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Evaluation of Feed Stimulants in Diets for Sea Bream (*Sparus aurata*)

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Abstract

Six isoprotein and isolipidic diets were formulated to investigate the effect of dietary additives on growth and feed efficiency of sea bream (*Sparus aurata*) fry fed a fishmeal-based diet for 80 days. The additives (protorsan, hydrolyzed fish protein, squid meal, krill meal, and betaine + inosine-5'-monophosphate) were added to the diets at the expense of fishmeal. The specific growth rates of the fish ranged from 2.17-2.18% per day for the protorsan and control groups to 2.42% per day for the group fed the squid additive. Significant differences ($p<0.05$) in final body weight and specific growth rate were detected only between the protorsan and control groups and the squid additive group. The feed conversion ratio ranged from 1.04 in the group fed hydrolyzed fish protein group to 1.24 in the group fed krill with no statistically significant differences ($p>0.05$) between treatments. The feed stimulating action of taurine was tested by observation. Pellets coated in a taurine solution were more actively consumed than control pellets during the morning feeding but consumption did not differ during the afternoon feeding.

Introduction

As part of the never-ending process of improving performance and profitability in aquaculture, there are attempts to produce feeds that are not only nutritive but also organoleptically satisfactory to fish. Such feeds result in the improvement of feeding time and subsequent reduction of feed wastage. Feed additives can improve palatability and nutritional value and, thus, play a decisive role in the replacement of fishmeal by less expensive plant-protein sources.

Indeed, feed additives acting as stimulants have been successful in improving feed intake in striped bass *Morone saxatilis* (Papatryphon and Soares, 2000) and yellowtail *Seriola quinqueradiata* (Kofuji et al., 2006). The inclusion of feeding stimulants can be applicable in cases where fish exhibit reduced appetite such as while weaning to inert diets (Koven et al., 2001), consuming medicated feeds (Toften and Jobling, 1997), being exposed to changes in water temperature (Kasumyan and Doving, 2003) or salinity (Stradmeyer, 1994), or when new species are

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introduced to aquaculture and are reluctant to feed on conventional feeds, such as yellowtail *Seriola dumerilli* (Papadakis et al., 2008).

The inclusion of additives with feed stimulation action in diets based on fishmeal (which is itself an attractant) may be beneficial. In this context we investigated the feasibility of supplementing a fishmeal-based diet with five feeding additives: protorsan (Sopropeche®), hydrolyzed fish protein, squid meal, krill meal, and betaine + inosine -5'-monophosphate (IMP). In a separate experiment, we observed the ability of taurine to act as an attractant.

Materials and Methods

Fish rearing. The experiment was conducted at the Institute of Aquaculture of the Hellenic Center for Marine Research in Crete, Greece. Sea bream (*Sparus aurata*) fry (2.78±0.04 g) from a genetically homogenous stock obtained by mesocosm hatchery technology were weaned and adapted to a compound diet (Aqua Start; Perseus Specialty Food Products S.A., Zevgolatio, Greece).

Diet preparation. Six isoprotein and isolipidic diets were formulated and prepared in the laboratory (Table 1). Five additives were added to the diets at the expense of fishmeal: protorsan, hydrolyzed fish protein, squid meal, krill meal, betaine + inosine-5'-monophosphate (IMP). The ingredients were thoroughly mixed in a feed mixer, moistened by the addition of 50% (w/v) water, and converted to pellets by a mincing machine. The pellets were cut manually, dried in an air-drier at 35°C for 24 h, and stored in a freezer at -15°C until used. Diets were analyzed for crude protein by the Kjeldahl method (N x 6.25) and crude fat by extraction with diethyl ether using the Soxlet Tecator system.

Growth experiment. The diets were fed to triplicate groups of 30 fish in 50-l tanks. The tanks were supplied with biologically filtered sea water of 39‰ salinity, renewed at 200% per hour, pumped from 4 m beneath the surface of the sea, and oxygenated to >70% saturation by an air supply. The temperature was the natural water temperature and ranged from 15°C at the start of the experiment in March to 20°C at the end of the experiment in June. Dissolved oxygen was 6.6±0.2 mg/l. The photoperiod was maintained at 12 h light/12 h dark throughout the experimental period. Tanks were cleaned as necessary. Before starting the experiments, the fish were acclimated for a week to the experimental conditions and fed the control diet. Three tanks were randomly assigned to each diet. The fish were fed to apparent satiation for 80 days. The fish were weighed every 30 days after starvation for 24 h and weak anesthetization with an ethyl glycol monophenyl ether solution (1:2000 v/v).

Taurine feeding experiment. In a separate feeding experiment, 30 fish (19±2 g) were placed in each of two 500-l tanks. The fish in the control tank were fed pellets (LT-Power, Perseus Specialty Food Products S.A., Zevgolatio, Greece) previously soaked in a predetermined amount of water so as to obtain a final 20% moisture content. Fish in the second tank were fed pellets soaked in water in which taurine was dissolved at a concentration of 2 g taurine per kg feed (dry wt). In both tanks the pellets were offered to the fish at a progressively reduced rate of four pellets per 15 s at the beginning of feeding and one pellet per min or less as the fish reached satiation. The pellets were offered in two meals (9:00 and 15:00). At the end of each meal, the time needed for the fish to reach satiation was recorded as well as the total number of pellets and amount of feed consumed. The meals were offered for five consecutive days.

Evaluation. Data were used to determine fish growth and feed utilization. Specific growth rate was calculated as SGR = (ln final BW - ln initial BW) x 100/time, feed conversion ratio as FCR = dry feed consumed/wet BW gain, and daily feed consumption as DFC = dry feed consumed x 100/(initial BW + final BW)/2 x days, where BW indicates the total body weight of the sea bream.

Statistical analysis. Data were analyzed by factorial analysis of variance ANOVA. When tests for normality and equal variance were rejected, a log transformation was performed. Differences among treatment means were identified by the Holm-Sidak test. Results are presented as

Table 1. Composition of experimental sea bream diets.

	Diet					
	Control	*Protorsan*	*Fish hydrolyzed protein*	*Squid meal*	*Krill meal*	*Betaine+ Inosine*
Ingredient (g/kg diet)						
Fishmeal	694.4	650.0	636.8	637.0	654.0	694.4
Fish oil	87.5	88.5	87.7	90.0	82.1	87.5
Protorsan[1]	-	50.0	-	-	-	-
Hydrolyzed fish protein[2]	-	-	50.0	-	-	-
Squid meal[3]	-	-	-	50.0	-	-
Antarctic krill meal[4]	-	-	-	-	50.0	-
Betaine + inosine (11:1)[5]	-	-	-	-	-	11.0
Corn starch	166.6	166.6	166.6	166.6	166.6	166.6
Vitamin-mineral premix	20.0	20.0	20.0	20.0	20.0	20.0
Gelatin	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose	11.0	4.4	18.0	16.0	6.7	-
Proximate analysis (%)						
Crude protein	49.5	50.8	48.6	49.3	48.9	50.4
Crude fat	15.2	15.8	14.9	15.3	14.4	14.9

[1] Sopropeche, France. 64% crude protein, 6% crude fat. Amino acids (% of protein): aspartic acid 4.96, threonine 2.96, serine 2.19, glutamic acid 12.32, glycine 2.42, alanine 6.04, valine 2.88, isoleucine 2.16, leucine 3.14, tyrosine 1.26, phenylalanine 1.91, lysine 2.34, histidine 0.92, arginine 3.11, methionine 0.87, cystine 0.20, proline 1.59, tryptophan 0.25, diaminopimelic acid 1.60.

[2] Sopropeche, France. 83% crude protein, 10% crude fat. Amino acids (% of protein): lysine 6.8-7.5, methionine 2.8-3.1, cystine 0.8-1.0, tryptophan 0.7-0.9, arginine 5.8-6.8, threonine 3.9-4.4, isoleucine 3.5-4.0, leucine 6.0-6.5, valine 1.0-1.2, histidine 1.9-2.6, phenylalanine 2.6-3.7, tyrosine 2.5-2.9, glycine 9.0-11.0, glutamic acid 13.0-14.0, aspartic acid 9.0-9.5, proline 4.3-6.5, serine 4.5-4.9, alanine 6.6-7.3.

[3] Sopropeche, France. 83% crude protein, 5% crude fat. Composition (mg/100 g): L-aspartic acid 18, L-threonine 44, L-serine 33, L-glutamic acid 53, L-valine 36, L-methionine 36, L-isoleucine 29, L-leucine 55, L-tyrosine 22, L-phenylalanine 29, L-lysine-HCl 29, L-histidine-HCl 15, L-proline 1456, L-alanine 273, L-arginine 228, taurine 337, glycine 892, betain-HCl 910, trimethylamine-HCl 91, *t* trimethylamine n-oxide HCl 1138, hypoxanthine 47, inosine 25, adenosine-5-monophosphate 40, L-(+)-lactic acid 91, alpha-cellulose 80.

[4] Sopropeche, France. 58% crude protein, 18% crude fat. Amino acids (% of protein): alanine 3.5, arginine 4.0, aspartic acid 6.6, cystine 0.7, glutamic acid 8.3, glycine 2.9, histidine 1.5, isoleucine 3.0, methionine 2.4, leucine 4.9, lysine 4.9, phenylalanine 3.1, proline 2.4, serine 2.7, threonine 2.8, tyrosine 2.7, valine 3.2.

[5] Sigma-Aldrich Chemie, Germany. Betaine 10 g/kg, Inosine 5'-monophosphate disodium salt (Grade III, 98-100%), 1 g/kg.

means of three replicates±standard deviation. Statistical analyses were performed with the SigmaStat statistical package (Systat Software Inc., Point Richmond, CA, USA).

Results

The fish grew well relative to their size and water temperature (Table 2). All the diets were well accepted except the betaine+IMP diet. The sea bream responded positively to pellets coated with taurine during the morning meal but not during the afternoon meal (Fig. 1).

Discussion

Protorsan is a brown meal and a byproduct of L-glutamic acid fermentation, composed by bacterial substances from *Corybacterium melassecola*. It is often recommended for use as an attractant in fish and shrimp feeds, pet foods, and feeds for piglets and cattle (Sopropeche, product specification sheet) due to its glutamic acid content (12.32%) which is a palatability enhancer in fish (Carr et at., 1996). In the present study, protorsan did not improve the growth rate or the feeding efficiency of sea bream. Presumably, any feed-stimulating action of protorsan was masked by the already high palatability of the fishmeal-based diet. Similarly, thin distillers' solubles were ineffective in improving the growth performance and feed utilization of rainbow trout *Oncorhynchus mykiss* fed diets containing canola or air-classified pea protein (Thiessen et al., 2003).

The enzymatic hydrolysis of fish protein produces soluble low molecular weight substances which may stimulate feed intake of fish (Liaset et al., 2000). Hydrolyzed fish protein increased feed intake in Atlantic salmon *Salmon salar* (Refstie et al., 2004) and was used effectively in the first feeding of sea bass *Dicentrarchus labrax* (Cahu et al., 1999). However, in the present study, the partial dietary replacement of fishmeal with fish protein hydrolysate did not improve growth or feed utilization, as in turbot *Scophthalmus maximus* (Oliva-Teles et al., 1999).

The squid extract supplement significantly increased the growth of the sea bream as found in yellow tail (*S. quinqueradiata*; Kofuji et al., 2006) and gibel carp (*Carassius auratus*; Xue and Cui, 2001). The stimulatory action is associated with active substances found in squid extracts such as free amino acids, nucleosides, nucleotides, and quaternary ammonium bases (Carr et al., 1996). Squid meal has excellent digestibility (Eusebio et al., 2004) and, at least in yellow tail, promotes growth by increasing protein digestion at low temperatures (Kofuji et al., 2006). Nevertheless the stimulant action of squid meal may not be evident if masked by other dietary

Table 2. Specific growth rate (SGR), feed conversion ratio (FCR), and daily feed consumption (DFC) of sea bream (*Sparus aurata*) fry fed diets with different feed additives for 80 days (n = 3).

	Initial wt (g)	*Final wt (g)*	*SGR (% per day)*	*FCR*	*DFC*
Control	2.82±0.04	16.22±0.99^{a}	2.18±0.08^{a}	1.10±0.06	3.73±0.24^{a}
Protorsan	2.78±0.00	15.78±0.32^{a}	2.17±0.03^{a}	1.16±0.15	3.72±0.21^{a}
Hydrolyzed fish protein	2.85±0.00	17.37±1.32^{ab}	2.26±0.09^{ab}	1.04±0.16	3.69±0.22^{a}
Squid meal	2.77±0.00	19.26±1.02^{b}	2.42±0.07^{b}	1.12±0.06	3.65±0.14^{ab}
Krill meal	2.74±0.08	16.59±0.0^{ab}	2.25±0.03^{ab}	1.24±0.03	3.33±0.12^{ab}
Betaine + inosine	2.75±0.02	17.61±1.50^{ab}	2.32±0.10^{ab}	1.13±0.07	3.19±0.06^{b}

Values in a column with the same letter do not differ significantly (p>0.05).

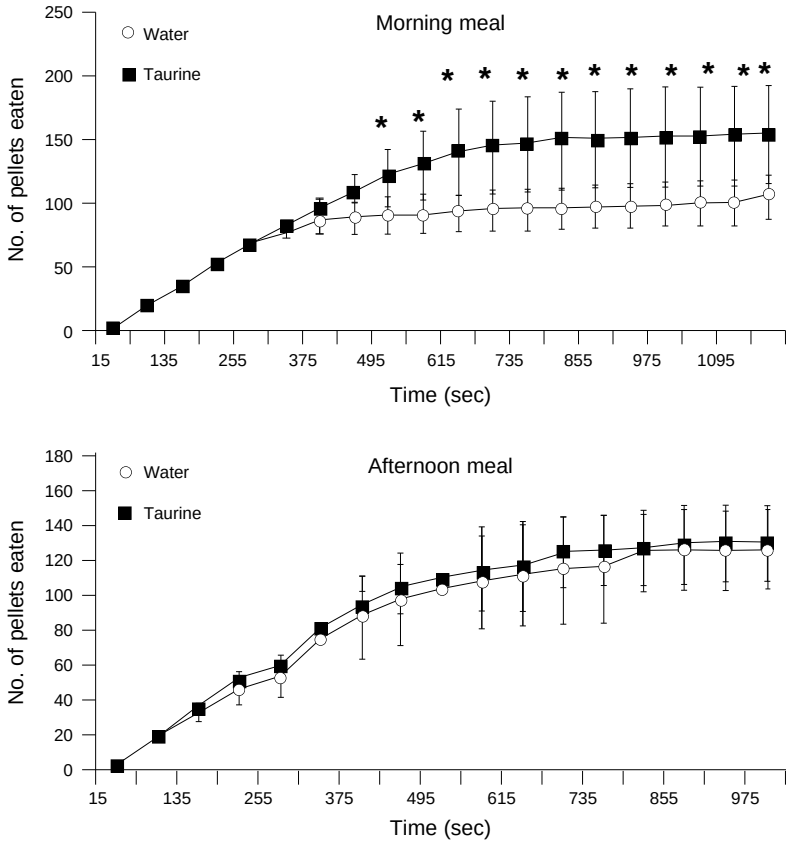

Fig. 1. Time needed for 30 sea bream (avg wt 19 g) to reach satiation during (a) morning (09:00) and (b) afternoon (15:00) meals of a diet soaked with water or taurine. Values are averages±standard deviation (n = 5). Significantly different values are marked with an asterisk (p<0.05).

ingredients, e.g., repulsive dietary additives, or if diets do not fulfill the nutrient requirements of the fish (Berge et al., 1999; Xue and Cui, 2001).

The krill meal not only did not improve the growth rate but produced a high (though not significantly different) FCR. This may be due to the chitin content of krill meal, which has low digestibility. Similar to fishmeal, it is suggested that krill meal acts as a feeding stimulant (Kubitza and Lovshin, 1997) and improves feeding rates of yellow tail (Sato, 2003) and freshwater species such as yellow perch *Perca flavescens* and walleye *Stizostedion vitreium* (Kolkovski et al., 2000). The stimulant action of krill meal is usually attributed to amino acids such as glycine, proline, and glucosamine as well as nucleotides and nucleosides (Kolkovski et al., 2000). Although the feeding stimulant action of krill meal is undisputed, not all investigations documented growth promoting effects. Similar to the present study on sea bream, krill meal was not effective in improving long-term growth in Atlantic salmon *Salmon salar* (Olsen et al., 2006).

Although betaine and IMP are feeding stimulants for striped bass (Papatryphon and Soares, 2000), gibel carp (Xue and Cui, 2001), and Atlantic halibut *Hippoglossus hippoglossus* (Berge et

al., 1999), under our experimental conditions betaine and IMP did not significantly promote growth in sea bream. Betaine also showed no significant stimulatory action on Nile tilapia *Oreochromis niloticus* (Yacoob et al., 2001) and even depressed feed intake in chinook salmon *Oncorhynchus tshawytscha* (Hughes, 1993). Betaine and IMP were effective in stimulating feeding when they acted in synergy with other substances or in cases where the diet had a low fishmeal content (Xue and Cui, 2001).

In addition to the 80-day feeding trial with dietary additives, we conducted a short-term monitoring of feed consumption of pellets coated in taurine. This was based on observation of pellets consumed by fish and is one of three methods (the others are the recording of feeding activity and the quantitative method of gastrointestinal content) described by Jobling et al. (1995). Taurine acted as an attractant to sea bream during the morning meal but not in the afternoon meal, presumably because the fish had a reduced appetite after being fed to satiation in the morning. The stimulatory effect of taurine on olfactory organs of fish has been observed in a number of species such as Arctic charr *Salvelinus alpinus* (Doving et al., 1980). Similarly, through a behavioral approach, it was estimated that the European glass eel *Anguilla anguilla* can be attracted by taurine at concentrations as low as 10^{-12} M (Sola and Tosi, 1993). Although it is a nonessential nutrient, taurine is often included in diets because it can increase the daily feeding rate of fish (Brotons-Martinez et al., 2004).

In conclusion, the replacement of fishmeal by the tested dietary additives was beneficial only in the case of squid meal, which improved the growth rate of the sea bream fry. The replacement of fishmeal by protorsan, hydrolyzed fish protein, krill meal, and betaine-IMP did not produce any observable effects on growth, possibly due to the already high palatability of the fishmeal-based diet. In addition, the feed stimulating action of taurine in sea bream was observed.

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