Effects of mannan oligosaccharide and inulin on sharpsnout seabream (Diplodus puntazzo) in the context of partial fish meal substitution by soybean meal

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Abstract

One hundred forty-four sharpsnout seabream of about 100 g initial body weight were randomly stocked in 12 experiment tanks (180 L). Testing conditions included 12 fish per tank, with triplicate tanks for treatment. The experimental period lasted 150 days. Average water temperature was 21.9±1.6°C, salinity was 30.0% and pH ranged from 7 to 8, throughout the experiment. A control diet (FM) was made from fish meal. One similar diet (SBM) was made with approximately 40% of the protein supplied by soybean meal. The remaining two diets (SBM-MOS and SBM-INU) were formulated adding 8 g of mannanoligosaccharide (MOS) and inulin (INU) per kg of the SBM diet, respectively. The results showed that mean final weight (average values 234.4 g), specific growth rate (average values 0.585), feed conversion rate (average values 2.05) and protein efficiency ratio (average values 1.01) were unaffected by MOS or INU supplementation to SBM diet. Body proximate composition was affected by MOS and INU supplementation. Fish fed SBM-MOS and SBM-INU diets showed the highest moisture level and the lowest lipid content. Also the total polyunsaturated fatty of the lipids was reduced by MOS and INU in comparison to SBM diet alone.

Introduction

In the last decade, Mediterranean aquaculture has attempted to select new species of marine fish in order to diversify its production. On the other hand, species diversification is today considered as a major topic for the sustainable development of the Mediterranean aquaculture. The potential success of a species is based on consumer acceptance, growth performance and on the availability of juveniles. Sharpsnout seabream (Diplodus puntazzo) is a promising fish with many qualities that make it an excellent new species for marine aquaculture in the Mediterranean Sea. The techniques used for larval rearing are the same as those used for Sparus aurata (Marangos, 1995) and relative high hatching and survival rates at first feeding permit obtaining appropriate percentage of juveniles. The results of research are quite encouraging, and indicate a considerable increase in sharpsnout seabream production in the near future (Orban et al., 2000; Hernàndez et al., 2001; Favaloro et al., 2002; Bonaldo et al., 2004; Piccolo et al., 2011).

In addition, because it is an omnivorous species (Sala and Ballesteros, 1997), could make a more efficient use of high levels of dietary vegetable protein, helpful for a sustainable aquaculture. However, the research of alternative proteins in diets for sharpsnout seabream conducted in recent years (Hernàndez et al., 2007; Chatzifotis et al., 2006; Nogales Merida et al., 2010) did not confirm this theory. The use of prebiotics could improve the sanitary status of the digestive system, increase the nutrient digestibility of vegetable feeds and thus permit a more easy replacement of fish meals. Prebiotics, such as mannan oligosaccharides (MOS) or inulin (oligosaccharide naturally occurring in many plants and commercially produced from the chicory root) 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).

The objective of this experiment was to study the introduction of soybean meal protein in sharpsnout seabream diets and the effects of supplementation with MOS or inulin (INU) on growth and body composition in sharpsnout seabream.

Materials and methods

Culture system and fish

The experiment presented in this paper represents the final phase of a trial whose preliminary results have been already published (Piccolo et al., 2011). Therefore, the experimental conditions have been already described. In particular, the trial was carried out in the indoor partially-recirculating water system (total volume 8 m3) of the Dipartimento di Medicina Veterinaria e Produzioni Animali, University of Naples Federico II (Italy), using 144 sharpsnout seabream of about 100 g initial body weight obtained from the company Maricultura Mattinatese s.r.l. (Mattinata, Italy). After a period of adaptation (15 days), fish were randomly distributed in 12 fiberglass tanks (180 L each). The system was provided with thermostatic control and regulation of water temperature, mechanical sand-filter, biological filter and UV lamp apparatus and a constant and optimal environmental quality was ensured to sharpsnout seabream (daily water renewal=5%, artificial day length=12 h, temperature=21.9±1.6°C, salinity=30.0±2 g L−1, pH 7.5±0.5, total ammonia nitrogen<0.15 mg L−1, nitrite-nitrogen<0.05 mg L−1). Testing conditions included 12 fish per tank, with each diet being experimentally tested in triplicate. The experimental period lasted 150 days. For further
Diets and feeding

Four isolipidic (crude lipid about 14% as fed) and isoproteic diets (crude protein about 49% as fed), were formulated using commercial ingredients. In the control diet (FM), 999 fish meal was the sole protein source. In the second diet (SBM) approximately 40% of fish meal protein was replaced by 44% CP soybean meal. SBM-MOS and SBM-INU (inulin) diets were prepared by adding 8 g kg⁻¹ of mannanoligosaccharides (ECHOMOS; Mazzoleni Prodotti Zootecnici, Cologno al Serio, BG, Italy) and fructooligosaccharides in the form of inulin (INULINA, FOS, Method Chemicals, Novellara, RE, Italy) to the SBM diet, respectively. For further details on ingredient composition and proximate analysis of the tested diets please refer to the Table 1 reported in Piccolo et al. (2011). Fish were daily hand-fed with two meals (9:00 and 16:00) to visual satiety (i.e. until the first feed item was refused). The feed was administered over the whole water surface in the tanks in order to be accessible simultaneously for all the fish.

Sampling procedures and growth parameter evaluation

Fish were weighed at the beginning of the experiment, and then on a monthly basis. Feed intake was monitored for each experimental group, in order to measure their daily intake rate (DIR). DIR, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated according to the following formulae:

\[
\text{DIR} = \left( \frac{\text{feed intake/mean weight}}{\text{no. days}} \right) \times 100;
\]

\[
\text{SGR} = 100 \times \left( \frac{\ln[\text{final body weight}] - \ln[\text{initial body weight}]}{\text{no. days}} \right);
\]

\[
\text{FCR} = \frac{\text{feed intake}}{\text{weight gain}};
\]

\[
\text{PER} = \frac{\text{weight gain}}{\text{protein intake}} \times 100;
\]

At the end of the experimental period, six fish per group (two for tank) were sacrificed (anaesthetised with phenoxethanol, 250 ppm) in order to collect data on fish length and total weights, as well as liver, mesenteric fat and visceral weights. These data were used to calculate the animals’ condition factor (CF), hepatosomatic index (HSI), mesenteric fat index (MFI) and viscerosomatic index (VSI) using the following formulae:

\[
\text{CF} = \frac{\text{body weight/total length}^2}{100};
\]

\[
\text{HSI} = \frac{\text{liver weight/body weight}}{100};
\]

\[
\text{MFI} = \frac{\text{mesenteric fat weight/body weight}}{100};
\]

\[
\text{VSI} = \frac{\text{visceral weight/body weight}}{100};
\]

Analytical methods

Proximate analyses of diets and whole bodies of fish (after grinding and freeze-drying) were based on the procedures from the AOAC (2000). All fish samples (six fish per group) were frozen at -20°C until analyzed. The total lipids of fish were extracted according to the Folch et al. (1957) method.

The fatty acid composition of the fillets was determined by gas chromatographic separation of the fatty acid methyl esters (FAMEs), using a 30 m x 0.32 mm capillary column (Omegawax, Supelco Inc., Bellefonte, PA, USA), hydrogen as carrier gas and flame ionization detection (ThermoQuest TRACE GC). The FAMEs were identified by comparison of retention times of known standards. The individual fatty acid concentrations were expressed as percentages of the total content (g of each fatty acid per 100 g of total fatty

Table 1. Fatty acid profile of the diets.

| Control diet | Experimental diets |
|--------------|-------------------|
| FM           | SBM               |
| SBM-MOS      | SBM-INU           |

| Fatty acids, g/100 g of total fatty acids | FM | SBM | SBM-MOS | SBM-INU |
|------------------------------------------|----|-----|---------|---------|
| 14:0                                    | 6.3| 7.6 |
| 15:0                                    | 0.4| 0.4 |
| 16:0                                    | 18.8| 18.6|
| 17:0                                    | 0.3| 0.3 |
| 18:0                                    | 5.4| 4.8 |
| 18:1 n-7                                | 6.6| 6.6 |
| 17:1                                    | 0.1| 0.1 |
| 18:1 n-9                                | 16.4| 17.0|
| 22:1 n-11                               | 7.0| 6.1 |
| 24:1 n-9                                | 0.7| 0.5 |
| 18:2 n-6                                | 13.0| 11.4|
| 18:3 n-3                                | 1.8| 1.5 |
| 18:4 n-3                                | 3.6| 5.5 |
| 20:3 n-3                                | 0.4| 0.2 |
| 20:4 n-6                                | 0.9| 0.7 |
| 20:5 n-3                                | 5.5| 6.1 |
| 22:5 n-3                                | 1.2| 1.0 |
| 22:6 n-3                                | 11.2| 11.3|
| Total saturated                         | 32.2| 31.7|
| Total monounsaturated                   | 31.1| 30.6|
| Total polyunsaturated                   | 37.6| 37.7|
| Total n-3                               | 23.7| 25.6|
| Total n-6                               | 13.9| 12.1|
| n-3/h-6                                 | 1.7| 2.1 |

Table 2. Growth parameters of sharpsnout seabream (n=3 per treatment) fed the experimental diets.

| Control diet | Experimental diets |
|--------------|-------------------|
| FM           | SBM               |
| SBM-MOS      | SBM-INU           |

| Parameter | FM | SBM | SBM-MOS | SBM-INU | SEM | P       |
|-----------|----|-----|---------|---------|-----|---------|
| Initial weight, g | 101.3 | 96.5 | 96.7 | 100.6 | 28.2 | 0.588   |
| Final weight, g    | 238.1| 234.2| 229.8| 235.4| 104.9| 0.798   |
| Weight gain, g     | 136.8| 137.7| 133.1| 134.8| 50.2 | 0.830   |
| SGR                 | 0.60| 0.59| 0.58| 0.57| 0.006| 0.968   |
| DIR                 | 1.11| 1.12| 1.11| 1.10| 0.008| 0.994   |
| FCR                 | 2.06| 2.01| 2.05| 2.06| 0.009| 0.909   |
| PER                 | 1.00| 1.02| 1.00| 1.00| 0.004| 0.970   |

FM, control diet; SBM, diet with approximately 40% of the protein supplied by soybean meal; SBM-MOS, diet with addition of 8 g of mannanoligosaccharide (MOS); SBM-INU, diet with addition of 8 g of inulin (INU).
acids). Twenty different fatty acids were determined and grouped into saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), PUFA n-3 and PUFA n-6. The fat quality indexes were calculated according to Ulbricht and Southgate (1991) as follows:

Atherogenic index (AI) = \[\{C12:0 + (4 \times C14:0) + C16:0\}/(0.5 \times C18:1) + (0.5 \times \text{sum of other MUFA}) + (3 \times \text{PUFA n-3}) + (\text{PUFA n-3}/\text{PUFA n-6})\].

Since lauric acid (C12:0) was not detected in the samples, it was not taken into account for the calculations.

Thrombogenic index (TI) = \[\{C14:0 + C16:0 + C18:0\}/(0.5 \times \text{sum of MUFA})\].

Statistical analysis
All the data were processed by ANOVA using a general linear model procedure of SAS (2000), according to the following model:

\[Y_{ij} = \mu + S_i + \varepsilon_{ij}\]

where \(Y\) is the single observation; \(\mu\) is the general mean; \(S\) is the diet effect (i = diets FM, SBM, SBM-MOS, SBM-INU) and \(\varepsilon\) is the error. The differences among means were tested for significance using Tukey’s multiple range test. Differences among treatments were considered significant at the P<0.05 level.

Results

Growth performance
During the experimental period, mortality rate was 6.9% and there was no statistical difference among groups. Growth performance of fish is reported in Table 2. Weight gain ranged from 133.1 to 137.7 g and was not significantly different among groups. Growth performance of SBM diets (72.7%) was significantly higher than in fish fed the FM (68.9%) and the SBM (69.9%) diets. The lipid concentration was significantly lower in fish fed SBM-MOS (5.8%) and SBM-INU (6.1%) than in fish fed FM (8.0%) and SBM (7.9%) diets. The ash concentration in fish fed FM diet was higher than in fish fed SBM-MOS and SBM-INU diets.

Fatty acids
Table 4 shows the fatty acid composition of the lipids in the fillets of sharpsnout seabream, that reflected that of the feed. Palmitic acid (16:0) and oleic acid (18:1 n-9) were the pre-

\[
\text{AI} = \frac{(16:0) + 4 \times (18:1\ n-9)}{(0.5 \times \text{sum of MUFA}) + (3 \times \text{PUFA n-3}) + (\text{PUFA n-3}/\text{PUFA n-6})}.
\]

\[
\text{TI} = \frac{(16:0) + (18:1\ n-9)}{\text{sum of MUFA}}.
\]

Table 3. Effect of different diets on sharpsnout seabream somatic parameters and body composition (g/100 g wet wt).

|                | FM       | SBM      | SBM-MOS  | SBM-INU  | SEM | P      |
|----------------|----------|----------|----------|----------|-----|--------|
| Condition factor | 2.01     | 2.02     | 2.01     | 2.01     | 0.013 | 0.993  |
| Viscerosomatic index | 6.14a | 5.89b | 5.51b | 5.53b | 0.290 | 0.046  |
| Hepatosomatic index | 1.35c | 1.02c | 1.13b | 1.19b | 0.079 | 0.005  |
| Mesenteric fat index | 1.85a | 1.72ab | 1.43b | 1.49b | 0.128 | 0.012  |
| Moisture | 68.9a | 69.9a | 72.7b | 72.3b | 0.580 | 0.001  |
| Crude protein | 17.0     | 16.4     | 16.3     | 16.9     | 0.142 | 0.125  |
| Crude fat | 3.9b     | 3.9b     | 3.8b     | 3.7b     | 0.025 | 0.003  |

Table 4. Fatty acid profiles of total lipids in fillets of Diplodus puntazzo from different diets (g of fatty acid/100 g of total fatty acids).

|                | FM       | SBM      | SBM-MOS  | SBM-INU  | SEM | P      |
|----------------|----------|----------|----------|----------|-----|--------|
| 14:0 | 7.3a | 7.7b | 7.2b | 8.5a | 0.065 | 0.001 |
| 15:0 | 0.4b | 0.4b | 0.2b | 0.2b | 0.001 | 0.001 |
| 16:0 | 19.5c | 19.4c | 22.2c | 20.5c | 0.532 | 0.005 |
| 17:0 | 0.2c | 0.2c | 0.3c | 0.3c | 0.002 | 0.014 |
| 18:0 | 4.7c | 3.8b | 4.7b | 4.0c | 0.055 | 0.021 |
| 20:0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.002 | 0.009 |
| 14:1 | 0.4c | 0.4c | 0.1c | 0.1c | 0.001 | 0.001 |
| 16:1 n-7 | 7.0 | 6.6 | 6.7 | 6.6 | 0.055 | 0.182 |
| 17:1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.003 | 0.003 |
| 18:1 n-9 | 15.8b | 15.9b | 18.4b | 17.3ab | 0.608 | 0.010 |
| 22:1 n-11 | 8.1c | 6.5b | 6.5b | 7.0c | 0.053 | 0.001 |
| 24:1 n-9 | 0.4 | 0.4 | 0.3 | 0.4 | 0.001 | 0.144 |
| 18:2 n-6 | 7.7 | 8.4 | 7.0 | 7.3 | 0.575 | 0.206 |
| 18:3 n-3 | 1.5 | 1.5 | 1.3 | 1.7 | 0.078 | 0.429 |
| 18:4 n-3 | 6.4ab | 6.8ab | 6.1b | 6.9b | 0.076 | 0.027 |
| 20:3 n-3 | 0.2b | 0.1b | 0.2b | 0.2b | 0.001 | 0.002 |
| 20:4 n-6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.019 | 0.075 |
| 20:5 n-3 | 9.1c | 8.9c | 6.2c | 6.3c | 0.352 | 0.003 |
| 22:5 n-3 | 0.6 | 0.6 | 0.6 | 0.6 | 0.016 | 0.955 |
| 22:6 n-3 | 9.9 | 11.6 | 11.2 | 11.3 | 0.564 | 0.098 |
| Total saturated | 32.2 | 31.3 | 34.7 | 33.6 | 1.445 | 0.051 |
| Total monounsaturated | 31.8 | 30.2 | 32.1 | 31.5 | 1.350 | 0.170 |
| Total polyunsaturated | 36.0ab | 38.5b | 33.2b | 34.9b | 1.511 | 0.004 |
| Total n-3 | 27.7b | 29.5b | 25.6b | 27.0b | 0.740 | 0.003 |
| Total n-6 | 8.3 | 9.0 | 7.6 | 7.9 | 0.315 | 0.070 |
| n-3h-6 | 3.34 | 3.28 | 3.37 | 3.42 | 0.102 | 0.097 |
| AI | 0.72 | 0.73 | 0.72 | 0.82 | 0.007 | 0.045 |
| TI | 0.28 | 0.26 | 0.31 | 0.29 | 0.002 | 0.591 |
dominant saturated and monounsaturated fatty acids in all groups while, among polyunsaturated fatty acids, C18:2 n-6, C20:5 n-3 and C22:6 n-3 were prevalent. Among the total fatty acids, a lot of differences were recorded due to the diet: fillets from SBM-INU group had a higher proportion of C14:0 (8.5%) than the other groups; SBM-MOS fillets showed a higher concentration of both C16:0 and C18:1 n-9 than the other groups. SBM-MOS and SBM-INU had a lower concentration of C20:5 n-3 than the other groups. A higher proportion of polyunsaturated fatty acids (about 39% of the total) and total n-3 fatty acids (about 30%) were found in the SBM group than SBM-MOS and SBM-INU groups. However, these differences did not make changes in AI and TI.

**Discussion**

From previous studies, the effects of MOS or inulin on growth performance of aquatic species seem to be contradictory. In this experiment, we tested the effect of nondigestible oligosaccharides in improving the growth performance, health and body composition of sharpsnout seabream. The present study demonstrated that dietary MOS or inulin did not significantly affect the growth performance of fish. This was also observed in the first part of the trial (Piccolo et al., 2011). Taking into account the SGR, it resulted obviously similar to the previous results. The comparison with the data reported in literature is not easy because of the differences regarding experimental conditions and initial body weights of the fish (Rondan et al., 2004; Hernandez et al., 2007; Piedecausa et al., 2007; Nogales Mérida et al., 2010). However, by utilizing the equation proposed by García García Nogales Mérida (2011) it is possible to estimate SGR values for sharpsnout sea bream as a function of temperature and body weight. By applying the equation to our data we obtain an SGR of 0.71, higher than our values.

The lack of growth response to the MOS is in agreement with results from studies on gulf turbot larvae (Mahious et al., 2006), Atlantic salmon (Refstie et al., 2006; Grisdale-Helland et al., 2008) and juvenile red drum (Buenello et al. 2010). The similar growth registered in FM and SBM shows a great potential for soybean meal usage in sharpsnout seabream. On the other hand, sharpsnout seabream is an omnivorous species that feeds on seaweeds in addition to worms, mollusks and shrimps (Bauchot and Hureau, 1986; Sala and Ballesteros, 1997) and this probably make easier the digestive utilization of soybean meal. In this regard, Nogales Mérida et al. (2010) showed similar growth rate in sharpsnout seabream in the weight interval 14-100 g between diets containing fish meal and 30% of sunflower meal.

Body proximate analysis in the present study showed that body protein was not affected by MOS or inulin supplementation. Also Grisdale-Helland et al. (2008) reported that neither MOS nor galactooligosaccharide affected body protein in Atlantic salmon. In contrast, 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. In this regard, the protein content is considered to be pre-determined by the genetic characteristics of the species (Shearer, 1994) and unaffected by the diet (Morris, 2001). The present study demonstrated that dietary MOS or inulin reduced body lipid level, and this was coupled with a parallel increase in water content. On the contrary, previous studies with gilthead seabream (Dimitroglou et al., 2010) and Atlantic salmon (Grisdale-Helland et al., 2008) have shown that MOS, fructooligosaccharide or galactooligosaccharide did not affect body lipid levels. However, reduction of fat percentage is reported in broilers fed a diet containing 3.75 g kg\(^{-1}\) fructooligosaccharide respect to that fed the control diet (Flickinger et al., 2003). Also, studies showed a reduction in fat deposition of the carcass of rabbits fed mannanoligosaccharides (Piccolo et al., 2009; Bovena et al., 2012), in triglycerides of poultry (Bokzurt et al., 2012) and in hepatic lipogenesis and plasma triacylglycerol of humans (Letexier et al., 2003). In the present study, MOS and INU supplementation reduced also mesenteric fat. A lower percentage of fat in the groups SBM-MOS and SBM-INU, if confirmed in further researches, is very important because could allow to obtain farmed fish with a fat percentage similar to that of wild fish.

In general, fatty acid composition of fish body reflects the dietary fatty acid composition and this is well documented in previously studied (Nicolosi Asmudo et al., 1993; Izquierdo et al., 2005; Mnari et al., 2007). In our trial, as the diets had a similar fatty acid profile, the differences in fatty acids of the fillets could be tied to the supplementation of prebiotics. Compared to SBM alone, SBM-MOS diet seemed to improve the synthesis of some fatty acids as C16:0, C18:0 and C18:1n-9; the supplementation of inulin seemed to improve the synthesis of C14:0. The lack of available data make very difficult a comparison with the literature and did not allow to present definitive conclusions; however, it suggests as very important to increase the number of research on this topic to verify a possible repeatability of these results. However, fatty acid profiles determined in this trial were in agreement to the data reported in sharpsnout seabream by Piedecausa et al. (2007) and Orban et al. (2000). AI and TI are not different among diets and were in general, very favourable and similar to those reported for other fish species (Poli et al., 2003; Rondan et al., 2004) and also to those of other foodstuffs that were considered suitable for human consumption (Perez-Llamas et al., 1998).

**Conclusions**

The results of the current study show that fish meal can be replaced by soybean meal in sharpsnout seabream diets at 40% of substitution rate (on protein basis) without negatively affecting fish performance and body composition. The results of this trial also indicate that supplementation of SBM diets with MOS or INU did not significantly affect growth performance, but reduced body lipid level and mesenteric fat index. The supplementation with MOS induced a different fatty acid profile of total lipids. However, further studies, possibly involving a larger number of fish, are needed in order to determine the appropriate inclusion level and to evaluate the effects of MOS and INU on health with attention to the intestinal microbiota and histology.

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