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Effect of short chain fructooligosaccharides (scFOS) on immunological status and gut microbiota of gilthead sea bream (*Sparus aurata*) reared at two temperatures

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ABSTRACT

The effects of dietary short chain fructooligosaccharides (scFOS) incorporation on hematology, fish immune status, gut microbiota composition, digestive enzymes activities, and gut morphology, was evaluated in gilthead sea bream (*Sparus aurata*) juveniles reared at 18 °C and 25 °C. For that purpose, fish with 32 g were fed diets including 0, 0.1, 0.25 and 0.5% scFOS during 8 weeks. Overall, scFOS had only minor effects on gilthead sea bream immune status. Lymphocytes decreased in fish fed the 0.1% scFOS diet. Fish fed the 0.5% scFOS diet presented increased nitric oxide (NO) production, while total immunoglobulins (Ig) dropped in those fish, but only in the ones reared at 25 °C. Red blood cells, hemoglobin, bactericidal activity and NO were higher at 25 °C, whereas total white blood cells, circulating thrombocytes, monocytes and neutrophils were higher at 18 °C. In fish fed scFOS, lymphocytes were higher at 18 °C. Total Ig were also higher at 18 °C but only in fish fed 0.1% and 0.5% scFOS diets. No differences in gut bacterial profiles were detected by PCR-DGGE (polymerase chain reaction denaturing gradient gel electrophoresis) between dietary treatments. However, group's similarity was higher at 25 °C. Digestive enzymes activities were higher at 25 °C but were unaffected by prebiotics incorporation. Gut morphology was also unaffected by dietary prebiotic incorporation.

Overall, gut microbiota composition, digestive enzymes activities and immunity parameters were affected by rearing temperature whereas dietary scFOS incorporation had only minor effects on these parameters. In conclusion, at the tested levels scFOS does not seem worthy of including it in gilthead sea bream juveniles diets.

1. Introduction

Prebiotics can be defined as non-digestible fibers that potentially increase specific-health promoting gut bacteria in the host [1]. Thus, prebiotics can positively affect host's health either indirectly, through by-products produced during bacterial prebiotic fermentation, or directly, through prebiotics interaction with pattern recognition receptors [2].

Fructooligosaccharides (FOS) are one of the most studied prebiotics in humans, farm animals and fish [2–5]. Short-chain fructooligosaccharides (scFOS) are similar to FOS but with a lower degree of polymerization, ranging from 1 to 5 fructose oligomers [6]. ScFOS are however much less studied than FOS. In fish, scFOS has been only evaluated in turbot (*Scophthalmus maximus*), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), common carp (*Cyprinus carpio*), and hybrid tilapia (*Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂) [7–14].

FOS is known to support growth and survival of gastrointestinal tract autochthonous bacteria, such as members of the genus *Lactobacillus*, which possess β-fructosidase activity and thus can hydrolyse FOS β-(2-1) glycosidic bonds [3]. *Lactobacillus* is known to interact with the host immune system, but the precise mechanisms involved are not completely clarified. Nonetheless, it seems that *Lactobacillus*, or its end-metabolic products, interact with gut

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epithelial cells, macrophages, dendritic cells, and lymphocytes [15]. For instance, in mice given *Lactobacillus* as a probiotic, the gut mucosal immune system was affected mainly through activation of innate immune response cells [16]. FOS is also considered an immunosaccharide [2] as it has a direct signaling capacity on human's immune cells, by activating toll-like receptors, mainly TLR2 and, to a lesser extent, TLR4 [17]. Although the benefits of prebiotics to animals are well known, as well as the relationship between gut bacteria and host's immune system, the majority of studies on prebiotics effects in fish immune status does not simultaneously evaluate gut microbial composition [18–23]. It was, however, recently reported an increase in gut cultivable lactic acid bacteria (LAB) population and a stimulation of several immune parameters upon incorporation of FOS in the diets for stellate sturgeon (*Acipenser stellatus*) and common carp (*C. carpio*) [24,25].

Prebiotics effects on fish immunity, particularly of FOS, are reported as immunomodulatory [2–5]. For instance, blunt snout bream (*Megalobrama amblycephala*) fed a diet with 0.4% FOS presented lower levels of plasma cortisol and higher levels of immunoglobulin, lysozyme, plasma acid phosphatase, alternative complement activity and nitrogen monoxide than fish fed the control diet [23]. Triangular bream (*Megalobrama terminalis*) fed a diet with 0.6% FOS had increased leucocyte counts, plasma alternative complement activity and immunoglobulins compared to fish fed the control diet [22]. Red drum (*Sciaenops ocellatus*) fed a diet with 1% FOS had increased plasma lysozyme [26]. In Pacific white shrimp (*Litopenaeus vannamei*), diets including scFOS led to alterations in gut microbiota and enhanced total hemocyte count and hemocyte respiratory burst [27].

In gilthead sea bream, prebiotics effects on immune parameters were so far only evaluated for inulin and mannanoligosaccharides (MOS). MOS did not affect fish health indicators, whereas leucocytes phagocytic capacity was decreased in fish fed inulin for 1 week [18,21]. In another study, complement activity, leukocyte phagocytic ability and capacity increased after 2 weeks of feeding an inulin supplemented diet [28]. However, after 4 weeks of feeding the same diet, differences in leucocytes phagocytic ability and capacity disappeared while gut bacteria richness was reduced [28,29].

Dietary supplementation with FOS has been associated with an increase in digestive enzymes activity in some fish species, which may be correlated with alterations in gut microbiota [30–32]. Although, the effect of FOS on gilthead sea bream digestive enzymes was not yet evaluated, MOS was associated with increased protein, carbohydrates and energy digestibility's [33].

Prebiotic effects on fish gut morphology are extensively studied. MOS was reported to increase gut absorptive area through increased microvilli length and density [34–38]. FOS was also reported to induce changes in morphology of fish intestine, such as increased microvillus height [26,32]. However, inulin was reported to induce significant damage in gilthead sea bream gut [29].

Fish, as heterothermic animals, are heavily influenced by environmental conditions and, as suggested by Ringø et al. [3], temperature may have greater effects than diet in fish health. This may turn difficult an evaluation of prebiotics effect. Indeed, water temperature was already reported to influence immunological parameters and gut bacterial community [9,12,39–42].

Several studies on the effects of prebiotics [2,9,43] and rearing temperature [9,41,42] on fish immune status are already available. However, studies simultaneously evaluating both parameters and their potential interactive effects on fish immune status are very scarce [9].

Since prebiotics are reported to promote gastrointestinal health and immunological status [44], the study of prebiotic effects on gut function and integrity, and on immunological parameters are particularly important. Therefore, the aim of this study was to evaluate the effect of dietary scFOS supplementation in the hematological profile, fish immune status, allochthonous gut microbiota composition, digestive enzymes activities, and gut morphology of gilthead sea bream juveniles reared at two temperatures: 18 and 25 °C.

2. Material and methods

2.1. Diets composition

Four diets were formulated to be isolipidic (18% lipid) and isonitrogenous (46% protein). Fish meal and plant feedstuffs (soybean meal, wheat gluten, corn gluten and wheat meal) were used as protein sources (circa 50% protein from fish meal and 50% from plant feedstuffs), and fish oil was the main lipid source. The experimental diets included 0% (diet D0 – control diet), 0.1% (diet D0.1), 0.25% (diet D0.25), and 0.5% (diet D0.5) of scFOS (PROFEED Maxflow, Jefo, France) replacing α-cellulose. All ingredients were thoroughly mixed and dry pelleted in a laboratory pellet mill (California Pellet Mill, CPM Crawfordsville, IN, USA), through a 2.0 mm die. Pellets were dried in an oven at 40 °C for 48 h, and then stored in a freezer in airtight bags until use. Ingredients and proximate composition of the experimental diets are presented in Table 1.

Chemical analyses of the diets were performed following the Association of Official Analytical Chemists methods [45]. Dietary starch content was determined according to Beutler [46].

2.2. Growth trial

The experiment was performed at the Marine Zoology Station, Porto University, Portugal, with gilthead sea bream (*S. aurata*) juveniles obtained from a commercial fish farm (Maresa S.A., Ayamonte, Huelva, Spain). The trial was performed in 2 identical recirculating water systems, each equipped with 12 cylindrical fiberglass tanks of 100 L water capacity, and thermo-regulated to 18.0 ± 0.5 °C and 25.0 ± 0.6 °C, respectively. The tanks were supplied with a continuous flow of filtered seawater (2.5–3.5 L min^{-1}) of 35 ± 1 g L^{-1} salinity, and dissolved oxygen was kept near saturation (7 mg L^{-1}). After a quarantine period of 1 month, fish were transferred to the experimental systems and adapted to the experimental conditions for 15 days. During quarantine and adaptation periods, fish were fed a commercial diet (48% protein and 17% lipids; Sorgal, S.A. Ovar, Portugal). A total of 528 fish with an initial mean body weight of 32.0 ± 0.01 g were randomly distributed by the tanks, 22 fish per tank. The experimental diets were randomly assigned to triplicate groups within each temperature, hence 6 tanks per dietary treatment, with 3 tanks at each temperature. The trial lasted 8 weeks, and during that period fish were fed by hand, twice daily, 6 days a week, until apparent visual satiation. Utmost care was taken to avoid feed losses. The experiment was performed by accredited scientists (following FELASA category C recommendations) and was conducted according to the European Union directive 2010/63/EU on the protection of animals for scientific purposes.

2.3. Sampling

At the end of the trial, a total of 8 fish per tank were randomly sampled 4 h after the morning meal. Blood from 3 fish was collected from the caudal vein using heparinized syringes and placed in heparinized tubes. One aliquot was used for hematological assessment while the remaining blood was centrifuged at 3000 × g for 10 min at room temperature. The resulting plasma was