

The Effect of Mannan-Oligosaccharide (MOS) as a Feed Supplement on Growth and Some Blood Parameters of Gilthead Sea Bream (*Sparus aurata*)

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Abstract

The study investigated the effect of increasing dietary inclusion of Mannan oligosaccharide (MOS) on growth and blood parameters of sea bream (*Sparus aurata*). Varying concentrations of MOS were added to commercial sea bream feed at 0‰ (control), 1, 2, 3 and 4‰. At the end of the 15 week trial, control group had the highest final weight, specific growth rate, feed conversion ratio and protein efficiency ratio ($P < 0.05$), although these did not differ among the MOS containing groups ($P > 0.05$). Survival rates were higher in the MOS treatment groups. Blood parameters, did not differ among treatment groups ($P > 0.05$). The lowest Alkaline phosphatase (ALP) value was measured in the control group whereas the highest was recorded in the 4‰ MOS group. The lowest level of triglycerides was measured in the 4‰ MOS group, while the highest level of triglycerides was measured in the control group. Cholesterol level was lowest in the 2‰ MOS group ($P < 0.05$). The study showed that the inclusion of dietary MOS has no negative effects on growth and blood parameters in sea breams. However, dietary MOS inclusion led to higher survival rate in trial fish.

Key words: Mannan-oligosaccharides (MOS), sea bream (*Sparus aurata*), growth, blood parameters

Introduction

Aquaculture has shown a fast development in Turkey. Fishery production was 672.241 ton, while aquaculture products were 240.334 tons. In particular, production of commercial sea bream (*Sparus aurata*) reached 51.844 tons in 2015 due to high protein quality and the great taste of the produce (TUIK, 2015). However, during intense aquaculture, increased stress conditions cause a decrease in host resistance against bacteria, virus and parasites in the environment and therefore the prevalence of diseases increases in a short time. Rapid growth in aquaculture is mainly designed to support fish growth free from diseases, avoiding intensive antibiotic, pesticide and other chemical use. Excessive protection oriented antibiotic use can cause the development of antibiotic resistant microorganisms which create harmful effect to aquatic and human life. For this reason, the use of antibiotics in aquaculture as a feed supplement is limited. Recently, the use of functional feed ingredients such as probiotics, prebiotics and immunostimulants has become widespread since they help to improve species growth and product quality without a harmful effect to the environment. Prebiotics are good examples of food supplements that hinder pathogen bacterium colonizing intestines of the host as well as strengthen the hosts' immune system (Newman, 1994; Yıldız & Akan, 2004; Dimitrioglou, Merrfield, Spring, Sweetman, Moate, & Davies, 2010).

Mannan-oligosaccharides (MOS) is one of the structural cell wall component of *Saccharomyces cerevisiae* (also known as bread yeast). MOS is a natural alternative product for anti-bacterial growth factors, which is known for connecting pathogen microorganisms and toxins to their chemical structure (Newman, 1994; Patterson &

Burkholder, 2003). In this way, pathogenic bacterial growth is prevented and consequently the harmful effect of microflora metabolites is decreased. The use of MOS as a feed supplement include its inhibitory impacts on pathogenic bacteria and can augment the immune response of the animal. As a result, the health and performance of the animal may improve if MOS is included in the diet (Bovera, Lestingi, Iannaccone, Tateo, & Nizza, 2012). MOS was first used as a food supplement for chicken and turkey. Additionally, it was given to the other types of animals such as poultry, dogs, pigs, horses, and ruminants. When MOS was used as a food supplement, animal weight and survival ratio were significantly increased. (Savage & Zakrzewska, 1996; Qigley, Drewry, Murray & Ivey, 1997; Kaufhold, Hammon, & Blum, 2000; Heinrichs, Jones, & Heinrichs, 2003; Sarıkaya & Küçük, 2009; Kahraman, Mızrak, Yenice, Atik, & Tunca, 2010; Güçlü, 2001, Yalçınkaya, Gungor, Bafialan, & Erdem, 2011). MOS has also been used in aquaculture by many authors. They have been added to the feed of sea bass, *Dicentrarchus labrax*, (Torrecillas, Makol, Caballero, Montero, Robaina, Real, Sweetman, Tort, & Izquierdo, 2007; Torrecillas, Makol, Caballero, Montero, & Gines, 2011), sea bream, *Sparus aurata* (Gultepe, 2007), Nile tilapia, *Oreochromis niloticus* (Samrongpan, Areechon, Yoonpundh, & Srisapome, 2006), rainbow trout, *Oncorhynchus mykiss* (Yılmaz, Genç & Genç, 2007; Staykov, Spring, Denev, & Sweetman, 2007), green tiger shrimp, *Penaeus semisulcatus* (Genc, Aktas Genc, & Yilmaz, 2007b), catfish, *Clarias gariepinus* (Bogut, Milakovic, Brkic, Novoselic & Bukvic, 2000; Ikizdogan, 2006), carp, *Cyprinus carpio* (Staykov, Denev & Spring, 2005; Culjak, Bogut, Has-Schon, Milakovic, & Canecki, 2006). In these studies, increased growth performance, survival rates and body weight were reported (Staykov et al., 2007; Burriel, 2006). In addition, the positive impact of haematological parameters of MOS has been tested on European bass, *Dicentrarchus labrax* (Torrecillas et al., 2011), Seabream, *Sparus aurata* (Dimitrioglou, Merrfield, Spring, Sweetman, Moate, & Davies, 2010) and channel catfish, *Ictalurus punctatus* (Welker, Lim, Yildirim-Aksoy, & Klesius, 2011).

In this study, the effects of MOS supplementation on growth performance and blood hematology and biochemistry in sea bream (*Sparus aurata*), an important Mediterranean marine fish species was investigated.

Materials and Methods

Feed Ingredients

In this study, 5 mm commercial sea bream feed (Çamlı Yem Inc., İzmir, Turkey) was milled with hammer. One portion of the feed (control feed) was stored without MOS addition and designated as the control feed. The rest of the feed was mixed with dry MOS (Sentiguard, Belgium) in increasing concentrations: 1, 2, 3, and 4‰ for the experimental study. After MOS addition, feed were homogenized and pressed as dry pelleted feed (2mm diameter) with pellet machine (Beysan Makina ve Torna, Rize, Turkey) and dried at room temperature, after which feed was stored at 4 °C. Feed composition is shown in Table 1.

Experimental design and Feeding Protocol

In the study, sea bream with initial weight 4.07 ± 0.03 g (mean \pm standard error, S.E) from the first mating season in 2013 were randomly stocked in 15 fibre glass tanks (5 groups with triplicates) at 50 fish per tank in the hatchery of Mediterranean Fisheries Research Production and Training Institute, Beymelek. Feeding was carried out twice a day at 08:30 and 15:30 respectively for 15 weeks. Prior to the feeding trial, 25 fish were randomly selected to determine the body composition. DO, temperature, pH, and salinity were maintained at 11 ± 0.16 mg/L,

24.77±0.18 °C, 7.68±0.04 and 37.35±0.1 % throughout the trial. Fish were anaesthetised with a 0.2 mL/L dose of phenoxyethanol to obtain accurate measurements in the stress-free environment.

Growth Parameters

Growth performance was evaluated every three weeks. At the end of the study, fish growth and feed intake were evaluated with the following formulas:

Weight Gain (wg) = Final weight (g) - initial weight (g)

Feed Conversion Ratio (FCR) = Feed consumption (g) / weight gain (g) (Santihna, Gomes, & Coimbra 1996)

Specific Growth Ratio (SGR) = {(In Final Weight -In Initial Weight) / time (days)} x 100 (Hossu, Korkut, & Firat, 2001)

Protein efficiency ratio (PER) =Live weight gain (g)/protein intake (g), (Skalli & Robin, 2004)

Viscerosomatic index (VSI) = 100 x (viscera weight (g)/body weight (g)) (Metailler, 1987)

Hepatosomatic index (HSI) = 100 x (liver weight (g)/body weight (g)) (Metailler, 1987)

Carcass yield (CY) = [Eviscerated fish weight (g)/Body weight (g)] × 100 (Metailler, 1987)

Condition factor (CF) = [Body weight (g)/(fish fork length (cm))³] × 100 (Hossu *et al.*, 2001)

Blood Analyses

At the end of the trial, blood samples were collected for analyses. Trial fish were not fed for 24 hours prior to blood sample collection. Blood samples were withdrawn from the tail of fish using 1.5-2 ml sterile tubes including EDTA (2.5 mg/ml). Blood analyses were conducted in accord with the Alan (2006) method. Consequently, all the samples were delivered to a hospital for the blood analyses. The following parameters were measured; hemoglobin (HGB), leucocyte (WBC), erythrocyte (RBC), haematocrit (HCT), mean erythrocyte volume (MCV), mean hemoglobin per erythrocyte (MCH), mean hemoglobin concentration (MCHC) and thrombocyte (PLT). In addition, cholesterol glucose, triglyceride, alkaline phosphate (ALP) glutamate oxaloacetate (GOT), glutamate pyruvate transaminase (GPT) values were checked.

Statistical Analyses

The normality and homogeneity of all data were tested using Kolmogorov-Smirnov and Levene's tests, respectively. The data were subjected to one-way ANOVA followed by Duncan multiple comparison test at a significance level of P<0.05. The results were expressed in the format mean ± standard error (mean ± S.E). Data were analysed using SPSS 15 (SPSS, Chicago, IL) statistical software.

Results

Growth parameters were given in Table 2. At the end of experiment, weight was highest (89.81 g) in the control group whereas fish fed MOS-diets had the lowest PER (P<0.05). Total length was lowest in the 1% MOS fed group whereas the control group had the highest value. However, SGR and FCR did not differ among treatment groups (P>0.05). In comparison to the control group, a higher survival rate was observed in fish fed MOS-added feed groups.

Viserosomatic Index (VSI), Carcass Yield (CY), Condition Factor (CF), and Hepatosomatic Index (HSI) were given on Table 3. No statistical difference was observed among groups in terms of VSI, CR, HSI ($P>0.05$). CF was lowest in MOS-added dietary groups ($P<0.05$).

Hematological and biochemical parameters of trial groups were shown in Table 4. MOS-addition did not present any statistical difference in WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT among trial groups ($P>0.05$).

Discussion

There are contradictory results about effect of MOS supplementation on fish growth. Dimitrioglou et al. (2010) did not find any difference ($P>0.05$) between control group and MOS supplemented groups in juvenile sea bream (*Sparus aurata*). In adult sea bream, Gültepe (2007) reported an increase in growth rates within the MOS-fed groups. In other studies, Bio-Mos was added (up to 4‰) to the juvenile sea bass (*Dicentrarchus labrax*) feed and percentage of growth and survival were affected positively (Burriel, 2006; Torrecillas et al., 2007). Similarly, MOS addition had a positive impact on growth performance of rainbow trout (*Oncorhynchus mykiss*) (Staykov et al., 2007; Yılmaz et al., 2007). It is well reported that no variations ($P>0.05$) were recorded in growth parameters between MOS-fed groups and the control groups in young common carp (*Cyprinus carpio*) and Belugas (*Huso huso*) (Genç, Şengül, & Genç, 2013, Akrami, Razeghi, Mansour, Chitsaz, & Ziaei, 2012; Mansour, Akrami, Ghobadi, Denji, Ezatrahimi, & Gharaei, 2012). The current study confirmed results reported by Dimitrioglou et al. (2010). In the study by Dimitrioglou et al. (2010) dietary MOS inclusion did not affect SGR, FCR and PER in sea bream, while another study showed that 4‰ and 6‰ Bio-Mos addition to feed reduced the FCR (Torrecillas et al., 2011). Moreover, 2‰ MOS addition to rainbow trout feed reduced the FCR (Staykov et al., 2007). On the other hand, Yılmaz et al. (2007) did not report any differences in FCR and PER among groups with 1.5‰, 3‰ and 4.5‰ MOS supplementation (Yılmaz et al., 2007). So far, different fish species were used to evaluate the effect of MOS supplementation on aquaculture. However, no exact relationship between MOS supplement and growth was found. We believe that the different results can be related to the different acclimation periods, selected species, differences in initial weight and the source and concentration of MOS.

Similar to our study, VSI did not differ ($P>0.05$) in hybrid tilapia (Genç, Yılmaz, Genc, & Aktas, 2007a) and rainbow trout (Yılmaz et al., 2007) with dietary MOS addition among trial groups. Dimitrioglou et al. (2010) found lower CF in groups fed with MOS-added feed than the control. On the contrary, according to Ye, Wang, Li, & Sun, 2011 and Pryor, Royes, Chapman, & Miles, 2003 that feeding flounder and sturgeon with MOS-added feed did not show any statistical difference in CF ($P>0.05$). In addition, the studies, carried out in black cod (Genç et al., 2006), rainbow trout (Yılmaz et al., 2007) and hybrid tilapia (Genç et al., 2007a) did not show any statistical difference ($P>0.05$) in HSI with MOS-addition to feed. Result of the current trial showed that HSI did not vary among feed groups, confirming results of previous studies.

Plasma biochemistry parameters play an important role in providing an insight into the nutritional, physiological and health status of the animals and may help in determining the suitability of feeding practices, husbandry conditions, presence of acute or chronic stressors and pathogenic manifestations (Peres, Santos, & Oliva-Teles, 2013). No difference ($P>0.05$) in blood parameters was observed among trial groups. Welker et al. (2007) observed no difference in blood parameters for *Ictalurus punctatus* after feeding with MOS supplemented feeds. Gültepe (2007) reported similar results for the Bio- MOS fed sea bream in 2‰ and 4‰ ($P>0.05$). Different MOS levels (0, 2, 4, 6, 8, 10 g/kg) did not affect the hematological parameters in juvenile Nil tilapia and *Huso huso* (Sado, Almedia

Bicudo, & Cyrino, 2008; Mansour et al., 2012). In terms of GOT (AST), all groups were found lower than control group. Whereas cholesterol level in groups fed on diet supplemented with MOS was found different from control which was first, 3‰ and 4‰ respectively, it showed similarity with fed on diet supplemented with 1‰ MOS. While triglyceride was observed lowest in 4‰ MOS group, groups fed on diet supplemented with 2‰ and 3‰ MOS showed similarity with 4‰, the highest value was measured in control group and this was followed by 1‰ MOS group. While ALP was obtained minimum with 250,67 (UI/l) and maximum was obtained from 4‰ MOS group.

In our study, the urine values were higher than reported values for *Mugil cephalus* (Francesco, Satheeshkumar, Senthil, Caterina, & Giuseppe, 2012), but they showed similarity with the results for *Synodontis membranacea* (Owolabi, 2011). Peres et al. (2013) reported that AST ($U L^{-1}$) level as 64.0 (28-134), $U L^{-1}$ for juvenile sea bream which were not fed for 24 hours. AST values in our study were comparable with Peres et al. (2013). Although Peres et al. (2013) reported cholesterol level in juvenile sea bream (left hungry for 24 hours) as 361.6 mg/dl (341-407), the highest cholesterol level in our study was 336.67 mg/dl (control group). In this phase, cholesterol level of the sea bream, fed with 2‰ MOS-addition was significantly lower ($P>0.05$) than the normal range. ALT level in the present trial was lower than that reported by Kanyılmaz (2012) for sea bream, although it was found compatible with values for *Mugil aeratus* (Ozretic & Ozretic, 1987). ALP ranged from 250-334 UI/l in the present trial was higher than the reported value (58-125 UI/l) by Peres et al (2013). After 24 hours of food deprivation in the current trial, glucose levels in test fish were similar to levels reported in previous studies (Peres et al., 1999, Sitja-Bobadilla, Pena-Llopis, Gomez-Requeni, Medale, Kaushik, Perez-Sanchez, 2005; Peres et al., 2013). This was attributed to the ability of sea bream to quickly restore basal glucose levels after feeding (Peres et al., 2013).

ALP, ALT and AST enzymes are indicative of tissue damage and their increase in blood is indicative of necrosis in liver, tissue degeneration and reflection on changes in protein metabolism (Kopp & Hetesa, 2000; Çelik, 2006). Blood parameters are affected by fish related factors (age, sex and genetic traits) and environmental factors (seasonal changes, food availability, environmental stress and laboratory techniques). Since the blood parameters are extensively affected by these with great fluctuations, the interpretation of the results is highly difficult (Vazquez & Guerrero, 2007, Peres et al., 2013, Oliva-Teles, 2012).

Conclusions

We found that MOS did not cause significant changes on blood parameters except some of the growth parameters, ALP, cholesterol, and triglycerides. At the end of our study, the use of MOS as an additive did not present any negative effect in sea bream. The inclusion of MOS in the diets of test fish led to higher survival rates than control group. However, further research is needed to determine the effect of MOS use in lower dosages for different sizes and larval stages. Moreover, the effect mechanism of MOS should be identified for economically valuable species for aquaculture.

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Table 1. Feed composition of the trial groups (% dry matter).

	Control	1‰ MOS	2‰ MOS	3‰ MOS	4 ‰MOS
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

Table 2. Growth performance of gilthead seabream (*Sparus aurata*).

	Control	1‰ MOS	2‰ MOS	3‰ MOS	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 3. The values belonging to Viserosomatic Index (VSI), Carcass Yield (CY), Condition Factor (CF), and Hepatosomatic Index (HSI)

	Control	1‰ MOS	2‰ MOS	3‰ MOS	4 ‰MOS
VSI	6.59±0.21 ^a	6.45±0.22 ^a	6.58±0.16 ^a	6.31±0.21 ^a	6.32±0.15 ^a
CY	91.80±0.23 ^a	91.83±0.29 ^a	92.07±0.20 ^a	92.45±0.52 ^a	92.43±0.18 ^a
CF	1.78±0.04 ^b	1.66±0.02 ^a	1.70±0.04 ^{ab}	1.64±0.03 ^a	1.72±0.05 ^{ab}
HSI	1.88±0.07 ^a	1.93±0.11 ^a	1.86±0.07 ^a	1.85±0.06 ^a	1.99±0.10 ^a

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 4. Hematological and biochemical parameters of trial groups

Parameters	Control	1‰ MOS	2‰ MOS	3‰ MOS	4‰ MOS
WBC (adet/mm ³)	36.46±1.20 ^a	35.80±1.00 ^a	35.56±6.76 ^a	32.23±5.38 ^a	35.56±1.01 ^a
RBC (x10 ⁶ /mm ³)	3 33.01±0.04 ^a	3.17±0.21 ^a	2.95±0.57 ^a	2.83±0.18 ^a	2.96±0.58 ^a
Hgb (g dl ⁻¹)	11.86±0.15 ^a	11.53±0.11 ^a	11.63±0.32 ^a	10.83±1.55 ^a	11.6±0.3 ^a
Hct (%)	61.33±2.01 ^a	55.03±1.71 ^a	53.2±4.32 ^a	50.63±8.19 ^a	57.6±4.91 ^a
MCV (fL)	204.17±6.76 ^a	181.63±2.76 ^a	179.9±11.17 ^a	176.3±18.37 ^a	194.5±8.96 ^a
MCH (pg)	39.63±0.23 ^a	38.03±0.7 ^a	39.36±0.52 ^a	38.03±0.76 ^a	39.1±0.51 ^a
MCHC (g dl ⁻¹)	19.33±0.54 ^a	20.93±0.53 ^a	22.06±1.47 ^a	22.06±2.22 ^a	20.13±0.68 ^a
PLT (x10 ³ /mm ³)	12.66±1.45 ^a	11.66±2.90 ^a	7.00±1.00 ^a	10.00±0.00 ^a	12.00±2.08 ^a
Ure (mg/dl)	28.66±0.88 ^{a*}	26.33±2.33 ^a	25.66±3.33 ^a	29.00±1.00 ^a	29.66±0.88 ^a
GOT (AST) (U/100ml)	16.16±0.63 ^c	8.33±1.33 ^a	10.76±0.29 ^{ab}	9.63 ±0.87 ^a	13.03±0.78 ^b
Cholesterol (mg/dl)	336.67±11.72 ^b	292.00±2.5 ^{ab}	219.00±44.1 ^a	301.33 ±8.25 ^b	310.00±28.7 ^b
ALT (GPT) (U/100ml)	1.36±0.13 ^a	1.06±0.88 ^a	1.63±0.58 ^a	1.16±0.12 ^a	1.66±0.13 ^a
Triglycerides (mg/dl)	461.67±35.13 ^c	399.6±19.65 ^{bc}	377.67±24.26 ^{ab}	343.33±15.3 ^{ab}	317.33±10.86 ^a
ALP (U/l)	250.67±29.97 ^a	296±2.88 ^{ab}	330.33±21.37 ^b	331.33±13.38 ^b	334.33±11.34 ^b
Glucose (mg/dl)	87.00 ±8.08 ^a	78.00±4.00 ^a	78.00±2.08 ^a	94.00±10.7 ^a	89.33±7.44 ^a

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