Effects of the partial substitution of fish meal by soy bean meal with or without mannanoligosaccharide and fructooligosaccharide on the growth and feed utilization of sharpsnout seabream, Diplodus puntazzo (Cetti, 1777): preliminary results

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Abstract

The present study was carried out in order to investigate the effects of the mannanoligosaccharide (MOS) and fructooligosaccharide (FOS) in sharpsnout seabream, Diplodus puntazzo, in the context of partial fish meal substitution by soybean meal. One-hundred-forty-four sharpsnout seabream of about 100 g initial body weight were randomly divided in 12 experimental tanks (180 litre each). Testing conditions included 12 fish per tank, with triplicate tanks for treatment. The experimental period lasted 114 days. Average water temperature was 21.9±1.6°C, salinity was 30.0‰ and pH ranged from 7 to 8. Four isonitrogenous-isolipidic diets were tested: a control diet (FM) with fish meal as the sole protein source; a second diet (SBM) with approximately 40% of the protein supplied by soybean meal. The remaining two diets were formulated adding 8 g of MOS and FOS per kg of the SBM diet respectively. Average final weight, specific growth rate, feed conversion ratio and protein utilization of the diets decreased, as an effect of the smaller digestibility coefficient observed for the diets containing soybean meal. The possible use of soybean meal as a substitute for fish meal in sharpsnout seabream diets was investigated by Hernandez et al. (2008) by progressively increasing its inclusion level. These authors found a decrease in final weights as the soybean meal content increased starting from 40% protein substitution rate. Similarly, as the soybean meal content increased, feed efficiency and protein utilization of the diets decreased, as an effect of the smaller digestibility coefficient observed for the diets containing soybean meal.

Materials and methods

Culture system and fish

The trial was carried out in the indoor partially-recirculating water system (total volume 8 m³) of the Department of Animal Science and Food Control (University of Napoli Federico II, Italy), using 144 sharpsnout seabream of about 100 g (98.8±2.5 g) initial body weight obtained from the Maricultura Mattinatese s.c.r.l. company (Mattinata, Italy). After a short period of adaptation (15 days) in the quarantine tanks, fish were randomly distributed in 12 fiberglass tanks (180 L each). The system was provided with thermostat control and regulation of water temperature, mechanical sand-filter, biological filter and UV lamp apparatus and a constant and optimal environment quality was ensured to sharpsnout seabream (daily water...

Introduction

Mediterranean marine aquaculture is still currently focused on sea bass (Dicentrarchus labrax) and gilthead seabream (Sparus aurata). The high increase of production and subsequent price drop of these species during the last decade has led the need to find new fish species suitable for farming. Among the possible candidates for mariculture, sharpsnout seabream (Diplodus puntazzo) is one of the most interesting species to spawn in captivity and to be managed in hatchery conditions (Micale et al., 1996; Firat et al., 2005; Garcia-Garcia et al., 2010). The results of recent research are quite encouraging, and indicate a considerable increase in sharpsnout seabream production in the near future (Orban et al., 2000; Hernández et al., 2001; Favaloro et al., 2002; Bonaldo et al., 2004).

Since dehulled oil-extracted soybean meal has high protein content, availability and competitive price, it represents a major dietary alternative to fish meal in diets for several fish species (Tibaldi et al., 2006). Soybean meal has been the focus of a number of studies which show large variability among various fish species in the utilization of this ingredient, with growth performance, feed and nutrient conversion efficiencies being generally impaired when it was used to replace high proportions of dietary fish meal (FM) protein (Shimeno et al., 1992; Watanabe et al., 1992; Robaina et al., 1995; Krogdahl et al., 2003).

The possible use of soybean meal as a substitution for fish meal in sharpsnout seabream diets was investigated by Hernandez et al. (2007) by progressively increasing its inclusion level. These authors found a decrease in final weights as the soybean meal content increased starting from 40% protein substitution rate. Similarly, as the soybean meal content increased, feed efficiency and protein utilization of the diets decreased, as an effect of the smaller digestibility coefficient observed for the diets containing soybean meal.

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renewal, 5%; artificial day length, 12 h; temperature, 21.9±1.6°C; salinity, 30.0±2 g L⁻¹; dissolved oxygen, 6.4±1.5 mg L⁻¹; pH, 7.5±0.5; total ammonia nitrogen, <0.15 mg L⁻¹; nitrite-nitrogen, <0.05 mg; nitrate-nitrogen <40 mg L⁻¹). Testing conditions included 12 fish per tank, with each diet being experimentally tested in triplicate. The experimental period lasted 114 days. Water temperature, pH and dissolved oxygen were measured daily using a mercury thermometer, Orian digital pH meter and oxygen meter (WTW, OXI 330, Weilheim, Germany). Total ammonia nitrogen (N-NH₃), nitrite nitrogen (NO₂-N) and nitrate nitrogen (NO₃-N) were determined biweekly by colorimetric methods using commercial kits and a spectrophotometer (Hanna Instruments, C-203, Leighton Buzzard, UK). The tanks were inspected once daily for mortalities and dead fish were removed immediately from the tanks after detection.

### Diets and feeding

Four isolipidic (crude lipid about 14% as fed) and isoproteic diets (crude protein about 49% as fed), whose composition and proximate analysis are reported in Table 1, 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 soybean meal. MOS and FOS diets were prepared by adding 8 g kg⁻¹ of mannooligosaccharide (ECHOMOS; Mazzoleni Prodotti Zootecnici, Cologno al Serio, BG, Italy) and fructooligosaccharide in the form of ECHOMOS (CHO and Kaushik, 1990; Tulli and Tibaldi, 2001).

#### Digestibility trials

Protein and energy apparent digestibility coefficients (ADC) of diets were measured in vivo in a separate trial using the indirect method and settling columns for faecal collection (Cho and Kaushik, 1990; Tulli and Tibaldi, 2001).

Acid-insoluble ash (AIA) was used as indigestible marker. The digestibility measurements were carried out using a tank system developed by the University of Guelph (Guelph CYAQ-2; Cho, 1992) consisting of three-tanks units each fitted with a common drain pipe connected to a settling column for collecting faecal material. The tank apparatus was connected with the indoor, partially-recirculating water system. Each 60 L tank within a unit was stocked with 10 sharpnout seabream (average body weight 130±3 g; 3.9 kg biomass per unit). During the trial, temperature was kept at 22±1°C and salinity at 30±1 g L⁻¹. Fish were left to adapt to the culture conditions over 30 days before starting measurements. Each diet was then tested in triplicate units. Fish were fed two meals daily (9:00 and 16:00) to visual satiety (until the first feed item was refused) and adapted over 3 weeks to the diet prior to faeces collection. Faeces were collected over 12-16 days, i.e. as long as a suitable amount of material (130-150 g fresh weight) was obtained for subsequent analysis. After each meal, the tanks and settling columns were cleaned to avoid faeces contamination by uneaten pellets. Faeces were collected daily from the settling column and immediately separated from the surrounding water by centrifugation (10,000xg; 20 min; 5°C). They were stored at -20°C until the end of the collection period, when the daily amounts of each unit (diet) were pooled and freeze-dried before analysis.

The ADC of DM, crude protein and gross energy of the diets were measured according to Maynard and Loosly (Cho, 1992):

ADC (%) = \[(\% \text{ nutrient in the diet} - \% \text{ nutrient in faeces}) / (\% \text{ nutrient in tested diet} - \% \text{ nutrient in faeces})\] × 100.

### In vivo measurements and performance indexes

Fish were group-weighted at the beginning of the experiment and then on a monthly basis after a 24-h fast and under moderate anaesthesia (Tricaine methanesulphonate, 50 mg L⁻¹). Feed intake was monitored for each experimental group, in order to measure 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:

DIR = (feed intake/mean weight)/no. days) x 100;

SGR = 100 x (ln[final body weight] – ln[initial body weight])/no. days;

FCR = feed intake/weight gain;

PER = weight gain (g)/protein intake (g)

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**Table 1. Ingredients and proximate composition of experimental diets.**

| Ingredients                  | FM  | SBM  | MOS  | FOS  |
|------------------------------|-----|------|------|------|
| Fish meal, herring, g kg⁻¹  | 695.0 | 415.0 | 415.0 | 415.0 |
| Soybean meal, g kg⁻¹         | -   | 408.0 | 408.0 | 408.0 |
| Fish oil, g kg⁻¹             | 85.0 | 98.0  | 98.0  | 98.0  |
| Starch, g kg⁻¹               | 180.0 | 35.0  | 27.0  | 27.0  |
| Vitamin mix, g kg⁻¹          | 17.5 | 17.5  | 17.5  | 17.5  |
| Mineral mix, g kg⁻¹          | 2.5  | 2.5   | 2.5   | 2.5   |
| Methionine, g kg⁻¹           | -    | 4.0   | 4.0   | 4.0   |
| Celite, g kg⁻¹               | 10.0 | 10.0  | 10.0  | 10.0  |
| Binder, g kg⁻¹               | 10.0 | 10.0  | 10.0  | 10.0  |
| Mannanoligosaccharide, g kg⁻¹| -    | -     | -     | -     |
| Fructooligosaccharide, g kg⁻¹| -    | -     | -     | 8.0   |

**Proximate composition**

| Dry matter, g kg⁻¹          | 926.0 | 919.2 | 916.0 | 923.1 |
| Crude protein, g kg⁻¹       | 485.8 | 488.7 | 488.1 | 486.8 |
| Crude lipid, g kg⁻¹         | 135.5 | 148.5 | 145.0 | 145.9 |
| Gross energy, MJ kg⁻¹       | 20.10 | 20.47 | 20.30 | 20.43 |

FM, fish meal diet; SBM, soybean meal diet; MOS, mannanoligosaccharide diet; FOS, fructooligosaccharide diet.
Statistical analysis

All data were processed by one-way analysis of variance (ANOVA) using a general linear model procedure of SAS (2000), and 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. The results are presented as mean values followed by the standard deviation (M±SD).

Results

Growth performance

During the experimental period, mortality was 5.5% (P>0.05) and there was no statistical difference among groups. Performance data are shown in Table 2. Weight gain was good (ranged from 91.2 to 99.2 g for groups MOS and SBM respectively) and was not significantly affected by dietary treatment. Similarly, no effect of diet was observed on final weight, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. SGR fluctuated from 0.46 (groups MOS and FOS) to 0.49 (group SBM) and DIR ranged from 1.13 (groups FM and FOS) to 1.17 (group SBM). Also FCR (average values 2.0) and PER (average values 1.02) showed low fluctuations among the groups.

Digestibility

The ADC values for organic matter are reported in Table 3 and ranged from 86.8%, for the MOS diet, to 97.1%, for the FM diet and were not significantly affected by dietary treatment. Similarly, no significant effect of diet was found for ADC of protein (ranged from 96.0% to 97.1%, with the fish-meal diet having the lowest), lipid (ranged from 96.0% for the SBM diet to 97.2% for the FOS diet) and energy (ranged from 94.5% for the MOS diet to 94.9% for the FOS diet).

Discussion

In the present study, sharpsnout seabream’s growth performance has to be considered quite acceptable and similar to those previously observed by other authors (Orban et al., 2000; Rondan et al., 2004; Piedecausa et al., 2007; Nogales Mérida et al., 2010). The SGR values in this trial were lower than those reported by Hernández et al. (2001) for sharpsnout seabream of 47.7-120.6 g, Piedecausa et al. (2007) for sharpsnout seabream of 14.8-72.9 g and Nogales Mérida et al. (2010) for sharpsnout seabream of 14.1-103 g. On the other hand, when daily weight gains are equivalent, fish with lower initial body weight have a higher SGR value. Moreover, as it is obvious, the growth performance depends on water temperature and in our trial water temperature (average 21.9°C) was lower than that registered in the cited literature. Also DIR and FCR values resulted similar to those reported by other authors (Hernández et al., 2001; Rondan et al., 2004) for sharpsnout seabream of similar weight. Protein efficiency ratio resulted lower than that reported by Torrejon-Atienza et al., (2004) for sharpsnout seabream of 10-30 g.

Currently, soybean meal represents one of the most commonly alternative protein source employed by aquaculture due to its worldwide presence, relatively low cost and sufficient digestibility in different species. It has a satisfactory amino acid profile and high protein content but it is deficient in the sulphur-containing amino acids and in tryptophan (Spinelli, 1980; Storebackken et al., 2000). However, some time the use of large quantities of soybean meal to replace fishmeal may lead to a decrease in growth performance compared to that obtainable with the use of fish meal as the sole protein source. This is more true for carnivorous fish species and at high levels of substitution (Chou et al., 2004). However, Hernández et al. (2007) reported a decrease in final weights, feeding efficiency, protein utilization and smaller digestibility coefficients using soybean meal as substitute for fish meal in diets for sharpsnout seabream.

Finally, soybean meal can has negative effect on the performance of farmed fish with its anti-nutritional factors (Francis et al., 2001).

In this trial feed intake was not impaired in fish fed soy-containing diets relative to the control and the replacing of FM protein with soy bean meal protein did not lead to a significant growth decrease, inducing to hypothesize that sharpsnout seabream is able to well-utilize this ingredient.

In this regard it has to be reminded that sharpsnout seabream is reported to be an omnivorous species that feeds on seaweeds in addition to worms, molluscs and shrimps (Bauchot and Hureau, 1986; Sala and Ballesteros, 1997) and this aspect probably influenced diets digestion and growth performance of fish and suggest a better utilization of soybean meal in this species. Nogales Mérida et al. (2010) observed similar growth performance in sharpsnout seabream from 14 to 100 g fed with a diet containing sunflower meal as partial substitute for fish meal (30% on protein basis) compared to a fish meal based control diet.

In this experiment, we tested if the addition of non-digestible oligosaccharides to the diet may improve the performance of sharpsnout seabream. The results showed there was no statistical difference on growth, DIR, FCR and PER by supplementing the diet with MOS or FOS. From previous studies it appears that the effects of MOS on growth performance of aquatic species are contradictory. Our results, showing dietary MOS does not significantly improve growth performance, should be considered in the context of the numerous studies that have demonstrated the benefits of using MOS as a growth promoter in aquaculture.

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

| Diets      | FM    | SBM   | MOS    | FOS    |
|------------|-------|-------|--------|--------|
| Initial weight, g | 101.3±4.1 | 96.5±4.2 | 96.7±5.0 | 100.6±6.6 |
| Final weight, g   | 198.1±9.3 | 195.7±10.5 | 187.9±10.8 | 195.2±8.2 |
| Weight gain, g    | 96.8±5.5  | 99.2±6.4  | 91.2±7.0  | 94.6±6.1  |
| SGR               | 0.47±0.06 | 0.49±0.05 | 0.46±0.04 | 0.46±0.05 |
| DIR               | 1.13±0.08 | 1.17±0.07 | 1.14±0.09 | 1.13±0.06 |
| FCR               | 2.00±0.07 | 1.97±0.09 | 2.02±0.09 | 2.02±0.08 |
| PER               | 1.03±0.05 | 1.04±0.03 | 1.01±0.04 | 1.02±0.06 |

Table 3. Apparent digestibility coefficients of experimental diets.

| Diets      | FM    | SBM   | MOS    | FOS    |
|------------|-------|-------|--------|--------|
| Dry matter, % | 87.1±0.40 | 87.0±0.42 | 86.8±0.45 | 87.0±0.34 |
| Protein, %    | 97.1±0.50 | 96.8±0.44 | 96.9±0.55 | 96.8±0.66 |
| Lipid, %      | 96.5±0.71 | 96.0±0.80 | 97.1±0.50 | 97.2±1.01 |
| Energy, %     | 94.8±0.59 | 94.6±0.71 | 94.5±0.65 | 94.9±0.82 |

FM, fish meal diet; SBM, soybean meal diet; MOS, mannanooligosaccharide diet; FOS, fructooligosaccharide diet; SGR, specific growth rate; DIR, daily intake rate; FCR, feed conversion ratio; PER, protein efficiency ratio.
Conclusions

This study shows that fish meal can be replaced by soybean meal in 100-200 grams sharpsnout seabream diets at 40% of substitution rate (on protein basis) without negatively affecting fish performance. Moreover, these results confirm the possibility of breeding this species with the use of dry feeds currently available in the market and that better growth rates probably can be obtained by increasing water temperature. The effect of this substitution on fish fillet quality and composition also has to be investigated and will be the subject of a forthcoming paper.

The results of this trial also indicate that supplementation of diets with MOS or FOS did not significantly affect growth performance or diet digestibility. However, further studies, possibly involving a larger number of individuals, are needed in order to determine the appropriate inclusion level and to evaluate the effects of MOS and FOS on health with attention to the intestinal microbiota and histology.

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