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Alterations in lipid metabolism and use of energy depots of gilthead sea bream (*Sparus aurata*) at low temperatures

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Abstract

Gilthead sea bream cultured along the northern Mediterranean coast are affected by the winter season when low temperatures reduce fish feed intake and growth. The coldest episodes can provoke a fish pathology known as 'winter disease'. The effects of low temperatures, as well as concurrent fasting, have been studied by transferring three groups of gilthead sea bream from 16 °C to 14 °C, 12 °C and 8 °C. Fish at 12 °C and 8 °C refused food, whereas those at 14 °C were not fed following the temperature drop. Changes in body indices, organ composition, liver metabolism, and in particular, lipid fractions and their fatty acids were analysed on days 7 and 20 after the temperature shift. Only the rapid reduction of non-polar lipids in muscle was common for the three conditions. Fasting effects were linked to the maintenance temperature, being maximal after 20 days at 14 °C where fish body weight, hepatosomatic index, and perivisceral fat were reduced by 18%, 40%, and 60%, respectively. In this group, liver lipids did not change, as was the case for the enzymatic activities of liver glucose-6-phosphate and phosphogluconic acid dehydrogenases (G6PDH and PGADH) and lipoprotein lipase (LPL). In contrast, the liver of sea bream submitted to 8 °C accumulated large amounts of non-polar lipids (from 80 mg to 125 mg in 20 days), changing in size and aspect (bigger, pale, and friable). Simultaneously, liver LPL and hepatic lipase (HL) activities decreased. After 20 days at 8 °C, sea bream exhibited incipient acclimation responses to low temperatures: rising levels of unsaturation ratio in gill and liver polar lipids, of docosahexaenoic acid (DHA) in muscle polar lipids, and of G6PDH and PGADH hepatic activities.

Fish at 12 °C presented some changes similar to those of the group at 14 °C (e.g., in morphological indices, and LPL and HL activities) and others like the group at 8 °C (increases in G6PDH and PGADH), suggesting a temperature threshold for gilthead sea bream (below 13 °C).

Keywords: Cold adaptation; Fasting; Fatty acid profile; Lipid composition; Liver enzymes

1. Introduction

The culturing of gilthead sea bream has been vastly improved by optimizing farming conditions. Nevertheless, in northern areas of the Mediterranean Sea production of this valuable fish is subject to significant water temperature fluctuations throughout the year. During the cold season, food intake is drastically reduced and fish growth comes to a stop (Tort et al., 1998). In particularly cold periods, sea bream can develop a pathological condition known as 'winter syndrome' or 'winter disease' (Bovo et al., 1995; Doimi, 1996; Tort et al., 1998; Sarusic, 1999; Gallardo et al., 2003), resulting in grave economic losses. Symptomatic animals present histopathological changes (hepatomegalia, fatty livers,

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and distended digestive tracts), as well as physiological alterations (increased amino acidaemia, low plasma albumin levels, increased plasma ASAT activity) (Gallardo et al., 2003). Some of these symptoms have also been observed when sea bream are subjected to cold water temperatures such as 12 °C or 8 °C (Ibarz et al., 2003, 2005; Sala-Rabanal et al., 2003). This species stops feeding below 13 °C under experimental conditions (Sánchez et al., 1999; Ibarz et al., 2003), constituting a temperature-induced fasting. Water temperatures below 13 °C are not unusual along the northwest Mediterranean coasts during winter.

Few studies are available on fasting in gilthead sea bream and even fewer still on the simultaneous effects of low temperature and fasting. Sea bream fasted for three weeks at 14 °C exhibited losses in body and liver mass, in addition to lower stores of protein, lipids, and glycogen (Power et al., 2001). This rather typical response to fasting contrasts with the hepatomegalia and liver lipid deposition observed in sea bream following a fall in temperature (Ibarz et al., 2005), or as presented by winter-affected animals (Gallardo et al., 2003).

Understanding the effects of low water temperatures, with the simultaneous induced fasting, would provide important insight into how gilthead sea bream cultured in winter can exhibit a particular pathological condition without a specific agent. In fact, this is the aim of the present study: analysing the physiological alterations that sea bream undergo following three different temperature changes, based on an initial value of 16 °C; specifically, from 16 °C to 14 °C (food-deprived), 12 °C (threshold temperature where animals refuse feeding) and 8 °C. Lipids proved to be the stores most affected by cold or fasting. Therefore, both their tissue distribution and lipid fraction fatty acid profiles (polar and non-polar) were analysed after 7 and 20 days of temperature drop. In addition, the activities of several hepatic enzymes related to lipid metabolism were examined. This study also provides data on the capacity of sea bream to adapt to low water temperatures, as shown by increases in certain enzyme activities, as well as by changes in the fatty acid profiles of polar lipid fractions.

2. Materials and methods

2.1. Animals and experimental conditions

Juvenile gilthead sea bream, obtained during the month of March from a local fish farm (Ametlla de Mar, Tarragona), were acclimated indoors over 15 days at 16 °C under a natural photoperiod (11.5:12.5 — Light: Dark, February). Thereafter, fish were randomly distributed, and adapted for three additional weeks, in three 600 l conical tanks (25 animals in each, at an approximate density of 3–4 $kg \cdot m^{-3}$) connected to a semi-closed recirculating system, with biological and solid filters, an ozone protein skimmer, and a U.V. sterilizer. Water was periodically assessed for nitrate, nitrite, and ammonia concentrations, pH and salinity. Fish were fed commercial pellets (Mistral 3 mm, ProAqua, Dueñas, Valencia, Spain) once a day to apparent satiation. Diet composition comprised: 47% protein, 21% lipids, 11% ash, and 1.5% fibre with an equivalent energy of 19.8 $MJ \cdot kg^{-1}$. Fatty acid composition of the diet was the same as that reported in a previous study (Ibarz et al., 2005).

Samples from four fish per tank were extracted prior to the beginning of the experiment ('Initial' values, $n=12$, including a temperature of 16 °C). In one tank, the water was cooled to 8 °C at a rate of 2.5 °C day^{-1} (8 °C-group). In a second tank, the water was cooled to 12 °C at a rate of 2 °C day^{-1} (12 °C-group). Fish from the third tank were submitted to forced fasting at a temperature of 14 °C (14 °C-group). Samples from 12 animals from each condition were taken 7 and 20 days after the start of water cooling. Tank water temperature was adjusted manually with an EA-1200 (CUBIJEI, Bologna, Italy) cooling unit. Except during temperature drops, the control of water temperature was very precise, with the maximum daily oscillation never exceeding 0.5 °C. Throughout the experimental period no mortality occurred. Animal maintenance and sacrifice were done in accordance with current legislation on animal use for experimentation by the Generalitat de Catalunya (Diari Oficial de la Generalitat de Catalunya, DOGC no. 2450, 214/1997).

2.2. Sampling and analysis

Fish body weight and total length were recorded individually. Blood samples were taken from the caudal vessels using EDTA-Li as anticoagulant. Animals were euthanized by severing their spinal cord. Liver and total perivisceral fat were removed and weighed, while samples of epaxial white muscle and gills were dissected, with all tissues immediately frozen in liquid nitrogen and stored at −80 °C for later analysis. Plasma samples obtained following centrifugation of blood at 13,000 *g* at 4 °C for 5 min were also stored at −80 °C.

The lipid content and fatty acid composition of liver, muscle, or gill samples of 5 animals from each experimental condition were measured. Briefly, total lipid contents were purified following the method of Folch et al. (1957). After the separation of lipid samples