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Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*)

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ABSTRACT

Two experiments were conducted in order to investigate the effect of dietary mannan oligosaccharides (MOS) on gilthead sea bream (*Sparus aurata*). Experiment I was designed to assess the effect of dietary MOS (0%, 0.2% and 0.4%) on fish fed diets containing fishmeal (FM) as the only protein source. Experiment II was designed to assess the effect of MOS (0% and 0.4%) on fish fed soybean meal (SBM) as a partial replacement of FM (SBM inclusion 31% of diet). After 9 weeks feeding on the experimental diets growth parameters, body composition, liver and intestinal histology and intestinal microbial diversity were assessed. The results showed that mean final weight, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) remained unaffected by MOS supplementation of fish fed FM or SBM diets.
However, compared to the control group (FM0), condition factor (*K*) and hepatosomatic index (HSI) were significantly lower in fish fed 0.2% MOS (FM02) and 0.4% MOS (FM04), respectively. These parameters were unaffected in SBM-fed fish. Body proximate composition remained unaffected by MOS supplementation in fish fed either FM or SBM diets ($P > 0.05$). Histological evaluation revealed that MOS had no effect on glycogen deposition in liver and no effect on gross villi morphology in the anterior intestine in either Experiment I or II. However, relative to the control groups (FM0) dietary MOS appeared to improve gross morphological absorptive surface area in the posterior intestine in Experiment I. Electron microscopy revealed that dietary MOS had a pronounced effect at the ultrastructural level in both experiments, as microvilli density and length were elevated in both intestinal regions in fish fed both the FM and SBM based diets. No significant histological differences were found between respective FM0 and SBM0 groups.
DGGE analysis revealed that both SBM and MOS affected the intestinal microbial species richness and diversity. However, the effect of dietary MOS on the gastrointestinal microbiota was more pronounced in FM-based diets (Experiment I) as was reflected by increased species richness and diversity and reduced similarity between microbial profiles of the different FM groups. The effect of MOS in Experiment II on SBM-fed fish was marginal, as species richness and diversity remained unaffected and similarity between microbial profiles of the SBM groups and replicates remained high (i.e. >80%). Dietary SBM exerted a greater effect on gut microbiota than dietary MOS.

1. Introduction

Gilthead sea bream (*Sparus aurata*) is an important cultivated fish species with great economic interest. However, with the recent ban on the use of antibiotic growth promoters in aquafeeds within the EU (Regulation, 2005) alternative nutraceutical products to enhance production and health status is a topic of concerted interest. Prebiotics, such as mannan oligosaccharides (MOS) have proved to be effective at enhancing health and growth performance of fish (Staykov et al., 2007; Torrecillas et al., 2007; Burr et al., 2008), improve gut morphology (Salze et al., 2008; Dimitroglou et al., 2009) and modulate the intestinal microbiota (Dimitroglou et al., 2009). Despite the progress made with other species, the effect of MOS on gilthead sea bream remains limited.

Soybean meal (SBM), an important plant protein source in aquafeeds due to its competitive price and relative availability (Gatlin

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et al., 2007), has been demonstrated to induce histological changes of the fish gastrointestinal (GI) tract which include enteritis, increased susceptibility to bacterial infection, increased presence of inflammatory cells, villi shortening and reduced microvilli density and length (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2000; Krogdahl et al., 2003; Sitja-Bobadilla et al., 2005; Heikkinen et al., 2006; Bakke-McKellep et al., 2007; Merrifield et al., 2009a). Several studies have assessed the feasibility of including SBM in diets for gilthead sea bream (for a summary refer to Martínez-Llorens et al., 2009); however, to the authors' knowledge only Bonaldo et al. (2008) have provided a histological examination of the GI tract. Bonaldo and co-authors evaluated the effect of SBM on gut histology of sea bream using light microscopy following the criteria suggested by Krogdahl et al. (2003). The results showed the sea bream could tolerate dietary levels of up to 300 g SBM kg^{-1} without significant negative effects on growth performance or posterior intestinal morphology. However, the effect on the gut (anterior and posterior) ultrastructure in terms of enterocyte morphology and the apical brush border are yet to be elucidated. To the authors' knowledge the effect of SBM on the gut microbiota of sea bream has also not been assessed. Given the importance of the gut microbiota in terms of gastric development, health and nutrition (Bates et al., 2006; Gómez and Balcázar, 2008), this topic is worthy of investigation.

As was recently highlighted by Gatlin et al. (2007), the approach of utilising prebiotics to improve utilisation of plant proteins should be a topic of high priority. Therefore, the aim of the present research was to assess the effect of MOS (incorporated into diets with or without SBM) on gilthead sea bream growth performance, intestinal histology and intestinal microbiota.

2. Methodology

2.1. Dietary formulation

Diets were prepared at the research facilities of the University of Plymouth, U.K. Five diets were formulated (Table 1). Mannan oligosaccharide (MOS; Bio-Mos®, Alltech Inc. USA) was supplemented at levels of 0%, 0.2% and 0.4% in Experiment I. In Experiment II, 31% FM was replaced with SBM and supplemented with either 0% or 0.4% MOS (Table 1). Each diet was produced by mechanically stirring the ingredients into a homogenous mixture using a Hobart food mixer (Hobart Food Equipment, Australia). Warm water was added to produce a consistency suitable for cold extrusion to form 2 mm pellets (PTM Extruder system, Plymouth, UK). Diets were dried in a hot air oven at 45 °C for 48 h. All diets were analysed for proximate composition according to AOAC (1995) protocols. Gross energy was measured in an adiabatic bomb calorimeter (Parr 1356 Bomb Calorimeter).

Table 1
Dietary formulations (g kg^{-1}) and proximate composition analysis (%).

	Experiment I			Experiment II	
	FM0	FM02	FM04	SBM0	SBM04
Treatments					
Fishmeal (LT-94)	640	640	640	427	427
SBM solv.ext. (decortic)[a]	0	0	0	313	313
Marine fish oil[b]	73.2	73.2	73.2	73.2	73.2
Corn starch[c]	110	110	110	110	110
Dextrin[d]	55	55	55	55	55
Vitamin mix[a]	10	10	10	10	10
Mineral mix[a]	5	5	5	5	5
α-cellulose[b]	106.8	104.8	102.8	6.8	2.8
MOS[e]	0	2	4	0	4
Proximate analysis					
Dry matter (%)	92.95	93.05	93.24	93.26	93.42
Moisture (%)	7.05	6.95	6.76	6.74	6.58
Protein (%)	43.73	43.65	43.77	45.59	46.77
Lipids (%)	10.83	11.04	10.96	11.08	11.81
Ash (%)	12.24	12.19	12.17	10.58	10.53
NFE[f](%)	26.16	26.15	26.34	26.01	24.31
Energy (MJ kg^{-1})	19.28	19.69	19.62	19.64	19.87

[a] Interfish Ltd, U.K.
[b] Sigma-Aldrich.
[c] Dextrin type II from corn, Sigma-Aldrich.
[d] Skretting, U.K.
[e] Bio-Mos®, Alltech Inc.
[f] Nitrogen free extracts (NFE) = dry matter − (crude lipid + crude ash + crude protein).

2.2. Feeding trial

The studies were conducted at the research facilities of the University of Plymouth, U.K. Gilthead sea bream fry, imported from a commercial hatchery in France (Aquastream, Ploemeur), were acclimated and grown on for approximately 2 months prior to the start of the trial. Thereafter, 49 fish (~24 g) were distributed into 15 × 120 L fibreglass tanks. Experiments I and II were both conducted simultaneously in aerated re-circulated marine water at a rate of 360 L h^{-1}. Each diet was randomly assigned to fish in three replicate tanks. Fish were fed 2.7–3.0% biomass day^{-1}, provided in equal rations at 09.00 and 17.00 h for a period of 9 weeks. Fish were weighed on a weekly basis following a 24-h starvation period. Water temperature was maintained at 22 ± 1 °C, pH between 7.0 ± 0.3 and salinity between 33 and 34 mg L^{-1} with a 12 h light/12 h dark photoperiod. Ammonia and nitrate concentrations were always found to be less than 1.6 and 1.1 mg L^{-1}, respectively.

2.3. Growth parameters and calculations

Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K) and hepatosomatic index (HSI) were calculated according to the following formulae: $SGR = 100 \times (\ln W_{fin} - \ln W_{in})/d$, $FCR = FI/W$, $PER = W/PI$, $K = 100 \times (W/FL^3)$ and $HSI = 100 \times (LW/W)$. Where W_{fin} is the final mean weight, W_{in} is the initial mean weight, d is the duration of feeding (days), FI is the feed intake (g), W is the live weight gain (g), PI is the protein intake (g), FL is fish fork length (cm) and LW is liver weight (g).

Additionally, 5 fish per tank were pooled at the end of the trial ($n = 3$) and analysed for body composition according to AOAC (1995) protocols.

2.4. Histology

Liver and intestinal samples from 3 fish per tank ($n = 9$) were retained for histological examination by light and electron microscopy. Intestinal sections from the middle of the anterior and posterior regions were taken for both light and electron microscopy analysis. Liver samples were analysed using light microscopy (LM).

Samples for LM were fixed in 4% saline formalin. The tissue samples were dehydrated in graded ethanol before equilibration in xylene and embedded in paraffin wax. Then, 8-μm transverse sections were cut and stained using Alcian blue periodic acid-Schiff staining technique (AB-PAS; Kiernan, 1981). At least five images from each sample were analysed. Liver images were analysed for glycogen deposition in the hepatocytes by the ratio of a stained area (glycogen) and the unstained area, producing arbitrary units (AU). Intestinal images from light microscopy were analysed to determine the perimeter ratio (PR) between the internal perimeter (IP) of the intestine lumen and the external perimeter (EP) of the intestine (PR = IP/EP, arbitrary units AU; after Dimitroglou et al., 2009). A high PR value indicates high absorptive surface area brought about by high villi length and/or increased mucosal folding. Additionally, intestinal LM samples were assessed for mucus pH (Alcian blue stains the acidic mucus blue and PAS stains the neutral to alkaline mucus magenta).

Samples for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were processed and analysed as described by Merrifield et al. (2009a). Briefly, TEM micrographs