In the present study, plasma proteins and albumin were analyzed. The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish (Swain et al., 2007). In this study there was a significant effect on the amount of serum proteins produced, when fed with 3% GroBiotic-A in the feed. Since, the complements fraction makes up the larger portion of the globulin, it can be inferred that GroBiotic-A might be responsible for the cidal activity of the serum. This is similar to the results of Siwicki et al. (1994) and Rairakhwada et al. (2007) who found the elevation of total protein content after feeding glucans and levan, respectively. Tasti et al. (2011) supplemented great sturgeon’s (Huso huso) diet with commercial prebiotic, immunostar but they didn’t find significant difference between experimental and control groups (p>0.05).

To conclude, the assessment of serumic levels of biochemical factor could be used not only for the diagnosis of disease, but also to obtain information which could be of use for taking preventive measures during aquaculture. It would be very useful to measure the levels of this parameter at different times to ascertain the possible beneficial effects of treatment with prebiotic and immunostimulants, as well as to detect the occurrence of any potential negative effect of stressors involved in aquacultural management.

REFERENCES


