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Effect of Mannan-oligosaccharide Supplementation on Body Growth, Fatty Acid Profile and Organ Morphology of Gilthead Seabream, *Sparus aurata*

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SG, MAG and EG designed the experiment. SG performed experimental work and analyzed the data. SG, YY, MAG and EG wrote the article.

Key words
Mannan-oligosaccharide (MOS), Gilthead seabream (*Sparus aurata*), Growth, Fatty acid profile, Histology.

ABSTRACT

This study was conducted to assess the impact of mannan-oligosaccharide (MOS) on growth performance, body physiology and tissue morphology of gilthead seabream (*Sparus aurata*). Treatment of fish with MOS-feed shown a significant increase in live weight and protein efficiency rates when were directly compared with mock-treated fish control. However, there was no statistically supported level of significance was observed for growth rates and feed conversion rates among groups. Improved live weight and protein efficiency rates reflected positively on the survival rate in MOS-fed fish. Interestingly, the whole body and fillet fatty acid composition shown no-correlation between treated and control groups ($p>0.05$). During the course of whole body examination, a positive correlation between MOS-fed and control-fed fish was observed for monounsaturated fatty acids and polyunsaturated fatty acids. However, these observations were not apparent in fillet samples. Profiling of the hepatic fatty acid clarified insignificant differences between MOS or mock treated groups for saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids values. Histological examination of fish that were fed on a diet supplemented with MOS shown no adverse effects on investigated organs, intestine and the liver. Taken together, it is plausible to state that a diet supplemented with MOS has positive effects on the survival rate and the fatty acids profile without any observable negative impact on body tissues and thus support the safe use of MOS in fish feed.

INTRODUCTION

Global human population is exponentially increasing and it is expected that the world's population will reach 9.1 billion (34 percent higher than today) by 2050. Current sources of food security are insufficient and thus warrant necessary investments and improved policies in the agricultural production systems. Aquaculture is a promising and rapidly growing sector by contributing approximately 40 percent of total fishery production, around the world (FAO). Specifically, aquaculture products yielded a total of 537.345 tons, which constitute 43.8% of the total fishing industry contribution to the food security in Turkey (TUIK, 2014).

Increasing demands of aquaculture have pressed the need to raise health-standards of fish industry to not only improve productivity but also to provide high-quality food products. Due to intensive production systems, there are higher stresses by the bacterial and viraldiseases with diverse and unexpected pathological outcomes. Using antibiotics, pesticides and other chemical substances for the purpose of enhanced protection and pest controls favor the development of antimicrobial resistance. However, due to global consensus on the restricted use of antibiotics, the use of natural, and environmental-friendly feed additives such as probiotics and prebiotics are receiving higher appreciations to ensure the healthy development of aquaculture (Dimitroglu *et al*., 2010; Genc *et al*., 2011; Akrami *et al*., 2012). One of these feed additives, prebiotics, are defined as oligosaccharide-structured, indigestible nutrient elements that have a positive effect on the host health by temporarily activating proliferation

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and/or activity of one or several species of microorganisms in the intestinal flora. In other words, probiotics change the flora in the favour of benign bacteria and to limit the growth of pathogens (Gibson and Roberfroid, 1995; Burr and Gatlin, 2005; Bavington and Page, 2005; Sang *et al.*, 2011). In recent years, one of the most important types of prebiotic oligosaccharide additives being used in the feed is mannan-oligosaccharide (MOS). The MOS, obtained from the cell walls of bread yeast (*Saccharomyces cerevisiae*), is glucomannoprotein, which is a natural alternative additive. The yeast cell wall is comprised of 30% mannan, 30% glucan and 12.5% protein, and carries strong antigenic stimulation effects. Main motivations for the use of MOS as a feed additive include its inhibitory impacts on pathogenic bacteria, stimulation of the immune system, potential to promote growth and to improve feed conversion (Newman, 1994; Patterson and Burkholder, 2003). Due to these positive effects, MOS has been used in diets of poultry and farm animals in order to promote health and growth in recent years (Savage, 1996b; Quigley *et al.*, 1997; Kaufhould *et al.*, 2000; Guclu, 2001; Heinrichs *et al.*, 2003; Sarıkaya and Kucuk, 2009; Kahraman *et al.*, 2010; Yalcınkaya *et al.*, 2011). The positive impact of MOS has been tested on European bass, *Dicentrachus labrax* (Torrecillas *et al.*, 2007, 2011), Nile tilapia, *Oreochromis niloticus* (Samrongpan *et al.*, 2006; Sado *et al.*, 2008), hybrid tilapia, *Oreochromis mossambicusx Oreochromis niloticus* (Genc *et al.*, 2007a), channel catfish, *Ictalarus punctatus* (Welker *et al.*, 2007), African catfish, *Clarias gariepinus*, (Genc *et al.*, 2006), rainbow trout, *Oncorhynchus mykiss* (Staykov *et al.*, 2007; Yılmaz *et al.*, 2007; Estrada *et al.*, 2013), carp, *Cyprinus carpio* (Staykov *et al.*, 2005; Culjak *et al.*, 2006; Genç *et al.*, 2013), Japanese flounder, *Paralichthys olivaceus* (Ye *et al.*, 2011) European sturgeon, *Huso huso* (Mansour *et al.*, 2012) and gilthead seabream, *Sparus aurata* (Gultepe et al., 2011). Diverse studies have concluded that MOS carry positive effects on growth performance, survival rate and live weight gain (Dimitroglou *et al.*, 2010; Gultepe *et al.*, 2011, 2012).

In this study, it was aimed to investigate the effects of MOS containing feed on growth, live weight gain, body composition, fatty acids profiles, and intestinal and hepatic histology of gilthead seabream, *Sparus aurata*.

MATERIALS AND METHODS

Feed material

Commercial bream feed (5mm, Camlı Yem Inc., İzmir, Turkey) was crushed in a hammer mill (Hammer mill, Kocamaz Tarim, İzmir, Turkey) and perior to addition of MOS the feed of one group (0% group) was separated. The feeds for rest of groups were supplemented with 0.1%, 0.2%, 0.3% and 0.4% MOS (Sentiguard, Belgium). All feeds were homogenised by a shovel and a hand mixer (Sahin Torna, Antalya, Turkey). The homogenised mixture was pressed into 2 mm diameter pellets with a research-type pelleting machine (Beysan Makina ve Torna, Rize, Turkey) and stored in feedbags post-cooling pellets. The feed was placed in a refrigerator until use. Contents of the feed used in the study are briefly outlined in Table I.

Table I.- Ingredients of feed used in the trial groups (% from dry matter).

	M0	M1	M2	M3	M4
Dry matter	92.43	91.76	91.39	91.92	93.23
Ash	12.51	12.73	12.68	12.8	12.37
Protein	45.39	46.1	46.5	46.98	45.35
Lipid	20.34	19.09	19.29	19.4	20.17
Carbohydrate	14.19	13.84	12.92	12.74	15.34
Energy(Kcal/Kg)	5083	4990	4993	5023	5113

Experimental design and sampling period

Gilthead seabream, produced during the first period of 2013 at the Mediterranean Fisheries Research Production and Training Institute (Beymelek Hatchery) with initial weights between 4.06 g and 4.09 g were used in this study. The fish were randomly distributed between 15 experimental tanks (350L) in groups of 50 fish per tank. Before the study was commenced, fish were fed with control feed in the morning and afternoon (4% of body weight per day) for an adaptation period of two weeks. The study was conducted with 5 groups and 3 recurrences per group according to the random parcel testing pattern. In order to ensure accurate and stress-free weighing, the fish were anaesthetised with a 0.2 mL/L dose of phenoxyethanol. For all groups, feed was given two times per day (in the morning and afternoons) at 08:30 A.M. and 03:30 A.M., respectively. Fish were fed by a free feeding method until they were sated. The water parameters including pH (Hanna HI98127, Germany), temperature (Dostmann, Germany), salinity (Atago, Japan), dissolved oxygen (OxyGuard, Denmark) and the amount of consumed feed were recorded daily. The average measurements for temperature, dissolved oxygen, pH and salinity values were, 24.77 ± 0.18 °C, 11 ± 0.16 mg/L, 7.68 ± 0.04 and 37.35 ± 0.1 ppt, respectively.

Measurement and analysis

The study was conducted for fifteen weeks with measurement intervals of three weeks. Individual fish was investigated for length and weight and before termination of the study, the live weight gain (LWG), feed conversion ratio (FCR), specific growth ratio (SGR) and protein efficiency ratio (PER) were calculated as suggested by Santihna *et al*. (1996), Hossu *et al*. (2001) and Skalli and Robin (2004).

Dry matter, crude ash and protein analyses were conducted in accord with the AOAC (1990) method whereas Bligh and Dyer (1959) method was used for lipid and fatty acid analysis. Samples placed in GC tubes were read in a GC device (Agilent Technologies 7820A GC System, USA) to determine fatty acid contents of each sample. Tissue samples taken from the liver and the anterior sections of small intestines of the fish were used in the study. Tissue samples from livers and small intestines of the fish were fixed in 10% neutral formaldehyde for 48 h before transferring to a graded alcohol (70%, 80% and 96%) series, made transparent in xylol and embedding in paraffin. Finally, 6-7 µm thick sections were cut from the paraffin block using a Leica RM 2125 RT microtome, and the sections were treated with the Haematoxylin-Eosin stain to determine the general structure of the liver. Tissue samples were examined at 40x under an Olympus BX53 microscope and recorded with an Olympus DP72 camera (Takashima and Hibiya, 1995; Pryor *et al*., 2003; Roberts and Smail, 2004; Genc *et al*., 2006). The normality and homogeneity of all data were tested using SPSS 15 (SPSS, Chicago, IL) statistical packet software. The variables were first subjected to a normality assessment and in the absence of normal distribution; the data were subjected to a non- parametric one-sample Kolmogorov-Smirnov test. Finally, the data were analysed with SPSS statistics unilateral variance analysis ANOVA. The Duncan multiple comparison test was used at a significance level of P<0.05 to determine the level of significance between groups. The results were expressed in the format 'average values ± standard error' (avg. ± S.E).

RESULTS

At the end of 105 days of examination, live weight values and protein efficiency rates of the groups fed with MOS-added feed were found to be statistically lower in comparison to the control group (P<0.05). All groups were found to be similar to each other with regard to live weight gain (LWG), specific growth ratio (SGR) and feed conversion ratio (FCR) (P>0.05). However, a higher survival ratio (SR) was observed in the fish fed with MOS-added feed in comparison to the control group (Table II).

The protein ratio in fillet samples of groups fed with MOS-added feed was found to be higher and statistically different in comparison to the control group. In regard to lipid contents, a statistical difference was found between the control group and groups fed with 0.1% MOS and 0.4% MOS-added feed, respectively (P<0.05). The dry matter was found to be similar in all groups (P>0.05) and in regard to crude ash a statistical difference was found between the control group and group fed with 0.3% MOS (P<0.05) (Table III).

According to the whole body fatty acid profile, results of bream fed with MOS-added feed at the end of testing (Table IV), the fatty acids found in all groups at high levels were C16:0 (palmitic acid), C18:1n-9 (oleic acid), C18:2n-6 (linoleic acid) and C22:6n-3 (DHA).

No statistically significant difference was found between test groups for the saturated fatty acids (SFA) value (P>0.05). Monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and ∑n-3 and ∑n-6 fatty acids were found at higher levels in bream fed with MOS-added feed compared to the control groups.

Table II.- Effect of mannan-oligosaccharide (MOS) on growth performance of gilthead seabream (*Sparus aurata*).

	0%	0.1% MOS	0.2% MOS	0.3% MOS	0.4%MOS
IW (g)	4.09±0.03[a]	4.08±0.02[a]	4.07±0.03[a]	4.06±0.03[a]	4.06±0.02[a]
FW (g)	89.81±1.14[b]	83.48±1.16[a]	85.84±1.17[ab]	86.79±1.27[a]	84.28±1.26[a]
IL (cm)	6.83±0.02[a]	6.83±0.02[a]	6.85±0.02[a]	6.85±0.02[a]	6.84±0.02[a]
FL (cm)	17.26±0.07[b]	16.87±0.08[a]	17.03±0.07[a]	17.07±0.08[ab]	16.95±0.08[a]
LWG (g)	85.69±1.67[a]	79.39±1.13[a]	81.78±0.39[a]	82.73±4.24[a]	80.24±1.76[a]
SGR	2.94±0.02[a]	2.87±0.01[a]	2.90±0.00[a]	2.92±0.05[a]	2.89±0.02[a]
FCR	1.28±0.02[a]	1.30±0.01[a]	1.35±0.04[a]	1.35±0.02[a]	1.33±0.05[a]
PER	1.73±0.02[b]	1.68±0.01[ab]	1.59±0.05[a]	1.58±0.02[a]	1.66±0.06[ab]
SR	97.33±0.02[a]	100.00±0.00[b]	100.00±0.00[b]	98.67±0.67[ab]	99.33±0.67[ab]

Data are expressed as mean values ± standard error. The groups which are shown with different letters at the same line are highly different from each other (P<0.05). IW, initial weight; FW, final weight; IL, initial length; FL, final length; LWG, live weight gain; SGR, specific growth ratio; FCR, feed conversion ratio; PER, protein efficiency ratio; SR, survival ratio.

Table III.- Effect of mannan-oligosaccharide (MOS) on fillet dry matter, raw ash, protein and lipids ratio of gilthead seabream (%)*.

	0%	0.1% MOS	0.2% MOS	0.3% MOS	0.4% MOS
Protein	20.60±0.23^{a*}	21.83±0.27^{b}	21.80±0.57^{ab}	21.01±0.38^{ab}	21.22±0.18^{ab}
Lipid	7.94±0.44^{a}	7.67±0.27^{a}	8.64±0.32^{ab}	8.75±0.26^{ab}	10.07±0.91^{b}
Dry matter	31.32±0.22^{a}	32.57±0.42^{a}	33.28±0.90^{a}	31.35±2.44^{a}	34.27±0.63^{a}
Raw ash	3.48±0.26^{b}	3.30±0.57^{ab}	2.56±0.30^{ab}	2.30±0.14^{a}	2.58±0.06^{ab}

Data are expressed as mean values ± standard error. The groups which are shown with different letters at the same line are highly different from each other (P<0.05).

Table IV.- Effect of mannan-oligosaccharide (MOS) on whole body fatty acid composition of gilthead seabream.

Fatty acids (mg/g)	0 %	0.1% MOS	0.2% MOS	0.3% MOS	0.4% MOS
14:00	2.34±0.03^{ab}	2.31±0.00^{a}	2.39±0.03^{b}	2.32±0.02^{ab}	2.37±0.02^{ab}
16:00	13.29±0.18^{ab}	13.03±0.06^{b}	13.10±0.03^{ab}	13.01±0.01^{a}	13.29±0.01^{ab}
18:00	3.64±0.06^{a}	3.52±0.14^{a}	3.65±0.04^{a}	3.63±0.01^{a}	3.74±0.03^{a}
∑SFA	19.27±0.27^{a}	19.16±0.20^{a}	19.15±0.04^{a}	18.95±0.03^{a}	19.40±0.00^{a}
16:1n-7	3.68±0.04^{ab}	3.65±0.01^{a}	3.72±0.03^{ab}	3.79±0.06^{a}	3.76±0.11^{b}
18:1n-9c	31.70±0.32^{a}	32.37±0.13^{b}	31.84±0.16^{ab}	32.15±0.03^{ab}	31.95±0.08^{ab}
18:1n-9t	3.17±0.03^{a}	3.28±0.02^{b}	3.24±0.01^{ab}	3.23±0.00^{ab}	3.23±0.01^{ab}
20:1n-9	0.64±0.01^{a}	0.65±0.01^{ab}	0.65±0.00^{ab}	0.65±0.00^{ab}	0.67±0.00^{b}
∑MUFA	39.19±0.41^{a}	39.95±0.15^{b}	39.47±0.14^{ab}	39.69±0.04^{ab}	39.62±0.07^{ab}
18:2n-6 t	14.92±0.13^{a}	15.31±0.02^{bc}	15.42±0.04^{c}	15.39±0.01^{c}	15.15±0.01^{b}
18:3n-3	2.33±0.09^{a}	2.40±0.01^{a}	2.35±0.04^{a}	2.47±0.02^{a}	2.36±0.02^{a}
20:2n-6	0.36±0.00^{a}	0.40±0.02^{a}	0.39±0.32^{a}	0.35±0.01^{a}	0.39±0.02^{a}
20:3n-6	1.51±0.09^{a}	1.53±0.14^{a}	1.39±0.01^{a}	1.41±0.00^{a}	1.38±0.01^{a}
20:4n-6	0.79±0.01^{a}	0.79±0.01^{a}	0.79±0.00^{a}	0.80±0.00^{a}	0.79±0.00^{a}
20:3n-3	0.42±0.00^{a}	0.40±0.01^{a}	0.41±0.01^{a}	0.43±0.00^{a}	0.41±0.00^{a}
20:5n-3	2.90±0.02^{a}	2.89±0.03^{a}	3.00±0.04^{b}	2.94±0.00^{ab}	3.00±0.01^{b}
22:5n-3	1.96±0.02^{a}	2.00±0.00^{ab}	2.08±0.02^{c}	2.08±0.00^{c}	2.02±0.00^{b}
22:6n-3	5.60±0.08^{a}	5.67±0.09^{ab}	5.94±0.10^{c}	5.76±0.02^{abc}	5.88±0.03^{bc}
24:1n-9	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
∑PUFA	30.18±0.17^{a}	31.42±0.04^{b}	31.76±0.11^{c}	31.65±0.01^{bc}	31.40±0.02^{b}
18:3n-3	2.33±0.09^{a}	2.40±0.01^{a}	2.35±0.04^{a}	2.47±0.02^{a}	2.36±0.02^{a}
20:3n-3	0.42±0.00^{a}	0.40±0.01^{a}	0.41±0.01^{a}	0.43±0.00^{a}	0.41±0.00^{a}
20:5n-3	2.90±0.02^{a}	2.89±0.03^{a}	3.00±0.04^{b}	2.94±0.00^{ab}	3.00±0.01^{b}
22:5n-3	1.96±0.02^{a}	2.00±0.00^{ab}	2.08±0.02^{c}	2.08±0.00^{c}	2.02±0.00^{b}
22:6n-3	5.60±0.08^{a}	5.67±0.09^{ab}	5.94±0.10^{c}	5.76±0.02^{abc}	5.88±0.03^{bc}
∑n-3	13.23±0.15^{a}	13.37±0.14^{ab}	13.79±0.10^{c}	13.68±0.00^{bc}	13.69±0.02^{bc}
18:2n-6 t	14.92±0.13^{a}	15.31±0.02^{bc}	15.42±0.04^{c}	15.39±0.01^{c}	15.15±0.01^{b}
20:2n-6	0.36±0.00^{a}	0.40±0.02^{a}	0.39±0.32^{a}	0.35±0.01^{a}	0.39±0.02^{a}
20:3n-6	1.51±0.09^{a}	1.53±0.14^{a}	1.39±0.01^{a}	1.41±0.00^{a}	1.38±0.01^{a}
20:4n-6	0.79±0.01^{a}	0.79±0.01^{a}	0.79±0.00^{a}	0.80±0.00^{a}	0.79±0.00^{a}
∑n-6	17.58±0.01^{a}	18.04±0.11^{b}	17.97±0.03^{b}	17.96±0.00^{b}	17.71±0.01^{a}
n-3/n-6 rates	0.75±0.00^{a}	0.74±0.01^{a}	0.76±0.00^{b}	0.76±0.00^{ab}	0.77±0.00^{b}

Each line on the mean±SE is expressed in different letters that show the difference is important (P<0.05). ΣSFA, total saturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣPUFA, total polyunsaturated fatty acid.

Table V.- Effect of mannan-oligosaccharide (MOS) on fillet fatty acid composition of gilthead seabream.

Fatty acids (mg/g)	0%	0.1% MOS	0.2% MOS	0.3% MOS	0.4% MOS
14:00	2.62±0.02[a]	2.74±0.03[ab]	2.79±0.01[b]	2.73±0.05[ab]	2.79±0.04[b]
16:00	13.19±0.08[a]	13.15±0.03[a]	13.22±0.22[a]	13.31±0.06[a]	13.23±0.19[a]
18:00	3.32±0.05[a]	3.22±0.02[a]	3.22±0.04[a]	3.33±0.02[a]	3.34±0.07[a]
∑SFA	19.13±0.15[a]	19.11±0.04[a]	19.23±0.25[a]	19.37±0.09[a]	19.36±0.28[a]
16:1n-7	4.03±0.04[a]	4.28±0.04[c]	4.23±0.05[bc]	4.07±0.06[ab]	4.11±0.03[ab]
18:1n-9c	31.46±0.45[a]	32.78±0.47[b]	31.44±0.05[a]	31.99±0.03[ab]	32.18±0.30[ab]
18:1n-9t	2.68±0.03[a]	2.73±0.06[a]	2.72±0.05[a]	2.69±0.04[a]	2.81±0.01[a]
20:1n-9	2.29±0.02[c]	2.24±0.02[bc]	2.18±0.00[ab]	2.21±0.02[ab]	2.15±0.02[a]
22:1n-9	0.38±0.00[ab]	0.37±0.00[a]	0.36±0.01[a]	0.40±0.00[b]	0.37±0.01[ab]
∑MUFA	40.15±0.14[c]	41.01±0.03[d]	40.09±0.06[bc]	39.74±0.11[a]	39.79±0.12[ab]
18:2n-6 t	14.55±0.02[a]	14.71±0.05[a]	14.89±0.20[a]	14.78±0.07[a]	14.84±0.05[a]
18:3n-3	6.94±0.04[b]	6.88±0.07[ab]	6.84±0.0[ab]	6.81±0.03[ab]	6.72±0.05[a]
20:2n-6	0.29±0.00[b]	0.26±0.00[a]	0.30±0.00[b]	0.29±0.00[b]	0.28±0.00[ab]
20:3n-6	0.65±0.00[b]	0.64±0.01[b]	0.62±0.01[ab]	0.63±0.00[b]	0.59±0.01[a]
20:4n-6	0.20±0.00[b]	0.19±0.00[ab]	0.18±0.00[ab]	0.18±0.00[ab]	0.17±0.00[a]
20:3n-3	0.42±0.00[a]	0.44±0.00[a]	0.42±0.01[a]	0.43±0.00[a]	0.43±0.00[a]
20:5n-3	2.87±0.01[ab]	2.75±0.02[a]	2.91±0.06[b]	2.91±0.04[b]	2.87±0.05[ab]
22:5n-3	2.02±0.02[a]	3.09±0.04[b]	3.09±0.00[b]	2.04±0.04[a]	2.15±0.08[a]
22:6n-3	5.74±0.07[ab]	5.45±0.07[a]	5.85±0.08[b]	6.08±0.05[b]	5.93±0.18[b]
24:1n-9	0.23±0.00[a]	0.23±0.01[a]	0.23±0.00[a]	0.24±0.00[a]	0.23±0.00[a]
∑PUFA	34.30±0.17[a]	35.05±0.26[bc]	35.72±0.09[c]	34.80±0.18[ab]	34.66±0.29[ab]
18:3n-3	6.94±0.04[b]	6.88±0.07[ab]	6.84±0.0[ab]	6.81±0.03[ab]	6.72±0.05[a]
20:3n-3	0.42±0.00[a]	0.44±0.00[a]	0.42±0.01[a]	0.43±0.00[a]	0.43±0.00[a]
20:5n-3	2.87±0.01[ab]	2.75±0.02[a]	2.91±0.06[b]	2.91±0.04[b]	2.87±0.05[ab]
22:5n-3	2.02±0.02[a]	3.09±0.04[b]	3.09±0.00[b]	2.04±0.04[a]	2.15±0.08[a]
22:6n-3	5.74±0.07[ab]	5.45±0.07[a]	5.85±0.08[b]	6.08±0.05[b]	5.93±0.18[b]
∑n-3	17.99±0.21[a]	18.61±0.19[bc]	19.12±0.16[c]	18.29±0.11[ab]	18.12±0.24[ab]
18:2n-6 t	14.55±0.02[a]	14.71±0.05[a]	14.89±0.20[a]	14.78±0.07[a]	14.84±0.05[a]
20:2n-6	0.29±0.00[b]	0.26±0.00[a]	0.30±0.00[b]	0.29±0.00[b]	0.28±0.00[ab]
20:3n-6	0.65±0.00[b]	0.64±0.01[b]	0.62±0.01[ab]	0.63±0.00[b]	0.59±0.01[a]
20:4n-6	0.20±0.00[b]	0.19±0.00[ab]	0.18±0.00[ab]	0.18±0.00[ab]	0.17±0.00[a]
∑n-6	15.71±0.03[a]	15.81±0.07[a]	15.99±0.18[a]	15.89±0.07[a]	15.89±0.05[a]
n-3/n-6 rates	1.14±0.01[a]	1.17±0.00[ab]	1.19±0.02[b]	1.15±0.00[a]	1.14±0.01[a]

Each line on the mean±SE is expressed in different letters that show the difference is important (P<0.05). ΣSFA, total saturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣPUFA, total polyunsaturated fatty acid.

According to fillet sample fatty acid profile results (Table V), the dominant fatty acids in all groups were C16:0 (palmitic acid), C18:1n-9 (oleic acid), C18:2n-6 (linoleic acid), C22:6n-3 (DHA) and C18:3n3 (linolenic acid).

SFA values in the groups were found similar to each other (P>0.05). PUFAs and ∑n-3 fatty acids were found at higher levels in fish fed with 0.1% and 0.2% MOS-added feed, while the MUFA level was found higher in fish fed with 0.1% MOS-added feed (P<0.05).

According to the hepatic fatty acids profiling (Table VI), fatty acids found at high levels in all groups were C16:0 (palmitic acid), C18:00 (stearic acid), C18:1n-9 (oleic acid), C18:2n-6 (linoleic acid), C18:3n-6 (alfa-linolenic acid) and C22:6n-3 (DHA).

Table VI.- Effect of mannan-oligosaccharide (MOS) on liver fatty acid composition of gilthead seabream.

Fatty acids (mg/g)	0 %	0.1% MOS	0.2% MOS	0.3% MOS	0.4%MOS
14:00	1.67±0.12[a]	1.72±0.14[a]	1.65±0.01[a]	1.78±0.09[a]	1.76±0.12[a]
16:00	12.97±0.41[a]	13.11±0.41[a]	12.34±0.13[a]	12.44±0.58[a]	12.65±0.62[a]
18:00	5.56±0.04[a]	5.65±0.29[a]	5.47±0.22[a]	5.23±0.37[a]	5.80±0.35[a]
∑SFA	20.20±0.28[a]	20.48±0.30[a]	19.45±0.28[a]	19.45±0.81[a]	20.20±0.84[a]
16:1n-7	2.69±0.03[a]	2.70±0.10[a]	2.66±0.03[a]	2.80±0.06[a]	2.75±0.08[a]
18:1n-9c	33.10±0.97[a]	32.55±0.58[a]	32.15±0.35[a]	31.37±0.36[a]	33.43±1.42[a]
18:1n-9t	3.71±0.06[b]	3.65±0.06[ab]	3.70±0.04[b]	3.49±0.01[a]	3.70±0.07[b]
20:1n-9	0.41±0.01[a]	0.39±0.07[a]	0.47±0.03[a]	0.39±0.03[a]	0.47±0.07[a]
∑MUFA	39.92±1.04[a]	39.29±0.53[a]	38.99±0.38[a]	38.05±0.36[a]	40.52±1.39[a]
18:2n-6 t	14.08±0.63[a]	14.53±0.44[a]	14.91±0.16[a]	15.51±0.04[a]	14.19±1.06[a]
18:3n-3	2.19±0.04[c]	1.82±0.16[ab]	2.03±0.03[abc]	1.66±0.14[a]	2.06±0.08[bc]
18:3n-6	5.12±0.32[a]	5.04±0.08[a]	5.20±0.17[a]	5.36±0.09[a]	5.02±0.35[a]
20:2n-6	0.50±0.01[a]	0.51±0.03[a]	0.48±0.02[a]	0.46±0.05[a]	0.51±0.10[a]
20:3n-6	0.61±0.08[a]	1.30±0.16[b]	1.19±0.05[b]	1.31±0.09[b]	1.11±0.08[b]
20:4n-6	1.14±0.04[b]	1.14±0.11[b]	0.48±0.01[a]	1.12±0.06[b]	0.49±0.00[a]
20:3n-3	0.74±0.24[ab]	0.61±0.08[a]	1.12±0.01[b]	0.62±0.16[a]	1.11±0.02[b]
20:5n-3	2.40±0.09[a]	2.69±0.22[a]	2.65±0.02[a]	2.78±0.10[a]	2.43±0.15[a]
22:5n-3	3.01±0.21[a]	2.98±0.11[a]	2.98±0.12[a]	3.35±0.21[a]	2.79±0.13[a]
22:6n-3	7.24±0.04[a]	9.04±0.42[b]	8.05±0.20[a]	9.59±0.39[b]	7.44±0.28[a]
∑PUFA	37.05±1.45[a]	39.69±0.65[ab]	39.13±0.47[ab]	41.80±0.18[b]	37.19±1.95[a]
18:3n-3	2.19±0.04[c]	1.82±0.16[ab]	2.03±0.03[abc]	1.66±0.14[a]	2.06±0.08[bc]
20:3n-3	0.74±0.24[ab]	0.61±0.08[a]	1.12±0.01[b]	0.62±0.16[a]	1.11±0.02[b]
20:5n-3	2.40±0.09[a]	2.69±0.22[a]	2.65±0.02[a]	2.78±0.10[a]	2.43±0.15[a]
22:5n-3	3.01±0.21[a]	2.98±0.11[a]	2.98±0.12[a]	3.35±0.21[a]	2.79±0.13[a]
22:6n-3	7.24±0.04[a]	9.04±0.42[b]	8.05±0.20[a]	9.59±0.39[b]	7.44±0.28[a]
∑n-3	15.59±0.50[a]	17.15±0.40[bc]	16.21±0.26[ab]	18.03±0.13[c]	15.23±0.47[a]
18:3n-6	5.12±0.32[a]	5.04±0.08[a]	5.20±0.17[a]	5.36±0.09[a]	5.02±0.35[a]
18:2n-6 t	14.08±0.63[a]	14.53±0.44[a]	14.91±0.16[a]	15.51±0.04[a]	14.19±1.06[a]
20:2n-6	0.50±0.01[a]	0.51±0.03[a]	0.48±0.02[a]	0.46±0.05[a]	0.51±0.10[a]
20:3n-6	0.61±0.08[a]	1.30±0.16[b]	1.19±0.05[b]	1.31±0.09[b]	1.11±0.08[b]
20:4n-6	1.14±0.04[b]	1.14±0.11[b]	0.48±0.01[a]	1.12±0.06[b]	0.49±0.00[a]
∑n-6	21.46±0.95[a]	22.53±0.55[a]	22.91±0.26[a]	23.77±0.15[a]	21.95±1.49[a]
n-3/n-6 rates	0.72±0.01[a]	0.76±0.02[a]	0.70±0.00[a]	0.75±0.01[a]	0.70±0.03[a]

Each line on the mean±SE is expressed in different letters that show the difference is important (P<0.05). ΣSFA, total saturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣPUFA, total polyunsaturated fatty acid.

While no statistical difference was observed between trial groups with reference to SFA and MUFA values (P>0.05), the PUFA value was found to be at higher levels in groups fed with MOS-added compared to the 0% group. ∑n-3 fatty acids were found at the highest level in the group fed with 0.3%MOS-added feed (P<0.05). No difference was observed between groups in regards to ∑n-6 fatty acid levels (P>0.05).

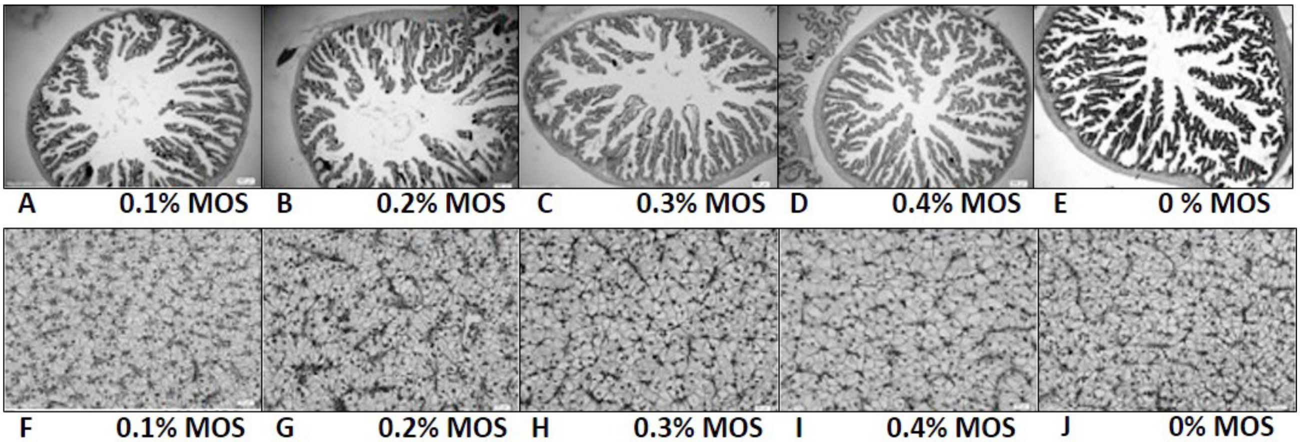

Fig. 1. Effect of mannan-oligosaccharide (MOS) on intestine and liver hitological structures: it is identified that extensions which are villus like intestine and intestine epithelium morphology are normal in A and E sections, vascular structure and minimal lipid vacuolization of hepatic tissue is at normal level in F and J sections (H&E, Bar; A-E: 200 µm, F-J: 20 µm).

According to the results of the examination of tissue slides from liver, as an organ deemed to have high vitality in histologic sections, the lipid vacuolisation levels of liver tissues representing different groups, which can be deemed typical for fish in aquaculture conditions, were found to be normal. The presence of villus-like extensions, crypts, intestinal internal epithelia cells and a small number of goblet cells confirm the status of small intestines, and all are at the levels and in the order sufficient to ensure healthy absorption, and no anomaly was caused in gilthead seabream fed with MOS-added feed (Fig. 1).

DISCUSSION

Results presented in this study confirm previous investigations performed by Dimitroglou *et al*. (2010) where bream fed with MOS-added feed showed no effect on LWG, SGR, FCR and PER (P>0.05). These results are aligned with the LWG, SGR and FCR results of this study. However, on the other hands, Torrecillas *et al*. (2011) found that feeding European seabass, *Dicentrachus labrax,* feed with the Bio-Mos additive (0.4% and 0.6% Bio-Mos), a commercial preparation, caused a positive effect. In addition, Gultepe *et al*. (2011) reported that gilthead seabream, *Sparus aurata* fed diet with the Bio-Mos additive, a commercial preparation, increased growth performance. Akrami *et al*. (2012) and Dimitroglou *et al*. (2011b) reported that MOS did not affect the FCR and PER values in Atlantic salmon, *Salmo salar* and Genc *et al*. (2013) reported the same with regard to carp, *Cyprinus caprio*. Piccolo *et al*. (2013) reported that in a similar manner, MOS does not affect the FCR, SGR and PER values in sharpsnout seabream, *Diplodus puntazzo*. Similar to the results of this study, it was reported that addition of MOS in the feed increases the survival rate in European seabass, *Dicentrachus labrax* (Burriel, 2006), rainbow trout, *Oncorhynchus mykiss* (Staykov *et al*., 2007), gilthead seabream, *Sparus aurata* (Gultepe *et al*., 2011), Nile tilapia, *Oreochromis niloticus* (Samrongpan *et al*., 2006) and catfish, *Ictalarus punctatus* (Bogut *et al*., 2000).

It has been reported earlier that addition of MOS in the feed fail to cause any difference in raw protein level in African catfish, *Clarias gariepinus* (Genc *et al*., 2006) and fresh water lobster, *Astacus leptodactylus* (Mazlum *et al*., 2011) (P>0.05). It was reported that addition of 4.5% MOS in the feed caused an increase in the raw protein level in the flesh of rainbow trout, *Oncorhynchus mykiss* (Yilmaz *et al*., 2007), and that the amount of the MOS additive in the feed increases (1.5%, 3%, 4.5%), the raw protein level in hybrid tilapia, *Oreochromis mossambicus x Oreochromis niloticus* also increases (Genc *et al*., 2007a). Similar to the results of this study in regards to dry matter content, it was reported that MOS addition to the feed did not exhibit a statistical difference between trial groups in fresh water lobster, *Astacus leptodactylus* (Mazlum *et al*., 2011), while studies on African catfish, *Clarias gariepinus* (Genc *et al*., 2006), rainbow trout, *Oncorhynchus mykiss* (Yilmaz *et al*., 2007) and hybrid tilapia, *Oreochromis mossambicus x Oreochromis niloticus* (Genc *et al*., 2007a) have found that MOS addition in the feed exhibit a statistical difference between trial groups in regard to dry matter content. Previous studies on hybrid tilapia, *Oreochromis mossambicus x Oreochromis niloticus* (Genc *et al*., 2007a), rainbow trout, *Oncorhynchus mykiss* (Yılmaz *et al*., 2007), African catfish, *Clarias gariepinus* (Genc *et al*., 2006) and carp, *Cyprinus caprio* (Genc *et *al*., 2013) also reported that the addition of MOS to the feed did not exhibit any statistical difference between the trial groups in regard to crude ash content (P>0.05). Up-to-date, no complete similarity has been found between studies on various fish species in regard to MOS effects on fish growth performance (Genc *et al*., 2011). However, it is believed that differences in effects of MOS on growth originate from differences in species, differences in initial weights, trial time and trial condition, the level of MOS use and differences in the MOS source.

Results for whole body dominant fatty acids based the conclusion of this study are similar to other studies related to sea fish (Torrecillas *et al*., 2007). The SFA values we found as a result of this study were lower than the values reported by Torrecillas *et al*. (2007) while the MUFA values were found to be higher. The reason for the MUFA values found in this study was higher compared to oleic acid value, whereas the reason for the lower SFA value is the lower palmitic acid value. Similar to the fillet dominant fatty acids, other studies on sea fish including Grigorakis *et al*. (2002), Pinto *et al*. (2007) and Lenas *et al*. (2011) reported the same fatty acids as dominant. The SFA and MUFA values found in this study are close to the SFA and MUFA values reported in the Lenas *et al*. (2011) study. In rabbits it has been reported that MOS increased the MUFA and PUFA values in a similar fashion as was investigated in this study (Bovera *et al*., 2012), while it was also reported that MOS increases the MUFA value in Japanese quail (Bonos *et al*., 2010). The MUFA and PUFA values found by Piccolo *et al*. (2013) in their MOS-added feed group in their study on sharpsnout seabream (*Diplodus puntazzo*) were close to the values found in this study. When compared with the SFA values in whole body and fillet samples, the SFA value in the liver was found to be higher. Similarly, the PUFA values in the liver were found to be higher than the PUFA values found in whole body and fillet samples. It is seen that the SFA, MUFA and PUFA values found in this study are congruent with the SFA, MUFA and PUFA values observed by Guerrero *et al*. (2011), wherein hepatic fatty acid profiles of 12 different sea fish species were examined. In their study on hepatic fatty acids in Gilthead seabream, Nogueira *et al*. (2013) found SFA, MUFA and PUFA values close to the results of this study. As can be ascertained from the studies mentioned above, the amount and profiles of fatty acids in fish can change according to species, size, age, gender and body section of the fish, as well as type and volume of feed, feeding pattern, geographical region, reproduction status, environmental conditions and the season (Nettleton, 1985; Ackman, 1989; Saito *et al*., 1999; Lenas *et al*., 2011). Therefore, results of studies conducted under different trial conditions and on different fish species will naturally provide different conclusions.

The intestinal and hepatic histological results of this study were found to be congruent with the results of Genc *et al*. (2006). In fact, many studies reported that feeding with MOS-added feed has no negative effect on intestinal and hepatic tissue histology (Genç *et al*., 2007a) regarding the addition of 0%, 1.5%, 3% and 4.5% MOS in feed for hybrid tilapia, *Oreochromis mossambicus x Oreochromis niloticus*, Genç *et al*. (2013) restuls regarding the addition of 0%, 1.5%, 3% and 4.5% MOS in feed for carp fingerlings, *Cyprinus caprio*, Genç *et al*. (2007b) study regarding green tiger prawn, *Penaeus semisulcatus* and Yılmaz *et al*. (2007) study regarding rainbow trout, *Oncorhynchus mykiss*. The histologic findings of this study are congruent with findings in these studies in the literature. In conclusion, it was certified that the addition of MOS to feed does not cause any negative effect on high vitality tissues involved in digestion in gilthead seabream.

CONCLUSIONS

This study investigate the potential of MOS as an alternative fish feed additive in the aquaculture of Gilthead seabream of the family Sparidae, which is a natural species of Aegean and Mediterranean regions and is important in Turkish aquaculture system. With the increasing importance of healthy food , replacing harmful substances that might leave residues with natural products for increasing yield becomes desirable. As one such product, mannan-oligosaccharide (MOS), the subject of this study, was tested on gilthead seabream fingerlings for the first time (approximate initial weight of 4 g). In sectorial and commercial assessments of results of this study and according to the relevant market investigations, we have concluded that the cost of MOS is relatively low. Feeds containing varying amounts of MOS were evaluated on bream for a period of 15 weeks. Taken together, it is determined that the use of MOS as a feed additive can't negatively effect the health of gilthead seabream. We believe that the effects of this product at lower doses on gilthead seabream of different sizes and in larval stages should also be investigated in future, and studies to determine its mechanisms on economical aquaculture species are also required to be investigated. Even though aquaculture benefits from various healthy alternatives, feed additive products in use today have been a subject of research for a long time; studies regarding the use of such products in our country, especially in the field of aquaculture, are relatively new and limited in scope. We believe conducting future studies on the use of these additives, determining their effects and increasing their field of application in protecting animal health and to increase productively will provide benefits for producers of aquaculture feeds as well as sector stakeholders.

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Conflict of interest statement

We declare that we have no conflict of interest.

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