Aquaculture 307 (2010) 49–55

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary restriction on *post-mortem* changes in white muscle of sea bream (*Sparus aurata*)

M.D. Suárez [a], T.F. Martínez [a,*], M.I. Sáez [a], A.E. Morales [b], M. García-Gallego [b]

[a] *Departamento de Biología Aplicada, E.P.S., Universidad de Almería, 04120 Almería, Spain*
[b] *Departamento de Biología Animal, Universidad de Granada, 18071 Granada, Spain*

ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 29 June 2010
Accepted 3 July 2010

Keywords:
Feed restriction
Firmness
Muscle physical properties
SDS-PAGE
Sea bream
Water holding capacity

ABSTRACT

Four lots of sea bream were fed with different ration levels (R100, R75, R50, and R25, corresponding to 100, 75, 50 and 25%, respectively, of the standard ration fed in the fish farm), in order to assess the effects of a 30-day pre-slaughtering period of feed restriction on the textural characteristics of the edible muscle of farmed sea bream (*Sparus aurata*). Results showed that food restriction over 50% influenced negatively total body and individual organ weights. Fish in a good nutritional condition presented lower muscle pH and poorer muscle characteristics, showing soft texture and decreased water holding capacity (WHC). When the intake was moderately restricted (R75), a positive influence on pH and WHC of muscle was found. A 50% reduction of the ration size also delayed muscle softening during cold storage; whereas, a more severe restriction (R25) did not improve this parameter. Electrophoretic studies showed minor changes in the relative optical density of sarcoplasmic and myofibrillar protein fractions attributable to the different ration sizes assayed. These results suggest that the main contributing factor to the observed enhancement in muscle texture due to feed restriction is likely the decrease of lipid deposits in the structural components of the muscle.

1. Introduction

The use of high energy diets in aquaculture allows considerable dietary protein sparing and improves conversion indexes (Alsted, 1990), although, simultaneously, undesirable effects on the quality of the product have been observed. For instance, the increase in carbohydrate or lipid dietary levels has been reported to cause higher fat accumulation in the viscera, this giving reduced slaughter yields (Alexis et al., 1986). An additional undesirable consequence of this feeding strategy is the excess of fat deposited in the carcass, this affecting negatively aspects such as the textural characteristics of the edible muscle (Thakur et al., 2002, 2003), and processing characteristics (Gjedrem, 1997). These facts might explain why cultured fish are less desirable to consumers than wild fish (Johnston et al., 2006), although complex interactions among subjective and objective factors determine consumers' preferences (Verbeke et al., 2007). However, compared with extractive fishing, aquaculture enables influencing the characteristics of the final product by means of different feeding strategies, with the purpose of limiting those factors responsible for lesser commercial appreciation. In this sense, one of the feasible strategies aimed to minimize the disadvantages of high energy diets is the restriction of feed intake prior to slaughtering.

Complete starvation has been suggested by some authors as an approach aimed to improve fish texture (Ginés et al., 2002; Suárez et al., 2009a), although the dramatic effects of starvation on fish yields, together with animal welfare concerns, likely limit the practical application of such strategy on farm. Partial restriction might overcome these undesirable effects associated to complete starvation, whilst maintaining the beneficial effects on texture characteristics. Studies carried out on salmonids showed that a decrease in feed intake caused lower condition factor and fat content (Storebakken et al., 1991), and positive influence in muscle texture (Johansson et al., 2000, in rainbow trout and Einen et al., 1999, in Atlantic salmon).

However, the modifications in textural properties of fish muscle due to diet changes suggest disparate results among different species. Thus, Faegermand et al. (1995) found that fat content in fillet of rainbow trout may increase up to 20% without affecting the texture, whilst Andersen et al. (1997) found that trout fed on high-lipid diets were softer than those fed on low fat diets. Lanari et al. (1999) found differences in the texture of sea bass fed on diets with high-lipid content, and Thakur et al. (2002, 2003) reported a decrease in the firmness of fish due to increased muscle fat.

Besides the influence of lipid contents on muscle texture, the characteristics of muscle proteins decisively determine the *post-mortem* changes in fish muscle. The *post-mortem* evolution of muscle tissue is characterized by successive biochemical modifications resulting in disorganisation of the muscular structure. Proteolysis of cytoskeletal components causes myofilament degradation, and this phenomenon influences the textural changes taking place in fish

* Corresponding author. Tel.: +34 950 015267; fax: +34 950 015476.
E-mail address: tomas@ual.es (T.F. Martínez).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.07.006

muscle during cold storage (Ofstad et al., 1996). Protein changes during *post-mortem* storage can vary depending on a range of factors such as species, physiological conditions, stress prior to death, and *post-mortem* storage temperature. A wide variety of approaches aimed to assess *post-mortem* changes in muscle proteins have been suggested. Recently Caballero et al. (2009) evaluated muscle structural modifications in fillets during cold storage by means of inmunohistochemical techniques in *Sparus aurata*. Previous studies have also shown that changes in the electrophoretic profile of muscle proteins of fish during storage have been virtually non-existent or very subtle, even after long periods (Verrez-Bagnis et al., 2002; Ladrat et al., 2003; Tejada et al., 2003; Suárez et al., in press). On the other hand, changes in the electrophoretic pattern of sarcoplasmic and myofibrillar proteins of fish muscle during storage have been described, and thus, Verrez-Bagnis et al. (2001) found slight changes in sea bass sarcoplasmic proteins during storage; Auburg et al. (2005) suggested a protein marker of degradation process in turbot (*Psetta maxima*) muscle; and Chéret et al. (2006) detected two protein fractions in sea bass after 2-day storage.

The objective of this work was to assess the possible influence of different feed intake levels on the *post-mortem* properties (firmness, water holding capacity, and pH) of sea bream muscle, monitored by physical, biochemical and electrophoretical procedures.

2. Material and methods

2.1. Animals, experimental conditions and sampling

Commercial-sized sea bream (average weight of 303 ± 10 g) (Predomar, S.L. fish farm, Almería, Spain) were distributed in four lots and stocked in polyethylene tanks (4.5 m^3 each) in groups of 100 fish per tank. The tanks were continuously supplied with 1.5 L s^{-1} of filtered and aerated seawater kept at 18 ± 1 °C.

Animals were subjected to a 15-day adaptation period, followed by a 30-day experimental period. Throughout both periods fish were fed twice a day on a commercial feed (47% protein, 21% fat on dry-matter basis).

Control lot was fed daily with an amount equivalent to 2% of fish body weight (100% of the standard ration, designated as R100). The other three lots were fed at different ration sizes: 75% (R75); 50% (R50) and 25% (R25) of control lot. All the animals were fed with R100 ration during the adaptation period.

At the end of the experimental period, after a 24-h fasting period, 25 fish were randomly withdrawn from each tank, and killed according to the requirements of the Council Directive 86/609/EEC (overdose of metacaine, approved euthanasia protocol number C.2.2 of the experimental aquarium AL/2/U, Universidad de Almería, Spain). Once in the laboratory, whole fish were wrapped up in aluminium foil, and stored at 4 °C in covered polypropylene containers for *post-mortem* changes and biometric parameters determinations.

2.2. Biometric parameters and muscle chemical composition

All fish (n = 25 per lot) were weighed and submitted to linear measurements. After muscle firmness and pH determinations, the muscle (after skin removing) and the viscera (whole viscera and separated liver) were dissected and weighed. The hepato-somatic index (HSI) = [100 × (liver weight (g)/body weight (g))], viscero-somatic index (VSI) = [100 × (total viscera weight (g)/body weight (g))] and fillet yield = [100 × (fillet weight/body weight)] were computed. Morphometric indexes such as condition factor=[100×(body weight/total length^3] where weight in g and length in cm and relative profile (maximum height (cm)/total length (cm)) were also calculated.

Additionally, in fish used as the initial point of *post-mortem* evolution study (2 hpm; n = 5), the right side of muscle was removed for chemical composition determinations following AOAC (2000) standard procedures: water content by desiccation in an oven at 105 °C for 24 h; crude protein by Kjeldhal's method (crude protein = N × 6.25) and crude fat extraction by Soxhlet's method.

2.3. Post-mortem changes in muscle

Samples were withdrawn at 2, 10, 24, 48 and 96 h after slaughtering (hpm; n = 25 per lot; 5 fish × 5 sampling time points) for the study of *post-mortem* changes. In each fish, muscle firmness and pH were determined, and then samples of muscle were removed from the dorsal anterior left part of the body. A portion was used immediately for water holding capacity (WHC) determinations. The rest of muscle sampled was frozen in liquid nitrogen and stored at −80 °C until being used for protein extraction and protein hydrolysis studies.

2.4. Firmness

Whole fish firmness was measured by compression of the anterior area to the dorsal fin, above the lateral line, on the left lateral wall of the fish using a Texture Analyser (TXT2 plus "Stable Mycro System"), equipped with a 20-mm cylindrical probe with a load cell of 5 kN, controlled with Texture Expert Exceed 2.52 software (from Stable Micro Systems, Surrey, England). The probe was pressed downwards at a constant speed of 1 mm/s into the fish, until a penetration depth of 5 mm (prior to the breakage of muscle fibres). The maximum force (measured in Newtons) at which muscle fibres were opposed to the pressure was recorded. During the tests, fish were kept in a tray of ice.

2.5. pH

The pH of the flesh was determined using a penetration electrode (Crison, model GLP 21; sensitivity 0.01 pH units) after carrying out a lateral incision in the dorsal muscle in order to place the tip of the electrode deep in the muscle mass (approx. 5 mm depth), according to Ginés et al. (2002).

2.6. Water holding capacity (WHC)

A modification of the original method of Pastoriza et al. (1998), specially designed for fish muscle, was used. The WHC was calculated as the difference between the initial percentage of water in the muscle (determined by oven drying at 105 °C until constant weight) and the percentage of water released after centrifugation (630 g, 30 min, 10 °C), using a tube inside of which another tube was fitted, in such a way that the centrifuged muscle sample (2 g approx.) was never in contact with the water released.

2.7. Extraction of muscle sarcoplasmic (SPP) and myofibrillar (MFP) protein fractions

For the extraction of sarcoplasmic protein fraction (SPP) (only at 2 and 96 hpm) freeze-dried portions of muscle were homogenized (1.5 mg muscle/mL) in chilled low ionic strength buffer (10 mM $NaHCO_3$, 1 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N′N′-tetraacetic acid (EGTA), pH 7.2) by using a mechanical homogenizer (Polytron PT-2100, Kinematica AG, Lucerne, Switzerland) at 10,000 rpm for 1 min. Heating of the homogenates was prevented by keeping samples on ice during the process. The homogenates were centrifuged (10,000 *g*; 20 min; 4 °C) and supernatants were collected. Pellets were resuspended in the same low ionic strength buffer and re-extracted as described above. Supernatants of both consecutive centrifugations were pooled, and together constituted the SPP fraction.

Remaining pellets were again extracted twice in chilled myofibrillar buffer (8 M urea, 50 mM Tris–HCl, 2% v/v Triton X-100, 2 mM