PREBIOTIC EFFECT OF FRUCTO-OLIGOSACCHARIDES ON GROWTH AND PHYSIOLOGICAL STATE OF RAINBOW TROUT, ONCORHYNCHUS MYKISS (WALBAUM)

J. Řehulka, B. Minařík, D. Cink, J. Žalák

Received: April, 4, 2011

Abstract


Rainbow trout at an average weight of 240 g were examined for the effect of dietary fructo–oligosaccharides in the diet on their growth and physiological state through selected biochemical parameters of the blood plasma. The prebiotic product Profeed® (experimental group, EG) was administered on a continuous basis at a rate of g kg⁻¹ of pellets for 105 days. The best growth performance for the EG was found in 42 days (363 ± 34.7 g vs. 340 ± 36.7 g, P = 0.003) and in 63 days (387 ± 35.6 g vs. 364 ± 42.3 g, P = 0.011). SGR of the fish from the EG was 0.69% and from the control group (CG) was 0.70%. The feed conversion level was 0.82 in the EG and 0.86 in the CG. Survival rate was 99% (EG) and 98% (CG). The results of the biochemical examination indicate significant differences in the creatinine (28 ± 5.5 vs. 22 ± 3.0 μmol L⁻¹) and the sodium cation (157.9 ± 1.66 vs. 155.7 ± 1.49 mmol L⁻¹) level and in the catalytic concentration of alkaline phosphatase (5.18 ± 1.57 vs. 3.43 ± 0.78 μkat L⁻¹). The positive results of the growth and biochemical tests as well as the favourable feed conversion suggest that it would be worthwhile to test higher concentrations of the Profeed® prebiotic product.

Profeed®, prebiotics, rainbow trout, growth performance, haematology, blood plasma biochemistry

Protection of salmonids in intensive culture against infections of bacterial origin is among the key preventive measures within the range of key principles of veterinary health management to minimise the morbidity of the fish. Frequent use of antibacterial products to control the much–feared pathogens colonising the intestinal environment involves the risk of simultaneously killing the useful intestinal micro biota needed for optimal utilisation of feed nutrients. It is therefore useful to use prebiotics to restore the beneficial intestinal micro biota and encourage its growth. According to Gibson & Roberfroid (1995), a prebiotics is defined as a non–digestible food ingredient that beneficially effect the host by selectively stimulating the growth and/or the activity of specific health–promoting bacteria that can improve the host's health. Oligosaccharides promoting beneficial bacterial growth within the gastrointestinal tract are the main components of prebiotics (Yazawa, Imai & Tamura, 1978; Gibson, Rastall & Fuller, 2003). A review of prebiotics in aquaculture was drawn up by Ringø, Olsen, Gifstad, Dalmo, Amlund, Henn & Bakke (2010) and the current status and future focus of prebiotic and probiotic applications for salmonids was described by Merrifield, Dimitroglou, Foey, Davies, Baker, Begwald, Castex & Ringø (2010). The potential prebiotic applications for salmonids include mannanoligosaccharides (MOS) (Yilmaz, Genc & Genc, 2007; Staykov, Spring, Denev & Sweetman, 2007; Rodriguez–Estrada, Satoh, Haga, Fushimi & Sweetman, 2009; Dimitroglou, Merrifield, Moate, Davies, Spring, Sweetman & Bradley, 2009), GroBiotic®–A (Sealey, Barrows, Johansen, Overturf, LaPatra & Hardy, 2007) and inulin (Reistle, Bakke–
McKellep, Penn, Sundby, Shearer & Krokodahl, 2006; Bakke–McKellep, Penn, Salas, Refstie, Sperstad, Landsverk, Ringø & Krokodahl, 2007; Olsen, Myklebust, Kryvi, Mayhew & Ringø, 2001; Ringø, Sperstad, Myklebust, Mayhew & Olsen, 2006, and fructooligosaccharides (FOS) (Grisdale–Helland et al., 2008). Of all these prebiotics, only MOS and GroBiotic®–A have been tested in rainbow trout, and the parameters investigated included the gut microbiota (Dimitroglou et al., 2009), gut histology (Dimitroglou et al., 2009; Yilmaz et al., 2007), growth performance (Yilmaz et al., 2007; Staykov et al., 2007; Rodriguez–Estrada et al., 2009; Sealey et al., 2007), feed utilisation (Yilmaz et al., 2007; Staykov et al., 2007), immune response (Staykov et al., 2007; Rodriguez–Estrada et al., 2009; Sealey et al., 2007), body composition (Rodríguez–Estrada et al., 2009; Sealey et al., 2007). It follows from the above information on the use of prebiotics in salmonids that further information on the effects of prebiotics on blood biochemical profiles is required, particularly in respect of FOS.

The objective of the present study was to examine the effect of a commercial FOS feed product Profeed® on growth performance and intermediary metabolism through selected biochemical parameters of the blood plasma. Besides haematocrit, the biochemical tests were complemented by examination of the profiles of nitrogen, carbohydrate, lipid and mineral metabolism and activities of the major enzymes.

MATERIAL AND METHODS

Description of the product being tested

Profeed® is a natural regulator of the intestinal flora and the digestive function of animals. This product by Beghìn–Meiji consists of a specific mixture of the short–chain fructo–oligosaccharides that are formed by interlinkage of one three–fructose molecules to one molecule of saccharose (= sucrose). The manufacturing process guarantees a contact between the fructose molecules. FOS contribute to the development and maintenance of an intestinal flora which is responsible for the production of volatile fatty acids (VFAs) and organic acids, which help to preserve a normal intestinal pH.

Feeding experiment

The trials were conducted on a trout farm (652 m above sea level) in flow–through round metallic tanks. Four thousand rainbow trout (weighing 240 ± 349 g) all of the same origin, in good condition and good health were distributed into four tanks, each containing 1000 fish with a stockling density of 50 kg m⁻³. Fish were acclimated to the testing environment for 10 days before the start of the trials for adaptation to the feeding system. Aller Safir pellets (4 mm diameter), produced by Aller Aqua, Denmark, were used as the experimental feed; their composition and formulation was as follows: crude protein (N × 6.25) 45%, crude fat 20%, nitrogen free extract 16%, crude fibre 2%, ash 8%, P 1%, metabolised energy (MJ/kg) 17.3. Supplementary substances per kg: vitamin A 2,500 IU, vitamin D$_3$ 500 IU, vitamin E (α–tocopherol acetate) 100 mg, ethoxyquin antioxidant 100 mg. The fish were fed a diet containing 1 g Profeed® in powder form per kg of pellets, applied to the pellets using rape–seed oil (experimental diet) or Profeed® free diet with rape–seed oil (control diet). The fish were fed twice daily at the rate recommended by the feed producer.

During the trial, which lasted from May to August, the water had the following physical and chemical characteristics: water temperature 9–14 ºC, pH 6.5–7.4, total hardness 4.5–5.2 “N, dissolved O$_2$ 1.9–2.9 mg L⁻¹, chemical oxygen demand (COD$_5$) 0.4–0.5 mmol L⁻¹, base neutralizing capacity (BNC$_4.5$) 0.4–0.5 mmol L⁻¹, acid neutralizing capacity (ANC$_4.5$) 0.02–0.09 mmol L⁻¹, NH$_4$+ 0.16–0.43 mg L⁻¹, NO$_3$− 0.024–0.055 mg L⁻¹, NO$_2$− 4.1–20.2 mg L⁻¹, Cl − 2.1–2.6 mg L⁻¹, PO$_4$³− mg L⁻¹, SO$_4$²−, total iron 0.10–0.22 mg L⁻¹, Ca$^{2+}$ 12–18 mg L⁻¹, Mg$^{2+}$ 9.1–12.9 mg L⁻¹, total dissolved substances 81–112 mg L⁻¹.

Growth

Random selection and returning were used to study the growth of the fish. In an interval of 21 days within a 105–day period (from 17 May to 30 August), 40 fish out of the total stock were caught in each test (experimental) group (EG) and control group (CG) and were individually weighed and measured (total body length, standard length, body height, body width). There were no statistical differences in the mean body weights of fish at the start of the experiment (EG 235 ± 36 g, CG 245 ± 33 g). Before weighing and measuring, the fish were starved for 24 h and were anaesthetised with 3–aminobenzoic acid ethylester natrium hydrogen sulphate, C$_6$H$_{12}$NaNO$_6$S) at a concentration of 0.06 g L⁻¹ (Král., 1988) to enable easy handling. Specific growth rate (% body weight/day) was calculated as: 

\[
SGR = \left[ \frac{\ln W_f - \ln W_i}{t} \right] \times 100
\]

where $W_i$ is average initial weight and $W_f$ is average weight of the final weight and $t$ is time (days) between $W_i$ and $W_f$.

Assessment of condition indices

To evaluate condition, Fulton’s condition factor (\(\text{condition} = \frac{\text{weight} \times 100}{\text{length}^3}\)) was calculated.

Preparation of the blood samples

Blood was sampled from ten experimental and ten control fish caught at random 24 hours after the last feeding (at day 108). Blood samples were collected from 11:00 to 14:00 hours. The fish were anaesthetised with Menocaine and the blood samples were taken by puncturing the caudal

J. Řehulka, B. Minařík, D. Cink, J. Žalák
vessels. Sodium heparin (5,000 IU in a 1 ml injection) was used as an anticoagulant. Centrifuging the blood at 400 g for 10 min and then removing it with a plastic syringe obtained plasma. During blood sampling, water temperature was 13 °C, dissolved oxygen content 9.6 mg L\(^{-1}\) and the photoperiod was 15:9 (light:dark hours).

**Haematology and blood chemistry**

Haematocrit values (Hct) were determined in microhaematocrit–heparinised capillaries within 40 min after blood sampling in duplicate, using a microhaematocrit centrifuge (15,300 g/3 min).

The biochemical indices of the blood plasma were determined within 24 h of storage at 4 °C; a Hitachi 717 multiparametric analyser (Tokyo, Japan) was used for the determinations. These included: total protein (TP, in g L\(^{-1}\)), blood urea nitrogen (BUN, in mmol L\(^{-1}\)), urea (UA, in μmol L\(^{-1}\)), creatinine (CREA, in μmol L\(^{-1}\)), glucose (GL, in mmol L\(^{-1}\)), triacylglycerol (TGL, in mmol L\(^{-1}\)), total calcium (Ca, in mmol L\(^{-1}\)), inorganic phosphate (P, in mmol L\(^{-1}\)), alanine aminotransferase (ALT, in μkat L\(^{-1}\)), aspartate aminotransferase (AST, in μkat L\(^{-1}\)), alkaline phosphatase (ALP, in μkat L\(^{-1}\)), lactate dehydrogenase (LD, in μkat L\(^{-1}\)). The values of the Na\(^+\), K\(^+\) and Cl\(^-\) ions were determined by the ISE, Nova 5 analyser (USA). Kits produced by PLIVA–Lachema, a. s. Brno (Czech Republic) and DIALAB, Vienna (Austria) and Prague (Czech Republic) were used for the determination of all indices. For controls, BIO–LA–Test® LYONORM HUM N, PRECINORM U and PRECIPATH U were used.

**Statistical analysis**

During the mathematics–statistical processing of the results, the selected sets of the experimental and control groups were treated on a one–dimensional and multidimensional basis. Correlation and regression analyses were used in studying the growth dynamics, and the time variable “day” was chosen as the independent variable.

Statistically, it is a regression model with replications, that makes it possible to test the null hypothesis “Population regression is linear” against the alternative hypothesis “Population regression is not linear”, using the test statistic of

\[
F = \frac{\text{Regression Error MS}}{\text{Within Groups MS}},
\]

which has a Fisher–Snedecor distribution with \(k - 1\) and \(n - k\) degrees of freedom (Zar, 1999).

The goodness of fit in the parameters of regression lines between the test group and control group was tested, using the heterogeneity of regression test after calculation with a pooled (p), common (c) and total (t) regression (Armitage & Berry, 2001).

**RESULTS AND DISCUSSION**

Figures 1 to 5 give a clear idea of the trend of development over time. Figure 6 shows the variability of the Fulton index. The parameters of regression equations and correlation coefficients are shown in Table I. The fact that the hypothesis concerning the goodness of fit of regression line slopes was not rejected shows that, regardless of diet composition, both the EG and CG had the same growth rate. The hypothesis of the goodness of fit of regression line slopes was only rejected in the case of body height: with higher body height observed in the experimental group. The best growth performance for the EG was found in 42 days (363 ± 34.7 g vs. 340 ± 36.7 g, \(P = 0.003\)) and in 63 days (387 ± 35.6 g vs. 364 ± 42.3 g, \(P = 0.011\)). SGR of the fish from the EG was 0.69% and from the CG was 0.70%. The average weight for the entire period was 370 ± 93.1 g (EG) and 366 ± 96.8 g (CG). The feed conversion level was 0.82 in the EG and 0.86 in the CG. Survival rate was 99% (EG) and 98% (CG).
2. Dynamic of total body length in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines.

3. Dynamic of standard length in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines.

4. Dynamic of body height in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines.
Prebiotic effect of fructo-oligosaccharides on growth and physiological state of rainbow trout

5: Dynamic of body width in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines.

6: Dynamic of Fulton’s condition factor in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines.

I: Parameters of regression and correlation on time variable (day) of rainbow trout

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Parameters of regression line type $y' = B_0 + B_1x$</th>
<th>Coefficient of correlation $R$</th>
<th>Coefficient of determination $R^2$</th>
<th>Statistical significance of $R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight g</td>
<td>Day</td>
<td>244.22, 2.35</td>
<td>0.897</td>
<td>80.4</td>
<td>44.388, 0.0000</td>
</tr>
<tr>
<td>Total body length mm</td>
<td>Day</td>
<td>267.82, 0.52</td>
<td>0.937</td>
<td>70.1</td>
<td>33.515, 0.0000</td>
</tr>
<tr>
<td>Standard length mm</td>
<td>Day</td>
<td>242.88, 0.48</td>
<td>0.832</td>
<td>69.2</td>
<td>32.830, 0.0000</td>
</tr>
<tr>
<td>Body height mm EG</td>
<td>Day</td>
<td>66.75, 0.18</td>
<td>0.849</td>
<td>72.1</td>
<td>24.859, 0.0000</td>
</tr>
<tr>
<td>Body height mm CG</td>
<td>Day</td>
<td>66.52, 0.16</td>
<td>0.836</td>
<td>70.0</td>
<td>23.581, 0.0000</td>
</tr>
<tr>
<td>Body width mm</td>
<td>Day</td>
<td>34.9, 0.08</td>
<td>0.830</td>
<td>68.9</td>
<td>32.615, 0.0000</td>
</tr>
<tr>
<td>Fulton</td>
<td>Day</td>
<td>1.73, 0.002</td>
<td>0.526</td>
<td>27.6</td>
<td>13.507, 0.0000</td>
</tr>
</tbody>
</table>

Note: EG = experimental group; CG = control group
The results of the biochemical examination, documented in Table II, indicate significant differences in the CREA level and Na⁺ and in the catalytic concentration of ALP. However, the values of these parameters did not go beyond the physiological range, computed as 2.5 and 97.5% quantiles for the physiological values (n = 350) of the same age category of trout under these conditions (CREA: 16–42; Na⁺: 146.6–163; ALP: 2.6–9.8).

The final examination of the health of the fish showed no clinical signs of disease, with no significant parasite infections (Ergens, 1992; Lom & Dyková, 1992) of the fish, and with no pathological and/or bacterial examination (Inglis, Roberts & Bromage, 1993; Whitman, 2004) of the post-mortem examination showed no pathological and anatomical change (Roberts & Shepherd, 1997; Ferguson, 2006) and/or bacterial examination (Inglis, Roberts & Bromage, 1993; Whitman, 2004) of the fish. There is still little information about the impact of MOS on growth performance in salmonid fish. Grisdale–Helland et al. (2008) is the only researcher who has evaluated the effect of 1% dietary MOS supplementation on growing Atlantic salmon, Salmo salar, after feeding for 16 weeks. The present authors have found that MOS–fed salmon displayed some improvement of feed efficiency ratio. More information about the effects of prebiotics on growth performance is available for MOS. In trials with rainbow trout reared either in fresh water net cages or fresh water raceways, Stakarov et al. (2007) found that 0.2% dietary MOS supplementation increased final body weight and reduced feed conversion ratio and mortalities in both net cage– and raceway–reared trout. In another rainbow trout study Yilmaz et al. (2007) examined the effect of dietary MOS (1.5%, 3.0% and 4.5%). They found an increased growth performance at 1.5% dietary MOS supplementation. Rodrigues–Estrada et al. (2009) reported, that in an in vivo study on rainbow trout fingerlings the administration of 0.4% MOS for 12 weeks stimulated growth and other characteristics such as haemolytic and phagocytic activity, mucosa weight and improved survival when the fish were challenged with V. (L.) anguillarum. Other positive properties of prebiotics in Atlantic salmon were described by Refstie et al. (2006) and Bakke–McKellep et al. (2007) and in other fishes by Mathious, Gatesoupe, Hervi, Metailler & Ollevier (2006) and Li & Gatlin (2004, 2005).

Mathious et al. (2006) in their preliminary experiments tested the effect of dietary inulin (Raftiline ST), oligofructose (Raftilose P95) and lactosucrose on the growth and intestinal bacteria of the marine carnivorous turbot, Psetta maxima. The final mean weight of the group weaned with Raftilose P95 was significantly higher than that of observed with other diets. Of the total load of bacterial isolates from turbot weaned on oligofructose, 14% consisted of a strain of Bacillus spp. This strain could use Raftilose P95 as a single source of carbon, and it might play a role in the beneficial effect of oligofructose on turbot growth. Li and Gatlin (2004) conducted two separate feeding experiments, in which they focused on evaluating the graded levels of a commercial prebiotic, GroBiotic®–A, a mixture of partially autolysed brewers yeast, dairy ingredient components and dried fermentation products, in the diet of hybrid striped bass (Morone chrysops × M. saxatilis), as compared to partially autolysed brewers yeast (Brewtech Reg.). Enhanced growth performance was generally observed in fish fed the diets supplemented with GroBiotic®–A or brewers yeast, compared to the basal diet after 7 weeks of...
feeding. A significantly higher feed efficiency was observed in fish fed diets supplemented with 1% and 2% GroBiotic−A. All the groups of fish fed brewers yeast and GroBiotic−A showed a significantly enhanced survival (73.3–90%) after bath exposure to *Streptococcus iniae*, compared to fish fed the basal diet (53.3%).

In the prebiotics research programme, the knowledge gap has to be filled, as to the effect of prebiotics on physiological state evaluated according to the haematological and biochemical parameters of peripheral blood. Our previous studies, in which rainbow trout responded sensitively to the composition of feed with different lipid and protein contents (Rehulka & Párová, 2000a, b) or diets with different synthetic pigments to produce the desired flesh colour (Řehulka & Žák, 1986; Řehulka, 2000), provided enough evidence of the suitability of these methods. The first study of some haematological parameters of juvenile beluga (*Huso huso*) fed oligofructose at varying levels (1, 2 or 3%) was performed by Hoseinifar, Mirvaghefi, Merrifield, Amiri, Yelghi & Bastami (2010). They found significant differences not only in comparison with the control group (Hct values, proportion of lymphocytes, cholesterol level) but also between experimental groups with different oligofructose levels (haemoglobin values, leucocyte level, proportion of lymphocytes, cholesterol level). Their results, together with ours, indicate that research in this area should continue and causal relationships should be sought between dietary prebiotics and some haematological and serum plasma biochemical parameters of fish.

**CONCLUSIONS**

The good state of health of the fish during the trial corresponded to the results of the haematological and biochemical examination of the blood plasma, whose values did not exceed the physiological range. The expected better digestibility of nutrients, accompanied by increased VFA production, requires further validation and calls for examining higher concentrations (the manufacturer recommends up to 3 kg per tonne) or combinations of CFOS with probiotics to make them act synergistically to speed up the growth of lactobacilli.

**SUMMARY**

Rainbow trout at an average weight of 240 g were examined for the effect of dietary fructo−oligosaccharides in the diet on their growth and physiological state through selected biochemical parameters of the blood plasma. The prebiotic product Profeed® (experimental group, EG) was administered on a continuous basis at a rate of 5 g kg$^{-1}$ of pellets for 105 days. The best growth performance for the EG was found in 42 days (363 ± 34.7 g vs. 340 ± 36.7 g, P = 0.003) and in 63 days (387 ± 35.6 g vs. 364 ± 42.3 g, P = 0.011). SGR of the fish from the EG was 0.69% and from the control group (CG) was 0.70%. The feed conversion level was 0.82 in the EG and 0.86 in the CG. Survival rate was 99% (EG) and 98% (CG). The results of the biochemical examination indicate significant differences in the creatinine (28 ± 5.5 vs. 22 ± 3.05 μmol L$^{-1}$) and the sodium cation (157.9 ± 1.66 vs. 155.7 ± 1.49 mmol L$^{-1}$) level and in the catalytic concentration of alkaline phosphatase (5.18 ± 1.57 vs. 3.43 ± 0.78 μkat L$^{-1}$). The positive results of the growth and biochemical tests as well as the favourable feed conversion suggest that it would be worthwhile to test higher concentrations of the Profeed® prebiotic product.

**Acknowledgements**

The authors gratefully acknowledge D. L. Merrifield, Aquaculture and Fish Nutrition Research Group, The School of Biomedical and Biological Sciences, The University of Plymouth, Plymouth, UK, for critical reading of the manuscript.

**REFERENCES**


REHULKA, J., 1989: Determining the optimum doses of Kurasan (ethoxyquinolin) and butylhydroxytoluol (BHT) in dry pellets: Effect of both antioxidants on some haematological and condition parameters of rainbow trout *Salmo gairdneri*. *Aquaculture and Fisheries Management* 20: 295–310.


Address