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Full Length Research Paper

The effect of fructo-oligosaccharides on blood RBC count and digestive enzyme activities of oxyeleotris lineolatus

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This study was designed to evaluate the effect of fructo-oligosaccharides (FOS) on blood RBC (red blood cell) count and digestive enzyme activities of oxyeleotris lineolatus. 60 oxyeleotris lineolatus were randomly divided into three groups with 20 fish in each group: control, low and high dose of FOS-treated groups. Animals in the control group consumed basic feed, animals in FOS-treated groups consumed the feed containing 1.5 and 3% FOS, respectively. After 20 days of FOS administration, growth rate, blood RBC count and hemoglobin (Hb) level of oxyeleotris lineolatus were dose-dependently significantly enhanced (P < 0.05) compared to control group, in addition, digestive enzyme activities in stomach and intestine of oxyeleotris lineolatus were dose-dependently significantly enhanced (P < 0.05) compared to control group. The present data indicated that supplementation of FOS could improve nutritive value of food through altering its digestibility and intestinal morphology. In other words, the addition of an appropriate level of digestible carbohydrates to the diet made with this raw material improved the nutritive utilization of the dietary protein and of the diet as a whole.

Key words: RBC, Hb, oxyeleotris lineolatus, digestive enzyme, FOS.

INTRODUCTION

Oligosaccharides are water soluble and typically 0.3 - 0.6 times as sweet as sucrose. In fact, the sweetness depends on chemical structure, the degree of polymerization of the oligosaccharides present and the levels of mono and disaccharides in the mixture (Crittenden and Playne, 1996; Xu et al., 2009). According to Ducasse et al. (2010), the sweetness decreases with longer the oligosaccharide chain length. This low sweetness intensity is quite useful in the various kinds of foods where the use of sucrose is restricted by its high sweetness property.

An increase in the number of bifidobacteria is considered as a prebiotic effect. A prebiotic effect of FOS has been demonstrated *in vitro* with pure bacterial cultures (Kaplan and Hutkins, 2000) and with human faecal slurries (Probert and Gibson, 2002; Wang and Gibson, 1993). Likewise, a prebiotic effect of FOS was shown in *in vivo* studies with mice (Dou et al., 2009) and human (Kleesen et al., 1997). Dietary FOS has also been shown to have a prebiotic effect on humoral immunity and serum lysozyme activity in chickens (Mountzouris et al., 2006). Effects of FOS on the number of intestinal flora, e.g. bifidobacteria, have been shown in pigs (Lykkegaard et al., 2005). Recent advances have insighted into the health benefits of FOS, including lowering of blood cholesterol, lowering of high blood pressure, protective effects against infections, controlling arthritis and enhancing antitumor properties. They are expected to be utilized as functional foods, medical supplies and biologically active substances (Xu et al., 2009; Giacco et al., 2004; Shuai et al., 2010).

However, pharmacological function of FOS in the gastrointestinal tract of fishes is scarcely reported. The objective of this study was to evaluate the pharmacological properties of FOS in aquatic animal and its potential in feed industry. In this study, we examined the effect of FOS on growth rate, blood RBC count and digestive enzyme activities of oxyeleotris lineolatus.

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Table 1. Ingredient and chemical composition of the basic diet.

Feedstuff	Ingredients	Proportion (%)	Content
fish meal	50	crude protein	42.36
soybean meal	10	crude lipid	8.81
corn meal	7	ash	12.52
α-starch	20		
yeast powder	6		
fresh fish oil	5		
vitamin premix [a]	1		
mineral premix [b]	1		

(a) The mineral premix provides the following (per kg): manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg. (b) The vitamin premix provides the following (per kg): all-trans-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-rac-a-tocopherol acetate, 12.5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B12, 0.02 mg; folic acid, 0.55 mg; day biotin, 0.1 mg.

MATERIALS AND METHODS

Materials

Oxyeleotris lineolatus were purchased from a local fish farm. "Fructo-oligosaccharides were kindly offered by the Yinhua Company (Dongguan, Guangdong, china)".

Feeding to fish

Fish were maintained in 75 l tanks that were part of a re-circulating system using biological filtration. Water temperature was maintained at 22 - 27°C. Dissolved oxygen, nitrate, nitrite, ammonia and pH were monitored daily and the photoperiod used was a 14 h light/10 h dark cycle. A total of 60 fish weighing 45.0 g on average were randomly assigned to 3 tanks (20 fish per tank) and were randomly assigned to one of three experimental diets: (i) control group (ii) oligosaccharides (I) group and (iii) oligosaccharides (II) group.

The basic diet was based on fish meal (Table 1). Fish in control group were fed with the basic diet. Fish in oligosaccharides (I) group were fed with the basic diet containing 1.5% oligosaccharides. Fish in oligosaccharides (II) group were fed with the basic diet containing 3% oligosaccharides. The diets were manufactured as described above. The fish were fed to satiation twice daily for the entire 30 days of experimental period. The total body weight of the fish was recorded on day 30 and feed intake was recorded daily. Growth performance was determined by weight gain (g fish^{-1}), and feed conversion ratio (feed gain^{-1}).

Enzyme assay

At the end of the experiment, for the samplings fish were anesthetized with benzocaine (50 mg/l), and heparinized blood was drawn from the caudal veins for assays of Hb (Sovcikova et al., 2002) and red blood cell count (RBC) (Dietrich et al., 2002). The entire alimentary tract was quickly removed and placed on ice. Individual atomaches and small intestines were washed thoroughly with chilled saline (0.89 g NaCl dl^{-1}), dried quickly over a piece of filter paper and weighed. As the intestines were very thin, whole gut was homogenised and the volume was made to 6 ml with normal saline as reported earlier (Onosaka and Cherian, 1981). The extract was centrifuged at 4500 × g at 4°C. The supernatant was stored at -20°C for analysis of -amylase (Jang et al., 1996), Lipase (Kim et al., 1996) and proteases (Ko et al., 1996). Enzyme activity was expressed min^{-1} g^{-1} of wet tissue.

Statistical analysis

All data for body weight gain were statistically analyzed using the General linear model procedure of the statistical analysis system. All statements of significance were based on probability of P 0.05. Results are presented as means ± SEM and data were analyzed by SAS software.

RESULTS

Effect of FOS on weight gain and feed conversion rate

Table 2 shows effect of FOS administration on weight gain and feed conversion rate. ANOVA revealed significant effect ($p < 0.05$) of the FOS administration on weight gain and feed conversion rate in fish. Compared with control group, the administration of oligosaccharides significantly increased weight gain and feed conversion rate in group II and III fish.

Effect of FOS on RBC and Hb

The effect of FOS administration on RBC number and Hb in fish was shown in Table 3. The number of RBC was significantly elevated ($P < 0.05$; $P < 0.01$) in fish (groups ii and iii) compared with that of control group (i). The same occurred with the Hb value in fish's blood, an increase ($P < 0.01$) in the two FOS groups compared with control group.

Table 2. Effect of FOS on weight gain and feed conversion rate.

Group	Initial body weight (g)	Final body weight (g)	weight gain (g)	Feed conversion rate
I	45.32 ± 1.17	82.44 ± 5.18	37.04 ± 1.09	1.71 ± 0.21
II	46.41 ± 2.05	89.49 ± 2.01	44.11 ± 1.27c	2.21 ± 0.31c
III	44.96 ± 3.12	91.37 ± 3.05	45.23 ± 1.42c	2 ± 0.38c

C = P<0.05, compared to group I (control group).

Table 3. Effect of FOS on RBC and Hb.

Group	RBC (10^4/mm)	Hb (g/ml)
I	182.47 ±11.43	19.31 ± 1.37
II	207.53 ± 13.09c	28.45 ± 1.64d
III	243.74 ± 15.03d	32.43 ± 2.05d

C = P < 0.05, d = P < 0.01, compared to group I (control group).

Effect of oligosaccharides on the activities of digestive enzymes in fish's stomach

The effect of FOS treatment on the activities of digestive enzymes (protease, lipase and amylase) in fish's stomach was assessed. FOS treatment caused a marked (P < 0.01) effect on activities of stomach digestive enzymes in group ii and iii fish (Table 4). According to results, activities of stomach digestive enzymes (protease, lipase and amylase) were significantly increased in a dose-dependent manner.

Effect of FOS on the activities of digestive enzymes in fish's small intestine

The effect of FOS treatment on the activities of digestive enzymes (protease, lipase and amylase) in fish's small intestine was assessed. Activities of digestive enzymes were significantly increased in small intestine of fish treated with FOS (Table 5) (P < 0.05, P < 0.01) compared to those of control group.

DISCUSSION

Fructooligosaccharides (FOS) also sometimes called oligofructose or oligofructan, is a class of oligosaccharides used as an artificial or alternative sweetener. FOS use emerged in the 1980s in response to consumer demand for healthier and calorie- reduced foods. FOS serves as a substrate for microflora in the large intestine, increasing the overall gastrointestinal tract (GI Tract) health. It has also been touted as a supplement for preventing yeast infections (Sangeetha et al., 2005).

Red blood cells are the most common type of blood cell and the vertebrate body's principal means of delivering oxygen to the body tissues via the blood. The cells are filled with hemoglobin, a molecule that can bind to oxygen. Hemoglobin (also spelled haemoglobin and abbreviated Hb or Hgb) is the iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates. Hemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases the oxygen for cell use. It also has a variety of other roles of gas transport and effect-modulation which vary from species to species and are quite diverse in some invertebrates (Vedernikov et al., 1998).

This study has found that FOS can dose-dependently promote oxyeleotris lineolatus's growth rate, blood RBC and Hb number, indicating its significantly immunity-stimulating properties.

Protein is one of the three major food groups needed for proper nutrition. Proteases refer to a group of enzymes whose catalytic function is to hydrolyze (break-down) proteins. They are also called proteolytic enzymes or proteinases. Proteolytic enzymes are very important in digestion as they breakdown the peptide bonds in the protein foods to liberate the amino acids needed by the body (Marsman et al., 1995; Veeru et al., 2009).

Lipase is an enzyme necessary for the absorption and digestion of nutrients in the intestines. This digestive enzyme is responsible for breaking down lipids (fats), in particular triglycerides, which are fatty substances in the body that come from fat in the diet. Once broken down into smaller components, triglycerides are more easily absorbed in the intestines. Lipase is primarily produced in the pancreas but is also produced in the mouth and stomach. Most people produce sufficient amounts of pancreatic lipase (Kusunoki et al, 2005).

Amylase is an enzyme which breaks down (hydrolyzes) starch, the reserve carbohydrate in plants and glycogen, the reserve carbohydrate in animals into reducing fermentable sugars, mainly maltose, and reducing nonfermentable or slowly fermentable dextrins (Yamamoto et al, 1999).

We investigated the changes in the levels of protease, lipase and amylase in fish's stomach and small intestine that were administrated by FOS for 30 days. To our knowledge, this is the first demonstration of the activities of digestive enzymes in oxyeleotris lineolatus fed with FOS. These data are of particular interest in the fish breeding industry. We observed that the activities of digestive enzymes in oxyeleotris lineolatus was

Table 4. Effect of FOS on activities of digestive enzymes in fish's stomach.

Group	Protease (U/mg ptot)	Lipase (U/mg ptot)	Amylase (U/mg ptot)
I	0.49 ± 0.03	0.032 ± 0.008	0.091 ± 0.005
II	0.81 ± 0.02d	0.059 ± 0.004d	0.144 ± 0.009d
III	0.99 ± 0.05d	0.083 ± 0.003d	0.173 ± 0.016d

d : P<0.01, compared to group I (control group).

Table 5. Effect of FOS on activities of digestive enzymes in fish's small intestine.

Group	Protease (U/mg ptot)	Lipase (U/mg ptot)	Amylase (U/mg ptot)
I	0.53 ± 0.04	0.091 ± 0.013	0.194 ± 0.011
II	0.87 ± 0.02d	0.114 ± 0.010c	0.261 ± 0.013d
III	1.04 ± 0.08d	0.132 ± 0.017d	0.295 ± 0.021d

C : P < 0.05, d : P<0.01, compared to group I (control group).

significantly increased 30 days after the FOS was administrated. Long-term FOS administration may, therefore be necessary for the oxyeleotris lineolatus growth. To conclude, some FOS products affect the digestion of nutrients in oxyeleotris lineolatus positively. This could possibly be associated with nutritional promotion effects of the FOS. The gastrointestinal tract is very heavily populated with bacteria, mainly of strictly anaerobic bacteria. This microbial community is extremely complex, both in terms of the number of organisms, approximately 10^{13} in total, and in its diversity, with over 400 - 500 different species reported. Most of these organisms are benign to the host, however, certain gut species are pathogenic and may be involved in the onset of acute and chronic disorders (Isoulari et al, 2005; Sun et al., 2009; Zhou et al., 2009). Due to their chemical structure, FOS are substrates that can only be consumed by a limited number of bacteria, stimulating thus their growth. Among the group of bacteria present in the gastrointestinal tract, the bifidobacteria and lactobacilli are those that most utilize oligosaccharides being con-sidered as the only microorganisms able to beneficially affect the host's health " beneficially effect the host's health (Bielecka et al., 2002; Wu et al., 2009; Hassan et al., 2009). In addition, the increase of the bifidobacteria and lactobacilli in the gastrointestinal tract results in the production of some nutrient elements and digestive enzymes. The latter was confirmed by the increase activities of digestive enzymes in oxyeleotris lineolatus."

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