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Meal timing affects protein-sparing effect by carbohydrates in sea bream: Effects on digestive and absorptive processes

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ABSTRACT

A commercial diet (C) (48% protein and 20% lipids) and a high-digestible carbohydrate diet (CH) (37% protein, 12.5% lipids and 40% high-digestible carbohydrates) were used to feed sea bream juveniles for an 8-week period. In the commercial diet, more than 60% of ingredients were of plant origin from various sources, whereas the only component of plant origin in the CH diet was wheat. To determine the best time to administer carbohydrates and the possible protein-sparing effect, three different dietary regimes were established: C, CH-M and CH-A, and the corresponding diet was fed to sea bream in the morning (1.6% of bw) and in the afternoon (1% of bw), calculating quantities according to the amount of feed that fish ate during the acclimatization period. After the growth trial, specific growth rate (SGR), relative intestinal length, intestinal pH content, gastric and pancreatic digestive enzyme activities and nutrient absorption capacities were studied 5 h post-feeding after each meal (morning and afternoon). The acid protease activity measured was anticipatory and was higher when the next meal would have more protein. No differences in relative intestinal length or feed buffering capacity were found. The smaller ration given to sea bream in the afternoon led to a lower pancreatic release of alkaline protease and α-amylase and an up-regulation of D-Glc and L-Ala absorption capacity. A higher transit rate was measured when sea bream were fed the CH diet. When high-digestible carbohydrates were administered in the morning and the commercial diet in the afternoon, we observed a better assimilation of both diets due to compensatory mechanisms such as an increase in L-Lys, D-Glc and L-Ala absorption capacity after the morning feed, and a higher pancreatic release of alkaline protease and amylase after the afternoon feed. In contrast, when high-digestible carbohydrates were given in the afternoon, only a significant up-regulation of the capacity to absorb L-Lys was detected. Thus, the inclusion of high-digestible carbohydrates in the diet improved digestion and absorption processes when administered in the morning, leading to a protein-sparing effect that yielded growth comparable to that of fish fed an exclusively commercial diet.

1. Introduction

Fish meal provides an adequate balance of amino acids, but increased demand and prices, as well as uncertain supply, render it necessary to identify alternative protein sources (SOFIA, 2012). At present, over 50% of the fish meal in commercial diets is replaced by protein of plant origin; thus, the possible presence of anti-nutritional factors (ANFs) and the limitation of some amino acids, such as lysine or methionine (Francis et al., 2001; Gatlin et al., 2007; Krogdahl et al., 2010), may promote changes in digestive and absorptive capacities (Santigosa et al., 2008, 2011a,b) in order to minimize the effects on metabolism (Metón et al., 1999) and compensate for those dietary changes.

The protein-sparing effect may possibly present a means to minimize the use of fish meal, by increasing the lipid and/or carbohydrate content of the feed. The use of dietary lipids instead of protein is well established in fish (Company et al., 1999; Vergara et al., 1999). A voluntary feed intake of at least 26% dietary lipids, and a diet of 48% protein and 22.5% lipids, has been established as providing optimal growth and protein gain (Lupatsch et al., 2001). However, despite these results, the high prices and limited supplies of fish oil (SOFIA, 2012) restrict its use, and it is often replaced by vegetable oils that diminish the quality of the final product (De Francesco et al., 2007; Fountoulaki et al., 2009; Izquierdo et al., 2005; Menoyo et al., 2004). On the other hand, the utilization of dietary carbohydrates for energy purposes in salmonids and other carnivorous species appears to be limited (Hemre et al., 2002; Stone et al., 2003; Wilson, 1994). Although several studies have indicated a protein-sparing effect by dietary carbohydrates in sea bream and sea bass (Couto et al., 2008; Dias et al., 1998; Enes et al., 2006; Fernández et al., 2007; Peres and Oliva-Teles, 2002), others have failed to demonstrate such an effect (Enes et al., 2008; Lanari et al., 1999; Moreira et al., 2008). Enes et al. (2011) have reported that European sea bass and gilthead sea bream juveniles can ingest up to 20% digestible carbohydrates without adverse effects on growth or feed utilization. Besides individual differences between species, the main factors affecting carbohydrate digestibility in fish are dietary inclusion level, molecular

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complexity, botanical origin, technological treatments applied, and water and temperature (Krogdahl et al., 2005; Stone, 2003; Venou et al., 2003; Wilson, 1994). In this respect, wheat has been described as a high-digestible carbohydrate source (Venou et al., 2003). Due to their lower amylose/amylopectin ratio and their structure, starch granules are more vulnerable to damage by industrial processing and attack by amylases (Bergot, 1993; Cousin et al., 1996).

Digestion and absorption processes are the key to successful utilization of a given diet and feed timing. Digestion processes begin in the stomach, where HCl converts pepsinogen into active pepsin (Wu et al., 2009; Yúfera et al., 2004). When the chyme passes into the pyloric caeca, cholecystokinin is secreted, in turn stimulating pancreatic secretions which include, among other substances, proproteases, α-amylase and lipolytic enzymes, as well as DNAase and RNAase (Bakke et al., 2011). The activity of pancreatic enzymes in fish has been studied in relation to the influence of diet composition, food quantity and the natural diet (Buddington et al., 1997; Hidalgo et al., 1999; Pérez-Jiménez et al., 2009; Reimer, 1982; Santigosa et al., 2008, 2011a,b; Zambonino Infante & Cahu, 2007). The activity of the main digestive enzymes, such as proteases, lipase and amylase, may be one of the most important parameters that determine the effectiveness of a given diet, optimizing growth and food utilization (Debnath et al., 2007; Lemieux et al., 1999; Mohanta et al., 2008).

Absorption processes occur throughout the entire intestine by means of diffusion, facilitated transport or active transport (Mailliard et al., 1995). In fish, the presence has been described of at least four Na^+-dependent and Na^+-independent amino acid transport systems (Storelli et al., 1989) and two peptide transporters (Hakim et al., 2009; Sangaletti et al., 2009; Terova et al., 2009). The apical translocation of lipolytic products in fish is not well understood and seems to occur by diffusion or facilitated transport, while D-glucose and D-galactose are transported by SGTL1 (Sala-Rabanal et al., 2004). Santigosa et al. (2011a,b) have described modifications in the absorption pattern after a short starvation period, or as a consequence of low availability of certain amino acids due to fish meal replacement.

Those processes are also affected by biological rhythms, which are driven by endogenous oscillators that affect physiological, behavioral, endocrine and metabolic variables, increasing the probability of success and optimizing energy use (Dardente and Cermakian, 2007; DeCoursey, 2004). The study of feeding behavior in several fish species has revealed that adjusting feeding times to match natural rhythms improves nutritional efficiency, feeding frequency and food conversion efficiency (Bolliet et al., 2001), and can also improve growth performance in many commercially cultured species such as rainbow trout (Boujard et al., 1995; Reddy et al., 1994), European sea bass (Azzaydi et al., 1999) and channel catfish (*Ictalurus punctatus*) (Noeske-Hallin et al., 1985). Food-anticipatory activity confers an adaptative advantage as it may improve food acquisition and utilization (Comperatore and Stephan, 1987).

In order to study the protein-sparing effect by carbohydrates and determine the best time for administration, we analyzed the effect on digestive and absorptive processes in sea bream fed a commercial diet of including one feed of a high-digestible carbohydrate diet in the dietary regime, administered in the morning or in the afternoon.

2. Materials and methods

2.1. Diets

Two diets were used, a commercial diet (C) (D-2 EXCEL 1P Skretting, Spain) and a high-digestible carbohydrate diet (CH) (University of Valencia, Spain). C diet contained 48% protein, 20% lipids, 8% ashes and 3.2% fiber, and had as ingredients: fish meal, soybean meal, fish oil, soybean oil and wheat. "EXCEL" diets contained 27% fish meal and 9% fish oil (García, 2012). The proximate composition of CH diet was 37% protein, 12.5% lipids, 8.5% ashes, 1.8% fiber and 40% high-digestible carbohydrates (calculated according Venou et al., 2003). The ingredients of CH diet were fish meal, fish oil and gelatinized wheat starch; that was the only source from vegetable origin.

2.2. Fish and sampling

Gilthead sea bream juveniles from Cripesa (Tarragona, Spain) were acclimatized for 2 weeks to the facilities at the University of Barcelona. After that period, 180 sea bream (±21 g body weight) were randomly distributed in 9 fiberglass tanks (400 L) equipped with a semi-closed recirculating system with physical and biological filters, ozone, and continuous aeration with a 35% weekly sea water renewal rate, and maintained at 24 °C with a 12 L/12D photoperiod. Water parameters such as temperature, oxygen content, pH, nitrate and nitrite content were recorded daily. The animals were fed twice a day (10:00 am and 17:00 pm) for an 8-week period (November–January).

Three experimental groups were studied in triplicate: commercial diet (C), a high-digestible carbohydrate diet in the morning (CH-M) and a high-digestible carbohydrate diet in the afternoon (CH-A). The daily ration was adjusted to 2.6% of total body weight, 1.6% in the morning and 1.0% in the afternoon. The quantity of the morning and afternoon ration was calculated according to the amount of feed that fish ate during the acclimatization period.

At the end of the 8-week experimental trial, two samples were collected at 5 h post-feeding, one after the morning and one after the afternoon meal. Fish were anesthetized (MS222 0.1 g L^{-1}), weighed and sacrificed by severing the spinal cord. The SGR was calculated as follows: $((\ln W_{fin}\,(g) - \ln W_{ini}\,(g))/t) \cdot 100$, where W_{fin} and W_{ini} were the final and initial weights respectively, and t was the number of feeding days. The digestive tract of eight fish per treatment was isolated, and relative intestinal length (RIL) was measured excluding pyloric caeca, expressed in relation to each animal's weight (Santigosa et al., 2008). Samples were also collected from stomach, pyloric caeca and proximal intestine, including the intestinal content. These were rapidly frozen in liquid nitrogen and maintained at −80 °C until enzymatic analyses. In addition, the intestinal tract of four animals per treatment was isolated and cut lengthwise, washed in an isosmotic saline solution containing 0.1 M protease inhibitor (phenyl-methyl-sulfonyl-fluoride) and frozen in liquid nitrogen until nutrient absorption experiments were performed. All fish-handling procedures complied with European guidelines on animal care (Directive 2010/63/EU).

2.3. Acid protease activity

Stomach samples were collected in order to determine acid protease activity according to Alarcón et al. (1998). Briefly, samples were individually homogenized (Politron 2000, Sorvall TC) at 4 °C to a final concentration of 50 mg mL^{-1} in 50 mM Tris–HCl buffer pH 7.5; this was centrifuged for 15 min (1100 g, 4 °C, Jouan CR 411) and supernatants were recovered and stored at −80 °C. For acid protease activity determination, homogenates from stomach were reacted with 50 mM glicine–HCl buffer at pH 2 containing 1% bovine hemoglobin at 20 °C for 30 min. After that, the reaction was stopped by adding 12% trichloroacetic acid. The samples were kept at 4 °C for an hour and centrifuged (7500 *g*, 5 min, 4 °C). Individual blanks for each sample were established. Supernatant absorbance was measured at 280 nm (UV-1603, Shimadzu). Pepsin from porcine gastric mucosa (Sigma Aldrich, Spain, 3440 U/mg solid) was used as standard and acid protease activity was measured as BAEE units.

2.4. Intestinal pH and digestive enzyme analysis

The pH of intestinal content was measured (Crison, micro pH 2000) in pyloric caeca (PC) and proximal intestine (PI). The pH in PC was