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Glucose tolerance in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*)

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Abstract

Glucose tolerance tests were performed with seabream and seabass. The fish were adapted to a practical diet (50% protein; 12% lipid) for three weeks and then, after being fasted for 24 h, injected intraperitoneally with 1 g glucose/kg body weight. Blood, liver and muscle samples were collected before and 1, 3, 6, 12, 24, 48 and 72 h after injection. In both species, an increase in plasma glucose level was observed after injection. In seabream, plasma glucose level reached a peak 1–3 h after injection, while in seabass the peak was reached 3–6 h after injection. The peak value was significantly higher in seabream than in seabass. In both species, plasma glucose levels returned to the initial values within 12 h after injection. In seabream, plasma triacylglyceride peaked 1 h after injection and thereafter started to decrease to the initial levels that were reached within 6 h after injection. In seabass, plasma triacylglyceride levels decreased after injection, starting to increase 3 h later. The peak value was attained 6–12 h after injection and the basal values were reached within 24 h after injection. During the first hour after injection liver glycogen content of seabream significantly decreased, while in seabass it significantly increased. In both species, 6 h after injection liver glycogen content started to increase to a peak value, which was reached 12 h and 24 h after injection in seabream and in seabass, respectively. The results of this study indicate that seabream and seabass were able to restore glucose levels within 12 h after being injected with 1 g glucose/kg body weight. These data also suggest that plasma triacylglyceride concentrations and liver glycogen content may be related to the mechanism of glucose regulation in both species. Glucose injection seemed to enhance catabolism of body stores in seabream and to stimulate anabolism in seabass.

Keywords: Glucose; Tolerance test; Plasma metabolites; Seabream; Seabass

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1. Introduction

It is now well established for the majority of the cultured fish species that the efficiency of protein utilization can be improved by increasing the proportion of conventional energy sources (lipid and carbohydrate) in the diet (Cho and Kaushik, 1990; Kaushik and Médale, 1994).

While the protein sparing effect of dietary lipid is well demonstrated, that of carbohydrate, particularly in some marine fish species, is still controversial. In the natural environment, fish have limited access to carbohydrate sources and are not well adapted at the digestive and metabolic levels to deal with high amounts of dietary carbohydrate. The digestive utilization of carbohydrate depends on the nature and complexity of the starch (Bergot, 1979; Spannhof and Plantikow, 1983), as well as the technological treatments applied to it (Bergot and Brèque, 1983). In rainbow trout, gelatinized starch has been shown to be as effective as lipid as an energy source (Pieper and Pfeffer, 1980). At the metabolic level, fish have a limited ability to metabolize glucose. High digestible carbohydrate intake results in a post-prandial hyperglycemia that remains for many hours (Bergot, 1979; Kaushik and Oliva-Teles, 1985; Brauge et al., 1994).

Glucose tolerance tests have been conducted in several fish species to study the metabolic utilization of glucose (Furuichi and Yone, 1981; Harmon et al., 1991; Garcia-Riera and Hemre, 1996). Although, in each case, glucose administration resulted in prolonged hyperglycemia, there were marked differences among species: carnivorous fish were less tolerant to glucose than omnivorous fish. Furuichi and Yone (1981) observed that glucose tolerance was lowest in yellowtail, followed by red sea bream and carp. However, even the most glucose tolerant fish species exhibited a much higher level of plasma glucose and a slower return to the basal levels than omnivorous mammals (Cowey, 1988).

The low glucose tolerance of fish was initially thought to be due to an insufficiency of insulin secretion (Furuichi and Yone, 1982). However, recent studies have shown that fish are not diabetic and that plasma insulin levels are similar to those observed in mammals (Mommsen and Plisetskaya, 1991). Nevertheless, the insulin response to glucose supply was shown not to be as rapid as in mammals, but to take several hours to develop (Furuichi and Yone, 1981; Harmon et al., 1991). Some studies have demonstrated that the delayed insulin secretion might be associated with somatostatin secretion that seems to be more sensitive to glucose than insulin; somatostatin appears to inhibit insulin secretion (Harmon et al., 1991; Sheridan et al., 1991). The low hepatic phosphorylation capacity of fish, initially pointed out as a limiting step of glucose metabolism, appears to be induced by digestible carbohydrate (Médale et al., 1998). The prolonged hyperglycemia in fish can also result from an impairment of one or more insulin post-receptors (Gutiérrez et al., 1991).

The aim of this study was to perform a glucose tolerance test in seabream and seabass in order to gain further knowledge on glucose regulation in marine fish species, as well as interactions between plasma glucose, plasma triacylglyceride concentrations and liver glycogen stores.

2. Material and methods

The animals used in this experiment were juveniles of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). The experiment was carried out at the Zoological Marine Station at Oporto, in a water recirculation system equipped with 16 cylindrical fiberglass tanks of 300-l capacity. During the trial, water temperature averaged 22 ± 1°C and salinity 32 ± 2‰. The tanks were stocked with eight groups of eight seabream with a mean body weight of 194 g and eight groups of eight seabass with a mean body weight of 133 g. Fish were allowed to adapt to the experimental facilities for three weeks, and during this period were fed by hand, once a day, to near satiety with an experimental diet. This diet had a protein content of 50% and a lipid content of 12%, and was presented as dry pellets, manufactured in the laboratory using a laboratory pellet mill (CPM).

After the acclimation period, fish were fasted for 24 h, lightly anaesthetized with ethylene glycol monophenyl ether (0.3 ml/l), immediately weighed and injected intraperitoneally with exactly 1 g of glucose per kg body weight. Due to differences in weight between species a glucose solution of 200 mg/ml was used for seabream and a glucose solution of 100 mg/ml was used for seabass. Fish were then placed back in the experimental tanks. In order to avoid stress during sampling, a different tank was used for each sampling time.

Blood, muscle and liver samples were collected just before (time 0) and 1, 3, 6, 12, 24, 48 and 72 h after glucose injection. At each sampling time, eight fish of each species were sampled and individually weighed. Blood was collected from the caudal vein, immediately centrifuged and the plasma frozen for analysis. Livers were weighed and frozen for analysis. Muscle samples were taken from the lateral dorsal part of the body behind the dorsal fin, and frozen for analysis.

Plasma glucose was determined using an enzymatic-colorimetric method (glucose kit, cod. 1001190; *Spinreact*), plasma triacylglycerides were determined with an enzymatic-colorimetric method (triacylglycerides kit, cod. 1001312, *Spinreact*), and total plasma amino acids according to Spies (1957). Liver and muscle glycogen was determined according to Plummer (1987), liver protein according to Lowry et al. (1951) and liver total lipid according to Folch et al. (1957).

Statistical evaluation of the data was done by one-way and two-way analysis of variance using a *Statgraphics* version 7 software package. The probability level for rejection of the null hypotheses was 0.05. Significant differences among means were determined by Duncan's multiple range test.

3. Results

3.1. Plasma metabolites

At time zero, plasma glucose level was similar in both species (66 mg/dl; Table 1). Immediately after injection, plasma glucose started to increase to a peak that was

Table 1
Evaluation of plasma glucose, triacylglycerides and total amino acids in seabream and seabass during the trial (mg/dl plasma)

Hour	0	1	3	6	12	24	48	72
Glucose								
Seabream	$67.9^{a} \pm 8.4$	$323.3^{bc} \pm 23.5$	$360.7^{c} \pm 26.2$	$311.0^{b} \pm 13.2$	$103.7^{a} \pm 11.8$	$79.0^{a} \pm 8.9$	$76.5^{a} \pm 11.3$	$57.0^{a} \pm 9.1$
Seabass	$63.9^{ab} \pm 10.8$	$188.3^{c} \pm 21.8$	$256.7^{d} \pm 23.6$	$279.6^{d} \pm 32.7$	$122.1^{b} \pm 23.0$	$75.9^{ab} \pm 90.0$	$55.2^{a} \pm 4.2$	$58.3^{ab} \pm 9.4$
Triacylglycerides								
Seabream	$124.5^{ab} \pm 16.2$	$279.9^{d} \pm 21.3$	$205.6^{c} \pm 22.5$	$84.7^{a} \pm 5.0$	$98.3^{a} \pm 10.6$	$99.0^{a} \pm 6.8$	$159.0^{b} \pm 8.4$	$126.1^{ab} \pm 7.3$
Seabass	$210.2^{b} \pm 20.8$	$145.1^{a} \pm 6.0$	$145.2^{a} \pm 11.2$	$249.6^{bc} \pm 25.1$	$304.8^{c} \pm 20.6$	$206.7^{b} \pm 17.5$	$238.1^{b} \pm 20.9$	$213.2^{b} \pm 24.2$
Amino acids								
Seabream	23.8 ± 2.1	21.1 ± 2.0	21.9 ± 0.8	21.3 ± 0.1	20.1 ± 0.1	23.1 ± 1.5	22.1 ± 0.7	23.8 ± 1.3
Seabass	21.6 ± 0.8	20.7 ± 1.2	24.3 ± 1.0	21.9 ± 0.8	21.8 ± 1.8	24.8 ± 1.1	23.0 ± 3.1	21.2 ± 3.3

Numbers in the same line with different superscript letters are significantly different ($P < 0.05$). Mean $\pm$ standard error.

Table 2
Evaluation of liver glycogen, protein and lipid (% wet weight) and muscle glycogen (% wet weight) in seabream and seabass during the trial

Hour	0	1	3	6	12	24	48	72
Liver								
Hepatosomatic index[1]								
Seabream	1.59 ± 0.1	1.42 ± 0.1	1.52 ± 0.1	1.47 ± 0.0	1.58 ± 0.1	1.46 ± 0.0	1.41 ± 0.1	1.29 ± 0.1
Seabass	0.91 ± 0.1	1.04 ± 0.1	1.19 ± 0.1	1.08 ± 0.1	0.97 ± 0.0	1.19 ± 0.1	1.08 ± 0.1	0.94 ± 0.1
Glycogen								
Seabream	$12.8^{bc} \pm 1.0$	$8.4^{a} \pm 0.5$	$10.0^{ab} \pm 08$	$8.8^{a} \pm 1.2$	$14.2^{c} \pm 1.0$	$13.4^{c} \pm 0.9$	$11.5^{abc} \pm 1.0$	$8.1^{a} \pm 1.8$
Seabass	$6.4^{a} \pm 0.1$	$11.7^{b} \pm 0.6$	$6.1^{a} \pm 0.2$	$6.0^{a} \pm 0.3$	$11.0^{b} \pm 1.3$	$16.3^{c} \pm 0.8$	$11.5^{b} \pm 0.6$	$4.6^{a} \pm 0.5$
Protein								
Seabream	$7.6^{ab} \pm 0.4$	$7.2^{a} \pm 0.2$	$7.8^{ab} \pm 0.1$	$8.9^{d} \pm 0.3$	$8.5^{cd} \pm 0.1$	$8.0^{bc} \pm 0.1$	$8.2^{bc} \pm 0.1$	$8.9^{d} \pm 0.1$
Seabass	$9.6^{d} \pm 0.1$	$9.0^{bc} \pm 0.1$	$8.6^{a} \pm 0.1$	$10.2^{e} \pm 0.1$	$10.1^{e} \pm 0.1$	$8.6^{ab} \pm 0.1$	$9.1^{c} \pm 0.1$	$9.3^{c} \pm 0.2$
Lipid								
Seabream	9.5 ± 0.7	9.5 ± 0.1	9.2 ± 0.2	9.7 ± 0.3	8.6 ± 0.5	8.7 ± 0.2	9.7 ± 0.8	8.6 ± 0.1
Seabass	32.3 ± 1.0	30.6 ± 5.0	35.3 ± 0.9	31.0 ± 1.9	33.7 ± 1.0	33.5 ± 1.6	35.3 ± 1.0	32.6 ± 0.8
Muscle								
Glycogen								
Seabream	0.74 ± 0.27	0.97 ± 0.04	0.72 ± 0.12	0.94 ± 0.13	0.76 ± 0.16	0.83 ± 0.14	0.73 ± 0.12	0.66 ± 0.13
Seabass	0.24 ± 0.03	0.29 ± 0.05	0.43 ± 0.18	0.30 ± 0.03	0.29 ± 0.03	0.51 ± 0.14	0.55 ± 0.21	0.61 ± 0.23

Numbers in the same line with different superscript letters are significantly different ($P < 0.05$). Mean $\pm$ standard error.
[1] HSI: (liver weight/body weight) $\times$ 100.

reached 1–3 h after injection in seabream and 3–6 h after injection in seabass. The peak value was significantly higher in seabream than in seabass. Thereafter, plasma glucose started to decrease to the basal levels, which were reached within 12 h after injection (Table 1). In both species, there were no significant differences in plasma glucose levels after 12 h.

Basal triacylglyceride levels were significantly higher in seabass than in seabream. In seabream, plasma triacylglycerides peaked 1 h after glucose injection, then they started to decrease to the basal values within 6 h after injection (Table 1). From this time onwards plasma triacylglyceride levels did not differ from the basal values. In seabass, plasma triacylglycerides first decreased until 3 h after glucose injection, then increased to a peak 12 h after injection. From time 24 h until the end of the trial, plasma triacylglycerides were not significantly different from the basal value (Table 1). No inter-species differences were observed in total amino acids in the plasma, which remained constant in both species, over the entire sampling period (Table 1).

3.2. Liver and muscle

Hepatosomatic indices were significantly higher in seabream than in seabass, and did not significantly change during the trial (Table 2).

Liver glycogen content was significantly higher in seabream than in seabass. In seabream, liver glycogen values were significantly lower than the basal values 1 and 6 h after injection. It remained similar to the basal values between 12 h and 48 h after injection and, 72 h after injection liver glycogen was again significantly lower than the basal values. On the contrary, liver glycogen in seabass was significantly higher than the basal value 1 h after injection. Thereafter it decreased to basal levels 3–6 h after injection, then it increased, peaked at 24 h after injection and basal levels were reached at time 72 h (Table 2).

Average liver protein levels in seabream were significantly lower than in seabass. In seabream significantly higher liver protein was found 6, 12 and 72 h compared to basal values (Table 2). In seabass, liver protein was significantly lower than the basal values 1 h and 3 h after injection, while at time 6 h and 12 h it was significantly higher (Table 2). Liver lipids were much higher in seabass than in seabream and remained unchanged during the trial in both species (Table 2).

Muscle glycogen was significantly higher in seabream than in seabass. During the trial muscle glycogen did not change significantly (Table 2).

4. Discussion

Both species showed a similar pattern of plasma glucose response to a glucose load as reported for other carnivorous fish (Bergot, 1979; Harmon et al., 1991; Hemre et al., 1991; Brauge et al., 1994; Garcia-Riera and Hemre, 1996), except that a shorter time to restore glucose levels after the glucose load was required as compared to oral glucose tolerance tests. The capacity of glucose regulation of carp, an omnivorous fish, is

considerably higher compared to carnivorous fish, as it only needed 5 h to restore plasma glucose levels (Furuichi and Yone, 1981; Shikata et al., 1994).

In fish, as in mammals, there is a significant relationship between plasma levels of glucose and pancreatic hormones (Pereira, 1996). Insulin has been shown to stimulate glucose and fatty acids removal from plasma and to enhance hepatic glycogen and lipid synthesis (Harmon and Sheridan, 1992; Harmon et al., 1993), while glucagon was shown to stimulate hyperglycemia and lipolysis (Plisetskaya et al., 1989; Harmon and Sheridan, 1992; Harmon et al., 1993). Somatostatin seems to have hyperglycemic and glycogenolytic effects (Eilertson and Sheridan, 1993).

In this trial, after glucose injection, the initial metabolic response differed between the two species. In seabream, 1 h after glucose injection plasma triacylglycerides significantly increased and hepatic glycogen significantly decreased. These results agree with the observations of Harmon et al. (1991), where glucose-injected rainbow trout resulted in an initial increase in plasma fatty acids, a decrease in hepatic glycogen, an increase in somatostatin and glucagon and a suppression of plasma insulin. In seabass the increase in plasma glucose was more gradual and the peak value was significantly lower than in seabream. Moreover, 1 h after injection, plasma triacylglycerides significantly decreased and hepatic glycogen significantly increased. These differences between species could result from different hormonal responses. According to Chaves et al. (1982) and Gutiérrez et al. (1984, 1986), insulin levels in seabass are among the highest to be found in teleost and this could, at least in part, contribute to explain the lower plasma glucose levels, the decrease in plasma triacylglycerides and the elevation of liver glycogen concentrations. In seabream, there was a shift in metabolic flux towards an enhanced catabolism of stored body reserves, while in seabass there was an enhancement of anabolism.

In fish, insulin secretion usually peaks only 2–3 h after a glucose load (Furuichi and Yone, 1981; Harmon et al., 1991; Sheridan et al., 1991). In this trial, the return to basal plasma glucose levels coincided with a significant increase in hepatic glycogen and protein levels. This is probably associated with an increase in insulin levels, as it is a potent anabolic agent that increases glucose uptake and glycogen synthesis.

As in this trial, a predominant increase in liver glycogen in relation to liver lipid after a glucose injection has also been reported in seabass (Rombaut, 1982, cited by Brauge et al., 1995). This author observed that radioactivity recovered as hepatic glycogen 12 h after a ^{14}C-glucose injection, represented 25–52% of total liver radioactivity, whereas the radioactivity recovered in hepatic lipids represented only 2–8%.

Liver lipid content of seabass was significantly higher than that of seabream. This agrees with McClelland et al. (1995) that showed that seabass is able to store 33% fresh weight as lipid in liver, while seabream stored only 5% of fresh weight.

From the results of this trial, it can be concluded that seabream and seabass were able to restore plasma glucose levels within 12 h after a peritoneal injection of 1 g glucose per kg body weight. Although there were differences between species in the patterns of some parameters analyzed, the basal values were restored within 24 h after glucose injection, except for liver glycogen in seabass. The variation of plasma triacylglycerides and liver glycogen, in both species, suggest that these components may have an important role in glucose regulation. Further studies, including the hormonal response to

a glucose load, are required for a better understanding of glucose metabolism in these marine species.

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