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Improved glucose and lipid metabolism in European sea bass (*Dicentrarchus labrax*) fed short-chain fructooligosaccharides and xylooligosaccharides

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ABSTRACT

The effects of short-chain fructooligosaccharides (scFOS) and xylooligosaccharides (XOS) on growth, feed utilization and liver activity of key enzymes of glycolytic, gluconeogenic, and lipogenic pathways were studied in European sea bass juveniles. This is the first study about the effect of prebiotics on fish glucose metabolism and few and contradictory studies are available about prebiotic effect on lipid metabolism.
Fish were fed isoproteic (46%) and isolipidic (15%) diets based on fish meal (FM diets) or plant ingredients (PP diets; 30 FM:70 PP) as main protein sources. Four other diets were formulated similar to the control diets (PPC; FMC) but including 1% scFOS or 1% XOS (PPFOS, PPXOS, FMFOS and FMXOS diets). Growth performance was higher in fish fed PPXOS diet than PPC diet. No effect of dietary prebiotics on feed efficiency was noticed. Glucokinase activity was higher in fish fed FMFOS and FMXOS diets than FMC diet. Lipogenic enzyme activities (malic enzyme, fatty acid synthetase, glucose-6-phosphate dehydrogenase) were lower in fish fed diets including XOS than in the other groups. Glycolytic (glucokinase, pyruvate kinase) and lipogenic enzyme activities were higher, and gluconeogenic (fructose-1,6-bisphosphatase) enzyme activity was lower in fish fed FM diets than the PP diets. Overall, dietary XOS decreased lipogenesis, independently of dietary protein source, and improved growth performance in fish fed PP diets. In fish fed FM diets, XOS and scFOS increased glycolytic activity. XOS seemed to have good potential to be used as prebiotic in European sea bass.

1. Introduction

Prebiotics by definition are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific bacteria present in the gastrointestinal tract (GI), improving host health (Gibson and Roberfroid, 1995). Therefore prebiotics are selectively fermented by specific-health promoting bacteria such as *Lactobacillus* and *Bifidobacterium*, leading to a decrease of pathogenic bacterial species in the GI tract and/or to the production of fermentation end products, mainly short-chain fatty acids (SCFAs), which can modulate host glucose and lipid metabolism (Delzenne et al., 2008; Gibson and Roberfroid, 1995; Qiang et al., 2009; Roberfroid et al., 2010). Compared with humans and farm animals, less information is available concerning the effects of prebiotics on aquatic animals (Dimitroglou et al., 2011; Merrifield et al., 2010; Ringø et al., 2010).

Fructooligosaccharides (FOS) are among the most well-established prebiotics for use in aquafeeds (Ringø et al., 2010). FOS are produced on a commercial scale by two different processes, either through enzymatic hydrolysis of chicory-root inulin or from sucrose. FOS are composed by long linear chains of fructose units linked by β-(2–1) bonds attached to a terminal glucose unit. Short-chain fructooligosaccharides (scFOS) have a chemical composition similar to that of FOS but a degree of polymerization of only 1 to 5 glucose units (Bornet et al., 2002). FOS are fermented in the GI tract by beneficial bifidobacteria and other lactic-acid producing bacteria which possess β-fructosidase that hydrolyses β-(2–1) glycosidic bonds, an enzyme lacking in mammals and fish digestive tracts (Roberfroid and Slavin, 2000). In several fish species, it was reported that FOS and scFOS improved growth performance and feed efficiency, enhanced non-specific immune responses and disease resistance, improved gut function and morphological status, and increased health-promoting bacteria in the intestine (Anguiano et al., 2013; Hui-yuan et al., 2007; Ortiz et al., 2013; Soleimani et al., 2012; Zhou et al., 2009, 2010).

Xylooligosaccharide (XOS) is an emerging prebiotic gaining importance as functional ingredient in pharmaceutics, feed and food formulation (Aachary and Prapulla, 2011). Industrially, XOS is produced by chemical or enzymatic hydrolysis of xylan, which is

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the major component of lignocellulosic raw materials (Vázquez et al., 2000). Although the chemical structure of XOS depends on the xylan source, it generally consists of chains of xylose linked by β-(1–4) bonds, with a degree of polymerization ranging from 2 to 10. Studies in mammals showed that XOS promoted activity of beneficial intestinal bacteria, mainly *Bifidobacterium* species, leading to an increase of caeca SCFAs (Broekaert et al., 2011). These two effects are related with a number of health benefits, including improvement of bowel function, mineral absorption, lipid and glucose metabolism, immunomodulatory activity, reduction of colon cancer risk, and to antioxidant, anti-inflammatory and anti-microbial functions (Aachary and Prapulla, 2011; Broekaert et al., 2011). Up to now, only two studies were performed to evaluate XOS potential in fish (Li et al., 2008; Xu et al., 2009). In allogynogenetic crucian carp (*Carassius auratus gibelio*) growth performance and digestive enzyme activities were higher in fish fed 100 mg XOS kg^{-1} compared to the control diet (Xu et al., 2009). Dietary XOS incorporation at 400 mg kg^{-1} enhanced growth performance and nonspecific immunity of juvenile turbot (*Scophthalmus maximus*) (Li et al., 2008).

It has been reported that prebiotics, namely FOS and XOS, can alter glucose and lipid metabolism in mammals, depending on colonic fermentation process (Broekaert et al., 2011; Delzenne et al., 2008; Roberfroid et al., 2010). Thus, several studies indicated that FOS and XOS improved glucose tolerance, by lowering plasma glucose levels and enhancing insulin sensitivity (Gobinath et al., 2010; Respondek et al., 2011; Sheu et al., 2008; Shinoki and Hara, 2011), and reduced hepatic lipogenesis, serum and liver cholesterol, and triacylglycerides levels and increased serum HDL/LDL ratio (Fiordaliso et al., 1995; Kok et al., 1996; Sheu et al., 2008; Wang et al., 2011). However, the effect of prebiotics on lipid metabolism in fish was scarcely studied (Guerreiro et al., 2014; Torrecillas et al., 2011b) and no studies are available concerning prebiotic effects on glucose metabolism.

In contrast to probiotics, few studies on prebiotic effects were conducted in European sea bass (*Dicentrarchus labrax*), and focused only on the effect of mannanoligosaccharides (MOS) on the immune system and disease resistance (Torrecillas et al., 2007, 2011a,b, 2012, 2013). The aim of the present study was thus to assess the role of scFOS and XOS, incorporated into plant protein or fish meal based diets, on European sea bass glucose and lipid metabolism, through measurement of activities of key liver enzymes of glycolytic, gluconeogenic and lipogenic pathways.

Table 1
Ingredient composition and proximate analysis of the experimental diets.

	Diets					
	PPC	PPFOS	PPXOS	FMC	FMFOS	FMXOS
Ingredients (% dry weight basis)						
Fish meal[a]	15.6	15.6	15.6	59.2	59.2	59.2
Soluble fish protein concentrate[b]	5.0	5.0	5.0	5.0	5.0	5.0
Soybean meal[c]	25.0	25.0	25.0	–	–	–
Wheat meal[d]	18.3	18.3	18.3	–	–	–
Wheat gluten[e]	15.0	15.0	15.0	–	–	–
Corn gluten[f]	5.2	5.2	5.2	–	–	–
Cod liver oil	11.5	11.5	11.5	8.9	8.9	8.9
Pregelatinized maize starch[g]	–	–	–	22.4	22.4	22.4
Fructooligosaccharide[h]	–	1.0	–	–	1.0	–
Xylooligosaccharide[i]	–	–	1.0	–	–	1.0
Vitamin premix[j]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[k]	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride (50%)	0.5	0.5	0.5	0.5	0.5	0.5
α-Cellulose	1.0	–	–	1.0	–	–
Binder[l]	1.0	1.0	1.0	1.0	1.0	1.0
Proximate analyses (% dry weight basis)						
Dry matter	89.5	89.1	89.8	88.6	93	96.1
Crude protein	45.7	45.9	46	46.2	46.7	47.4
Crude fat	14.9	15.4	15.2	15.4	15	15.1
Ash	6.9	6.9	6.9	12.5	12.6	12.7
Starch	12.6	14.3	13.2	19.7	20.2	19.0
Gross energy (kJ g^{-1} DM)	22.9	22.7	23.5	21.1	20.9	21.2

DM, dry matter; CP, crude protein; GL, gross lipid.
[a] Inproquise, Madrid, Spain (CP: 70.1% DM; GL: 8.8% DM).
[b] Sopropèche G, France (CP: 79.4% DM; GL: 19.7% DM).
[c] Sorgal, S.A. Ovar, Portugal (CP: 50.5% DM; GL: 1.7% DM).
[d] Sorgal, S.A. Ovar, Portugal (CP: 11.8% DM; GL: 1.9% DM).
[e] Sorgal, S.A. Ovar, Portugal (CP: 82.8% DM; GL: 1.9% DM).
[f] Sorgal, S.A. Ovar, Portugal (CP: 65.7% DM; GL: 3.5% DM).
[g] C-Gel Instant — 12016, Cerestar, Mechelen, Belgium.
[h] PROFEED Maxflow, Jefo, France.
[i] Qingdao FTZ United International Inc., Quingdao, China.
[j] Vitamins (mg kg^{-1} diet): retinol, 18000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); α-tocopherol, 35; menadione sodium bisulphate, 10; thiamine, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.
[k] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.44 (g kg^{-1} diet).
[l] Aquacube. Agil, England (guar gum, polymethyl carbamide, manioc starch blend, hydrate calcium phosphate).

2. Materials and methods

2.1. Diets

Two control diets were formulated to be isoproteic (46% crude protein) and isolipidic (15% crude lipid). One diet included fish meal (FM) as the main protein source (FMC diet) and the other diet included FM and plant ingredients (PP; soybean meal, wheat meal, wheat gluten and corn gluten) at a ratio of 30 FM:70 PP as protein sources (PPC diet). In both diets, cod liver oil was used as the main lipid source. Control diets also differ in terms of starch source. PPC diet contained approximately 13% of wheat starch and FMC diet contained approximately 20% of pregelatinized maize starch. In the experimental diets two commercial available prebiotics — scFOS (PROFEED Maxflow, Jefo, France) and XOS (Qingdao FTZ United International Inc., Quingdao, China) were added to the control diets at 1%, replacing α-cellulose (PPFOS, PPXOS, FMFOS and FMXOS diets). All dietary ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (California Pellet Mill, CPM Crawfordsville, IN, USA) through a 3 mm die. The pellets were then dried in an oven (40 °C) for 24 h and stored in plastic containers until used. The ingredients and proximate composition of the experimental diets are presented in Table 1.

2.2. Animals and experimental conditions

The experiment was conducted according to the recommendations of the European Union Directive 2010/63/EU on the protection of animals for scientific purposes. The growth trial was performed at the Marine Zoology Station, Porto University, Portugal, in a thermoregulated recirculating water system equipped with 18 fibreglass tanks (100 L capacity) supplied with continuous flow of filtered seawater (6.0 L min^{-1}), temperature regulated to 25.0 ± 1.0 °C, salinity of 36.0 ± 1.0 g L^{-1} and dissolved oxygen kept near saturation (7.0 mg L^{-1}).

European sea bass juveniles were obtained from a commercial fish farm (Maresa S.A., Ayamonte, Huelva, Spain) and after transportation to the experimental facilities fish were submitted to a quarantine period of 15 days. During that period fish were fed with a commercial diet (48% protein, 11% lipids, 5% starch). Thereafter, 18 groups of 20 fish with an initial mean body weight of 60 ± 0.01 g were established and each diet randomly assigned to triplicate tanks. The trial lasted 7 weeks and during that period fish were fed by hand, 6 days a week, until visual satiation. Utmost care was taken to assure that all feed supplied was consumed. No mortality occurred during the growth trial.