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Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles

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Abstract

We determined the effect of dietary starch on growth performance and feed utilization in European sea bass juveniles. Data on the dietary regulation of key hepatic enzymes of the glycolytic, gluconeogenic, lipogenic and amino acid metabolic pathways (hexokinase, HK; glucokinase, GK; pyruvate kinase, PK; fructose-1,6-bisphosphatase, FBPase; glucose-6-phosphatase, G6Pase; glucose-6-phosphate dehydrogenase, G6PD; alanine aminotransferase, ALAT; aspartate aminotransferase, ASAT and glutamate dehydrogenase, GDH) were also measured. Five isonitrogenous (48% crude protein) and isolipidic (14% crude lipids) diets were formulated to contain 10% normal starch (diet NS10), 10% waxy starch (diet WS10), 20% normal starch (diet NS20), 20% waxy starch (diet WS20) or no starch (control diet). Another diet was formulated with no carbohydrate, and contained 68% crude protein and 14% crude lipids (diet HP). Each experimental diet was fed to triplicate groups of 30 fish (initial weight: 23.3 g) on an equivalent feeding scheme for 12 weeks. The best growth performance and feed efficiency were achieved with fish fed the HP diet. Neither the level nor the nature of starch had measurable effects on growth performance of sea bass juveniles. Digestibility of starch was higher with waxy starch and decreased with increasing levels of starch in the diet. Whole-body composition and plasma metabolites, mainly glycemia, were not affected by the level and nature of the dietary starch. Data on enzyme activities suggest that dietary carbohydrates significantly improve protein utilization associated with increased glycolytic enzyme activities (GK and PK), as well as decreased gluconeogenic (FBPase) and amino acid catabolic (GDH) enzyme activities. The nature of dietary carbohydrates tested had little influence on performance criteria.

Keywords: Amino acid catabolic enzymes; Carbohydrates utilization; European sea bass; Fish nutrition; Glycolytic enzymes; Gluconeogenic enzymes; Intermediary metabolism; Lipogenic enzymes; Normal starch; Waxy starch

1. Introduction

Although the major enzymes for carbohydrate digestion are present in fish (Wilson, 1994), carnivorous fish present limited capacity of digesting polysaccharides. They also have low glucose tolerance and preferentially use body lipids instead of glycogen to derive energy even during starvation (Cowey and Walton, 1989). This poor utilization of dietary glucose by fish may be due to a dysfunction in the nutritional regulation of carbohydrate metabolic pathways in the liver (Panserat et al., 2001b). Fish seem to have low capacity to store excess glucose in the postprandial stage (through glycogen synthesis or lipogenesis) (Cowey and Walton, 1989; Wilson, 1994) and/or have a persistent high capacity of hepatic glucose production even when carbohydrates are provided in the diet (Panserat et al., 2000b, 2001a).

Sea bass is a carnivorous fish which in the natural environment thrives on food practically devoid of carbohydrates as also occurs in other carnivorous fish such as rainbow trout, eel, yellowtail and plaice (Walton and Cowey, 1982). Therefore, when carbohydrates are not provided in the diet, more proteins

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are catabolized for glucose synthesis (Suarez and Mommsen, 1987). This impairs protein retention and increases nitrogen releases into the environment (Cowey and Walton, 1989; Wilson, 1994). Under aquaculture conditions, it is important to provide appropriate levels of carbohydrate in the diets to ensure efficient utilization of the other nutrients (Wilson, 1994). It is generally considered that for marine or coldwater fish species incorporation of digestible carbohydrates should not exceed 20% of the diet (Kaushik and Oliva-Teles, 1985; Beamish and Medland, 1986; Kaushik et al., 1989; Hemre et al., 1995).

Knowledge on carbohydrate utilization by European sea bass is still scarce and discordant. Normal starch is considered a relatively poor energy source, mainly due to its low digestibility. However, differences in botanical origin or technological treatment of starch can improve starch digestibility by modifying carbohydrate granules in such a way that their susceptibility to enzymatic action increases (Watson and Johnson, 1965; Bergot, 1993). Although Gouveia et al. (1995) found no differences in growth performances and feed utilization efficiency of sea bass fed diets including either 15% or 25% raw or gelatinized starch, Dias et al. (1998) observed better growth and feed utilization of sea bass fed gelatinized compared to raw starch, at the two dietary inclusion levels tested (11% and 23%). On the other hand, according to Peres and Oliva-Teles (2002) performance of sea bass was better with a mixture of 12.5% raw starch plus 12.5% gelatinized starch than with either 25% gelatinized or 25% raw starch alone. As regards enzymes of glucose metabolism, there is practically no information available in European sea bass.

Contrary to normal maize, which contains 25–28% amylose, waxy maize only contains 1% (Pfeffer et al., 1991; Bergot, 1993). This affects nutritional availability of starch for animals. Indeed, Bergot (1993) measured the digestibility of starch of different botanical origins by rainbow trout and found that digestibility of waxy maize starch was significantly higher than that of amylomaize or normal maize. Pfeffer et al. (1991) also found that rainbow trout performed better with waxy than normal maize starch.

The aim of this study was to evaluate the effect of two levels (10% and 20%) and two forms (normal maize and waxy maize) of dietary starch intake on an equivalent feeding scheme on growth performance and nutrient utilization in European sea bass juveniles reared at 25 °C. In order to gain further information on metabolic control mechanisms of glucose, lipid and amino acid metabolism, the activities of selected key-enzymes of glycolytic, gluconeogenic, lipogenic and amino acid metabolism were measured in the liver of sea bass adapted to the different dietary regimes.

2. Material and methods

2.1. Diets

Five isonitrogenous (48% crude protein) and isolipidic (14% crude lipids) diets were formulated to contain 10% normal maize starch (diet NS10), 10% waxy maize starch (diet WS10), 20% normal maize starch (diet NS20), 20% waxy maize starch (diet WS20) or no starch (control diet). Normal maize starch (72% amylopectin, 28% amylose) from COPAM (Loures, Portugal) and waxy maize starch (99% amylopectin, 1% amylose) from Cerestar (Mechelen, Belgium). The diets were adjusted by manipulating the cellulose content. Another diet was formulated with no carbohydrate, and contained 68% crude protein and 14% crude lipids (diet HP). All dietary ingredients were finely ground, well mixed and dry pelleted in a laboratory pellet mill (CPM) through 2 and 3 mm dies. Ingredients and proximate composition of the experimental diets are presented in Table 1.

2.2. Fish rearing

Juvenile European sea bass (*Dicentrarchus labrax*) were reared at the Marine Zoological Station (Porto, Portugal) in a thermo-regulated recirculating water system equipped with 18 fiberglass cylindrical tanks (250 L water capacity each). During the trial, water temperature was 25±0.5 °C, salinity averaged 35±1‰, dissolved oxygen was 92% of saturation and ammonia ranged between 0.2 and 0.5 mg L^{-1}.

Table 1
Composition and proximate analyses of the experimental diets

	Diets					
	Control	NS10	WS10	NS20	WS20	HP
Ingredients (% dry weight)						
Fish meal [a]	57.3	57.3	57.3	57.3	57.3	85.5
Soluble fish protein concentrate [b]	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	8.6	8.6	8.6	8.6	8.6	6.0
Normal maize starch [c]	–	10.0	–	20.0	–	–
Waxy maize starch [d]	–	–	10.0	–	20.0	–
α-Cellulose	25.6	15.6	15.6	5.6	5.6	–
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
Lignin sulphate	1.0	1.0	1.0	1.0	1.0	1.0
Proximate analyses (% dry weight)						
Dry matter	96.5	93.1	95.0	96.0	94.0	96.4
Crude protein	49.0	48.7	48.3	48.3	48.4	69.4
Crude fat	14.2	13.9	14.2	14.0	14.0	13.8
Ash	11.9	11.9	11.9	11.8	11.9	16.8
Starch	0.8	10.6	9.6	19.0	18.5	0.7
Gross energy (kJ g^{-1} DM)	21.1	21.2	21.3	21.3	21.3	21.3

[a] Pesqueira Diamante, Steam Dried LT, Spain (CP: 72% DM; GL: 9.4% DM).
[b] Sopropêche G, France (CP: 74.7% DM; GL: 19.6% DM).
[c] COPAM (Loures, Portugal).
[d] Cerestar (Mechelen, Belgium).
[e] Vitamins (mg kg^{-1} diet): retinol acetate, 18000 (IU kg^{-1} diet); cholecalciferol, 2000 (IU kg^{-1} diet); alpha tocopherol acetate, 35; sodium menadione bisulphate, 10; thiamine–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).