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

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Abstract

ŘEHULKA, J., MINAŘÍK, B., CINK, D., ŽALÁK, J.: *Prebiotic effect of fructo–oligosaccharides on growth and physiological state of rainbow trout, Oncorhynchus mykiss (Walbaum).* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 5, pp. 227–236

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 ± 3.05 μ*mol 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 μ*kat 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, Hemre & Bakke (2010) and the current status and future focus of prebiotic and probiotic applications for salmonids was described by Merrifield, Dimitroglou, Foey, Davies, Baker, Bøgwald, Castex & Ringø (2010). The potential prebiotic applications for salmonids include mannanoligosaccharides (MOS) (Yilmaz, Genc & Genc, 2007; Staykov, Spring, Denev & Sweetman, 2007; Grisdale–Helland, Helland & Gatlin, 2008; 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 (Refstie, Bakke–

McKellep, Penn, Sundby, Shearer & Krokdahl, 2006; Bakke–McKellep, Penn, Salas, Refstie, Sperstad, Landsverk, Ringø & Krokdahl, 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 (Rodriguez–Estrada *et al.*, 2009; Sealey *et al.*, 2007) and disease resistance (Rodriguez–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, particularity 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 Beghin–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 composition for Profeed®, in terms of GF2 (1 kestose, 37%), GF3 (nystose, 53%), and GF4 (fructosyl nystose, 10%). The digestive enzymes of monogastric animals cannot break down the so–called ß 1–2 linkages 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 ± 34.9 *g*) all of the same origin, in good condition and good health were distributed into four tanks, each containing 1000 fish with a stocking 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 1 *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 °C, pH 6.5–7.4, total hardness 4.5–5.2 °N, dissolved O_2 9.1–10.8 $mg\ L^{-1}$, chemical oxygen demand (COD_{Mn}) 1.9–2.9 $mg\ L^{-1}$, acid neutralizing capacity ($ANC_{4.5}$) 0.4–0.5 $mmol\ L^{-1}$, base neutralizing capacity ($BNC_{8.3}$) 0.02–0.09 $mmol\ L^{-1}$, NH_4^+ 0.16–0.43 $mg\ L^{-1}$, NO_2^- 0.024–0.055 $mg\ L^{-1}$, NO_3^- 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, $C_9H_{12}NaNO_6S$) at a concentration of 0.06 $g\ L^{-1}$ (Král, 1988) to enable easy handling. Specific growth rate (% body weight/day) was calculated as: $SGR = [(\ln W_f - \ln W_i)/t] \times 100$, where W_f is average weight of the final weight and W_i is average weight of initial weight and t is time (days) between W_f and W_i.

Assessment of condition indices

To evaluate condition, Fulton's condition factor [weight × $100/S_L^3$] 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

vessels. Sodium heparin (5 000 *IU* in a 1 *ml* injection) was used as an anticoagulant. Centrifuging the blood at 400 *g* for10 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 µ*mol* L^{-1}), creatinine (CREA, in µ*mol* 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, µ*kat* L^{-1}), aspartate aminotransferase (AST, in µ*kat* L^{-1}), alkaline phosphatase (ALP, in µ*kat* L^{-1}), lactate dehydrogenase (LD, in µ*kat* 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

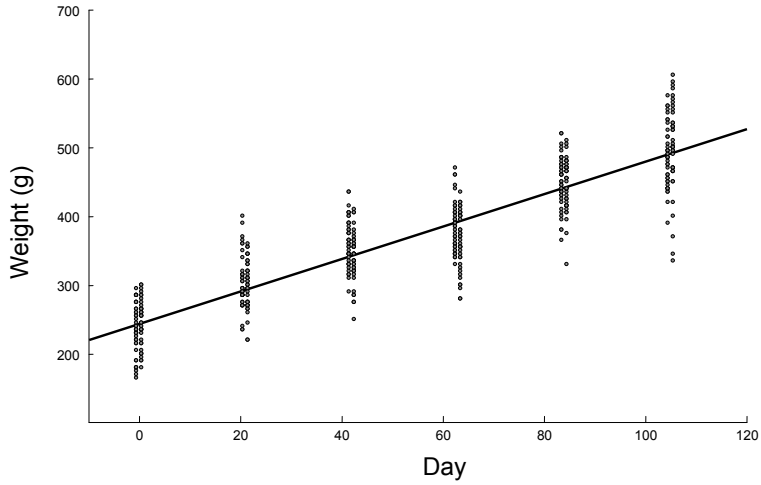
$$F = \frac{Regression\ Error\ MS}{Within\ Groups\ MS},$$

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

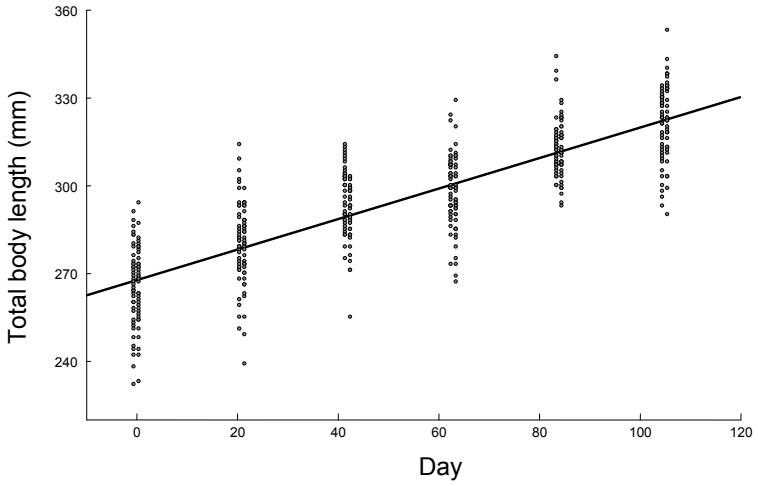
The goodness of fit in the parameters of regression lines between the test group and control group was tested, using the heterogeneity of regression test after calculation with a pooled (*p*), common (*c*) and total (*t*) regression (Armitage & Berry, 2001).

RESULTS AND DISCUSSION

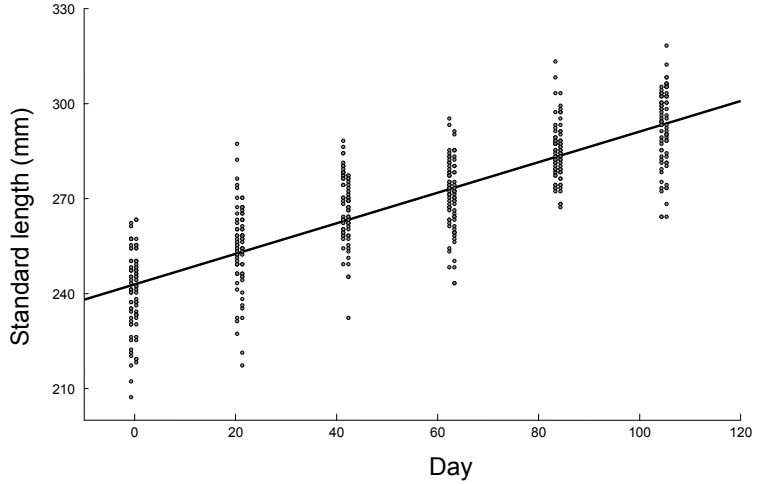
Figures 1 to 5 give a clear idea of the trend of development over time. Figure 6 shows the variability of the Fulton index. The parameters of regression equations and correlation coefficients are shown in Table I. The fact that the hypothesis concerning the goodness of fit of regression line slopes was 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).



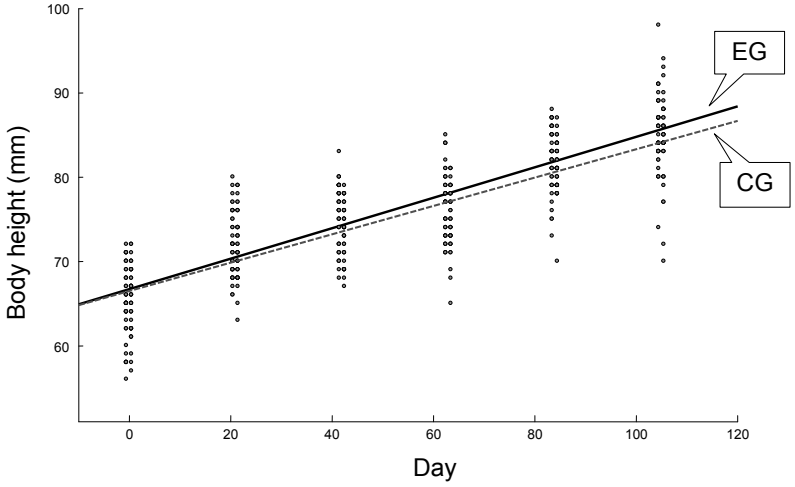
1: *Dynamic of weight in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines*



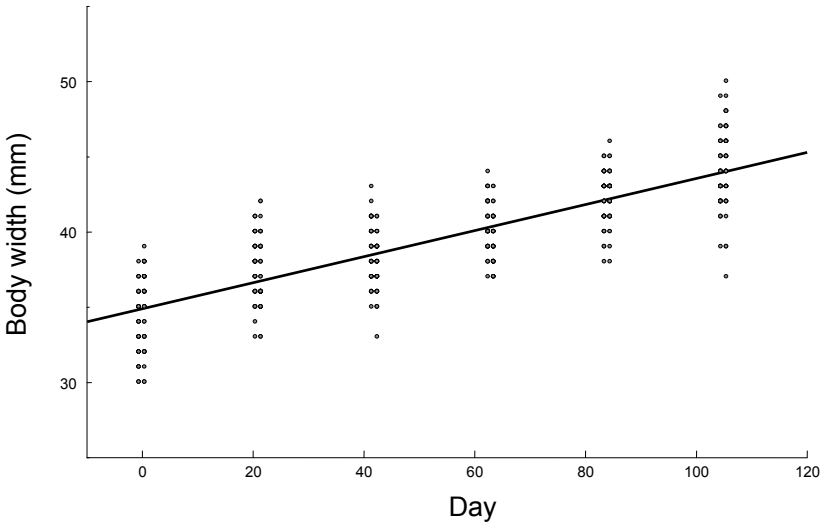
2: *Dynamic of total body length in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines*



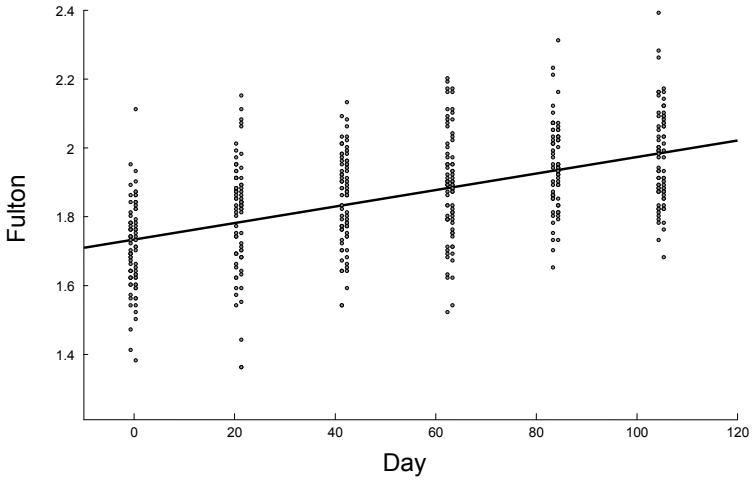
3: *Dynamic of standard length in the experimental group (first group of data: EG) and control group (CG) of rainbow trout, depending on time, represented by regression lines*



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*



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*

Dependent variable	Independent variable	Parameters of regression line type $y' = B_0 + B_1x$		Coefficient of		Statistical significance of R	
		B_0	B_1	correlation R	determination 100 R^2	t–value	Probability
Weight *g*	Day	244.22	2.35	0.897	80.4	44.388	0.0000
Total body lengtht *mm*	Day	267.82	0.52	0.837	70.1	33.515	0.0000
Standard length *mm*	Day	242.88	0.48	0.832	69.2	32.830	0.0000
Body height *mm* EG	Day	66.75	0.18	0.849	72.1	24.859	0.0000
Body height *mm* CG	Day	66.52	0.16	0.836	70.0	23.583	0.0000
Body width *mm*	Day	34.9	0.08	0.830	68.9	32.615	0.0000
Fulton	Day	1.73	0.002	0.526	27.6	13.507	0.0000

Note: EG = experimental group; CG = control group

II: *Haematocrit and biochemical parameters of blood plasma of control rainbow trout and rainbow trout (n = 10) fed Profeed® – containing diet for 108 days*

	Mean	SD	R	Mean	SD	R	*t*–value	Probability
Ht	0.443	0.0211	0.4–0.473	0.413	0.0408	0.324–0.436	1.864	0.083
TP *g L*$^{-1}$	48.3	5.03	42–56	45.6	5.95	38–55	1.095	0.287
BUN *mmol L*$^{-1}$	0.5	0.14	0.4–0.9	0.5	0.14	0.3–0.7	1.429	0.170
UA µ*mol L*$^{-1}$	8.2	3.39	5–16	10.7	2.83	6–15	–1.789	0.090
CREA µ*mol L*$^{-1}$	28	5.5	24–41	22	3.05	16–28	2.608	**0.017**
GL *mmol L*$^{-1}$	4.2	0.76	3.2–5.4	4.1	0.48	3.5–5.1	0.489	0.630
TGL *mmol L*$^{-1}$	3.6	1.36	1.7–5.8	3.3	1.16	2–5.3	0.549	0.589
P *mmol L*$^{-1}$	5.03	0.45	4.23–5.8	4.79	0.674	3.82–6.32	0.936	0.361
Ca *mmol L*$^{-1}$	3.09	0.233	2.81–3.55	3.02	0.297	2.54–3.5	0.603	0.553
Na^+ *mmol L*$^{-1}$	157.9	1.66	156–161	155.7	1.49	154–158	3.111	**0.006**
Cl^- *mmol L*$^{-1}$	124.3	2.31	119–127	124.6	2.07	120–127	–0.306	0.763
AST µ*kat L*$^{-1}$	5.38	1.414	3.36–7.56	5.71	1.554	3.73–7.8	–0.501	0.622
ALT µ*kat L*$^{-1}$	0.18	0.055	0.12–0.27	0.19	0.046	0.12–0.25	–0.660	0.517
ALP µ*kat L*$^{-1}$	5.18	1.567	2.7–7.7	3.43	0.787	2.5–5.0	2.984	**0.011**
LD µ*kat L*$^{-1}$	17.18	6.569	8.5–27.2	16.15	3.123	11.2–22.8	0.447	0.661
AST/ALT	29.07	5.623	21.67–36.73	30.13	5.963	19.89–39.56	–0.399	0.694
LD/AST	3.24	1.016	1.68–4.48	2.94	0.654	2.08–4.32	0.787	0.441

Note: *SD* = standard deviation; *R* = range

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, 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, and with no significant parasite infections (Ergens, 1992; Lom & Dyková, 1992).

There is still little information about the impact of FOS on growth performance in salmonid fish. Grisdale–Helland *et al.* (2008) is the only researcher to have evaluated the effect of 1% dietary FOS supplementation on growing Atlantic salmon, *Salmo salar*, after feeding for 16 weeks. The present authors have found that FOS–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, Staykov *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 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 (Řehulka & Párová, 2000a, b) or diets with different synthetic inhibitors of fat oxidation (Řehulka, 1989) or with pigmenting substances 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 and serum biochemical 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 *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 μ*mol 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 μ*kat 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.

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