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Adipose tissue and liver metabolic responses to different levels of dietary carbohydrates in gilthead sea bream (*Sparus aurata*)

Marta Bou [a], Marijana Todorčević [b], Ramón Fontanillas [c], Encarnación Capilla [a], Joaquim Gutiérrez [a], Isabel Navarro [a,*]

[a] Department of Physiology and Immunology, Faculty of Biology, University of Barcelona, Barcelona 08028, Spain
[b] Nofima, 1431 Ås, Norway
[c] Skretting Aquaculture Research Center (ARC), 4016 Stavanger, Norway

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ABSTRACT

This study analyzes the effects of replacing dietary lipids by carbohydrates and carbohydrates by fiber on gilthead sea bream growth, as well as lipid and glucose metabolism in adipose tissue and liver over the course of a 15-week feeding trial. Six different diets were formulated and fish were classified into two experimental groups sharing one diet. In the first group (LS), fish were fed four diets where lipids were reduced (23%–17%) by increasing carbohydrates (12%–28%) and, the second group (SF) consisted on three diets where the amount of carbohydrates (28%–11%) was exchanged at expenses of fiber (1%–18%). Differences in growth were not observed; nevertheless, the hepatosomatic index was positively related to dietary starch levels, apparently not due to enhanced hepatic lipogenesis, partly supported by unchanged G6PDH expression. In the LS group, lipogenic activity of adipose tissue was stimulated with low-lipid/high-carbohydrate diets by up-regulating G6PDH expression and a tendency to increase FAS, and promoted carbohydrate utilization *versus* fatty acid oxidation by modulating the transcription factors LXRα, PPARα and PPARβ expression. In the SF group, PPARs and LXRα increased parallel to fiber levels in adipose tissue. Furthermore, an adaptation of hepatic GK to dietary starch inclusion was observed in both groups; however, the lack of effects on G6Pase expression indicated that gluconeogenesis was not nutritionally regulated under the conditions examined. Overall, metabolic adaptations directed to an efficient use of dietary carbohydrates are present in gilthead sea bream, supporting the possibility of increasing carbohydrate or fiber content in diets for aquaculture sustainability.

1. Introduction

Aquaculture production has expanded by almost 12-fold in the last three decades (FAO, 2012). Aquafeed relies on fish meal and fish oil due to its high nutritional quality; however, the sustainability of this practice is not well accepted at present, especially over the long term (Watanabe, 2002). Hence, in order to reduce aquafeed costs and alleviate overexploited marine fisheries pressure, research efforts to find suitable alternatives are currently underway.

The use of raw plant materials as protein and lipid sources has been recognized as a sustainable alternative to fish products (Bouraoui et al., 2011; Nasopoulou and Zabetakis, 2012); nevertheless, protein continues to be the most expensive nutrient and in an attempt to spare protein energy in several fish species, lipid content is rising (Company et al., 1999; Watanabe, 2002; Li et al., 2012). The current trend to use high-lipid diets has been shown to induce undesirable increases in fat depots or even physiological alterations such as induction of oxidative stress (Kjaer et al., 2008). Thus, carbohydrates are attractive ingredients as they are considered to supply energy at a low cost; however, the inclusion of high amounts of dietary carbohydrates remains controversial. Carnivorous fish are considered to present a limited ability to use dietary carbohydrates (Moon, 2001; Hemre et al., 2002), and their effects on growth depend upon many factors such as the source, concentration, level of food intake and digestibility (Brauge et al., 1994). Gilthead sea bream has been reported to present excellent starch digestibility coefficients (Enes et al., 2008; Couto et al., 2012) and elevated activity of the

Abbreviations: eF1α, elongation factor 1α; F, fiber; FAS, fatty acid synthase; FFA, free fatty acids; GK, glucokinase; G6Pase, glucose 6-phosphatase; G6PDH, glucose-6-phosphate dehydrogenase; HSI, hepatosomatic index; HSL, hormone sensitive lipase; L, lipids; LPL, lipoprotein lipase; LXRα, liver X receptor α; MFI, mesenteric fat index; PCR, polymerase chain reaction; PPARs, peroxisome proliferator-activated receptors; RPL27, ribosomal protein 27; S, starch; SGR, specific growth rate.

* Corresponding author at: Department of Physiology and Immunology, Faculty of Biology, University of Barcelona, Av. Diagonal 643, Barcelona 08028, Spain. Tel.: +34 934021532; fax: +34 934110358.
E-mail address: mnavarro@ub.edu (I. Navarro).

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main enzymes of the glycolytic pathway (Panserat et al., 2000; Couto et al., 2008; Enes et al., 2008), suggesting a possible efficient utilization of dietary starch for energetic purposes in this species.

Most of the studies in cultured fish species regarding high dietary carbohydrate content have focused on the effects of starch origin and inclusion levels on nutrient digestibility and retention efficiency, as well as growth performance (Capilla et al., 2003; Capilla et al., 2004). The response of glucose, lipid or amino acid metabolism enzymes has been reported mainly in the liver as crucial active metabolic organ (Caseras et al., 2000; Panserat et al., 2000; Caseras et al., 2002; Panserat et al., 2002b; Metón et al., 2004; Enes et al., 2006, 2008; Couto et al., 2008). However, and despite its importance in controlling fish energy balance, scarce information concerning the effects of dietary carbohydrates on adipose tissue is available. It has been reported that the lipogenic activity of adipose tissue is hormonally regulated in rainbow trout and modulated by diet in gilthead sea bream (Bouraoui et al., 2011; Cruz-Garcia et al., 2011; Polakof et al., 2011a), suggesting its possible implication on glucose homeostasis, being glucose one of the main lipogenic precursors. Therefore, the study of both, adipose tissue and liver, as well as their inter-relation may help to better explain how fish use dietary lipids and carbohydrates to grow.

Few studies have analyzed the interactions between dietary lipids and carbohydrates and their effects on glucose metabolism in fish (Polakof et al., 2011b; Figueiredo-Silva et al., 2012). In mammals, it is known that high levels of fatty acids disrupt carbohydrate metabolism, eventually causing impaired glucose tolerance (Randle, 1998). A recent study demonstrated the existence of a similar alteration in rainbow trout, where fish developed high fat-induced persistent hyperglycemia and reduced insulin sensitivity (Figueiredo-Silva et al., 2012). The poor utilization of carbohydrates by rainbow trout was linked, at least to some extent, to the use of high-fat diets (Panserat et al., 2002a). These observations pointed out that a reduction in dietary fat content could improve the glycemic control in carnivorous fish fed high-carbohydrate diets, and highlighted the importance of studying the metabolic interactions between dietary macronutrients.

Although in humans it has been shown that some dietary fibers tend to reduce cholesterolemia and improve glucose tolerance (Johnson, 1990; Kishimoto et al., 1995), the effects of different fiber levels in fish diets are still not clear and some discrepancies between species have been reported. In Atlantic salmon, apparent digestibility of lipids was linearly reduced with the inclusion of cellulose, while starch or protein digestibility was not influenced (Aslaksen et al., 2007). However, no significant effects of cellulose inclusion were found on the digestibility of main nutrients in rainbow trout (Hansen and Storebakken, 2007). Most of the research effort has been focused on the effects of dietary fiber inclusion on growth, digestibility and feces characteristics, whereas little is known about its possible implications on lipid and glucose metabolism. In white sea bream, the use of guar gum in the diet had no effect apparently on glucose utilization but contributed to lower endogenous glucose production (Enes et al., 2013).

In order to characterize the lipogenic potential and glucose utilization capacity of adipose tissue and liver of gilthead sea bream, two experimental groups were established. One group was used to test 4 diets with different lipid-to-carbohydrate ratios, in order to know whether gilthead sea bream is able to efficiently use high levels of starch, and how lowering lipid levels affect the fish energetic status. A second group was used to test 3 diets with different fiber-to-carbohydrate ratios in order to detect specific metabolic changes triggered by modifications in starch levels together with the use of a high content of cellulose as a filler agent. To this end the expression of key enzymes and transcriptional factors involved in glucose and lipid metabolism was evaluated. We chose the enzymes lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) as markers of fatty acid uptake and lipolysis respectively, and enzymes related with lipogenesis, fatty acid synthase (FAS), and one of the main enzymes acting as NADPH donor, glucose-6-phosphate dehydrogenase (G6PDH), in order to assess whether dietary increases in carbohydrate-fiber or carbohydrate-lipid ratios might activate lipogenic processes. The expression of two enzymes, hepatic glucokinase (GK) and glucose 6-phosphatase (G6Pase) involved in glucose uptake and release, respectively, was expected to be regulated by diet composition. The lipid metabolism-related transcription factors determined were: liver X receptor α (LXRα) recently involved in triglyceride breakdown in fish adipose tissue (Cruz-Garcia et al., 2012) and peroxisome proliferator-activated receptors α, β and γ (PPARα, PPARβ, PPARγ), being the α and β isotypes promoters of fatty acids use in mammals (Kota et al., 2005) and PPARγ involved in lipid accumulation and adipogenesis also in fish (Bouraoui et al., 2008). We hypothesize that PPARs transcription levels could be related to the dietary lipid content.

All in all we aimed to elucidate the mechanisms involved in the activation of lipid and carbohydrate metabolic pathways at a transcriptional level, in both, adipose tissue and liver, in response to dietary macronutrient replacements. To generate a complete picture we also studied the effects of these dietary manipulations on growth performance, plasma metabolites and adipocyte size.

2. Materials and methods

2.1. Animals and feeding experiment

All animal handling and experimental procedures were conducted in compliance with the experimental research protocol approved by the Committee of Ethic and Animal Experimentation of the University of Barcelona (CEEA 239/09), and the Departament de Medi Ambient i Habitatge (DMAH permit number 5420, Generalitat de Catalunya, Spain) following regulations and procedures established by the European Union, and by the Spanish and Catalan Governments.

A total of 306 juvenile gilthead sea bream (*Sparus aurata* Linnaeus 1758) with an initial body weight of 115 g were maintained in IRTA-SCR facilities (Tarragona, Spain) in 18 cylindro-conical 400 L indoor tanks connected to a closed recirculation system with feed collectors to measure the food wasted to calculate feed intake, in groups of 17 fish per tank (3 tanks per each dietary condition). Water temperature ranged from 21 to 23 °C under natural photoperiod and fish were fed *ad libitum* twice daily with automatic feeders for 15 weeks. Six different diets were formulated by Skretting (Stavanger, Norway) and fish were classified into two experimental groups which were carried out simultaneously. The first group consisted on four isoproteic diets where lipids (L) were decreased by increasing carbohydrates (S) (LS group, diets $L_{23}S_{12}$, $L_{20}S_{16}$, $L_{19}S_{22}$, $L_{17}S_{28}$), and the second group consisted on three isolipidic and isoproteic diets where the amount of carbohydrates was changed at expenses of fiber (F) (SF group, diets $S_{28}F_1$, $S_{19}F_9$, $S_{11}F_{18}$) starting from $S_{28}F_1$, which was the same diet as $L_{17}S_{28}$ ($L_{17}S_{28}F_1$). Ingredients and proximate composition of the experimental diets are presented in Table 1. Subscripts indicate the percentage of inclusion of each component (L, S or F) in the diet. The ratios of distinct protein sources, calculated from the proportion between plant (corn gluten, wheat gluten and soya concentrate) and animal (fish meal) protein ingredients, were the same in all experimental diets (plant-to-animal ratio of 2.3).

After the 15-week feeding period all fish were fasted 24 h before sampling to avoid regurgitation of food and to obtain basal values of plasma metabolites and killed with a blow to the head under anesthesia (3-aminobenzoic acid ethyl ester, MS-222; 100 μg/ml). We sampled 9 fish for each dietary condition (3 fish from each of 3 tanks). Blood was collected and, mesenteric adipose tissue and liver excised, weighed, frozen in liquid nitrogen and stored at −80 °C until further analyses. Small pieces of adipose tissue were also taken for histological studies. Utmost care was taken to assure that all fish had consumed the feeds, which was checked by visual observation of intestinal tract content. Weight values from all fish from each tank were used to obtain the specific growth rate (SGR) and feed conversion ratio (FCR).