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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.

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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

(magnification ×20,000) were analysed to measure the microvilli length and SEM images (magnification ×20,000) were analysed in order to measure the density of the microvilli (MD) of the enterocytes on top of the villi. In order to calculate the density the ratio between the microvilli covered area (M, foreground) to the gaps between the microvilli (B, background) was calculated (MD = M/B, arbitrary units AU). All images were analysed using Image J version 1.36 (National Institutes of Health, USA).

2.5. Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE)

DNA was extracted from 2 intestinal samples (pooled from 6 fish) per treatment using QIAamp® Stool Mini Kit (Qiagen) with a lysozyme pre-treatment prior to PCR amplification of V3 16S rRNA genes as described elsewhere (Merrifield et al., 2009b). The DGGE was performed using a DGGE-2001 system (C.B.S. scientific, CA, USA). 10 μL of standardized PCR products were run on an 8% acrylamide gel with a denaturing gradient of 40–60% (where 100% denaturant is 7 M urea and 40% formamide). All samples were run on the same gel to avoid issues of reproducibility. The gel was run at 65 V for 17 h at 60 °C in 1 × TAE buffer (66 mM Tris, 5 mM Na acetate, 1 mM EDTA). Visualization of the DGGE bands was achieved by the optimized silver staining method of Benbouza et al. (2006). The gel was scanned in a Bio-Rad universal hood II (Bio-Rad laboratories, Italy) and optimized for analyses by enhancing contrast and greyscale.

2.6. Statistics

One-way ANOVA and independent sample *T*-test were applied to growth and intestinal histological data for Experiment I and Experiment II, respectively. Analyses were carried out on SPSS 15.0 (SPSS Inc., IL, USA) and significance was accepted at the $P<0.05$ level. The results are presented as mean values followed by the standard deviation (M ± SD). DGGE banding patterns were transformed into presence/absence matrices for similarity assessment between treatments using Quantity one® version 4.6.3 analyses software (Bio-Rad laboratories, CA, USA) after Schauer et al. (2000). Band intensities were measured and analysed using Primer v6 (Clarke and Gorley, 2006) and similarity percentages (SIMPER) were used for pairwise comparisons to determine differences between DGGE banding profiles. The diversity of the intestinal microbiota was assessed using the Shannon–Weaver index, calculated with the following formula: $H = -\Sigma(pi(\ln pi))$. Where *pi* is the proportion of the total number of individuals in the *i*th species.

3. Results

3.1. Growth analysis

Growth analysis is presented in Table 2. All groups grew well and average fish weight increased by over 200%. In both Experiments I and II final weight, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) remained unaffected by MOS supplementation. In Experiment 1 (FM-based diets) condition factor (*K*) was significantly reduced ($P=0.021$) from 2.01 ± 0.14 in control treatment to 1.89 ± 0.16 in FM02. FM04 had no effect on *K*. Hepatosomatic index (HSI) was significantly affected only by the higher dose of MOS supplementation in sea bream fed FM-based diets (Experiment I). These factors were unaffected by dietary MOS in SBM-fed fish (Experiment II).

Analysis of body proximate composition (Table 3) revealed that moisture, crude protein, lipids, ash and energy were unaffected ($P>0.05$) in both Experiments I and II.

Table 3
Fish body proximate composition analysis. Three fish from each of the three replicate tanks were pooled (thus $n=3$ per treatment). Values are presented as %.

	Experiment I			Experiment II	
Treatment	FM0	FM02	FM04	SBM0	SBM04
Moisture	68.56 ± 0.48	68.96 ± 0.38	69.27 ± 0.52	68.23 ± 0.69	69.59 ± 1.37
Protein	16.36 ± 0.38	16.29 ± 0.48	16.09 ± 0.08	16.64 ± 0.44	16.89 ± 0.40
Lipid	10.90 ± 0.39	9.93 ± 0.64	9.70 ± 0.74	11.00 ± 0.82	9.13 ± 1.11
Ash	3.23 ± 0.17	3.39 ± 0.37	3.24 ± 0.02	3.19 ± 0.23	3.34 ± 0.25
Energy (MJ kg^{-1})	23.76 ± 0.33	23.88 ± 0.76	23.52 ± 0.63	23.81 ± 0.21	23.07 ± 0.63

There were no significant differences between any groups.

3.2. Histological analysis

An overview of the histological analysis is presented in Table 4. Image analysis of PAS-Alcian Blue liver sections revealed that there was no significant effect of diet on glycogen deposition in either Experiment I or II. Light microscopy revealed that the dietary treatments had no effect on villi surface area (i.e. perimeter ratio) or mucus pH in the anterior intestinal region. In the posterior intestinal region the perimeter ratio was significantly increased from 3.92 ± 1.06 (AU: arbitrary units) in FM0 to 5.63 ± 1.67 AU to FM04 ($P=0.046$) in Experiment I. SEM analysis of the anterior and the posterior intestinal regions of fish from Experiment I revealed that MOS supplementation significantly ($P<0.050$) increased the microvilli density (Table 4 and Fig. 1). In the anterior intestinal region 0.2% MOS supplementation increased the microvilli density from 5.00 ± 1.97 AU in the control treatment (FM0) to >18 AU in MOS-fed fish (FM02 and FM04) ($P<0.001$). Similarly, the posterior intestinal microvilli density of the control fed group (FM0) increased from 6.69 ± 2.61 AU to >12 AU in MOS treatments (FM02 and FM04) ($P<0.02$). TEM analysis demonstrated that in Experiment I microvilli length was significantly increased with MOS supplementation in both intestinal regions (Table 4 and Fig. 2). The microvilli length of anterior intestinal region increased from 1.76 ± 0.14 μm in control groups (FM0) to over 2 μm in the MOS-fed groups (FM02 and FM04) ($P<0.01$). However, in the posterior intestinal region, only high level MOS supplementation significantly increased the microvilli length: from 1.68 ± 0.29 μm in

Table 2
Growth parameters ($n=3$ per treatment) and somatic indices ($n=9$ per treatment) of sea bream fed the experimental diets.

	Experiment I			Experiment II	
Treatments	FM0	FM02	FM04	SBM0	SBM04
Initial fish weight (g)	24.09 ± 0.45	23.44 ± 1.37	23.56 ± 1.77	23.61 ± 0.55	23.59 ± 1.04
Final fish weight (g)	74.91 ± 3.82	74.58 ± 3.15	73.59 ± 0.86	77.98 ± 4.78	79.34 ± 2.66
FCR	1.88 ± 0.05	1.86 ± 0.08	1.88 ± 0.05	1.84 ± 0.07	1.83 ± 0.05
SGR (% day^{-1})	1.80 ± 0.05	1.84 ± 0.04	1.82 ± 0.10	1.89 ± 0.06	1.93 ± 0.04
PER	1.22 ± 0.02	1.23 ± 0.05	1.22 ± 0.03	1.21 ± 0.02	1.18 ± 0.05
K (%)	2.01 ± 0.14[a]	1.89 ± 0.16[b]	1.96 ± 0.14[a,b]	1.96 ± 0.15	2.02 ± 0.15
HSI (%)	1.74 ± 0.17[a]	1.80 ± 0.23[a]	1.47 ± 0.25[b]	1.39 ± 0.26	1.48 ± 0.13

[a,b]Values within the same row, in the same Experiment (I or II), with different superscripts are significantly different ($P<0.05$).

Table 4
Histological analysis of liver and gut samples. Gut samples were divided in posterior and anterior regions ($n=9$ per treatment).

		Experiment I			Experiment II	
Treatments		FM0	FM02	FM04	SBM0	SBM04
Liver glycogen (AU)		1.41 ± 0.23	1.49 ± 0.10	1.39 ± 0.22	1.35 ± 0.26	1.33 ± 0.35
Anterior intestine	Absorptive surface (PR)*	7.34 ± 1.27	6.81 ± 2.59	6.97 ± 1.64	6.12 ± 1.18	6.33 ± 0.94
	Microvilli density*	5.00 ± 1.97^{a}	20.41 ± 8.70^{b}	18.06 ± 8.79^{b}	6.13 ± 2.71^{c}	13.97 ± 8.54^{d}
	Microvilli length (μm)	1.76 ± 0.14^{a}	2.12 ± 0.23^{b}	2.02 ± 0.20^{b}	1.81 ± 0.36	1.93 ± 0.37
Posterior intestine	Absorptive surface (PR)*	3.92 ± 1.06^{a}	$5.44 \pm 1.47^{a,b}$	5.63 ± 1.67^{b}	4.85 ± 0.98	5.36 ± 1.30
	Microvilli density*	6.69 ± 2.61^{a}	12.74 ± 5.71^{b}	13.81 ± 6.15^{b}	6.63 ± 2.43^{c}	16.19 ± 9.15^{d}
	Microvilli length (μm)	1.68 ± 0.29^{a}	$1.82 \pm 0.27^{a,b}$	2.07 ± 0.22^{b}	1.72 ± 0.29^{c}	2.15 ± 0.30^{d}

*Arbitrary units.
[a,b]Values within the same row, the same Experiment (I or II), with different superscripts are significantly different ($P<0.05$).
There were no significant differences between respective FM and SBM treatments (i.e. FM0 v SBM0).

the control group (FM0) to 2.07 ± 0.22 μm in the 0.4% MOS-fed fish (FM04) ($P<0.006$).

In Experiment II, LM analysis of the anterior and the posterior intestinal region revealed that MOS supplementation did not produce any significant effect on the villi morphology or mucus pH. However, SEM analysis demonstrated that microvilli density was increased in both examined intestinal regions. In the anterior intestine microvilli density was increased from 6.13 ± 2.71 AU in SBM0 to 13.97 ± 8.54 AU in SBM04, $P=0.018$. Similarly, in the posterior intestine density was increased from 6.63 ± 2.43 in SBM0 to 16.19 ± 9.15 in SBM04, $P=0.014$. Microvilli length was only affected by MOS supplementation in the posterior intestine ($P=0.008$). Additionally, no significant differences (independent sample *T*-test) of gut histological parameters were observed with the inclusion of dietary SBM (i.e. FM0 vs SBM0).

3.3. PCR-DGGE analysis

Fig. 3 displays the Bray–Curtis dendrogram and V3 16S rRNA DGGE fingerprints of bacterial communities from the intestine of sea bream from Experiment 1 and Experiment II. SBM-fed fish (Experiment II)

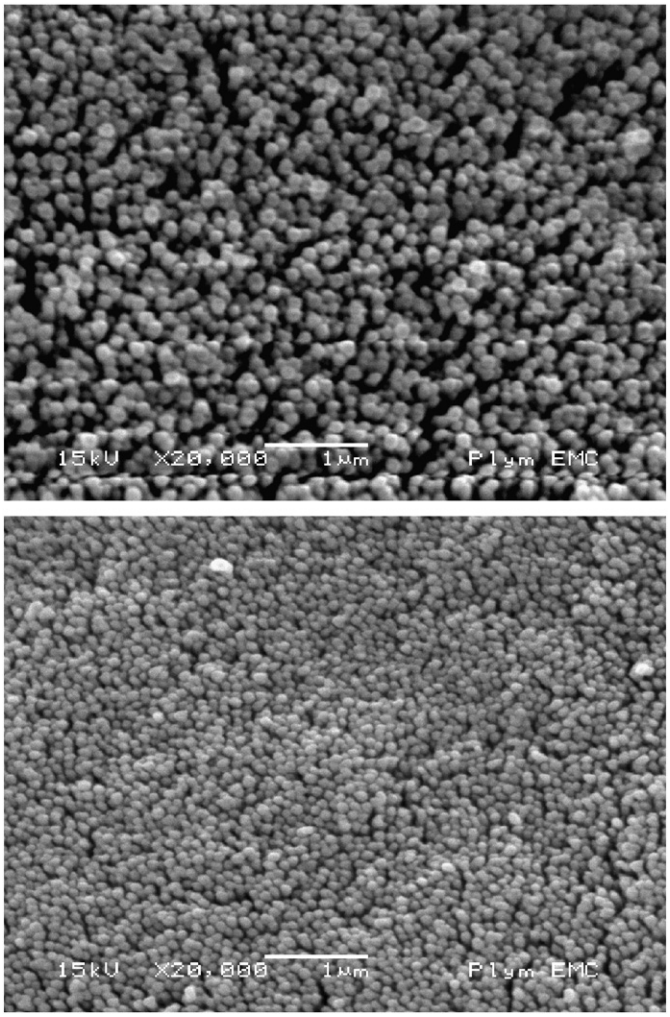

Fig. 1. SEM micrographs from the posterior intestinal region. Top micrograph is from a fish from group FM0 and the bottom micrograph from FM02, presenting the effect of MOS supplementation on FM-based diets. Scale bar = 1 μm.

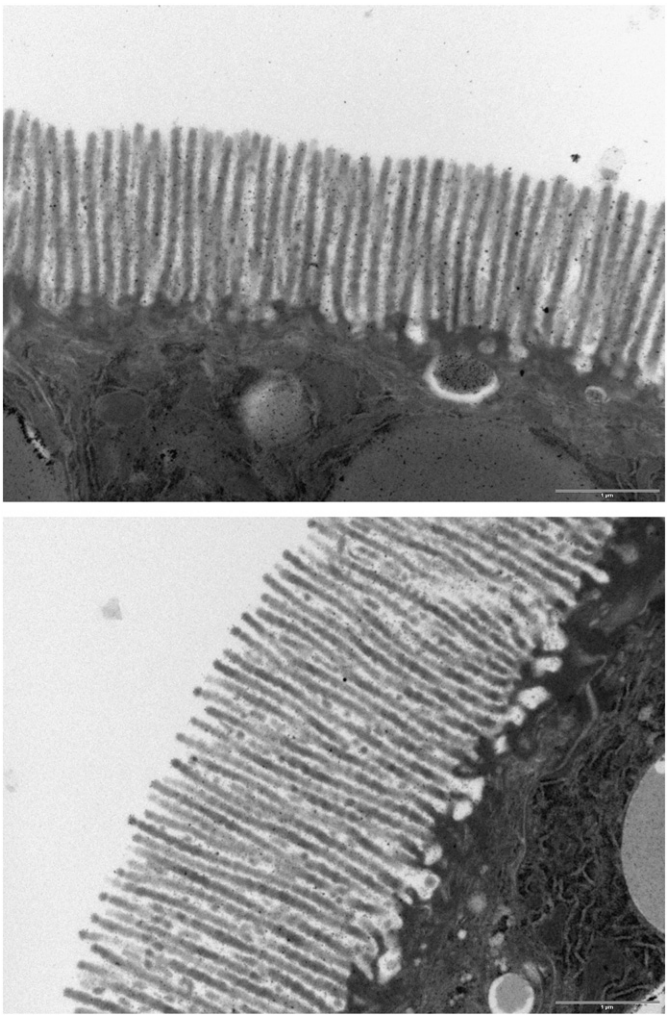

Fig. 2. Comparative TEM micrographs from the anterior intestinal region. Top micrograph is from FM0 and the bottom micrograph from FM02, presenting the effect of MOS supplementation in FM-based diets. Scale bar = 1 μm.

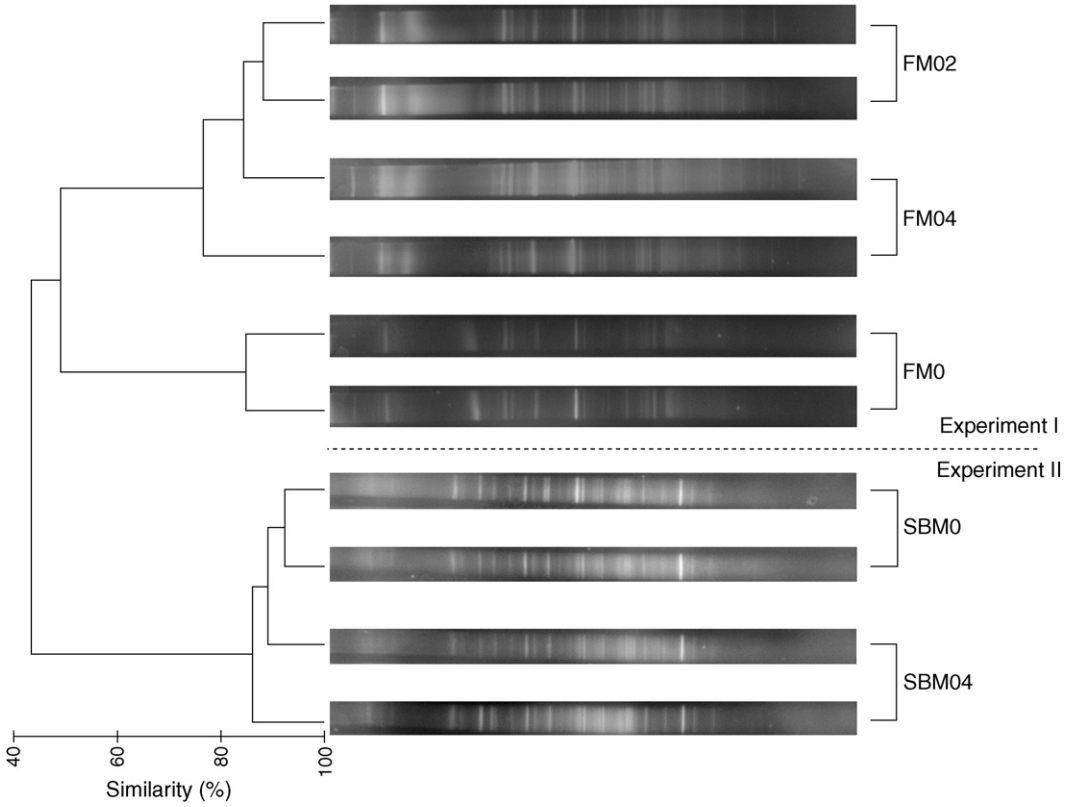

Fig. 3. Bray–Curtis dendrogram demonstrating the similarity of V3 16S rRNA DGGE fingerprints of bacterial communities in sea bream intestine.

displayed distinctly different profiles than fish fed FM (Experiment I). Dietary MOS also affected the intestinal microbiota and subsequently the microbial profiles were clustered into five distinct groups. However, the effect was greater in Experiment I than Experiment II with no clear dose response effect by the MOS supplementation. In Experiment I, dietary MOS increased species richness (from 15.5 ± 0.7 in FM0 to ca. 25 in FM02 and FM04) and diversity (Shannon Weaver Diversity Index increased from 2.65 ± 0.05 in FM0 to over 3 in FM02 and FM04) and reduced similarity between microbial profiles of FM-fed groups (Table 5; Fig. 3). However, MOS supplementation did not affect intestinal microbial species richness or diversity in fish fed SBM diets (Experiment II).

4. Discussion

From previous studies it appears that the effects of MOS on growth performance of aquatic species are contradictory. The present study demonstrated that dietary MOS did not significantly affect the growth performance of fish fed either FM-based diets or diets containing partial FM replacement with SBM. Similar to the results of the present study, previous investigations with Atlantic salmon (*Salmo salar*; Grisdale-Helland et al., 2008), cobia larvae (Salze et al., 2008), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*; Genc et al., 2007a) and Gulf sturgeon (*Acipenser oxyrinchus desotoi*; Pryor et al., 2003) showed that MOS supplementation did not improve growth performance. On the contrary, previous studies with rainbow trout (Staykov et al., 2007), European sea bass (*Dicentrarchus labrax*; Torrecillas et al., 2007) and green tiger prawn (*Penaeus semisulcatus*; Genc et al., 2007b) have shown that MOS can improve growth performance. Additionally, Burr et al. (2008) also demonstrated that dietary MOS improved red drum (*Sciaenops ocellatus*) protein, organic matter and energy ADC in SBM-rich diets. Body proximate analysis in the present study showed that none of the examined parameters were affected by MOS supplementation; whereas, previous studies on rainbow trout (Yilmaz et al., 2007) and hybrid tilapia (Genc et al., 2007a) using 0.4% MOS supplementation reported increased body protein levels.

HSI is directly related with metabolism because glycogen and lipids can be stored in the liver (Robaina et al., 1995; Krogdahl et al., 2004). High level of MOS supplementation reduced the HSI in fish fed FM-based diets, while HSI remained unaffected by dietary MOS in SBM-fed fish. However, histological evaluation of the glycogen deposition in the liver tissue revealed that none of the dietary treatments appeared to affect hepatocyte glycogen deposition.

The present study demonstrated that none of the dietary treatments appeared to affect gross villi structure. Contrary to these findings, previous studies showed that MOS supplementation at similar levels (0.15%–0.3%) increased villi length of rainbow trout (Yilmaz et al., 2007). Similarly, improvements of gut morphology have been reported in poultry and swine (Iji et al., 2001; Hooge, 2004; Peet-Schwering et al., 2007; Castillo et al., 2008) using low-level MOS supplementation (0.1–0.2%). However, similar to the present study, Torrecillas et al. (2007) reported that 0.4% MOS supplementation did not increase European sea bass intestinal villi length. The differences

Table 5
Summary of species richness, Shannon–Weaver diversity index and similarity analysis of the intestinal microbiota of sea bream fed different experimental diets.

Group	Species richness[a]	Shannon–Weaver diversity index[b]	SIMPER similarity[c] (%)
Experiment I			
FM0	15.5 ± 0.7	2.65 ± 0.05	84.79
FM02	25 ± 0	3.06 ± 0.02	88.16
FM04	24.5 ± 0.7	3.10 ± 0.05	81.34
Experiment II			
SBM0	25 ± 0	3.17 ± 0.01	92.25
SBM04	25 ± 0	3.17 ± 0.02	88.89

[a] Average number of bands/presumed species.
[b] Shannons diversity index: $H' = -\Sigma(pi(\ln pi))$.
[c] SIMPER = similarity within group replicates.

between the reported effects of MOS on villi structure to date might be due to the different doses used, different species assessed, different gut microbiota within these species, different rearing conditions or different methodological approaches used (i.e. villi length measurement vs perimeter ratio method). Future studies should help to reveal the complex mechanisms underpinning the potential improvements induced by prebiotics in the gut of fish. Dietary SBM incorporation of ~30% did not produce any gross histological alteration at the level of villi structures in the present study, which supports the findings of Bonaldo et al. (2008).

SEM analysis in the present study revealed that MOS supplementation increased microvilli density in both the anterior and posterior intestinal regions. SEM analysis suggests that MOS supplementation can produce more microvilli structures which should provide the potential to improve nutrient capture. TEM analysis of the intestine revealed that MOS supplementation could increase the microvilli length, which is in accord with Salze et al. (2008) who observed that 0.2% dietary MOS supplementation increased the microvilli length of cobia larvae. Despite the clear increase of both microvilli density and length these improvements did not translate into improved nutrient utilisation or growth performance in the present study. Indeed, no qualitative differences of endocytic activity were noted among the groups, although future quantitative studies are required to verify this. Despite this, the improvement of microvilli morphology may lead to improved apical brush border integrity which should be assessed by pathogenic challenge studies in the future. Previous studies with salmonids have demonstrated that dietary SBM decreased microvilli length and density (Bakke-McKellep et al., 2007; Merrifield et al., 2009a) which is in contrast to the findings of the present study. This suggests that gilthead sea bream is not as susceptible to SBM-induced enteritis as salmonids. The specific reason for this is currently unclear but may be due to the physiology of this omnivorous fish species compared to other carnivorous fish species which often face great difficulties in accepting plant proteins.

PCR-DGGE analysis in the present study suggests that both MOS and SBM can influence the intestinal microbiota in terms of microbial abundance and species richness. Shannon–Weaver diversity indices and species richness were increased with MOS supplementation in the FM groups. Additionally, cluster analysis demonstrated a clear shift of intestinal microbial profiles with each dietary treatment clustered into distinct groups. SBM has been shown to effect the gut microbiota of fish previously (Heikkinen et al., 2006; Ringø et al., 2006; Bakke-McKellep et al., 2007; Ringø et al., 2008; Merrifield et al., 2009a) and the present study indicates that the gut microbiota of sea bream are also sensitive to dietary SBM. In fact our findings suggest that dietary SBM exerted a greater effect on gut microbiota than dietary MOS as the clear differences in microbial profiles and diversity induced by dietary MOS in FM-fed fish appeared to be reduced or eliminated in diets containing SBM. The reason for this is not at present clear, however, this might be due to fermentable carbohydrates provided by SBM which typically comprise approx. 4–5% oligosaccharides on a dry-weight basis (Obendorf et al., 1998). Indeed, SBM oligosaccharides have been discussed in terms of prebiotic potential and microbial fermentability previously (Gibson et al., 2004) and thus these components of SBM may have exerted some effect on gut microbial populations. Future studies should incorporate 16S rRNA sequence analysis and quantitative culture independent techniques such as fluorescent *in-situ* hybridization (FISH) to fully elucidate these changes.

The findings of the present study suggest that MOS supplementation alters the intestinal microbiota and morphology of gilthead sea bream. Furthermore, gilthead sea bream can accept up to 30% dietary SBM without suffering from SBM-induced enteritis. According to the previous research with MOS, it appears that the fish size, species, rearing conditions and level of MOS supplementation are the factors which determine the effect of MOS in fish. Hence, further research is required in order to evaluate and optimize MOS applications especially with regard to the intestinal microbiota and intestinal histology.

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