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Rearing temperature enhances hepatic glucokinase but not glucose-6-phosphatase activities in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) juveniles fed with the same level of glucose

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ABSTRACT

The aim of this work was to elucidate if the previous results observed in hepatic glucokinase (GK) and glucose-6-phosphatase (G6Pase) activities in European sea bass and gilthead sea bream are due to temperature per se or to differences in feed intake at different water temperatures. For that purpose triplicate groups of fish (30 g initial body weight) were kept at 18 °C or 25 °C during two weeks and fed a fixed daily ration of a glucose-free or 20% glucose diet. At the end of the experimental period, plasma glucose levels in both species were not influenced by water temperature but were higher in fish fed the glucose diet. Higher hepatic GK activity was observed in the two fish species fed the glucose diet than the glucose-free diet. In the glucose fed groups, GK activity was higher at 25 °C than at 18 °C. Glucose-6-phosphatase activities in both species were not influenced by water temperature. In European sea bass and in contrast to gilthead sea bream it was observed an effect of dietary composition on G6Pase activities with surprising higher activities recorded in fish fed the glucose diet than in fish fed the glucose-free diet. Overall, our data strongly suggest that European sea bass and gilthead sea bream are apparently capable to strongly regulate glucose uptake by the liver but not glucose synthesis, which is even enhanced by dietary glucose in European sea bass. Within limits, increasing water temperature enhances liver GK but not G6Pase activities, suggesting that both species are more able to use dietary carbohydrates at higher rearing temperatures.

1. Introduction

Glucose plays a key role as energy source in mammals but its importance in fish appears limited (Wilson, 1994; Hemre et al., 2002; Stone, 2003). Although, fish have the enzymatic machinery and metabolic pathways required for carbohydrate metabolism (Cowey and Walton, 1989; Dabrowski and Guderley, 2002) a very high hyperglycemia is generally observed after feeding carbohydrate-rich diets or after the performance of glucose tolerance tests, particularly in carnivorous species (Wilson, 1994; Peres et al., 1999). Until now, the physiological basis for such apparent glucose intolerance is not fully understood (Moon, 2001; Hemre et al., 2002).

Hepatic glucokinase (GK, EC 2.7.1.2) and glucose-6-phosphatase (G6Pase, EC 3.1.3.9) are key enzymes playing major roles in the regulation of glycolytic and gluconeogenic pathways, respectively (Printz et al., 1993; van de Werve et al., 2000). Glucokinase catalyses the phosphorylation of glucose to glucose-6-phosphate and thus plays a key role in hepatic glucose utilization, whereas G6Pase catalyses the last step of hepatic glucose production, by hydrolysing glucose-6-phosphate into glucose. Thus, a lack of significant regulation of hepatic glucose utilization and production may possibly explain the relative inability of fish to efficiently utilize dietary glucose (Panserat et al., 2001). As in mammals, an increase of hepatic GK activity with the increase of dietary carbohydrate was reported in several fish species (Tranulis et al., 1996; Borrebaek and Christophersen, 2000; Panserat et al., 2000a) including gilthead sea bream (Panserat et al., 2000a; Caseras et al., 2002; Enes et al., 2008a) and European sea bass (Enes et al., 2006a; Moreira et al., 2008). Regarding G6Pase, although Shimeno et al. (1995) reported a decrease of hepatic enzyme activity with increasing dietary carbohydrate levels in common carp *Cyprinus carpio*, such effect was not observed in European sea bass (Enes et al., 2006a), gilthead sea bream (Caseras et al., 2002) and rainbow trout *Oncorhynchus mykiss* (Panserat et al., 2000b, 2001).

Water temperature is an important environmental factor that modulates fish growth. It also affects among other factors, feed intake, efficiency of carbohydrate utilization as energy source or the activity of intermediary metabolism enzymes (Médale et al., 1991, 1999; Brauge et al., 1995; Shikata et al., 1995; Peres and Oliva-Teles, 1999; Enes et al., 2006b, 2008b; Moreira et al., 2008). For instance, in common carp increasing water temperature improves starch

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digestibility (Médale et al., 1999), whereas activity of key enzymes of both glycolytic and gluconeogenic pathways increased at lower water temperatures (Shikata et al., 1995). In rainbow trout, efficiency of carbohydrates as energy source improved with the increase of water temperature (Médale et al., 1991; Brauge et al., 1995). In European sea bass and gilthead sea bream, growth performance, feed efficiency, protein utilization as well as liver glycolytic activity improved at higher water temperature (Peres and Oliva-Teles, 1999; Person-Le Ruyet et al., 2004; Enes et al., 2006b, 2008b; Moreira et al., 2008). Moreover, in European sea bass increasing water temperature improves starch digestibility and liver gluconeogenic and lipogenic capabilities (Enes et al., 2006b; Moreira et al., 2008).

However, analyses of the effect of water temperature on enzymatic activity, is generally confused by the different feed intake of fish kept at different water temperatures. Nonetheless, feeding rate was already shown to influence enzymatic activities in common carp, separately or cooperatively with water temperature (Shimeno and Shikata, 1993).

According to our knowledge, there are no studies available concerning the effect of dietary glucose and water temperature on hepatic GK/G6Pase activities in fish reared under the same feeding rate. Enes et al. (2006b, 2008b) already studied the effect of carbohydrate source and water temperature on growth performance and metabolic utilization of carbohydrates in European sea bass and gilthead sea bream. However, fish kept at each water temperature received different amounts of feed, therefore not allowing to clearly discriminating if the results observed were due to the effect of temperature or of feed intake. Thus, the aim of the present study is to elucidate the effect of water temperature (18 °C and 25 °C) in the regulation of hepatic GK and G6Pase activities in European sea bass and gilthead sea bream juveniles receiving the same amount of feed.

2. Materials and methods

Juveniles of European sea bass (*Dicentrarchus labrax*, Moronidae, Perciformes) and gilthead sea bream (*Sparus aurata* , Sparidae, Perciformes) were obtained from a commercial fish farm and transported to the experimental facilities at the Marine Zoological Station (Porto, Portugal). Before the trial fish were allowed to recover from transport and to adapt to the experimental conditions. Two isonitrogenous (49% crude protein) and isolipidic (18% crude lipids) diets were formulated to contain 20% of either α-cellulose (Control diet) or glucose (GLU diet). All dietary ingredients were finely ground, mixed thoroughly 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.

The trial was performed in two independent partial water recirculation systems, thermoregulated to 18.2±0.1 °C and 24.3±0.2 °C, respectively. Both systems contained a battery of 12 cylindrical fiberglass tanks of 100 L water capacity each. Tanks were supplied with a continuous flow of filtered seawater (2.5–3.0 L min^{-1}), salinity averaged 37.3±0.9‰ and dissolved oxygen was kept near to saturation. Groups of 15 fish with an average body mass of 30 g were randomly distributed to each tank. Each diet was assigned to triplicate groups of animals. Fish in both water systems were fed by hand, twice a day, 6 days a week, with a daily ration of 8 g per day per tank for European sea bass and 16 g per day per tank for gilthead sea bream. Within each fish species, these rations were equivalent to the satiation feed intake of the group that ingested less feed (control group at 18 °C) during the week preceding the beginning of the trial. The trial lasted 2 weeks as this period was considered long enough for fish to physiologically adapt to the diets and short enough to avoid physiological negative effects due to the restricted feeding regime imposed. No mortality was recorded during the trial. At the end of the trial, blood and liver from 3 fish per tank were sampled 6 h after the morning meal for plasma glucose analysis and measurement of liver enzyme activities. Blood was collected from the caudal vein with a heparinised syringe, immediately centrifuged and the plasma frozen at −20 °C until analysis. After collection livers were immediately frozen in liquid nitrogen and then stored at −80 °C until measurement of enzymatic activities.

Chemical analysis of the diets was conducted using the following procedures: dry matter after drying at 105 °C until constant weight; ash by incineration in a muffle furnace at 450 °C for 16 h; protein content (N×6.25) by the Kjeldahl method after acid digestion using a Kjeltec digestion and distillation units (Tecator Systems, Höganäs, Sweden; model 1015 and 1026, respectively); lipid by petroleum ether extraction in a SoxTec extraction system (Tecator Systems, Höganäs, Sweden; extraction unit model 1043 and service unit model 1046) and gross energy by direct combustion in an adiabatic bomb calorimeter (PARR Instruments, Moline, IL, USA; PARR model 1261). Plasma glucose was determined using an enzymatic-colorimetric method (Spinreact, Girona, Spain; glucose kit, cod. 1001191).

For measurement of glucokinase activity, a frozen sample of liver was homogenized (dilution 1/10) in ice-cold buffer (80 mM Tris; 5 mM EDTA; 2 mM dithiothreitol; 1 mM benzamidine; 1 mM 4-(2-aminoethyl) benzenesulphonyl fluoride, pH 7.6). Homogenates were centrifuged at 900 ×*g* for 10 min. The GK activities were measured using 100 mM of glucose as described previously (Tranulis et al., 1996; Panserat et al., 2000a) at 37 °C by coupling ribulose-5-phosphate formation from glucose-6-phosphate to the reduction of NADP using purified glucose-6-phosphate dehydrogenase (Sigma, Sintra, Portugal) and 6-phosphogluconate dehydrogenase (Sigma) as coupling enzymes. The assay for measuring GK activity on frozen samples necessitated correction by measuring glucose dehydrogenase (EC 1.1.1.47) activity as described by Tranulis et al. (1996). Glucose dehydrogenase is a moderately active microsomal enzyme in fish liver that can introduce significant bias in GK measurements on frozen tissues (Tranulis et al., 1996).

In order to measure glucose-6-phosphatase activity, microsomes were obtained from European sea bass and gilthead sea bream livers, as described previously (Panserat et al., 2000b). Microsomes were suspended in buffer (100 mM sodium dihydrogenphosphate; 25 mM disodium hydrogenphosphate; 2 mM EDTA; 1 mM dithiothreitol, pH 7), without further treatment. The procedure followed was that of Alegre et al. (1988),

Table 1
Composition and proximate analyses of the experimental diets

	Diets	
	Control	GLU
Ingredients (dry weight basis)		
Fish meal[a]	64.3	64.3
Soluble fish protein concentrate[b]	1.0	1.0
Cod liver oil	11.3	11.3
α-Cellulose	20.0	–
d-Glucose	–	20.0
Vitamin premix[c]	1.0	1.0
Mineral premix[d]	1.0	1.0
Choline chloride (60%)	0.5	0.5
Aquacube	1.0	1.0
Proximate analyses (dry matter basis)		
Dry matter (%)	90.6	86.5
Crude protein (%)	48.9	50.9
Crude fat (%)	17.7	18.7
Ash (%)	12.3	12.1
Gross energy (kJ g^{-1})	21.7	22.4

[a] TripleNine, Prime Quality, Denmark (CP: 74.5% DM; GL: 10.2% DM).
[b] Sopropèche G, France (CP: 80.7% DM; GL: 19.4%DM).
[c] 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.
[d] 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).