Comparative Biochemistry and Physiology, Part A 203 (2017) 17–23

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Dietary carbohydrates improve oxidative status of common dentex (*Dentex dentex*) juveniles, a carnivorous fish species

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 10 August 2016
Accepted 16 August 2016
Available online 21 August 2016

Keywords:
Antioxidant enzymes
Carbohydrate level
Common dentex
Dextrin
Maltodextrin
Pregelatinized starch
Oxidative status

ABSTRACT

Common dentex (*Dentex dentex*) is an appreciated carnivorous fish with high growth rate and life cycle adaptable to existing farming techniques. Since the use of carbohydrates is an economic and sustainable alternative for a protein-sparing effect, the study of how this macronutrient affects the welfare of carnivorous species must be studied. The aim of the present study was to evaluate the effects of different types and levels of carbohydrates on common dentex oxidative status. Nine isonitrogenous (43%) and isoenergetic (22 MJ kg^{-1}) diets were formulated combining three types (pregelatinized starch-PS, dextrin-Dx and maltodextrin-Mx) and three levels (12, 18 and 24%) of carbohydrates. The activities of catalase-CAT, superoxide dismutase-SOD, glutathione peroxidase-GPX, glutathione reductase-GR and glucose 6-phosphate dehydrogenase-G6PDH, SOD isoenzymatic profile, lipid peroxidation-LPO and protein oxidation-PO were determined in liver and white muscle. SOD and CAT were not affected. GPX in liver and white muscle and GR in liver increased at higher inclusion carbohydrates levels. The lowest levels of GR and G6PDH in both tissues and LPO in liver were observed in maltodextrin groups. No significant effects by carbohydrate source were observed in liver PO and white muscle LPO. Regarding carbohydrate level effect, 18% and 24% dietary inclusion level decreased LPO in white muscle and PO in liver. LPO in liver was also decreased at 24% inclusion level. Altogether, results indicate the use of carbohydrates as an alternative energy source does not produce negative effects on oxidative status of common dentex, on the contrary, even contribute to their oxidative protection.

1. Introduction

It is usually considered that carnivorous fish misused carbohydrates, although it is necessary to determine the maximum levels at which this macronutrient begin to be misused and how the type of carbohydrate affects its use and therefore, the food efficiency and growth performance. The use of carbohydrates is an economic and sustainable alternative for a protein-sparing effect. This possibility has been previously investigated in common dentex (*Dentex dentex*) and overall, good results were obtained by using different carbohydrates sources and levels, including starch, dextrin and maltodextrin in diets for juvenile dentex (Pérez-Jiménez et al., 2009a, 2015).

Common dentex is an appreciated and scarce sparid in the European markets, besides having a presence seasonally unstable. This species has a life cycle adaptable to existing farming techniques and its growth rate in captivity is superior to the natural environment (Riera et al., 1993; Rueda and Martínez, 2001; Stephanis and Divanach, 1993). In addition, common dentex growth rate is higher than that of other cultivated species such as sea bream (*Sparus aurata*) or sea bass (*Dicentrarchus labrax*), kept under the same conditions of temperature and experimental diets. However, despite the different studies performed in this species, there is still a knowledge gap about some parameters related to its culture, mainly regarding to the dietary optimum conditions that optimize both growth and welfare of animals (Pérez-Jiménez et al., 2009a, b, 2015; Rueda and Martínez, 2001).

When a diet is formulated, it has been taken into account the fact that dietary composition has a major influence on both the generation of oxidants and antioxidant defense mechanisms. In this regard, many tests aimed at determining the influence of amino acids, lipids, vitamins or minerals on the oxidative status of fish have been performed (Aminikhoei et al., 2013; Bañuelos-Vargas et al., 2014; Castro et al., 2012, 2015; Martínez-Álvarez et al., 2005; Pérez-Jiménez et al., 2009c, 2012a). However, few authors have studied the role played by different types or levels of dietary carbohydrates on the antioxidant defenses and

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http://dx.doi.org/10.1016/j.cbpa.2016.08.014
1095-6433/

oxidative damage of fish (Álvarez et al., 1999; Castro et al., 2015; Lygren and Hemre, 2001; Pérez-Jiménez et al., 2009c; Rueda-Jasso et al., 2004; Wang et al., 2014). The results of these studies show different responses depending on fish species and dietary formulation. So, although for the effect of the type of carbohydrate results are diverse, in general most of the studies have observed a decreased antioxidant enzymatic activity and oxidative damage when higher carbohydrate levels were included in the diet. Moreover, a link between the influence of carbohydrates and antioxidant defense mechanisms has been established through the pentose phosphate pathway. Thus, an increase in the activity glucose 6-phosphate dehydrogenase (G6PDH) when carbohydrates are used for feeding fish, could be a supply NADPH not only for lipid synthesis, but also for the necessary regeneration from oxidized glutathione to its reduced form (Lygren and Hemre, 2001). This connection between the G6PDH activity and glutathione reduction has been shown in several studies in mammals and fish (Morales et al., 2004; Pandolfi et al., 1995; Salvemini et al., 1999). Additionally, different authors have indicated that glucose can serve as "scavenger" of hydroxyl radicals (OH•) in human phagocytic cells and in fish, which could itself be a mechanism of antioxidant defense (Castro et al., 2015; Sagone et al., 1983).

In this context, the aim of the present study was to evaluate the effects of different types and levels of carbohydrates on the enzymatic antioxidant defenses and the possible lipid and protein oxidative damage in liver and white muscle of common dentex. Additionally, the possible influence of these dietary conditions on the liver and white muscle isoenzymatic pattern of SOD was also evaluated.

2. Materials and methods

2.1. Experimental diets

Nine experimental diets were formulated to combine the use of three carbohydrates with different complexity, pregelatinized starch (PS), dextrin (Dx) and maltodextrin (Mx), and at three levels (12, 18 and 24%) of dietary inclusion. The protein content (43%) remained constant while the proportion of dietary lipids varied according to the carbohydrate content (% lipid/% HC: 25.1/12, 22.5/18, 19.9/24) in order to maintain constant gross energy of the diet (22 MJ kg^{-1}). Dietary ingredients were thoroughly mixed and dry pelleted in a laboratory pellet mill through a 1.9 mm die. The pellets were dried at 35 °C for 24 h and stored in a refrigerator until use. The formulation and chemical composition of the diets, performed according to AOAC methods (AOAC, 2000), are reported in Table 1.

2.2. Animals and experimental conditions

This experiment was directed by trained scientists (following the Federation of Laboratory Animal Science Associations, FELASA, category C recommendations) and was conducted according to the European Union Directive 2010/63/EU on the protection of animals for scientific purposes. Both, University of Granada and Spanish Institute of Oceanography possessed all required licenses from the Animal Research Ethic Committees. Sexually immature common dentex (*Dentex dentex*) were raised at the Marine Culture Experimental Plant of the Spanish Institute of Oceanography in Mazarrón (Murcia, Spain). Fish with an initial body weight of 9.76 ± 0.05 g were randomly distributed into 9 triplicate groups (25 fish/lot) and maintained in cylindrical fiberglass tanks 230 L capacity, supplied with sea water (salinity 37‰) in open circuit with a continuous flow of 6.5 L min^{-1}. The photoperiod was 12 L/12 D. The average water temperature was 25.3 ± 2.0 °C and the average dissolved oxygen levels were not lower than 80% of the saturation level. After a week of adaptation, the experiment was initiated and fish were hand-fed to apparent visual satiation three times a day, during 9 weeks.

2.3. Sampling

Feed intake and mortality were recorded daily and fish were weighted at the beginning and at the end of the experiment (results published by Pérez-Jiménez et al., 2015). Fish feeding was discontinued 24 h

Table 1
Ingredients and proximate composition of the experimental diets.

	DIETS								
	PS_{12}	PS_{18}	PS_{24}	Dx_{12}	Dx_{18}	Dx_{24}	Mx_{12}	Mx_{18}	Mx_{24}
% L/% CH	25.1/12	22.5/18	19.9/24	25.1/12	22.5/18	19.9/24	25.1/12	22.5/18	19.9/24
Ingredients (% dry weight)									
Fish meal[a]	58.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3	58.3
Fish oil	18.8	16.2	13.6	18.8	16.2	13.6	18.8	16.2	13.6
Lecithin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Pregelatinized starch	12.0	18.0	24.0						
Dextrin				12.0	18.0	24.0			
Maltodextrin							12.0	18.0	24.0
Mineral premix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Ascorbyl palmitate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Hydroxypropyl-methyl cellulose	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Microcrystalline cellulose	7.0	3.6	0.2	7.0	3.6	0.2	7.0	3.6	0.2
Proximate analyses (% dry weight)									
Dry matter (DM)	95.4	96.3	95.5	94.9	94.2	93.7	93.8	93.6	91.9
Crude protein	42.8	42.2	43.0	43.1	43.1	43.7	44.5	42.6	44.3
Crude lipid	25.9	23.2	20.0	25.6	23.3	20.7	26.8	22.2	19.8
Ash	10.2	10.2	10.2	10.1	10.2	10.2	10.2	10.5	10.1
NFE[d]	21.1	24.4	26.9	21.1	23.4	25.5	18.5	24.6	25.8
Gross energy (MJ kg^{-1})	22.9	22.3	22.1	22.9	22.3	21.8	23.2	22.0	21.9

[a] Steam Dried LT, Harinas del Atlántico, S.A., Spain (CP: 71.5% DM; CL: 10.6% DM).
[b] Minerals (mg kg^{-1} diet): CO_3Ca, 600; ClK, 200; ClNa, 400; IK, 2; $MoO_4Na_2 \cdot 2H_2O$, 1; $(PO_4H_2)_2Ca \cdot H_2O$, 4000; PO_4H_2K, 3000; SeO_3Na_2, 0.4; $(SO_4)_3Al_2 \cdot 18H_2O$, 1.6; SO_4Co, 2; $SO_4Cu \cdot 5H_2O$, 10; $SO_4Fe \cdot 7H_2O$, 300; SO_4Mg, 1000; $SO_4Mn \cdot H_2O$, 30; $SO_4Zn \cdot 7H_2O$, 50.
[c] Vitamins (mg kg^{-1} diet): retinol, 14; cholecalciferol, 1.4; alpha tocopherol, 210; menadione, 28; thiamine, 40; riboflavin, 56; pantothenic acid, 105; niacin, 350; pyridoxine, 28; folic acid 10.5; cyanocobalamin, 0.1; biotin, 4.2; inositol, 1400; canthaxanthin, 50.
[d] Nitrogen free extract = 100 − (crude protein + crude lipid + ash).