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PAPER

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) on 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 efficiency ratio remained unaffected by partial fish meal substitution and by MOS or FOS supplementation. Also apparent digestibility coefficients values for organic matter, protein, lipid and energy were not significantly affected by dietary treatment.

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; Hernàndez *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 substitute 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, feeding efficiency and protein utilization of the diets decreased, as an effect of the smaller digestibility coefficient observed for the diets containing soy bean meal.

In the last years, a number of alternative products, as probiotics, prebiotics, organic acids, essential oils and oligosaccharides have been the subject of research to enhance health and growth performance (Ozduven *et al*., 2009).

Prebiotics, such as mannanoligosaccharide (MOS) or inulin (oligosaccharide naturally occurring in many plants and commercially produced from the chicory root) have proved to be effective for enhancing health and growth performance of fish (Staykov *et al*., 2007; Torrecillas *et al*., 2007; Burr *et al*., 2008), improving gut morphology (Salze *et al*., 2008; Dimitroglou *et al*., 2009) and modulating the intestinal microbiota (Dimitroglou *et al*., 2009).

The objective of this study was to investigate the effects of the partial substitution of fish meal protein by soy bean meal protein in sharpsnout seabream diets and to verify whether the supplementation with MOS or FOS in form of inulin could improve the growth, feed conversion ratio (FCR), feed and nutrients digestibility and reduce mortality of fish.

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Materials and methods

Culture system and fish

The trial was carried out in the indoor partially-recirculating water system (total volume 8 m^3) 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 Maricoltura 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 thermostatic 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 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 ($N\text{-}NH_3$), nitrite nitrogen ($NO_2\text{-}N$) and nitrate nitrogen ($NO_3\text{-}N$) 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.

Table 1. Ingredients and proximate composition of experimental diets.

Diets	FM	SBM	MOS	FOS
Ingredients				
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}	-	-	8.0	-
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.

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 mannanoligosaccharide (ECHOMOS; Mazzoleni Prodotti Zootecnici, Cologno al Serio, BG, Italy) and fructooligosaccharide in the form of inulin [INULINA (F.O.S), Methodo Chemicals, Novellara, RE, Italy] to the SBM diet respectively. Celite (Celite®, Prolabo, Fontenay-sous-Bois, France, i.e. purified silica powder from diatoms, containing >90% w/w of acid-insoluble ash) was added to each diet at 1% as inert indigestible marker. The diets were produced in the laboratories of the Department of Animal Science and Food Control, University of Napoli Federico II, Italy. All ingredients were ground through a 0.5 mm sieve before final mixing and dry pelletting through a 3 mm dye.

Diets proximate composition was determined after homogenization following the AOAC methods (AOAC, 2000): dry matter (105°C to constant weight), ash (incinerated at 550°C to constant weight), crude protein (N x 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden), crude lipid extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator). Gross energy was determined by adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA). All analyses were performed in triplicate. 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.

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 sharpsnout 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 pellet 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 (%)={[(% nutrient in the diet /% marker in the diet)−(% nutrient in faeces /% marker in faeces)] /(% nutrient in tested diet/ % marker in diet)}×100.

In vivo measurements and performance indexes

Fish were group-weighed at the beginning of the experiment and then on a monthly basis after a 24-h fast and under moderate anesthesia (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)

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 87.1%, 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 highest value and the MOS 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 Hernàndez *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 (Hernàndez *et al*., 2001; Rondan *et al*., 2004) for sharpsnout seabream of similar weight. Protein efficiency ratio resulted lower than that reported by Torrejòn-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, Hernandez *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

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

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

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	96.6±0.44	96.0±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, mannanoligosaccharide diet; FOS, fructooligosaccharide diet.

affect growth performance in sharpsnout seabream, are in agreement with those previously obtained on Gulf sturgeon (Pryor *et al*., 2003), hybrid tilapia (Genc *et al*., 2007a), Atlantic salmon (Grisdale-Helland *et al*., 2008) and gilthead seabream (Dimitroglou *et al*., 2010), and in contrast to the results on rainbow trout (Staykov *et al*., 2007), green tiger prawn (Genc *et al*., 2007b) and European sea bass (Torrecillas *et al*., 2007), that showed MOS improving growth performance in these species. The lack of growth response to the fructooligosaccharide in the form of inulin (FOS diet) is in agreement with the results on turbot larvae (Mahious *et al*., 2006), atlantic salmon (Refstie *et al*., 2006; Grisdale-Helland *et al*., 2008) and juvenile red drum (Buentello *et al*. 2010). However, the lack of any significant effect on growth, DIR, FCR and PER in this trial, could also be a consequence of the adopted culture system. While the use of a recirculating system allows a total control of the environmental parameters, on the other hand it includes the use of a limited number of individuals and replications. All four experimental diets had similar high ADC. The ADC values of dry matter, protein and lipid are in agreement with those reported by Piedecausa *et al*. (2007) for sharpsnout seabream fed fish meal/fish oil based commercial diets or soy bean based diets. In the present study, protein ADC values for fish meal control diet were also similar to those reported for other species such as European sea bass (Tibaldi *et al*., 2006) or rainbow trout (Glencross *et al*. 2005). Digestibility of protein, lipid and energy was not affected by dietary vegetable inclusion.

The ADC for protein, lipid and energy was similar in the prebiotic-supplemented diets compared to the SBM diet. Similar results on ADC of lipid, protein and energy were observed in Atlantic salmon fed on fish meal based diets supplemented with MOS or FOS (Grisdale-Helland *et al*., 2008). Instead, Burr *et al*. (2008) observed in red drum (*Sciaenops ocellatus*) that dietary MOS improved organic matter, protein and energy ADC in SBM diets. However, it isn't easy to compare the results obtained in the different few research carried on digestibility of MOS/FOS containing diets because of different variables involved such as fish species, dietary supplementation level, diet composition, trial length, temperature etc. However, more research is needed to fully elucidate the physiological mechanisms by which prebiotics may enhance the selected parameters as well as to provide a complete characterization of the effects and understanding of their potential applications in aquaculture.

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 has also 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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