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Growth performance and metabolic utilization of diets with native and waxy maize starch by gilthead sea bream (*Sparus aurata*) juveniles

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Abstract

The effect of dietary starch source and level on growth performance, feed utilization, apparent digestibility coefficients and liver enzyme activities involved in intermediary metabolism of gilthead sea bream juveniles was studied. Five isonitrogenous (47% crude protein) and isolipidic (15% crude lipids) diets were formulated to contain 10% native (diet NS10) or waxy (diet WS10) maize starch; 20% native (diet NS20) or waxy (diet WS20) maize starch or no starch (control). Diets were adjusted with α-cellulose. Another diet was formulated without carbohydrates, and contained 70% crude protein and 15% crude lipids (diet HP). Each diet was fed to triplicate groups of 30 fish (initial weight: 20 g) for 12 weeks. The HP group was fed to near satiation and the other 5 groups were fed on a pair-feeding scheme according to the group that ingested less feed (control diet group). The reduction of dietary protein level from 70% to 47% by the incorporation of 20% starch did not significantly affect gilthead sea bream growth performance or feed efficiency. Compared to the control diet, neither the level nor the nature of starch had any measurable effect on growth performance and feed efficiency. Digestibility of starch was unaffected by source or dietary inclusion level. Diet had no effect on plasma glucose levels, but liver glycogen was higher in diet groups NS20, WS20 and HP. Dietary carbohydrates increased GK and G6PD enzyme activities and decreased ALAT and GDH enzyme activities while had only a minor effect on FBPase activity. The nature of dietary starch tested (native or waxy) had little influence on performance criteria.

Keywords: Carbohydrate utilization; Digestibility; Enzyme activity; Gilthead sea bream; Gluconeogenic enzymes; Glycolytic enzymes; Growth performance; Native starch; Waxy starch

1. Introduction

Gilthead sea bream *Sparus aurata* L. is nowadays one of the most extensively cultured marine fish species in the Mediterranean countries (FAO, 2007). However, diets supplied to gilthead sea bream in culture are costly mainly due to its high protein content which is usually provided by fish meal. High dietary protein levels are also associated to negative environmental impacts due to potential nitrogenous losses which may result in water eutrophication. Therefore, both from an economical and environmental point of view, great deal of research has been conducted in gilthead sea bream, as well as in other species, aiming to evaluate the effects of replacing fish meal with alternative protein sources of plant origin (Robaina et al., 1995; Pereira and Oliva-Teles, 2002, 2003, 2004; Gómez-Requeni et al., 2003) or reducing dietary protein content by replacement with carbohydrates or lipids (Bonamusa et al., 1992; Metón et al., 1999; Venou et al., 2003; Fernández et al., 2007).

In fish, particularly in carnivorous species, the utilization of digestible dietary carbohydrates for energy purposes appears limited (Wilson, 1994; Hemre et al., 2002; Stone, 2003). In fact, and although fish have all the major enzymes and metabolic pathways involved in metabolism of carbohydrates (Hemre et al., 2002) a prolonged postprandial hyperglycemia has been observed after a carbohydrate-rich diet (Bergot, 1979; Cowey

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and Walton, 1989; Wilson, 1994; Moon, 2001). However, until now the mechanisms that may explain the distinct capacity of fish to metabolize glucose are not fully understood.

The relative efficiency of dietary carbohydrate utilization by fish is species-dependent and has been associated to factors such as dietary level and technological treatments applied (Wilson, 1994; Stone, 2003; Krogdahl et al., 2005). In general, it is assumed that dietary digestible carbohydrate level should not exceed 20% for carnivorous species, including salmonids and marine fish, whereas for warmwater herbivorous or omnivorous species levels up to 40% can be used (Wilson, 1994). It is well established that native starch is considered a relatively poor energy source, mainly due to its low digestibility. A possible explanation for that is the reduction of α-amylase activity due to its adsorption to the starch molecules and intestinal transit acceleration, thereby reducing time available for absorption (Spannhof and Plantikow, 1983). Technological processing of starches, which includes the application of moisture and heat are reported to improve starch digestibility and as a consequence its availability to the animals (Wilson, 1994; Hemre et al., 2002; Stone, 2003). In fact, gelatinized and extruded starch proved to be a suitable dietary ingredient for gilthead sea bream (Santinha, 1997; Metón et al., 1999; Venou et al., 2003; Fernández et al., 2007) and other species (Bergot and Brèque, 1983; Arnesen and Krogdahl, 1993; Peres and Oliva-Teles, 2002; Amirkolaie et al., 2006).

The major components of starch are the glucose polymers, amylose and amylopectin, composed of glucose units linked exclusively by α-glycosidic bounds. Amylose consists of a straight chain of glucose units that form tight helical structures making the α-glycosidic bounds less available to enzymatic cleavage whereas amylopectin is highly branched and thus more susceptible to enzymatic degradation. Thus, the amylose/amylopectin ratio affects nutritional availability of starch. Techniques using genetic engineering allowed obtaining modified starches with only 1% of amylose named waxy starches (Pfeffer et al., 1991; Hung et al., 2006). In fact, the digestibility of waxy maize starch was considerably higher than that of native starch in rainbow trout *Oncorhynchus mykiss* (Bergot, 1993) and European sea bass *Dicentrarchus labrax* (Enes et al., 2006).

The aim of the present study was to evaluated the effect of two levels (10% and 20%) and two forms (native and waxy) of maize starch on growth performance, nutrient digestibility and activity of key liver enzymes of intermediary metabolism in gilthead sea bream juveniles reared at 25 °C.

2. Materials and methods

2.1. Diets

Five isonitrogenous (47% crude protein) and isolipidic (15% crude lipids) diets were formulated to contain 10% native maize starch (diet NS10), 10% waxy maize starch (diet WS10), 20% native maize starch (diet NS20), 20% waxy maize starch (diet WS20) or no starch (control). Diet composition was adjusted by manipulating α-cellulose levels. A high protein diet (diet HP) without starch or α-cellulose was also formulated, and contained 70% crude protein and 15% crude lipids. Native maize starch (72% amylopectin, 28% amylose) was obtained from COPAM (Loures, Portugal) and waxy maize starch (99% amylopectin, 1% amylose) from Cerestar (Mechelen, Belgium). All dietary ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (CPM) through a 3 mm dye. Ingredients and proximate composition of the experimental diets are presented in Table 1.

2.2. Growth trial

Juvenile gilthead sea bream were obtained from a commercial fish farm and transported to the experimental facilities at the Marine Zoological Station (Porto, Portugal). After a period of quarantine, fish were transferred to the experimental rearing system, which consisted of a thermo-regulated recirculating water system equipped with 18 fiberglass cylindrical tanks (250 L water capacity each). During the trial, water temperature was 25 ± 0.3 °C, salinity averaged 36 ± 1‰ and dissolved oxygen was kept near saturation.

After 2 weeks of adaptation to the experimental conditions, 18 groups of 30 fish with an initial mean body weight of 20.0 g were established. Each diet was randomly assigned to triplicate groups of animals. Fish were fed by hand two times a day, 6 days/week. Groups receiving the isonitrogenous diets were fed on a pair-feeding scheme according to the group that ingested less feed (control group) in such a way that all groups received the same quantity of protein and lipids and a variable amount of carbohydrates. The HP diet was not include in the pair-feeding scheme as it was expected that feed intake of this group would be lower than in the other groups as previously observed in European sea bass (Enes et al., 2006), therefore imposing a severe restricted feeding regime.

Table 1
Composition and proximate analysis of the experimental diets

	Diets					
	Control	NS10	WS10	NS20	WS20	HP
Ingredients (% dry weight)						
Fish meal [a]	56.0	56.0	56.0	56.0	56.0	86.0
Soluble fish protein concentrate [b]	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	8.5	8.5	8.5	8.5	8.5	5.5
Normal maize starch [c]	–	10.0	–	20.0	–	–
Waxy maize starch [d]	–	–	10.0	–	20.0	–
α-Cellulose	27.0	17.0	17.0	7.0	7.0	–
Vitamin premix [e]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix [f]	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride (60%)	0.5	0.5	0.5	0.5	0.5	0.5
Carboxymethylcellulose	1.0	1.0	1.0	1.0	1.0	1.0
Proximate analyses (% dry weight)						
Dry matter	92.4	93.2	95.7	95.4	95.3	96.4
Crude protein	47.4	47.1	47.0	47.2	47.6	70.1
Crude fat	14.9	14.8	14.9	15.1	15.1	15.1
Ash	9.7	9.7	9.6	9.5	9.6	13.9
Starch	–	9.4	9.0	18.1	17.5	–
Gross energy (kJ g^{-1} DM)	22.0	22.1	21.8	21.9	21.8	22.7

[a] TripleNine, Prime Quality, Denmark (CP: 77.1% DM; GL: 10.0% DM).
[b] Sopropèche G, France (CP: 75.8% DM; GL: 18.8% DM).
[c] COPAM (Loures, Portugal).
[d] Cerestar (Mechelen, Belgium).
[e] Vitamins (mg kg^{-1} diet): retinol acetate, 18 000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); alpha tocopherol acetate, 35; sodium menadione bisulphate, 10; thiamin-HCl, 15; riboflavin, 25; calcium pantothenate, 50; nicotinic acid, 200; pyridoxine HCl, 5; folic acid 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbic acid, 50; inositol, 400.
[f] Minerals (mg kg^{-1} diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dibasic calcium phosphate, 5.93 (g kg^{-1} diet); potassium chloride, 1.15 (g kg^{-1} diet); sodium chloride, 0.40 (g kg^{-1} diet).