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Effects of the dietary supplementation with fructooligosaccharides on the excretion of nitrogen and phosphorus in *Miichthys miiuy* fries*

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Abstract: Effects of dietary supplementation with fructooligosaccharides on the excretion of nitrogen and phosphorus in *Miichthys miiuy* fries were investigated. Nine hundred *Miichthys miiuy* fries were divided into 3 groups, each with triplicates. The basal diet and the basal diet supplemented with carnitine groups were considered as the negative and positive controls respectively. Results showed that the nitrogen concentration in excreted feces decreased significantly in fries fed the diet supplementation with 1000×10^{-6} fructooligosaccharides and 200×10^{-6} carnitine ($P<0.05$). The ammonic-nitrogen concentration decreased significantly in the carnitine group only ($P<0.05$), indicating the decreasing tendency caused by the supplementation with fructooligosaccharides. Supplementation with both did not have significant effects on the concentration of phosphorus in feces of *Miichthys miiuy* fries.

Key words: Fructooligosaccharides, *Miichthys miiuy* fries, Nitrogen, Phosphorus, Carnitine
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INTRODUCTION

Aquaculture, one of fastest-growing food industries, has increased worldwide at an average rate of 9.2% per year since 1970, compared with only 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production system (FAO, 2002). Aquaculture has become the most advanced among agricultural fields. Simultaneously, due to intensive farming and lack of controlling guidelines, aquaculture's impact on the environment has become of increasing concern. Excessive nutrients (especially nitrogen and phosphorus) in food residue and feces released to the farm have resulted in water pollution (Hecky and Kilham, 1988; Wiesmann *et al*., 1988; Pillay, 1992; Ketola and Harland, 1993; Wu, 1995; Skonberg *et al*., 1997). Therefore, fishery technology to reduce culture environment pollution must be developed. Manipulations of the dietary components to balance nutrition and the use of feed additives are considered to be effective ways (Bergheim *et al*., 1984; Cho and Bureau, 1997; Cheng *et al*., 2003; Sajjadi and Carter, 2004).

Fructooligosaccharides originally used to supplement human diets (Gibson and Roberfroid, 1995; Coudray *et al*., 1997). It has attracted considerable interest in implement in animals (Delzenne *et al*., 1995; Manhart *et al*., 2003; Twomey *et al*., 2003).

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The function of fructooligosaccharides for fish has not been investigated. This study aimed at evaluating the influences of a dietary supplementation of fructooligosaccharides on the excretion of nitrogen and phosphorus from *Miichthys miiuy* fries originally produced in the west coast of north Pacific. The experiment was conducted in the Breeding Base in the Ningbo Institue of Aquacultural Research from April to July in 2004.

MATERIALS AND METHODS

Diet preparation and experiment design

The experimental diet components are presented in Table 1. The natural supply of nutrients is adequate for *Miichthys miiuy* fries (the nutrition requirement of *Miichthys miiuy* fries unpublished). The basal diet consisted of red fishmeal, soybean meal, corn protein meal, protein powder of blood corpuscles, fish oil and wheat meal. Three groups of fries were used in the research. Group I was fed with basal diet as the negative control, Group II basal diet was supplemented with carnitine (200 mg/kg) as the positive control and Group III basal diet was supplemented with fructooligosaccharides (1000 mg/kg) as the trial feed. The experimental diets were pelleted (diameter 2.5 mm) by Ningbo Tech-Bank Co. Ltd. and stored at –20 °C. The pellet could float in the static water for at least 20 min.

Fish and feeding

Miichthys miiuy fries were bought in a marine culture market with cages (Xibu Port, Xiangshan, Zhejiang Province, China). Nine hundred healthy *Miichthys miiuy* fries with average body weight of 1.5±0.5 g were used for this experiment. The fish were acclimated to laboratory conditions for 2 weeks in 80 cm wide, 90 cm long, 100 cm high PVC tanks and fed a commercial diet (Ningbo Tech-Bank Co. Ltd.). The laboratory conditions during the acclimation period were similar to those for the experiment. Oxygen concentration dissolved in each PVC tank was monitored daily and averaged at 8.3±0.5 mg/L throughout the experimental period. Salinity was averaged at 27.5‰±0.5‰. Seawater used for rearing was sieved through sand and sterilized by flowing calcium oxide immediately before the experiment in net cages (300 mesh). A photoperiod of 14-h light, 10-h dark was used. The water was completely changed twice daily. During the experimental period, the *Miichthys miiuy* fries were randomly divided into three triplicate groups of 100 fries each and were fed in 720-liter PVC tanks. After the acclimation period the *Miichthys miiuy* fries were fed their respective experimental diets at a rate of 4% of the body weight at 7:00, 13:00 and 18:00 each day.

Sampling and chemical analysis

1. Water sampling

The water samples were collected on the 16th and 17th day of the experimental period. The *Miichthys miiuy* fries were fed at 13:00; feed residue was removed 30 min after feeding and the first water samples were collected 100 min after feeding.

The second water samples were collected 240 min after feeding; water samples were collected again on the following day at the same time point without feeding.

2. Feces sampling

The feed residue was removed everyday immediately after fries stopped feeding. The feces samples were then collected using a siphon 45 min later. Afterwards salt in samples was removed using quantitative filter paper and the samples were dried using electric fan and stored in liquid nitrogen for subsequently laboratory tests which were conducted as soon as possible.

3. Chemical analysis

Chloroform (1/1000, *V*/*V*) was added to the filtrated water samples which were frozen immediately for future use. Nitrogen and phosphorous contents in the water samples were analyzed by a μMic-1000 nitrogen-phosphorus analysis device (CYSTEA Company). Total nitrogen in the feces was analyzed by a Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Sweden). Phosphorus content in the feces was determined by the conventional molybdenum colorimetry methods.

Table 1 Components of basal diet

Crude protein (%)	Crude fat (%)	Acid-insoluble ash (%)	Dry matter (%)	Calcium (%)	Phosphorus (%)	Gross energy (kJ/g)
46.41	9.85	1.49	94.24	1.25	1.89	19.30

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA). When the group differences were significant (P<0.05), multiple comparisons between the treatments were made using Duncan's multiple range test (SAS/PC program). Statistical significance was determined by setting aggregate type I error at 5% (P<0.05) for each set of comparisons.

RESULTS

Ammonia nitrogen concentration in the water

The ammonia nitrogen concentration in water sample for *Miichthys miiuy* fries fed the basal diet was significantly higher than the group with basal diet supplemented with carnitine (P<0.05) (Table 2). Although there was no significant difference from the basal diet group (P>0.05) (Table 2), the supplementation with fructooligosaccharides had a tendency of decreasing the ammonia nitrogen concentration in the water. The ammonia nitrogen concentration for the group with basal diet supplemented with fructooligosaccharides was higher than the group with basal diet supplemented with carnitine, but the differences were not statistically significant (P>0.05) (Table 2).

Nitrogen concentration in feces of *Miichthys miiuy* fries

The nitrogen digestibility for the basal diet group was 87.81%, lower than the Groups II and III with supplements (Table 3). The fecal nitrogen content for Group I fed the basal diet was 3.71%, significantly higher than the Groups II and III with supplements (P<0.05) (Table 3). There was no significant difference between Groups II and III (P>0.05) (Table 3).

Phosphorus concentration excreted in the water and the feces

Table 4 shows that there were no significant differences in the phosphorus concentration in the water for *Miichthys miiuy* fries fed the basal diet and with supplementations. Feces phosphorus contents did not differ between the groups (P>0.05) (Table 4).

Table 2 The changes in ammonia nitrogen concentration (mg/L) for *Miichthys miiuy* fries fed a basal diet supplemented with either carnitine or fructooligosaccharides

Group	100 min after feeding X_1-X_0	240 min after feeding X_2-X_0
Basal diet	9.14±1.14	9.36±1.16
Basal diet+carnitine	4.15±0.22*	4.62±0.23*
Basal diet+fructooligosaccharides	7.66±0.78	7.97±0.63

Values are means of the triplicates, each containing 100 *Miichthysi miiuy* fries; *Significant differences (P<0.05); X_0 is the ammonia nitrogen concentration value in the water sample before feeding; X_1, X_2 are the ammonia nitrogen concentration in the water samples collected 100 min and 240 min respectively after feeding

Table 3 The nitrogen content in feces of *Miichthys miiuy* fries with basal diet supplemented with either carnitine or fructooligosacharides

Group	Digestibility (%)	Fecal nitrogen (%)
Basal diet	87.81±0.62	3.71±0.11
Basal diet+carnitine	89.42±0.50*	3.66±0.02*
Basal diet+fructooligosacharides	89.23±0.28*	3.50±0.02*

*Significant differences (P<0.05)

Table 4 The changes in phosphorus concentration ($\times10^{-1}$ mg/L) in the water and phosphorous concentration in the feces of fries fed basal diet supplemented with either carnitine or fuctooligosaccharides

Group	100 min after feeding X_1-X_0	240 min after feeding X_2-X_0	Fecal phosphorus contents (%)
Basal diet	6.92±0.23	7.02±0.20	1.20±0.05
Basal diet+carnitine	6.99±0.12	7.04±0.10	1.18±0.11
Basal diet+fructooligosachrides	6.97±0.28	6.98±0.24	1.15±0.07

Significant difference (P<0.05); X_0 means the PO_4^{3-} concentration with water sample before feeding; X_1, X_2 are the PO_4^{3-} concentration in the water collected 100 min and 240 min after feeding

DISCUSSION

It is well known that intake of nitrogen equals to the nitrogen excreted in feces and urea plus nitrogen deposit in body for terrestrial animals. For most teleosts as well as the *Miichthys miiuy* they excrete predominantly ammonia together with a small amount of urea, accounting for approximately 20% of total nitrogen excretion. Gills are known to be the main excretion sites of both ammonia and urea (Mommsen and Walsh, 1991; Wood, 1993). Ammonia nitrogen is the catabolic product of protein in the body. The dissolution of excreted ammonia nitrogen in water increases the ammonia nitrogen in the aquaculture environment. This study showed that the ammonia nitrogen concentration in the water was reduced when the fries' basal diets were supplemented with carnitine ($P<0.05$), and that the supplementation of the basal diet with fructooligosaccharides tended to reduce ammonia nitrogen concentration in the water (Table 2). Fecal nitrogen from fries is another major pollutant to water (Wu, 1995). This study showed that supplementation of either carnitine or fructooligosaccharides to the fries' basal diet could reduce the fecal nitrogen excretion (Table 2). In brief, the total nitrogen in the water and feces showed decreasing tendency when the *Miichthys miiuy* fries' basal diet were supplemented with fructooligosarccharides. The reason is unclear, so further investigation must be conducted. The study on dietary fiber in dogs indicated that excretion of fecal and urinary N and dietary N intake tended to be low for the fructooligosaccharides diet (Howard *et al*., 2000). The present study showed that there were no significant differences in the ammonia nitrogen concentration in the water in response to a supplementation with fructooligosaccharrides to the basal diet of fries (Table 2). The possible reason could be that the *Miichthys miiuy* fries were at the growing stage during which more nitrogen was used for the body protein deposition. Fructooligosaccharides probably enhanced the nitrogen deposition in body protein as the protein digestibility increased in the present study. The reason for the effect of fructooligosaccharides supplementation on the nitrogen digestibility is not clear.

Phosphorus is another major pollutant in culture water environment (Wu, 1995). Additives such as phyate were investigated to reduce phosphorus waste from plant-meal-based diets for Atlantic salmon (Sajjadi and Carter, 2004). In the present study, there were no significant differences in the excreted phosphorus either into water or in the feces ($P>0.05$) (Table 4). A study in *Salmon* showed that the dissolved phosphorus accounted for 50% of total phosphorus and that the feces phosphorus dissolved also in water, and was 30% of the total phosphorus **(**Ketola and Harland, 1993). The phosphorus is rich in fish meal in the form of $n Ca_3(PO_4)_2 \cdot CaCO_3$. It was impossible for *Miichthys miiuy* fries to use fishmeal completely, so it was convenient to increase the phosphorus concentration of the culture water in the form of feces phosphorus. Fructooligosaccharides can enhance mineral apparent absorption (Lopez *et al*., 2000a); Delzenne *et al*.(1995) suggested that a diet supplemented with fructooligoccharrides led to an improvement of the absorption of Ca, Mg, Fe and Zn. Although interaction exists between the calcium and phosphorus absorption and the absorption of calcium can cause terrestrial animals' absorption of phosphorus. The influence of high dietary calcium intake on phosphorus utilization by Atlantic salmon (*Salmo salar*) reared in freshwater was not significantly different ($P>0.05$) (Vielma and Lall, 1998). Therefore, the supplementation of fructooligosaccharides probably did not reduce the feces phosphorus excretion.

Fructooligosaccharides supplementation to diets could enhance the absorption of minerals such as Ca, Mg, Fe and Zn (Delzenne *et al*., 1995). Furthermore, the presence of fermentable carbohydrates in diets could stimulate bacterial proliferation in the cecum, which could enhance divalent cation absorption (Lopez *et al*., 1998; 2000a; 2000b). In other words, intestinal bacteria could be important in enhancing absorption of certain minerals. Therefore, the addition of probiotics to fructooligosaccharides could together have better performance in promoting the absorption of minerals.

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