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

Inês Guerreiro^{a,b,*}, Aires Oliva-Teles^{a,b}, Paula Enes^a

^aCIIMAR/CIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal.

^bDepartamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal.

*Corresponding author:

Inês Guerreiro

Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

Rua do Campo Alegre s/n, Ed. FC4

4169-007 Porto, Portugal

Tel.: +351 220402736; Fax: +351 223401511;

imguerreiro@gmail.com

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 prebiotics effect on lipid metabolism. Fish were fed isoproteic (46%) and isolipidic (15%) diets based on fish meal (FM diets) or plant ingredients (PP diets; 30FM:70PP) 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 enzymes 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 enzymes activities were higher, and gluconeogenic (fructose-1,6- biphosphatase) enzyme activity was lower in fish fed the 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.

Keywords: European sea bass; Short-chain Fructooligosaccharides; Glucose metabolism; Lipid metabolism; Prebiotic; Xylooligosaccharides.

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; Zhou et al., 2010).

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

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., Qingdao, 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 fiberglass 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.

2.3 Sampling

Fish in each tank were bulk-weighed at the beginning and at the end of the trial, after 1 day of feed deprivation. For that purpose, fish were slightly anaesthetised with 0.3 ml L⁻¹ ethylene glycol monophenyl ether. After the final weighing fish continued to be fed for 3 more days to minimize manipulation stress and then 6 fish from each tank were sampled 6 h after the morning meal. Blood from 3 fish per tank was collected from the caudal vein with a heparinised syringe and immediately centrifuged at 10 000×g for 10 min. Plasma aliquots were frozen at -80°C until analysis of glucose, triacylglycerides, cholesterol and total lipids. Fish were then sacrificed with a sharp blow in the head and dissected on chilled trays. Livers were weighed for determination of hepatosomatic index (HSI) and then stored to quantify glycogen and lipid contents. Other 3 fish were sacrificed as above and livers collected for measurement of the activities of key enzymes of intermediate metabolism. After being collected livers were snap frozen in liquid nitrogen and stored at -80°C until analysis.

2.4 Proximate analysis of the diets

Chemical analyses of the diets were performed following Association of Official Analytical Chemists methods ([AOAC, 2000](#)). Energy content was determined by direct combustion in an adiabatic bomb calorimeter (PARR model 1261, PARR Instruments, Moline, IL, USA) and starch according to [Beutler \(1984\)](#).

2.5 Plasma metabolites and liver composition

Plasma glucose, cholesterol, triacylglycerides and total lipids were analyzed using enzymatic colorimetric kits from Spinreact, Girona, Spain (glucose kit, code 1001191;

cholesterol kit, code 1001091; triacylglycerides kit, code 1001312; total lipids kit, code 1001270). Hepatic glycogen and liver lipids were measured as described by [Plummer \(1987\)](#) and [Folch et al. \(1957\)](#), respectively.

2.6 Enzyme activity

In order to measure hexokinase (HK; EC 2.7.1.1), glucokinase (GK; EC 2.7.1.2), and L-type pyruvate kinase (PK; EC 2.7.1.40) activities, liver samples were homogenized (dilution 1:10) in ice-cold buffer (80 mM Tris-base; 5 mM EDTA; 2 mM dithiothreitol; 1 mM benzamidine; 1mM 4-(2-aminoethyl) benzenesulphonyl fluoride, pH 7.6).

Homogenates were centrifuged at 900×g for 10 min at 4°C and the resultant supernatant was separated for HK/GK and PK activity measurements. The HK (low Km HKs) and GK (high Km HK or HK IV) activities were measured using 0.5 mM and 100 mM of glucose, respectively, as described previously ([Panserat et al., 2000](#); [Tranulis et al., 1996](#)). To measure PK activities, the supernatant was centrifuged at 10 000×g for 20 min at 4°C and the resultant cytosolic fraction was used for enzyme activity measurements according to [Foster and Moon \(1985\)](#).

To measure the activity of fructose-1,6-bisphosphatase (FBPase; EC 3.1.3.11), liver samples were homogenized (dilution 1:10) in ice-cold buffer (20 mM Tris-HCl; 5 mM EDTA; 2 mM dithiothreitol; 0.24 M saccharose, pH 8). The homogenate was centrifuged at 900×g for 10 min at 4°C. Enzyme assays were performed on cytosolic fractions as previously described by [Tranulis et al. \(1996\)](#).

For glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), malic enzyme (ME; EC 1.1.1.40) and fatty acid synthetase (FAS; EC 2.3.1.38) activities, liver samples were homogenized (dilution 1:5) in ice-cold buffer (0.02 M Tris-HCl; 0.25 M sucrose; 2 mM EDTA; 0.1 M sodium fluoride; 0.5 mM phenyl methyl sulphonyl fluoride (PMSF); 0.01

M β -mercapto ethanol, pH 7.4) and the homogenate centrifuged at 30 000×g for 20 min at 4°C. G6PD activity was measured according to [Bautista et al. \(1988\)](#), ME activity according to [Ochoa \(1955\)](#) and FAS activity according to [Chang et al. \(1967\)](#) as modified by [Chakrabarty and Leveille \(1969\)](#).

Enzyme assays were carried out at 37°C and the changes in absorbance were monitored to determine the enzyme activity using a microplate reader (ELx808; Bio-Tek Instruments, Winooski, Vermont, USA). The reagents used in the enzymatic analysis were purchased from Sigma-Aldrich (Sigma-Aldrich Química, S.L., Sintra, Portugal) and were of reagent grade.

All enzyme activities were expressed per mg of hepatic soluble protein (specific activity). Protein concentration was determined according to [Bradford \(1976\)](#) using Sigma protein assay kit (ref. B6916) with bovine serum albumin as standard. One unit of enzyme activity was defined as the amount of enzyme that catalysed the hydrolysis of 1 μ mol of substrate per min at assay temperature.

2.7 Statistical analysis

Results are presented as means with their standard deviations. Statistical analysis was done by two-way ANOVA using the SPSS 21.0 software package for Windows (IBM® SPSS® Statistics, New York, USA). Data were tested for normality by the Shapiro-Wilk test and homogeneity of variances by the Levene's test. When normality was not verified values were transformed prior to ANOVA. In the case of interaction between factors a one-way ANOVA analysis was also performed for prebiotics within each protein source. The probability level of 0.05 was used for rejection of the null hypothesis. Significant differences among groups were determined by the Tukey's multiple range test.

3. Results

In fish fed the PP diets, but not on fish fed the FM diets, growth performance was improved by XOS supplementation (Table 2). Protein source had no effect on fish growth performance ($P=0.136$). Feed intake (FI) was affected by dietary protein source ($P=0.000$) being higher in fish fed PP diets. In contrast, feed efficiency (FE) ($P=0.000$) and protein efficiency ratio (PER) ($P=0.000$) were higher in FM-fed fish. Prebiotic supplementation had no effect on FI, FE, and PER, independently of dietary protein source ($P>0.05$).

Plasma glucose ($P=0.000$) and cholesterol ($P=0.000$) levels were higher in fish fed the FM diets while plasma total lipids ($P=0.001$) and triacylglycerides ($P=0.000$) levels were higher in fish fed the PP diets (Table 3). All plasma parameters measured were unaffected by prebiotic supplementation ($P>0.05$).

Hepatosomatic index ($P=0.000$) and liver glycogen content ($P=0.000$) were higher in fish fed the FM diets (Table 3) while liver lipid content ($P=0.307$) was not affected by dietary protein source. All above mentioned parameters were unaffected by dietary prebiotic supplementation ($P>0.05$).

All measured liver enzymatic activities were affected by the dietary protein source (Table 4). HK, GK, PK, G6PD, ME (all with $P=0.000$) and FAS ($P=0.002$) activities were higher in fish fed the FM diets whereas FBPase ($P=0.000$) activity was higher in fish fed the PP diets. HK, PK and FBPase ($P>0.05$) activities were unaffected by prebiotic supplementation. In contrast, G6PD ($P=0.000$), ME ($P=0.001$) and FAS ($P=0.009$) activities were decreased in fish fed the XOS supplemented diets. In fish fed the FM diets, but not the PP diets, GK activity was higher in the scFOS and XOS groups.

4. Discussion

Prebiotics were reported as growth promoters in some studies performed in fish ([Dimitroglou et al., 2011](#); [Merrifield et al., 2010](#); [Ringø et al., 2010](#)). The eventual positive effect of prebiotics on growth performance is dependent on prebiotic source, fish species, and other factors, including diet composition. In the present study, XOS improved growth performance in fish fed PP diets but not in the FM diets, and scFOS did not affect growth performance of the animals, independently of diet composition. The absence of XOS effect on growth performance of fish fed the FM diets was possibly related to the high quality and digestibility of the diet, comparatively to the PP diet ([Enes et al., 2006](#)) which might have masked potential prebiotic effects. Improved growth with dietary XOS supplementation was previously reported by [Li et al. \(2008\)](#) in turbot and [Xu et al. \(2009\)](#) in allogynogenetic crucian carp. The lack of growth response due to dietary scFOS supplementation is also in agreement with previous studies in Atlantic salmon (*Salmo salar*) ([Grisdale-Helland et al., 2008](#)), red drum (*Sciaenops ocellatus*) ([Buentello et al., 2010](#)), and turbot ([Guerreiro et al., 2014](#)).

Previous studies also reported improved FE in fish fed diets supplemented with prebiotics, including a study with dietary XOS supplementation in turbot ([Li et al., 2008](#)). This higher FE was attributed to increased digestibility that could be related to increased exogenous microbial activity ([Soleimani et al., 2012](#)). In the present study, however no FE improvement was noticed due to prebiotic supplementation, independently of dietary protein source.

In mammals it was reported that FOS and XOS may alter glucose and lipid metabolism ([Broekaert et al., 2011](#); [Delzenne et al., 2008](#); [Roberfroid et al., 2010](#)). To the authors knowledge this is the first study in fish assessing the potential of prebiotics in

modulating glucose metabolism. While in fish fed the FM diets it was observed an increase of GK activity due to dietary prebiotics supplementation no such effect was evidenced in fish fed the PP diets. These differences between diets are possibly related to the lower dietary starch content in the PP diets. In contrast to mammals, dietary prebiotic supplementation did not seem to have improved glucose tolerance, as plasma glucose levels were not different to that of the control groups. In line with the present results, no changes in plasma glucose levels were observed in juvenile beluga (*Huso huso*) fed with diets supplemented with oligofructose or inulin ([Hoseinifar et al., 2011](#); [Reza et al., 2009](#)). [Guerreiro et al. \(2014\)](#) also reported no effect of scFOS on turbot glycaemia. Although not measured in the present study the SCFAs that are produced through prebiotics fermentation by gut microbiota, namely propionate, might explain the GK activity enhancement observed in fish fed FM diets. Thus, in isolated rat hepatocytes propionate stimulated glycolysis whereas the opposite was observed with acetate and butyrate ([Anderson and Bridges, 1984](#)). The enhancement of glycolysis observed with propionate was related with a decrease of hepatic citrate concentrations which is a metabolic inhibitor of phosphofructokinase ([Blair et al., 1973](#)). Thus, further studies, with SCFAs measurements are needed to understand the mechanism by which these end-fermentation products might affect GK activity. In mammals, besides stimulating glycolysis propionate also decreased gluconeogenesis via inhibition of pyruvate carboxylase, which catalyses the conversion of pyruvate to oxaloacetate ([Anderson and Bridges, 1984](#); [Blair et al., 1973](#)). In contrast, acetate and butyrate increased glucose production from lactate in rat isolated hepatocytes ([Anderson and Bridges, 1984](#)). Present data on FBPase activity pointed to an absence of prebiotics effect on gluconeogenesis. On the other hand, in fish fed FM diets FBPase activity was lower than in fish fed the PP diets, which had lower starch levels. Such a result is

interesting, as previous studies with European sea bass have reported no effect of dietary carbohydrate level on hepatic FBPase activities ([Enes et al., 2006](#); [Moreira et al., 2008](#)).

In mammals, propionate also stimulated the production of the intestinal hormone glucagon-like peptide 1 (GLP-1), which in turn stimulated insulin secretion leading to an increase of liver glycogen synthesis and a decrease in plasma glucose levels ([Cani et al., 2005](#); [Delzenne et al., 2007](#); [Frost et al., 2003](#)). It is unlikely that such effects occurred in European sea bass as no effect of prebiotics on hepatic glycogen deposition and glycaemia was noticed in the present study.

As observed for the glycolytic enzymes activity, lipogenic enzymes activity were higher in fish fed the FM diets than the fed PP diets, indicating that lipogenesis was stimulated by the higher dietary starch levels presented in FM diets. This was also previously observed in this species ([Dias et al., 1998](#); [Enes et al., 2010](#)). Curiously, this higher lipogenesis is not reflected in liver lipid deposition or in plasma total lipids and triacylglycerides levels. Only plasma cholesterol levels were in line with the higher lipogenic enzyme activities recorded in fish fed FM diets. However, this might be related to the presence of soybean meal in the PP diets, as it is known that soybean components, such as phytosteroids have a hypocholesterolemic effect in fish ([Gómez-Requeni et al., 2004](#); [Kaushik et al., 1995, 2004](#)), including European sea bass ([Couto et al., in press](#)).

In mammals, several studies reported a reduction of hepatic lipogenesis, plasma cholesterol and triacylglycerides levels due to dietary prebiotic supplementation ([Causey et al., 2000](#); [Fiordaliso et al., 1995](#); [Gobinath et al., 2010](#); [Kok et al., 1996](#); [Letexier et al., 2003](#); [Sheu et al., 2008](#); [Wang et al., 2011](#)). Accordingly, in this study dietary XOS supplementation also decreased the activity of G6PD, ME and FAS in fish

fed both PP and FM diets. Similar results were observed by [Torrecillas et al. \(2011b\)](#) in European sea bass fed diets with MOS. In the present study, scFOS at a 1% incorporation level had no effect on lipogenic enzyme activities. This may be related to the dietary inclusion level, as [Guerreiro et al. \(2014\)](#) observed lower hepatic ME activity in turbot juveniles fed diets incorporating scFOS at 2%.

The lower hepatic lipogenesis due to dietary XOS supplementation could be related, as happened with GK, to the colonic production of SCFAs generated through XOS fermentation by fish gut microbiota ([Delzenne et al., 2002, 2008; Teitelbaum, 2009](#)). Thus, an increase of caecal SCFAs concentration namely acetate, propionate, butyrate and lactate was recorded in rats fed XOS supplemented diets ([Campbell et al., 1997; Imaizumi et al., 1991](#)). However, the modulation of lipid metabolism by the main SCFAs produced, acetate and propionate, is conversely. Thus, whereas acetate is a lipogenic substrate, propionate is a competitive inhibitor of the entrance of acetate into liver cells ([Delzenne et al., 2008; Teitelbaum, 2009](#)). Propionate was also reported to inhibit lipid synthesis in rat hepatocytes ([Nishina and Freedland, 1990; Wright et al., 1990](#)). Moreover, acetate supplied in the diet of diabetic mice at a dose of 0.3% activates liver AMP kinase, an enzyme related with the inhibition of lipogenesis ([Sakakibara et al., 2006](#)). In fact, in an *in vitro* study with hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) of around 200 g, incubation with GroBiotic®-A, MOS, and galactooligosaccharide (GOS) tend to produce lower acetate levels but higher butyrate levels at 48 h when compared to the diet alone ([Burr et al., 2010](#)). Of the SCFAs produced, acetate was the one produced in higher concentration after 24 h in inoculated samples, however after 48 h the concentration was reduced ([Burr et al., 2010](#)). On the other hand, in *in vitro* studies with rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*), incubation with various saccharides lead the microbiota to

produce higher levels of butyric and propionic acid (Kihara and Sakata, 2001; Kihara and Sakata, 2002). In *in vivo* studies, for instance in Siberian sturgeon (*Acipenser baerii*) fed arabinoxylan-oligosaccharides (AXOS) the production of acetate and butyrate was increased while propionate was produced in very low levels and similar to control diet (Geraylou et al., 2012; Geraylou et al., 2013).

Overall, it seems that the pattern of prebiotics fermentation by gut microbiota and the ratio of acetate to propionate reaching the liver will condition the potential lipid-lowering properties of prebiotics, moreover that ratio may change depending on the fish species. Therefore, in future studies the determination of SCFAs production should be measured in order to validate the hypotheses that in fish as in mammals the glucose and lipid metabolism might be mediated by bacteria SCFAs production. Even though XOS decreased hepatic lipogenesis, plasma total lipids, cholesterol and triacylglycerides, as well as liver lipid content, were unaffected by dietary prebiotic supplementation, which is contrary to what has been observed in mammals, where a reduction of these parameters is usually detected (Gobinath et al., 2010; Sheu et al., 2008; Wang et al., 2011).

In conclusion, diet supplementation with XOS improved growth performance in fish fed PP diets but not the FM diets. Independently of dietary protein source, supplementation with XOS decreased lipogenesis. In fish fed FM diets, XOS and scFOS increased glycolytic activity. Fish fed FM diets had increased glycolysis and lipogenesis and decreased gluconeogenesis, which seems to be related with the higher dietary starch content of these diets. Overall, use of XOS as prebiotic for European sea bass seems to have good potential and requires further investigation.

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References

- Aachary, A.A., Prapulla, S.G., 2011. Xylooligosaccharides (XOS) as an emerging prebiotic: microbial synthesis, utilization, structural characterization, bioactive properties, and applications. *Compr. Rev. Food Sci. Food Safety* 10, 2-16.
- Anderson, J.W., Bridges, S.R., 1984. Short-chain fatty acid fermentation products of plant fiber affect glucose metabolism of isolated rat hepatocytes (41 958). *Proc. Soc. Exp. Biol. Med.* 177, 372-376.
- Anguiano, M., Pohlenz, C., Buentello, A., Gatlin III, D.M., 2013. The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* x *M. saxatilis*). *Brit. J. Nutr.* 109, 623-629.
- AOAC, 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA. 1018 pp.
- Bautista, J.M., Garrido-Pertierra, A., Soler, G., 1988. Glucose-6-phosphate dehydrogenase from *Dicentrarchus labrax* liver: kinetic mechanism and kinetics of NADPH inhibition. *Biochim. Biophys. Acta* 967, 354-363.
- Beutler, H.O., 1984. Starch. In: Bergmeyer, H.U. (Ed.), *Methods of enzymatic analysis*, vol. 6. Verlag Chemie Weinheim, Basel, pp. 2-10.
- Blair, J.B., Cook, D.E., Lardy, H.A., 1973. Interaction of Propionate and Lactate in the Perfused Rat Liver. Effects of Glucagon and Oleate. *J. Biol. Chem.* 248, 3608-3614.
- Bornet, F.R.J., Brouns, F., Tashiro, Y., Duvillier, V., 2002. Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. *Dig. Liver Dis.* 34, S111-S120.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.

Broekaert, W.F., Courtin, C.M., Verbeke, K., De Wiele, T.V., Verstraete, W., Delcour, J.A., 2011. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides and xylooligosaccharides. *Crit. Rev. Food Sci. Nutr.* 51, 178-194.

Buentello, J.A., Neill, W.H., Gatlin III, D.M., 2010. Effects of dietary prebiotics on the growth, feed efficiency and non-specific immunity of juvenile red drum *Sciaenops Ocellatus* fed soybean-based diets. *Aquac. Res.* 41, 411-418.

Burr, G., Hume, M., Ricke, S., Nisbet, D., Gatlin III, D., 2010. In Vitro and In Vivo Evaluation of the Prebiotics GroBiotic®-A, Inulin, Mannanooligosaccharide, and Galactooligosaccharide on the Digestive Microbiota and Performance of Hybrid Striped Bass (*Morone chrysops* × *Morone saxatilis*). *Microb. Ecol.* 59, 187-198.

Campbell, J.M., Fahey, G.C., Wolf, B.W., 1997. Selected Indigestible Oligosaccharides Affect Large Bowel Mass, Cecal and Fecal Short-Chain Fatty Acids, pH and Microflora in Rats. *J. Nutr.* 127, 130-136.

Cani, P.D., Daubioul, C.A., Reusens, B., Remacle, C., Catillon, G., Delzenne, N.M., 2005. Involvement of endogenous glucagon-like peptide-1(7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. *J. Endocrinol.* 185, 457-465.

Causey, J.L., Feirtag, J.M., Gahaher, D.D., Tuqland, B.C., Slavin, J.L., 2000. Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutr. Res.* 20, 191-201.

Chakrabarty, K., Leveille, G.A., 1969. Acetyl-CoA carboxylase and fatty acid synthetase activities in the liver and adipose tissue of meal-fed rats. *Proc. Soc. Exp. Biol. Med.* 131, 1051-1054.

Chang, H.C., Seidman, I., Teebor, G., Lane, M.D., 1967. Liver acetyl CoA carboxylase and fatty acid synthetase: relative activities in the normal state and in hereditary obesity. *Biochem. Biophys. Res. Commun.* 28, 682–686.

Couto, A., Kortner, T.M., Penn, M., Østby, G., Bakke, A.M., Krogdahl, Å., Oliva-Teles, A., in press. Saponins and phytosterols in diets of European sea bass (*Dicentrarchus labrax*) juveniles: effects on growth, intestinal morphology and physiology. *Aquac. Nutr.* doi: 10.1111/anu.12146.

Delzenne, N.M., Daubioul, C., Neyrinck, A., Lasa, M., Taper, H.S., 2002. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *Br. J. Nutr.* 87, S255-S259.

Delzenne, N.M., Cani, P.D., Neyrinck, A.M., 2007. Modulation of Glucagon-like Peptide 1 and Energy Metabolism by Inulin and Oligofructose: Experimental Data. *J. Nutr.* 137, 2547S-2551S.

Delzenne, N.M., Cani, P.D., Neyrinck, A.M., 2008. Prebiotics and lipid metabolism. In: Versalovic, J., Wilson, M. (Eds.), *Therapeutic microbiology: probiotics and related strategies*. ASM Press, Washington, DC, pp. 183-192.

Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Corraze, G., Bautista, J.M., Kaushik, S.J., 1998. Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). *Aquaculture* 161, 169-186.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Güroy, D., Davies, S.J., 2011. Microbial manipulations to improve fish health and production - A Mediterranean perspective. *Fish Shellfish Immunol.* 30, 1-16.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2006. Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles. *Comp. Biochem. Physiol. A* 143, 89-96.

Enes, P., Sanchez-Gurmaches, J., Navarro, I., Gutiérrez, J., Oliva-Teles, A., 2010. Role of insulin and IGF-I on the regulation of glucose metabolism in European sea bass (*Dicentrarchus labrax*) fed with different dietary carbohydrate levels. *Comp. Biochem. Physiol. A* 157, 346-353.

Fiordaliso, M., Kok, N., Desager, J.-P., Goethals, F., Deboyser, D., Roberfroid, M., Delzenne, N., 1995. Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* 30, 163-167.

Folch, J., Lees, M., Sloane-Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* 226, 497-509.

Foster, G.D., Moon, T.W., 1985. Enzyme activities in Atlantic hagfish, *Myxine glutinosa*: changes with capacity and food deprivation. *Can. J. Zool.* 64, 1080-1085.

Frost, G.S., Brynes, A.E., Dhillon, W.S., Bloom, S.R., McBurney, M.I., 2003. The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *Eur. J. Clin. Nutr.* 57, 293-298.

Geraylou, Z., Souffreau, C., Rurangwa, E., D'Hondt, S., Callewaert, L., Courtin, C.M., Delcour, J.A., Buyse, J., Ollevier, F., 2012. Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (*Acipenser baerii*) performance, immune responses and gastrointestinal microbial community. *Fish Shellfish Immunol.* 33, 718-724.

Geraylou, Z., Souffreau, C., Rurangwa, E., Maes, G.E., Spanier, K.I., Courtin, C.M., Delcour, J.A., Buyse, J., Ollevier, F., 2013. Prebiotic effects of arabinoxylan

oligosaccharides on juvenile Siberian sturgeon (*Acipenser baerii*) with emphasis on the modulation of the gut microbiota using 454 pyrosequencing. Microbiol. Ecol. 86, 357-371.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125, 1401-1412.

Gobinath, D., Madhu, A.N., Prashant, G., Srinivasan, K., Prapulla, S.G., 2010. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. Br. J. Nutr. 104, 40-47.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S., Pérez-Sánchez, J., 2004. Protein growth performance, amino acid utilisation and somatotrophic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture 232, 493- 510.

Grisdale-Helland, B., Helland, S.J., Gatlin III, D.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture 283, 163-167.

Guerreiro, I., Enes, P., Merrifield, D., Davies, S., Oliva-Teles, A., 2014. Effects of short-chain fructooligosaccharides on growth performance and hepatic intermediary metabolism in turbot (*Scophthalmus maximus*) reared at winter and summer temperatures. Aquac. Nutr. doi: 10.1111/anu.12175.

Hoseinifar, S.H., Mirvaghefi, A., Merrifield, D.L., Amiri, B.M., Yelghi, S., Bastami, K.D., 2011. The study of some haematological and serum biochemical parameters of juvenile beluga (*Huso huso*) fed oligofructose. Fish Physiol. Biochem. 37, 91-96.

Hui-yuan, L.V., Zhi-gang, Z., Rudeaux, F., Respondek, F., 2007. Effects of dietary short chain fructo-oligosaccharides on intestinal microflora, mortality and growth performance of *Oreochromis aureus* ♂ × *O. niloticus* ♀. Chin. J. Anim. Nutr. 19, 1-6.

Imaizumi, K., Nakatsu, Y., Sato, M., Sedarnawati, Y., Sugano, M., 1991. Effects of Xylooligosaccharides on Blood Glucose, Serum Liver Lipids and Cecum Short-chain Fatty Acids in Diabetic Rats. Agric. Biol. Chem. 55, 199-205.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133, 257-274.

Kaushik, S.J., Covès, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391-404.

Kihara, M., Sakata, T., 2001. Influences of incubation temperature and various saccharides on the production of organic acids and gases by but microbes of rainbow trout *Oncorhynchus mykiss* in a micro-scale batch culture. J. Comp. Physiol. B 171, 441-447.

Kihara, M., Sakata, T., 2002. Production of short-chain fatty acids and gas from various oligosaccharides by gut microbes of carp (*Cyprinus carpio* L.) in micro-scale batch culture. Comp. Biochem. Physiol. A 132, 333-340.

Kok, N., Roberfroid, M., Robert, A., Delzenne, N., 1996. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. Br. J. Nutr. 76, 881-890.

Letexier, D., Diraison, F., Beylot, M., 2003. Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. *Am. J. Clin. Nutr.* 77, 559-564.

Li, Y., Wang, Y.J., Wang, L., Jiang, K.Y., 2008. Influence of several non-nutrient additives on nonspecific immunity and growth of juvenile turbot, *Scophthalmus maximus* L. *Aquac. Nutr.* 14, 387-395.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Børgwald, J., Castex, M., Ringø, E., 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302, 1-18.

Moreira, I.S., Peres, H., Couto, A., Enes, P., Oliva-Teles, A., 2008. Temperature and dietary carbohydrate level effects on performance and metabolic utilisation of diets in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* 274, 153-160.

Nishina, P.M., Freedland, R.A., 1990. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *J. Nutr.* 120, 668-73.

Ochoa, S., 1955. Malic enzyme. In: Colowick, S.P., Kaplan, N.O. (Eds.), *Methods in Enzymology*, vol. 1. Academic Press, New York, USA, pp. 739-753.

Ortiz, L.T., Rebole, A., Velasco, S., Rodriguez, M.L., Trevino, J., Tejedor, J.L., Alzueta, C., 2013. Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 19, 475-482.

Panserat, S., Médale, F., Blin, C., Brèque, J., Vachot, C., Plagnes-Juan, E., Gomes, E., Krishnamoorthy, R., Kaushik, S., 2000. Hepatic glucokinase is induced by dietary carbohydrates in rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*), and common carp (*Cyprinus carpio*). *Am. J. Physiol.* 278, R1164-R1170.

- Plummer, D.T., 1987. An Introduction to Practical Biochemistry. 3rd edn. McGraw-Hill Book, London, UK. p.332.
- Qiang, X., YongLie, C., QianBing, W., 2009. Health benefit application of functional oligosaccharides. *Carbohydr. Polym.* 77, 435-441.
- Respondek, F., Myers, K., Smith, T.L., Wagner, A., Geor, R.J., 2011. Dietary supplementation with short-chain fructo-oligosaccharides improves insulin sensitivity in obese horses. *J. Anim. Sci.* 89, 77–83.
- Reza, A., Abdolmajid, H., Abbas, M., Abdolmohammad, A.K., 2009. Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, *Huso huso* (Linnaeus, 1758). *J. World Aquac. Soc.* 40, 771-779.
- Ringø, E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.I., Bakke, A.M., 2010. Prebiotics in aquaculture: a review. *Aquac. Nutr.* 16, 117-136.
- Roberfroid, M., Slavin, J., 2000. Nondigestible oligosaccharides. *Crit. Rev. Food Sci. Nutr.* 40, 461-480.
- Roberfroid, M., Gibson, G.R., Hoyles, L., McCartney, A.L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, M.J., Leotoing, L., Wittrant, Y., Delzenne, N.M., Cani, P.D., Neyrinck, A.M., Meheust, A., 2010. Prebiotic effects: metabolic and health benefits. *Br. J. Nutr.* 104, S1-S63.
- Sakakibara, S., Yamauchi, T., Oshima, Y., Tsukamoto, Y., Kadowaki, T., 2006. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. *Biochem. Biophys. Res. Commun.* 344, 597-604.
- Sheu, W.H.-H., Lee, I.-T., Chen, W., Chan, Y.-C., 2008. Effects of Xylooligosaccharides in type 2 diabetes mellitus. *J. Nutr. Sci. Vitaminol.* 54, 396-401.

Shinoki, A., Hara, H., 2011. Dietary fructo-oligosaccharides improve insulin sensitivity along with the suppression of adipocytokine secretion from mesenteric fat cells in rats. *Br. J. Nutr.* 106, 1190-1197.

Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M., Abadi, Z.H., 2012. Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish Shellfish Immunol.* 32, 316-321.

Teitelbaum, J.E., 2009. Prebiotics and lipid metabolism. In: Cho, S.S., Finocchiaro, T. (Eds.), *Handbook of prebiotics and probiotics ingredients: Health Benefits and Food Applications*. CRC Press, USA, pp. 209-220.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., Izquierdo, M.S., 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. *Fish Shellfish Immunol.* 23, 969-981.

Torrecillas, S., Makol, A., Benitez-Santana, T., Caballero, M.J., Montero, D., Sweetman, J., Izquierdo, M., 2011a. Reduced gut bacterial translocation in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Fish Shellfish Immunol.* 30, 674-681.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Ginés, R., Sweetman, J., Izquierdo, M., 2011b. Improved feed utilization, intestinal mucus production and immune parameters in sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Aquac. Nutr.* 17, 223-233.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Dhanasiri, A.K.S., Sweetman, J., Izquierdo, M., 2012. Effects on mortality and stress response in European

sea bass, *Dicentrarchus labrax* (L.), fed mannan oligosaccharides (MOS) after *Vibrio anguillarum* exposure. J. Fish Dis. 35, 591-602.

Torrecillas, S., Makol, A., Betancor, M.B., Montero, D., Caballero, M.J., Sweetman, J., Izquierdo, M., 2013. Enhanced intestinal epithelial barrier health status on European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol. 34, 1485-1495.

Tranulis, M.A., Dregni, O., Christophersen, B., Krogdahl, Å., Borrebaek, B., 1996. A glucokinase-like enzyme in the liver of Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. B 114, 35-39.

Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C., 2000. Xylooligosaccharides: manufacture and applications. Trends Food Sci. Technol. 11, 387-393.

Wang, J., Cao, Y., Wang, C., Sun, B., 2011. Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. Carbohydr. Polym. 86, 1192-1197.

Wright, R.S., Anderson, J.W., Bridges, S.R., 1990. Propionates inhibits hepatocyte lipid synthesis. Exp. Biol. Med. 195, 26-29.

Xu, B.H., Wang, Y.B., Li, J.R., Lin, Q., 2009. Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiol. Biochem. 35, 351-357.

Zhou, Z.-G., He, S.X., Liu, Y.C., Shi, P.J., Huang, G.X., Yao, B., 2009. The effects of dietary yeast culture or short-chain fructo-oligosaccharides on the intestinal autochthonous bacterial communities in juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*. J. World Aquac. Soc. 40, 450-459.

Zhou, Q.-C., Buentello, J.A., Gatlin III, D.M., 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops Ocellatus*). Aquaculture 309, 253-257.

Table 1. Ingredients 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.

^aInproquise, Madrid, Spain (CP: 70.1% DM; GL: 8.8% DM).

^bSopropèche G, France (CP: 79.4% DM; GL: 19.7% DM).

^cSorgal, S.A. Ovar, Portugal (CP: 50.5% DM; GL: 1.7% DM).

^dSorgal, S.A. Ovar, Portugal (CP: 11.8% DM; GL: 1.9% DM).

^eSorgal, S.A. Ovar, Portugal (CP: 82.8% DM; GL: 1.9% DM).

^fSorgal, S.A. Ovar, Portugal (CP: 65.7% DM; GL: 3.5% DM).

^gC-Gel Instant - 12016, Cerestar, Mechelen, Belgium.

^hPROFEED Maxflow, Jefe, France.

ⁱQingdao FTZ United International Inc., Qingdao, China.

^jVitamins (mg kg⁻¹ diet): retinol, 18 000 (IU kg⁻¹ diet); cholecalciferol, 2 000 (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.

^kMinerals (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).

^lAquacube. Agil, England (guar gum, polymethyl carbamide, manioc starch blend, hydrate calcium phosphate).

Table 2. Growth performance and feed utilization efficiency of European sea bass fed the experimental diets^a.

	Diets					
	PPC	PPFOS	PPXOS	FMC	FMFOS	FMXOS
Final body weight (g)	98.4±2.1 ^a	101.0±6.4 ^{ab}	108.5±1.0 ^b	107.0±2.8	106.8±1.1	103.6±7.1
Weight gain (%IBW [†])	64.1±3.5 ^a	68.3±10.7 ^{ab}	80.8±1.7 ^b	78.3±4.6	78.0±1.9	72.6±11.7
Feed intake (g kg ABW ⁻¹ § day ⁻¹)	15.9±0.5	16.7±0.9	16.5±0.3	14.3±0.2	14.4±0.6	15.0±0.5
Feed efficiency ^b	0.66±0.03	0.61±0.03	0.69±0.02	0.79±0.05	0.80±0.03	0.76±0.02
Protein efficiency ratio ^c	1.44±0.07	1.34±0.07	1.47±0.04	1.70±0.11	1.72±0.07	1.59±0.05

Two-way ANOVA

	Variation	Source ^d	
	Protein	Prebiotic	Interaction
Final body weight (g)	0.136	0.410	0.038
Weight gain (%IBW)	0.135	0.408	0.038
Feed intake (g kg ABW ⁻¹ § day ⁻¹)	0.000	0.130	0.372
Feed efficiency ^b	0.000	0.744	0.039
Protein efficiency ratio ^c	0.000	0.531	0.026

[†]IBW: initial body weight.[§]ABW: average body weight (initial body weight + final body weight) / 2.^aMean values and standard deviation (± SD) are presented for each parameter (n=3).^bFE: (wet weight gain / dry feed intake).^cPER: (wet weight gain / crude protein intake).

^dTwo-way ANOVA: if interaction was significant, one-way ANOVA was performed for prebiotics within each protein source and mean in the same line with different superscript letters are significantly different ($P < 0.05$).

Table 3. Plasma glucose, cholesterol, tryacylglycerides (mmol l⁻¹) and total lipids levels (g dl⁻¹) and hepatosomatic index (HSI), liver glycogen (g 100 g⁻¹ liver) and lipid (g 100 g⁻¹ liver) contents in European sea bass fed the experimental diets^a.

	Diets					
	PPC	PPFOS	PPXOS	FMC	FMFOS	FMXOS
<i>Plasma</i>						
Glucose	5.4±0.6	5.1±1.6	5.3±0.9	6.1±1.4	7.0±0.8	8.2±3.4
Cholesterol	8.9±0.5	8.9±1.2	9.7±1.3	11.9±1.3	11.4±0.6	11.6±1.6
Total lipids	2.3±0.3	2.2±0.3	2.3±0.2	1.9±0.3	2.0±0.2	2.1±0.5
Tryacylglycerides	17.8±1.2	17.5±2.1	17.3±1.7	15.0±2.5	15.5±2.1	14.7±1.9
<i>Liver</i>						
HSI	1.6±0.2	1.6±0.3	1.5±0.3	2.2±0.3	2.4±0.2	2.5±0.4
Glycogen	6.8±2.1	5.6±1.6	6.7±1.9	8.0±2.9	9.6±1.9	9.8±2.2
Lipids	9.4±1.6	12.0±1.3	9.7±4.1	10.5±1.0	11.4±1.9	11.0±1.6
Two-way ANOVA						
	Variation source					
	Protein	Prebiotic	Interaction			
<i>Plasma</i>						
Glucose	0.000	0.236	0.168			
Cholesterol	0.000	0.390	0.340			
Total lipids	0.001	0.595	0.726			
Tryacylglycerides	0.000	0.723	0.802			
<i>Liver</i>						
HSI	0.000	0.559	0.132			
Glycogen	0.000	0.480	0.128			
Lipids	0.307	0.055	0.364			

^aMean values and standard deviation (± SD) are presented for each parameter (n=9).

Table 4. Hepatic glycolytic (hexokinase, HK, glucokinase, GK and pyruvate kinase, PK), gluconeogenic (fructose-1,6-bisphosphatase, FBPase) and lipogenic (glucose-6-phosphate dehydrogenase, G6PD, malic enzyme, ME and fatty acid synthetase, FAS) enzyme activities (mU mg protein⁻¹) in European sea bass fed the experimental diets^a.

	Diets					
	PPC	PPFOS	PPXOS	FMC	FMFOS	FMXOS
HK	0.38±0.18	0.42±0.11	0.41±0.15	0.64±0.15	0.80±0.16	0.62±0.25
GK	2.3±0.6	1.8±0.5	1.7±0.7	5.9±1.7 ^a	11.0±2.7 ^b	10.3±2.2 ^b
PK	81.4±6.7	81.9±3.5	80.8±5.9	115.0±15.6	112.1±18.4	99.0±8.5
FBPase	37.1±5.8	34.2±7.5	33.4±9.0	27.5±6.2	23.8±4.6	24.9±3.7
G6PD	146.7±22.1	144.0±27.3	120.2±22.3	222.3±22.3	232.7±29.5	184.0±25.7
ME	5.3±0.9	5.5±1.3	4.5±0.7	7.3±1.4	7.0±1.3	5.6±0.9
FAS	23.1±2.7	22.1±2.7	18.7±4.0	24.5±3.5	26.0±3.4	22.8±3.7

Two-way ANOVA

	Variation source ^b			Prebiotic		
	Protein	Prebiotic	Interaction	Control	FOS	XOS
HK	0.000	0.140	0.328			
GK	0.000	0.002	0.000			
PK	0.000	0.147	0.258			
FBPase	0.000	0.230	0.906			
G6PD	0.000	0.000	0.337	b	b	a
ME	0.000	0.001	0.426	b	b	a
FAS	0.002	0.009	0.405	b	b	a

^aMean values and standard deviation (± SD) are presented for each parameter (n=9).

^bTwo-way ANOVA: if interaction was significant, one-way ANOVA was performed for prebiotics within each protein source and mean in the same line with different superscript letters are significantly different (P<0.05).