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Patterns of gastric evacuation, digesta characteristics and pH changes along the gastrointestinal tract of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.)

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ABSTRACT

A comparative study of gastric evacuation rates (GERs) and digesta content, moisture and pH values along the gastrointestinal tract was performed between gilthead sea bream and European sea bass. In order to distinguish species-specific differences from diet-elicited effects, all parameters were determined under either a fishmeal diet or a carob seed germ meal diet that contained high levels of total and soluble non-starch polysaccharides. GERs were significantly different between species and they were not affected by diet. Similarly, species-specific patterns were revealed in the distribution of digesta and water content along the gastrointestinal tract. In sea bream, stomach digesta and water content decreased with time, whereas in sea bass stomach retained the highest digesta and water content throughout the sampling period. The anterior and distal intestine exhibited the lowest accommodating capacities of digesta and water in either species. Overall, sea bream performed stomach digestion at lower hydration levels and higher pH compared with sea bass. Diet affected stomach moisture in both species and pH of stomach digesta in sea bass and of all intestinal sections in sea bream. The results obtained indicated that water and inorganic ion exchanges through the gut may differentiate between the species and warrant further investigation.

1. Introduction

European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are the main Mediterranean cultured fish species. Both species are euryhaline, found in marine and brackish water environments such as coastal lagoons and estuarine areas, in particular during the initial stages of their life cycle (Pickett and Pawson, 1994; FAO, 1995). Both species are also carnivorous. Sea bream feed mainly on crustaceans, molluscs, polychaetes, echinoderms and teleosts (Wassef and Eisawy, 1985), while sea bass at juvenile stage feed chiefly on shrimps and molluscs and consume increasingly more fish with age (Hampel et al., 2005). Studies on the morphology and ontogeny of their digestive tract have revealed a similar mode of development from a straight undifferentiated tube to a complex structure comprising the oesophagus, stomach, pyloric caeca, intestine and the rectum (Elbal et al., 2004; Garcia Hernandez et al., 2001). A direct involvement of sea bass digestive tract to osmoregulation has been shown (Giffard-Mena, et al., 2006). Sea bream exhibits intense absorption in the lower part of the intestine (Cataldi et al., 1987) and the mucus cells of the oesophagus and the digestive goblet cells of the two species contain different mucosubstances, indicative of differences in absorption capacity (Garcia Hernandez et al., 2001; Sarasquete et al., 1995).

The digestive tract of marine teleosts has a dual role as a food-processing organ and as an osmoregulatory organ (Bucking and Wood, 2006a,b; Taylor and Grosell, 2006). Food ingestion is inevitably combined with water drinking and results in changes in the ionic balance of the intestine due to dietary salt intake and salt content of the water. In farmed species, dry diets impose an extra osmotic stress on the intestine, triggering post-prandial drinking and water influx in the chyme from the extracellular fluid in order to moisturize feed to an adequate level for its digestion (Windell et al., 1969; Ruohonen et al., 1997; Kristiansen and Rankin, 2001).

Previous works have dealt with digestion characteristics of sea bream and sea bass providing data on variations of pH levels and digestive enzymes activities along the digestive tract (Eshel et al., 1993; Deguara et al., 2003; Tibaldi et al., 2006; Yufera et al., 2004; Zambonino Infante and Cahu, 1999). Only few works have studied gastric evacuation rates in response to different dietary treatments (Adamidou et al., 2009; Venou et al., 2003) on the aforementioned species, and response of digestive enzyme secretion to diet and time after feeding (Fountoulaki et al., 2005; Venou et al., 2003). However,

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there is significant lack of comparative studies on digestion characteristics, in which both species are examined under the same rearing conditions. So far, the fish feed industry and the overall feeding practices applied do not differentiate between the species. Data on their digestive physiology and gastric evacuation rates will allow the prediction of optimal feeding frequencies and the design of species-specific diets increasing feed efficiency in both species.

Replacement of fishmeal and fish oil in fish diets is among the top priorities for a sustainable aquaculture production. Leguminous seeds provide a good alternative as dietary protein sources and their nutritional potential, especially that of soybean meal, has been extensively studied in farmed fish (reviewed by Alexis and Nengas, 2001; Glencross et al., 2007; Refstie, 2007). However, their inclusion in fish diets introduces a number of compounds, which are not common or exist in small levels in diets based mainly on fishmeal. Among these, non-starch polysaccharides (NSPs) and especially soluble NSPs have attracted considerable research attention since they are osmotically active compounds that can alter the physicochemical properties of digesta (Refstie et al., 1999; Amirkolaie et al., 2005; Leenhouwers et al., 2006) and affect gastric evacuation time (Storebakken, 1985; Shiau et al., 1988). However, not much information exists on changes in physicochemical characteristics of the digesta of fish elicited by plant raw materials (Refstie et al., 1999; Leenhouwers et al., 2007) or their components (Amirkolaie et al., 2005, 2006; Storebakken, 1985; Shiau et al., 1988; Fagberno and Jauncey, 1995; Leenhouwers et al., 2006) or on the osmoregulatory characteristics of the gut in the presence of different feed types (Taylor and Grosell, 2006). CSGM is a by-product of the carob gum producing industry with sufficient protein content for fish diets and a balanced amino acid profile (Del Re-Jimenez and Amandò, 1989; Maza et al., 1989; Dakia et al., 2007). Its reported content in NSPs is 18% (Batlle and Tous, 1997; Bengoechea et al., 2008) higher than that of other legumes such as peas (15.5%) and chickpeas (9.8–13.6%) (Adamidou et al., 2009, Nikolopoulou et al., 2006).

The aim of the present study was therefore to compare certain digestion characteristics of gilthead sea bream and European sea bass. The parameters compared concern gastric evacuation rates and the temporal patterns of digesta distribution and their moisture content and pH values in four parts along the digestive tract post-prandial. We hypothesize that species-specific differences in digestion physiology are unaffected by exogenous parameters, including diet characteristics. In order to test our hypothesis, all comparisons were made under two different feeding regimes, a fishmeal diet and a diet containing CSGM.

2. Materials and methods

2.1. Experimental diets

CSGM was supplied by 'LBG Sicilia' (Italy). It is a by-product of the production of galactomannan from carob seeds. The manufacturing process involves dehulling of the seeds by mechanical treatments followed by moderate crushing in which the germ is separated from the endosperm and sieving (www.lbg.it/manufactoring-process.html). The product used was further mechanically cleaned in order to increase its protein content, to be better suited as a raw material for fish feeds.

A fishmeal (FM) control diet was formulated using 60% of a low temperature (LT) FM, 27.3% wheat flour and 10.4% fish oil. CSGM diet was formulated by substituting 30% of the FM control diet with CSGM. Both diets were supplemented with 0.3% minerals and vitamins (Table 1). In preparing the test diets, all the dietary ingredients were blended thoroughly, moistened to produce a dense paste and passed through a mincer (Hobart food mixer, model no A 120, Hobart manufacturing). Diets were air dried in a forced air dryer (<40 °C) and stored frozen (−20 °C). The chemical composition and pH of the diets is given in Table 1.

Table 1
Composition of the experimental diets.

	Diets	
	FM	CSGM
pH	6.45	6.35
Dry matter	84.64	82.87
Chemical composition (% dry matter)		
Ash	12.54	11.20
Protein	47.76	44.83
Fat	17.43	11.84
Starch	20.05	14.93
Non-starch polysaccharides (NSP)	1.88	12.47
Insoluble NSP	1.25	7.10
Soluble NSP	0.63	5.37

Vitamins and minerals (per kg feed) provided by 'BIOMAR HELLENIC'.
Vitamin A: 5000 IU, Vitamin D3: 1000 IU, Vitamin C 100 mg, Vitamin E 175 mg Vitamin K3: 10 mg.
Vitamin B1: 10 mg, Vitamin B2: 20 mg, Vitamin B3: 50 mg, Vitamin B5: 40 mg, Vitamin B6: 10 mg.
Vitamin B8: 0.8 mg, Vitamin B12: 0.03 mg, folic acid: 10 mg, Co: 1 mg, Cu: 5 mg, I: 1.2 mg, Mn: 30 mg.
Se: 0.2 mg, Zn :100 mg.

2.2. Digestion characteristics

2.2.1. Experimental conditions—sampling

Eleven-month old gilthead sea bream (*Sparus aurata;* 150 ± 30 g mean mass) and European sea bass (*Dicentrarchus labrax;* 110 ± 25 g mean mass) were obtained from a commercial sea farm (SELONDA Aquaculture SA, Greece). Triplicate groups of 30 sea bass and 20 sea bream were assigned to 250 L cylindro-conical fiberglass tanks. Seawater of 26 ± 1 °C was supplied to the tanks with a continuous flow of 6 L min^{-1}. Photoperiod was 10 h light: 14 h dark. Water salinity was 38‰.

Experimental fish were acclimated to two different experimental diets for 4 weeks before initiation of the present study. Fish were fed a single meal per day at 1.4% of their biomass. At the end of this feeding period, samplings were performed at 2, 4, 6, 8, 10, 12 and 24 h post-prandial for sea bream and further at 26, 29 and 32 h for sea bass (until gastric evacuation). Four sea bream and five sea bass from each diet were sacrificed at each sampling point by immersion in ice water containing a slight dose of anaesthetic (phenoxyethanol/ethanol 1/1, 0.4 ml L^{-1}). This procedure was found most appropriate for avoiding defecation of the fish. Fish were sampled from consecutive tanks each time to avoid excessive stressing. Fish that did not contain feed in their digestive tract were rejected and immediately replaced by another during the sampling procedure.

The abdominal cavity was opened and the digestive tract was ligated at the oesophagus, pylorus and anus, which were then freed from connective and adipose tissue. The intestine was also ligated in two specific points to separate three segments; anterior intestine (AI), middle intestine (MI) and distal intestine (DI). The intestinal segments were separated as follows: AI: the section between the pyloric sphincter and the first intestinal loop of the digestive tract (Cataldi et al., 1987; Garcia Hernandez et al., 2001); MI: from the distal end of the AI to the valve separating the intestine from the rectum and DI: the section from the distal end of the MI to the anus. Stomach and intestinal contents from the above sections were collected in pre-weighed vials, weighed to the nearest 0.1 mg for each fish separately, freeze-dried, reweighed and the measured weights used for the calculations described below.

2.2.2. Rate of digestion

The geometric mean of the stomach digesta was regressed against time in order to examine the possible fit to a model for calculating gastric evacuation rate (GER). Data for both treatments and fish were