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Rapid metabolic adaptation in European sea bass (*Dicentrarchus labrax*) juveniles fed different carbohydrate sources after heat shock stress

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Abstract

A study was conducted to evaluate the effect of two dietary carbohydrate sources (waxy maize starch and glucose) on the metabolic adaptation of sea bass juveniles (initial weight: 24 g) to a heat shock treatment (temperature rise from 18 °C to 25 °C within 24 h). Two isonitrogenous and isolipidic diets were formulated to contain 20% waxy maize starch (WS diet) or 20% glucose (GLU diet). Triplicate groups of fish were fed to near satiation for 4 weeks at both temperatures (18 °C and 25 °C). Then, fish previously maintained at 18 °C were submitted to a heat shock (18 °C to 25 °C) and continued to be fed with the same diets during 1 more week. The higher water temperature significantly improved growth performance, feed efficiency, as well as protein efficiency ratio, independently of diet. At 25 °C, but not at 18 °C, growth of fish fed the WS diet was higher than that of fish fed the GLU diet. Plasma glucose levels were higher in sea bass fed the GLU diet and not influenced by water temperature. Fish fed a glucose diet or reared at high temperatures (25 °C) showed enhanced liver glycolytic, lipogenic and gluconeogenic capacities compared to fish fed a starch diet or reared at low temperatures (18 °C). For the majority of the enzymes studied, 1 week seemed to be enough time for metabolic adaptation in sea bass submitted to an acute heat shock.

Irrespective of carbohydrate source, HSP70 gene expression was similar in both cold water (18 °C) and warm water (25 °C) acclimated sea bass. A weak down regulation was observed after heat shock only in fish fed the GLU diet. This suggests that HSP70 gene expression is not affected by the rearing temperature *per se*.

Keywords: Biomarker; Carbohydrate utilization; European sea bass; Gluconeogenic enzymes; Glucose; Glycolytic enzymes; HSP70; Lipogenic enzymes; Temperature; Waxy maize starch

1. Introduction

Carnivorous fish require high-protein diets to obtain amino acids for use in protein synthesis, glucose synthesis and for energy purposes (Sánchez-Muros et al., 1998). However, protein is one of the most expensive components of diets and excess protein increases N excretion. Therefore, both from an economical and environmental point of view, it is advisable to spare protein for plastic purposes by increasing the utilization of conventional energy sources like lipids or carbohydrates (Cho and Kaushik, 1990; Kaushik and Médale, 1994).

Although carbohydrates are the cheapest energy source, most teleosts do not tolerate high dietary carbohydrate levels, and maximum dietary inclusion level depends on fish species. It is generally assumed that optimal dietary digestible carbohydrate level is less than 20% for carnivorous fish, but much higher (30–40%) for omnivorous fish (Wilson, 1994). For the European sea bass, evidence exists that a dietary incorporation of 20–25% of digestible carbohydrate does not affect growth or feed efficiency (Gouveia et al., 1995; Lanari et al., 1999; Peres and Oliva-Teles, 2002).

The complexity of the carbohydrate molecule also affects carbohydrate utilization. For example, starch has been demonstrated to be used more efficiently than glucose in both marine

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and fresh water species: carp, red sea bream (Furuichi and Yone, 1982), yellowtail (Furuichi et al., 1986), tilapia (Anderson et al., 1984; Shiau and Peng, 1993), white sturgeon (Deng et al., 2005). On the contrary, juvenile grass carp (Tian and Liu, 2004) and rainbow trout (Bergot, 1979a; Hung and Storebakken, 1994) appear to utilise glucose better than starch. However, in grouper (Shiau and Lin, 2001) starch seems to be used as efficiently as glucose.

Various factors affect the digestible energy provided by complex carbohydrates in fish diets (Bergot, 1993). Some of these factors are the source and treatment of starch. Contrary to normal maize, which contains 25–28% amylose, waxy maize only contains 1% (Pfeffer et al., 1991; Bergot, 1993). 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.

For the European sea bass, data on the utilization of different carbohydrate sources is still limited. Alliot et al. (1979) observed a protein sparing effect of glucose, although growth depression occurred at high dietary inclusion levels. Despite differences in carbohydrate digestibility, Alliot et al. (1984) observed similar growth performance of sea bass fed diets including either maltose or starch. Although the effect of starch gelatinization on growth performance is somewhat discordant (Gouveia et al., 1995; Dias et al., 1998; Peres and Oliva-Teles, 2002) it is well established that gelatinization of starch improves carbohydrate digestibility (Dias et al., 1998; Peres and Oliva-Teles, 2002). Also in sea bass, digestibility of waxy maize starch was higher than that of normal maize starch (Enes et al., 2006).

Water temperature is an important environmental factor affecting physiological and biochemical functions in fish (Jobling, 1994), as well as the activities of hepatic enzymes (Shikata et al., 1995). In sea bass, temperature plays an important role in governing growth via its effects on feeding rate and metabolism (Person-Le Ruyet et al., 2004). The increase of water temperature from 18 °C to 25 °C improved growth and feed efficiency of sea bass juveniles (Peres and Oliva-Teles, 1999; Person-Le Ruyet et al., 2004). Although protein utilization seems to be affected by temperature (Peres and Oliva-Teles, 1999), the optimum dietary protein inclusion level is independent of water temperature (Alliot et al., 1974; Hidalgo and Alliot, 1988; Peres and Oliva-Teles, 1999).

Heat shock proteins (HSP) are a wide family of conserved proteins, classified according to their molecular weight, present in all organisms including fish (Basu et al., 2002). HSP70 is known to assist the folding of nascent polypeptide chains, act as a molecular chaperone, and mediate the repair and degradation of altered or denatured proteins (Basu et al., 2002). Thus, HSP70 has been most widely used as a biomarker of stress. In fish, like in mammals, HSP70 is induced by heat and chemical shocks (Gornati et al., 2004). In sea bass, HSP70 was also shown to be inducible by rearing density (Gornati et al., 2004).

According to our knowledge, there are no previous studies with sea bass comparing starch and glucose utilization at different water temperatures. Thus, the purpose of the present study was to evaluate the effect of two water temperatures (18 °C and 25 °C) and two carbohydrate sources (waxy maize starch and glucose) on growth performance and activities of hepatic enzyme related to glycolysis, gluconeogenesis and lipogenesis in European sea bass juveniles.

2. Materials and methods

2.1. Diets

Two isonitrogenous (48% crude protein) and isolipidic (18% crude lipids) diets were formulated to contain 20% of either waxy maize starch (WS diet) or glucose (GLU diet). Waxy maize starch (99% amylopectin, 1% amylose) was purchased from Cerestar (Mechelen, Belgium) and D(+)-glucose from MERCK. All dietary ingredients were finely ground, mixed thoroughly and dry pelleted in a laboratory pellet mill (CPM) through a 3 mm die. Ingredients and proximate composition of the experimental diets are presented in Table 1.

2.2. Fish rearing

We used European sea bass (*Dicentrarchus labrax*) juveniles obtained from a commercial hatchery. The first part of the trial lasted 4 weeks and was performed in two independent partial water recirculation systems, thermoregulated to 18.3±0.5 °C

Table 1
Composition and proximate analyses of the experimental diets

	Diets	
	WS	GLU
Ingredients (% dry weight)		
Fish meal [a]	58.7	58.7
Soluble fish protein concentrate [b]	5.0	5.0
Cod liver oil	11.9	11.9
Waxy maize starch [c]	20.9	–
D-glucose	–	20.9
Vitamin premix [d]	1.0	1.0
Mineral premix [e]	1.0	1.0
Choline chloride (60%)	0.5	0.5
Carboxymethylcellulose	1.0	1.0
Proximate analyses (% dry weight)		
Dry matter	92.3	90.2
Crude protein	47.2	49.4
Crude fat	17.7	17.7
Ash	11.4	12.1
Gross energy (kJ g^{-1} DM)	22.4	23.1

[a] Pesqueira Diamante, Steam Dried LT, Spain (CP: 75.3% DM; GL: 8.8% DM).
[b] Sopropèche G, France (CP: 75.4% DM; GL: 19.2% DM).
[c] Cerestar (Mechelen, Belgium).
[d] 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; 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.
[e] 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).