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Liver and intestine oxidative status of gilthead sea bream fed vegetable oil and carbohydrate rich diets

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ABSTRACT

This study investigated the effects of dietary lipid source and carbohydrate content on liver and intestine oxidative status of gilthead sea bream juveniles, by assessing antioxidant defences enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPX; glutathione reductase, GR; and glucose-6-phosphate dehydrogenase, G6PD); total, reduced, and oxidized glutathione (GSH); and lipid peroxidation (LPO). Fish were fed for 13 weeks with four diets (2 × 2 factorial design) differing in lipid source (Fish oil- FO or a blend of vegetable oil-VO) and carbohydrate level (0 or 20% of digestible starch). Dietary VO reduced liver and intestine LPO, and enhanced liver GSH redox status (total and reduced GSH), GPX, GR, and G6PD activities, while in the intestine a decrease of G6PD activity and an increase of catalase activity and of reduced GSH (only in the carbohydrate diet) were recorded. Dietary carbohydrate promoted an increase of hepatic SOD and G6PD activities, and a decrease of CAT activity, total, oxidized, and reduced GSH, LPO, and oxidative stress index (OSI) (only in the FO diet). In the intestine, dietary carbohydrate did not induce alterations in LPO or enzymatic antioxidant defences but negatively affected GSH redox status (OSI, reduced and oxidized GSH). Overall, few interactions between dietary lipid source and carbohydrates were recorded. Dietary VO appeared to have a protective role against LPO in both tissues. Dissimilarities in liver and intestine susceptibility to LPO by dietary carbohydrate may reflect differences in glucose and GSH metabolism in the two tissues.

Statement of relevance: Plant related macronutrients improve fish oxidative status.

1. Introduction

Fisheries-related feedstuffs such as fishmeal (FM) and fish oil (FO) have traditionally been used as main sources of protein and lipids in aquafeeds for marine carnivorous fish (Oliva-Teles et al., 2015). However, given that FM and FO are expensive and limited resources, the current trend in aquaculture is towards their replacement with more cost-effective and sustainable feed ingredients, such as terrestrial plant feedstuffs (PF) and vegetable oils (VO).

Nevertheless, incorporation levels of PF in aquafeeds for carnivorous marine fish have several nutritional drawbacks, such as having high amounts of carbohydrates, non-starch polysaccharides, antinutrients, and lacking n − 3 LC-PUFA (long-chain polyunsaturated fatty acids). Although equipped with all enzymatic apparatus required for digestion and metabolic utilization of carbohydrates, carnivorous fish use carbohydrates inefficiently, and dietary carbohydrate inclusion levels above 20% generally lead to decreased growth performance and feed utilization (Enes et al., 2009, 2011). On the other hand, marine fish have limited ability for elongating and desaturating the C18 PUFA characteristics of VO into n − 3 LC-PUFA. Thus, diets for marine fish must include n − 3 LC-PUFA, namely eicosapentaenoic (EPA; 20:5 n − 3) and docosahexaenoic acid (DHA; 22:6 n − 3) (Turchini et al., 2009; Tocher, 2010). Besides being required for normal membrane structure and function, these fatty acids (FA) play vital roles on the inflammatory response as they are precursors of eicosanoids (Sargent et al., 2002; Bell and Koppe, 2010).

Research undertaken until now has demonstrated that feeds for marine carnivorous fish with PF and VO replacing up to 50% and 70% of dietary FM and FO, respectively, have minimal impacts on fish growth performance and feed utilization.

However, the shift in diet formulation from marine resources towards PF and/or VO promotes marked modifications in whole-body lipid deposits and in FA composition of tissues like liver, muscle and intestine (Rosenlund et al., 2010; Benedito-Palos et al., 2011; Torstensen et al., 2011). A decrease in dietary FO leads to an unavoidable decrease of tissue n − 3 LC-PUFA levels with a consequent loss of the beneficial effects of fish for human health. In recent years, research has been devoted to the impact of these new dietary formulas on the metabolic

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processes involved in lipid deposition and FA composition of fish tissues (Torstensen and Tocher, 2010). Changes of fish lipid composition may also affect membrane phospholipids and therefore affect structure, integrity, and function of cell and intracellular membranes. Among other effects, these changes may induce imbalances on fish oxidative status (Crockett, 2008).

It is well known that all aerobic organisms are susceptible to attack by oxidizing agents - free radicals or reactive oxygen species (ROS) - that are produced in the body primarily as result of aerobic metabolism but that are also promoted by external factors, including nutritional ones (Sargent et al., 2002; Mourente et al., 2007; Halliwell and Gutteridge, 2015). Fish are rich in n−3 LC-PUFA and are therefore particularly prone to ROS attack, which can lead to lipids peroxidation (LPO) and eventually to oxidative stress, if ROS attack is not prevented and cellular damage is not repaired (Sargent et al., 2002; Crockett, 2008; Halliwell and Gutteridge, 2015). Detrimental effects of LPO include decreased membrane structure or fluidity, increased membrane permeability to normally impermeable substances, and inactivation of membrane-bound enzymes, with potential pathological effects on cells, tissues, quality and palatability of the final product to consumers (Sargent et al., 2002; Crockett, 2008). To prevent oxidative injury, organisms developed an antioxidant protection system that involves low molecular weight antioxidants (vitamins and other molecules, such as glutathione, GSH) and enzymatic antioxidants (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPX; glutathione reductase, GR) (Halliwell and Gutteridge, 2015).

An adequate knowledge of dietary PF effects on fish's susceptibility to oxidative damage and on defence mechanisms to counteract it is of upmost importance to ensure that PF-rich aquafeeds do not compromise fish health and nutritional value to humans. However, potential interactions between VO and carbohydrates on processes that affect fish oxidative status are poorly known and available data is still apparently contradictory. For instance, there is growing evidence that high levels of dietary LC-PUFA increased LPO in the muscle of rainbow trout, European sea bass, and javelin goby (Álvarez et al., 1998; Luo et al., 2012). On the other hand, dietary substitution of FO by single VO decreased susceptibility to LPO in the liver of turbot, grouper, and black seabream (Stéphan et al., 1995; Lin and Shiau, 2007; Peng et al., 2008) and in the muscle of turbot, gilthead sea bream, Japanese sea bass, and tilapia (Stéphan et al., 1995; Menoyo et al., 2004; Gao et al., 2012; Ng et al., 2013). However, no differences in LPO due to dietary substitution of FO by single VO were reported in the liver Japanese sea bass, Atlantic cod, and European sea bass (Gao et al., 2012; Kjær et al., 2014; Castro et al., 2015a), or in the muscle of Atlantic salmon (Østbye et al., 2011).

Although dietary FA were reported to affect tissue LPO susceptibility in fish, their role in the regulation of enzymatic and non-enzymatic antioxidant defence mechanisms is not clearly understood, as studies simultaneously assessing enzymatic and non-enzymatic antioxidant responses and LPO damages are still scarce (Østbye et al., 2011; Kjær et al., 2014; Castro et al., 2015a).

The potential effects of dietary carbohydrate on fish oxidative status have also received limited attention. However, it seems that dietary carbohydrate protects tissues against oxidative damage (Sagone et al., 1983; Álvarez et al., 1999; Lygren and Hemre, 2001; Pérez-Jiménez et al., 2009; Castro et al., 2015a). This is possibly due to the nature of the glucose molecule, which can scavenge ROS by itself, or through increased activity of the pentose phosphate pathway, that leads to increased production of NADPH via glucose-6-phosphate dehydrogenase (G6PD) activity. NADPH is required for the generation of reduced glutathione (GSH) by GR, and GSH is required for GPX to reduce H_2O_2. Recently, it was shown that dietary carbohydrate increased GSH concentration and GR and G6PD activities, and promoted a decrease of LPO in the liver of European sea bass (Castro et al., 2015a).

Most studies on lipids effects in oxidative status focused on liver and muscle, the target tissues involved in lipid deposition. However, the intestine is also highly susceptible to oxidative stress (Olsvik et al., 2007; Gao et al., 2012; Morais et al., 2012; Castro et al., 2015a) as it has a high cell turnover, and therefore requires more attention regarding this subject.

Thus, the aim of the present study was to evaluate the potential effects of dietary lipid source, carbohydrate content and interactions between both on liver and intestine oxidative status in gilthead sea bream juveniles.

2. Methods

2.1. Experimental diets

Four diets were formulated differing in carbohydrate content (0 and 20% gelatinized starch, diets CH− and CH+, respectively) and lipid source (diets FO and VO) (Table 1). Carbohydrate inclusion in the diets was achieved by replacing protein, which was kept above requirement for the species in all diets (Oliva-Teles, 2000). In the four diets, FM was added as a major dietary protein source to isolate the impacts of dietary VO and to avoid the interference of dietary plant protein on the oxidative status related parameters. The major lipid source of FO diets was cod liver oil. In VO diets, 100% of the cod liver oil was replaced by a VO blend composed of 20% rapeseed, 50% linseed and 30% palm oils. All ingredients were finely ground, well mixed, and dry pelleted in a laboratory pellet mill (California Pellet Mill), through a 3-mm die. The pellets were air-dried for 24 h and stored in a refrigerator at 4 °C until use.

Table 1
Ingredient and chemical composition of the experimental diets.

	Experimental diets			
Lipid source	FO		VO	
Carbohydrates	CH−	CH+	CH−	CH+
Ingredients (% dry weight)				
Fish meal[a]	87.3	65.1	87.3	65.1
Starch[b]	0	20	0	20
Cod liver oil[c]	9.2	11.4	0	0
Vegetable oil blend[d]	0	0	9.2	11.4
Vitamins[e]	1.5	1.5	1.5	1.5
Minerals[f]	1.0	1.0	1.0	1.0
Binder[g]	1.0	1.0	1.0	1.0
Proximate analyses (% dry matter)				
Dry matter (DM)	87.0	86.8	87.2	87.6
Crude protein (CP)	66.3	50.3	66.3	50.4
Crude fat (CF)	18.4	18.4	18.2	18.3
Starch	–	16.8	–	18.0
Energy	22.7	23.3	23.3	22.7
Ash	14.1	11.2	14.3	11.1

Fish oil (FO). Blend of vegetable oils (VO); carbohydrate content: 0% (CH−) or 20% (CH+) gelatinized maize starch.

[a] Steam dried LT fish meal (Superprime), Inproquisa, Madrid, Spain (CP:74.6% DM; CL: 10.1% DM).

[b] C-Gel Instant-12018, Cerestar, Mechelen, Belgium.

[c] Labchem, Laborspirit Lda, Lisboa, Portugal.

[d] 30% palm oil (Colmi, Malaysia), 50% linseed oil (Sociedade Portuense de Drogas, S.A., Portugal) and 20% rapeseed oil (Huilerie Emile Noël S.A.S., France).

[e] 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, Premix, Viana do Castelo, Portugal.

[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), Premix, Viana do Castelo, Portugal.

[g] Aquacube (Guar gum, polymethyl carbamide, Manioc starch blend, hydrate calcium sulphate), Agil, England.