Comparative Biochemistry and Physiology, Part A 152 (2009) 314–321

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Use of different combinations of macronutrients in diets for dentex (*Dentex dentex*) Effects on intermediary metabolism

Amalia Pérez-Jiménez [a,*], M. Carmen Hidalgo [a], Amalia E. Morales [a], Marta Arizcun [b], Emilia Abellán [b], Gabriel Cardenete [a]

[a] Dpto. Biología Animal, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain
[b] Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, 30860 Puerto de Mazarrón, Murcia, Spain

ARTICLE INFO

Article history:
Received 8 September 2008
Received in revised form 4 November 2008
Accepted 4 November 2008
Available online 18 November 2008

Keywords:
Common dentex
Intermediary metabolism
Macronutrients
Plasma metabolites
Tissue composition

ABSTRACT

The influence of the dietary macronutrient balance on the intermediary metabolism of common dentex (*Dentex dentex* L.) was evaluated. Four experimental diets combining high and low levels of macronutrients were formulated. Dentex fed on 43% protein had higher liver and muscle lipid content, corresponding with an increased hepatic G6PDH activity. This "excess" of hepatic lipids at higher protein levels could be used to obtain energy as would be reflected by hepatic HOAD. In the liver, 43% of dietary protein induced higher AlaAT and FBPase activities. Similarly, dentex fed on the $P_{43}C_{28}$ and $P_{38}C_{28}$ diets showed an increased hepatic and muscular gluconeogenic pathways (higher FBPase activity) from amino acids (elevated AlaAT) and/or glycerol (elevated GK). However, changes in glycemia were not observed among dietary treatments. At coronary level, the use of lower dietary protein induced an increase in the activity of glycolytic (PK and HK-IV) and lipolytic (HOAD) enzymes. Considering the overall results and the experimental conditions, it could be suggested that dietary protein could be reduced until 38% without affecting negatively the normal physiology of dentex. Moreover, high dietary carbohydrate levels could not be used efficiently by dentex given that gluconeogenesis occurs.

1. Introduction

One of the most important requirements for the introduction of a new aquaculture species is the establishment of a specific feed that meets all nutritional needs with no negative repercussions in its metabolism. As well as considering nutrient quality, it is necessary to fix the relative proportion of energy contributed by each macronutrient: protein, lipids and carbohydrates. A correct balance of these three nutrients would result in their optimal use, with the consequent positive repercussions on growth and feed utilization. Moreover, this balance would involve the protein, the most expensive ingredient and the one that is incorporated in a higher proportion in foodstuffs for carnivorous fish, being utilized for growth and not for energy-production.

Although the influence of balanced dietary macronutrients on hepatic intermediary metabolism has been shown in several studies, there are not many studies focused to know the effect of dietary composition in white muscle and heart of fish.

Fish are reported to be able to adapt to new nutritional situations by changing their metabolic profile (Walton and Cowey, 1982; Metón et al., 1999; Lundstedt et al., 2004). In general, the main metabolic changes, generated by the nutritional status of the fish, imply that protein-rich diets stimulate the proteolytic and gluconeogenic pathways. However, the partial replacement of proteins by lipids would inhibit the lipogenic pathway, whereas the use of carbohydrates would stimulate glycolysis, glucogenesis and lipogenesis, reducing protein catabolism and gluconeogenesis.

The main effect of high dietary protein levels is to increase hepatic activity of AspAT, AlaAT and GDH enzymes, which represent amino acid catabolism (Suárez et al., 1995; Sánchez-Muros et al., 1998; Gallagher, 1999; Stone et al., 2003; Bibiano Melo et al., 2006). However, other authors have suggested that the activity of these enzymes is unaffected by dietary protein levels (Cowey and Walton, 1989; Kirchner et al., 2003). The partial replacement of proteins by carbohydrates or lipids seems to produce a reduction in the activity of these enzymes (Suárez et al., 1995). The increase of amino acid-degrading enzyme activities, resulting from a higher dietary protein level, is usually also parallel to a reduced glycolytic and increased gluconeogenic pathway activity, as has been observed in the increased hepatic FBPase activity in species like rainbow trout (*Salmo gairdneri*; now *Oncorhynchus mykiss*) (De la Higuera and Cárdenas, 1985; Kirchner et al., 2003, 2005), European eel (*Anguilla anguilla* L.) (Suárez et al., 1995, 2002) and gilthead sea bream (*Sparus aurata*) (Caseras et al., 2002). Similarly, the partial replacement of protein by carbohydrates in the diet induces a decrease in the activity of this enzyme (Suárez et al., 1995, 2002; Panserat et al., 2002). However, at the same level of protein and carbohydrates, increased dietary lipid levels show no significant

* Corresponding author. Tel.: +34 958243247; fax: +34 958243238.
E-mail address: calaya@ugr.es (A. Pérez-Jiménez).

1095-6433/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2008.11.002

Table 1
Composition and proximate analysis of the experimental diets

	Diet			
	$P_{43}C_{28}$	$P_{43}L_{24}$	$P_{38}C_{28}$	$P_{38}L_{24}$
% P/% L/% CH	43/16.7/28	43/24/4.1	38/19.5/28	38/24/13.4
Ingredients (% dry weight)				
Fish meal	58.89	58.89	52.04	52.04
Fish oil	9.68	16.97	13.28	17.73
Lecithin	0.50	0.50	0.50	0.50
Maltodextrin	28.00	4.15	28.00	13.42
Mineral premix[1]	1.00	1.00	1.00	1.00
Vitamin premix[2]	0.50	0.50	0.50	0.50
Choline chloride	0.30	0.30	0.30	0.30
Ascorbyl palmitate	0.10	0.10	0.10	0.10
Hydroxypropyl-methyl cellulose	0.50	0.50	0.50	0.50
Microcrystalline cellulose	0.53	17.09	3.78	13.91
Proximate analyses (% dry weight)				
Dry matter	87.88	95.54	88.94	96.01
Crude protein	44.68	42.80	39.11	38.67
Crude lipid	16.83	23.36	19.64	24.10
Ash	9.68	12.30	9.47	11.93
Nitrogen free extract[3]	28.81	21.55	31.78	25.29
Gross energy (MJ kg^{-1})	22.10	22.99	22.41	22.96

[1] 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.
[2] 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; canthaxantine, 50.
[3] Nitrogen free extract = 100 − (crude protein + crude lipid + ash).

variation in FBPase enzyme activity (Suárez et al., 1995). It has been reported that the activities of hepatic glycolytic enzymes, HK, HK-IV or PFK, increase with low dietary protein levels, fundamentally when this nutrient is partially replaced by carbohydrates (Metón et al., 1999, 2000; Caseras et al., 2002; Suárez et al., 2002; Kirchner et al., 2005). Similarly, low protein diets also seem to reduce hepatic NADPH-generating enzyme activity (Walton, 1986; Barroso et al., 1994), although the absence of this effect has been reported too (Lupiáñez et al., 1989).

In contrast, in several species of salmon, a lipid-rich feed has been reported to decrease lipogenic enzyme activity (Lin et al., 1977; Arnesen et al., 1993). Similarly, Suárez et al. (1995) found an inverse relation between liver G6PDH activity and the dietary lipid level when carbohydrate and protein content were held constant in diets for eels. Similar results were obtained in rainbow trout (Jürss et al., 1985; Gélineau et al., 2001; Suárez et al., 2002), when dietary proteins were replaced by lipids. Boujard et al. (2004) and Wang et al. (2005a) also reported that in European sea bass (*Dicentrarchus labrax*) and cobia (*Rachycentron canadum*), respectively, hepatic G6PDH and ME activity diminished on increasing dietary lipid level. Furthermore, high levels of carbohydrates in the diet stimulate the activities of enzymes involved in lipid synthesis (Likimani and Wilson, 1982; Fynn-Aikins et al., 1992; Suárez et al., 1995; Barroso et al., 2001).

The aim of the present study was to determine how different combinations of protein, lipids and carbohydrates affect metabolism, as in liver as in white muscle and heart, in order to characterize the intermediary metabolism in these tissues and to know the extent to which protein can be reduced, increasing the lipid or carbohydrate levels in diets for dentex, without producing a potential state that can affect the fish quality and production.

2. Materials and methods

2.1. Experimental diets

Considering the proposed aims in the present study and the macronutrient inclusion limits observed in previous studies for dentex (Riera et al., 1993; Tibaldi et al., 1996; Company et al., 1999; Espinós et al., 2003; Skalli et al., 2004; Pérez-Jiménez, 2008), four diets were formulated combining high and low levels of the different macronutrients. Dietary ingredients were thoroughly mixed and dry pelletted in a laboratory pellet mill through a 3 and 4.5 mm die. The pellets were dried at 35 °C for 24 h and stored in a refrigerator until use. Composition and proximate analysis of the experimental diets are presented in Table 1. Chemical composition analysis were performed according to AOAC methods (AOAC, 2000).

2.2. Animals and experimental conditions

Sexually immature common dentex (*Dentex dentex*, Sparidae, Perciformes) were raised at the Marine Culture Experimental Facilities of the Spanish Institute of Oceanography in Mazarrón (Murcia, Spain). Fish were randomly selected and distributed into four triplicate groups of 24 fish each (91.7 ± 1.4 g mean mass). Each group was maintained, at 18.0 ± 1.0 °C, in 500-L tanks continuously supplied with seawater (37‰) at a flow rate of 15 L min^{-1}. Oxygen saturation was always higher than 85%. The photoperiod was regulated as a 12:12 dark/light cycle. After an acclimation period, each experimental diet was randomly assigned to triplicate groups of animals, which were fed by hand, three times a day to apparent visual satiation, for thirteen weeks. Feed intake and mortality was recorded daily and fish in each tank were bulk weighed at the beginning and at the end of the experimental period.

2.3. Sampling

At the end of experimental period, fish were fasted 24 h and then three animals per tank (nine per treatment) were randomly sampled and killed with a sharp blow to the head. Blood samples were taken from the caudal vein with heparinized syringes. Plasma was recovered after centrifugation and stored at −20 °C. After this, liver, heart and white muscle samples were excised and immediately frozen in liquid nitrogen and thereafter stored at −80 °C.

2.4. Plasma metabolites, liver and white muscle glycogen and lipids

Commercial kits were used for the determination of plasma metabolites: glucose (Dipal, 28.160, Trinder, 1969), triglycerides (Dipal, 28.761, Buccolo and David, 1973), total cholesterol (Dipal, 28.601, Meiattini et al., 1978), LDL cholesterol (Dipal, 28.610, Okada et al., 1998), HDL cholesterol (Dipal, 28.628, Naito, 1989) and total lipid (Dipal, 28.215, Cottet and Etienne, 1965) in the plasma. The concentration of total amino acids and soluble protein present in the plasma was determined according to Spies (1957) and Bradford (1976), respectively.

To determine the hepatic and muscular glycogen content, a portion of liver and other of white muscle were homogenized in five volumes of iced-cold distilled water. The homogenate obtained was stored at −80 °C for further assays. The glycogen content was determined by

Table 2
Effect of feeding with different macronutrient levels in the lipid and glycogen content in liver and white muscle of common dentex

Diet	Glycogen (mg glucose g $tissue^{-1}$)		Lipids (mg g $tissue^{-1}$)	
	Liver	Muscle	Liver	Muscle
$P_{43}C_{28}$	120.0 ± 1.6	0.87 ± 0.06	48.91 ± 2.28	17.74 ± 1.19
$P_{43}L_{24}$	112.3 ± 6.8	0.80 ± 0.09	41.36 ± 3.05	18.35 ± 0.88
$P_{38}C_{28}$	101.0 ± 3.5	0.55 ± 0.04	35.26 ± 2.12	11.08 ± 0.68
$P_{38}L_{24}$	117.7 ± 2.3	0.92 ± 0.09	35.94 ± 1.74	14.01 ± 0.84
P	n.s.	n.s.	***	***
C-L	n.s.	n.s.	n.s.	n.s.
P × C-L	**	**	n.s.	n.s.

Values are means ± S.E. (n = 9). In the inferior moiety of the table, results from two way ANOVA are reflected where asterisks indicate significant differences as $^{**}P<0.01$ and $^{***}P<0.001$. Initials n.s. indicate non significant differences.