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Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*)

D. Rodríguez-Barreto [a], S. Jerez [b], J.R. Cejas [b], M.V. Martin [b], N.G. Acosta [a], A. Bolaños [a], A. Lorenzo [a,*]

[a] Departamento de Biología Animal (U.D.I. Fisiología), Facultad de Biología, Universidad de La Laguna, 38206 Santa Cruz de Tenerife, Spain
[b] Instituto Español de Oceanografía, Centro Oceanográfico de Canarias (I.E.O.), Vía Espaldón nº 1, Dársena Pesquera, 38120 Santa Cruz de Tenerife, Spain

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ABSTRACT

The aim of this study was to compare the total lipid (TL) content, the lipid class (LC) composition and their associated fatty acids from muscle, liver and ovary of wild and cultured mature females of greater amberjack (*Seriola dumerili*), in order to obtain information to formulate a more suitable diet for this species broodstock. TL content in muscle and liver was higher in cultured fish than in wild fish, mainly due to TG accumulation, while the ovary TL content was higher in wild fish. Regarding to fatty acids profile, the percentage of 18:1*n*-9 in TL and TG was lower in ovaries and muscle of cultured fish than in wild ones. Cultured fish displayed lower proportion of arachidonic acid (20:4*n*-6, ARA) and higher proportions of 18:2*n*-6 and eicosapentaenoic acid (20:5*n*-3, EPA) than wild specimens for all tissues in TL and LC. In contrast, differences in the proportion of docosahexaenoic acid (22:6*n*-3, DHA) between both groups were found only in some tissues and in some LC, being in those cases higher in wild fish. In consequence, cultured fish presented a lower DHA/EPA ratio and a higher EPA/ARA ratio with respect to wild fish. These results suggest that 18:1*n*-9, 18:2*n*-6 and essential fatty acids (EFA), especially EPA and ARA, are not supplied in the appropriate proportions in the diet of cultured fish and could negatively affect their reproductive performance.

1. Introduction

The culture of new high-value and fast growing species could be one of the keys to the future development of the aquaculture sector, and in this regard, the greater amberjack (*Seriola dumerili*, Risso 1810) is a leading candidate for marine aquaculture. This carangid fish, distributed worldwide in temperate and tropical waters, offers excellent flesh quality, high market price and high growth rates in the wild and in captivity (García and Díaz, 1995; Harris et al., 2007; Jerez et al., 2006; Mazzola et al., 2000; Nakada, 2002; Vidal et al., 2008; Yilmaz and Sereflisan, 2011). Regardless of its great potential for the aquaculture industry, the culture of this species is currently limited to the growth of fish captured from the wild (Hamasaki et al., 2009), mainly due to the difficulties for its reproduction. Most of the studies about reproduction in captivity for this species have focused on hormonal induction treatment of wild mature fish (García et al., 2001; Kozul et al., 2001; Lazzari et al., 2000; Papandroulakis et al., 2005; Pastor et al., 2000). Hormone induced spawn has been obtained from cultured fish according to Mylonas et al. (2004), and natural spawning has also been achieved in wild fish kept in captivity and fed raw mackerel (Jerez et al., 2006), however no spawns have been obtained from cultured fish born in captivity and fed by commercial diets (Jerez, unpublished data). The reproduction problems found in this species could be related to several factors, including the use of inadequate broodstock diets which do not fulfil the nutritional requirements of this species.

Regarding nutrients, lipids, fatty acids, and specifically highly unsaturated fatty acids (HUFA) play an important role in the reproductive processes, embryo ontogeny and the early stages of larval development in marine fish (Sargent et al., 2002). Eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are the major HUFA in cell membranes, involved in maintaining their structure and function, although EPA is selectively catabolised with respect to DHA to provide energy during ovary maturation prior to spawning (Tocher, 2003). EPA and arachidonic acid (ARA, 20:4*n*-6) are precursors of a group of highly biologically active compounds known as eicosanoids. ARA eicosanoids derivatives have a wide range of functions in fish reproduction, including pheromonal attraction (Stacey and Sorensen, 2005), steroidogenesis (Henrotte et al., 2011; Mercure and Van der Kraak, 1996; Van der Kraak and Chang, 1990), steroid transport (Hwang et al., 2008), or ovulation and oocyte maturation (Lister and Van der Kraak, 2008; Patiño et al., 2003; Sorbera et al., 2001). Since EPA and ARA compete for the same enzymatic complex to generate different series of prostanoids with different biological activities, the relative proportions of these two fatty acids are even more important than the level of each fatty acid in broodstock diet, as imbalances in the EPA/ARA ratio could lead to deregulated production of different mediators involved in reproduction.

* Corresponding author. Tel.: +34 922 318339; fax: +34 922 318311.
E-mail address: alorenhe@ull.es (A. Lorenzo).

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It is widely accepted that marine fish species have limited ability to synthesize HUFA from their 18C precursors, due a deficient activity of the Δ5 and Δ6 desaturases, enzymes involved in the conversion pathway from 18C to HUFA (Castro et al., 2012; Sargent et al., 2002). Thus, DHA, EPA and ARA are essential fatty acids (EFA) that must be obtained from the diet. It has been shown that the fatty acid composition of fish tissues is directly influenced by dietary profile of fatty acids (Almansa et al., 1999; Cejas et al., 2003; Regost et al., 2003; Torstensen et al., 2000), and comparisons of lipid composition between wild fish and their cultured counterparts have provided a good estimation of the suitability of the diet for lipid nutrition (Alasavar et al., 2002; Cejas et al., 2003, 2004; Oku et al., 2009; Rodríguez et al., 2004). Although total lipid (TL) content and fatty acid composition have been studied in muscle of *S. dumerili* juveniles (Haouas et al., 2010; Thakur et al., 2009), there are no studies on broodstock of this species.

The aim of this study was to compare the TL content, the lipid class (LC) composition and their associated fatty acid from muscle, liver and ovary of wild and cultured mature females of *S. dumerili*, in order to identify possible nutritional deficiencies in cultured fish and to obtain information to formulate a more suitable diet for this species broodstock.

2. Material and methods

2.1. Animal and experimental conditions

From a broodstock group born in captivity in the experimental culture facilities of the Spanish Institute of Oceanography (Tenerife, Canary Islands, Spain), a total of nine mature females of *S. dumerili* (average weight 6.75 ± 1.97 kg, 6 years old) were randomly selected during the second half of the spawning period. During the previous years, fish were kept in an outdoor 500 m^3 raceway tank with continuous water supply (6 renewals tank day^{-1}), oxygen level close to saturation, temperature ranged between 19.8 °C and 23.8 °C, and natural photoperiod with sunlight intensity attenuated by tank covers. Fish were fed a turbot commercial diet (R22, Skretting, Spain; proximate composition: crude protein 52%, crude fat 20%, crude ash 11.4%, crude cellulose 0.3%, carbohydrates 6%, total phosphorus 1.8%) supplied once a day and three days a week (1% of biomass day^{-1}). On the other hand, nine mature females (average weight 14.45 ± 5.12 kg) were captured from the wild during the same spawning period.

2.2. Sampling and assay methods

For both groups of females (cultured and wild), after the sacrifice by an anaesthetic overdose (2-phenoxiethanol, 600 ppm), gonadal maturity was confirmed by visual examination (Holden and Raitt, 1974), biometric parameters of length, and body, gonad and liver weight were measured. Samples of ovary, liver and muscle tissue were collected and stored at −80 °C for lipid analysis. A visual assessment of the organs external appearance and the degree of fat deposit in the peritoneal cavity was carried out.

Moisture content was determined in 300–500 mg samples by thermal drying of samples in an oven at 110 °C until constant weight, according to the Official Method of Analysis of the Association of Official Analytical Chemists (AOAC, 1990).

Total lipid (TL) was extracted from the tissues and diet by homogenization in chloroform/methanol (2:1, v/v) according to the method of Folch et al.(1957). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically (Christie, 1982) and stored in chloroform/methanol (2: 1), containing 0.01% butylated hydroxytoluene (BHT). Analysis of lipid class (LC) composition was performed by one-dimensional double development high-performance thin layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (w/v) KCl (5:5:5:2:1.8, by volume) as developing solvent system for the polar lipid classes and isohexane/diethyl ether/acetic acid (22.5:2.5:0.25, by volume), for the neutral lipid separation. Lipid classes were visualized by charring with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid, and quantified by scanning densitometry using a dual-wavelength flying spot scanner Shimadzu CS-9001PC (Shimadzu, Duisburg, Germany) (Olsen and Henderson, 1989). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and triacylglycerides (TG) were purified by thin layer chromatography (TLC) using the polar solvent system described before for PC and PE purification, and the neutral solvent system for TG. The separated classes were sprayed with 0.1% 2′, 7′-diclorofluorescein in methanol (98%) (w/v), containing BHT, and visualized under ultraviolet light. Bands were scraped off the plates into tubes for the subsequent analysis of fatty acids.

To determine the fatty acid profiles, TL extracts and PC, PE, and TG fractions were subjected to acid-catalyzed transmethylation with 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC) (Christie, 1982). During acid-catalyzed transmethylation, FAME are formed simultaneously with dimethyl acetals (DMA) which originate from the 1-alkenyl chain of plasmalogens. FAME and DMA were separated and quantified using a Shimadzu GC-14A gas chromatograph (Shimadzu, Duisburg, Germany) equipped with a flame ionization detector and a fused silica capillary column, Supelcowax TM 10 (Sigma–Aldrich, Madrid, Spain). Individual FAME and DMA were identified by reference to authentic standards. Prior to transmethylation, nonadecanoic acid (19:0) was added to the total lipid extract as an internal standard.

Results are reported as means ± SD (n = 9). Non-detected fatty acids were considered as 0 value for statistical analysis. Normal distribution was checked for all data with the one-sample Kolmogorov–Smirnoff test and homogeneity of the variances with the Levene test. When necessary, arcsin transformation was performed. Differences between pairs of means were tested using Student's t-test. In all statistical tests used, $p<0.05$ was considered significantly different. Statistical analysis was carried out using the SPSS package (version 15.0 for Windows).

3. Results

The fatty acid profile of the commercial diet used to feed cultured *S. dumerili* broodstock is shown in Table 1. TL content and LC composition of muscle, liver and ovary of wild and cultured greater amberjack are shown in Table 2. TL from muscle and liver in wild fish was significantly lower than in cultured fish. Conversely, TL from ovary was lower in cultured fish. No significant differences between the two groups were found in total polar lipid (TPL) content for muscle and liver, although some particular phospholipids displayed minor differences. Nevertheless, in these organs, the total neutral lipid (TNL) content was much higher in cultured specimens than in wild ones. The compound mainly responsible for the differences between the two groups was TG accumulation in cultured fish, since this lipid class was around 7 fold higher in cultured fish muscle and almost 10 fold higher in cultured fish liver. For both tissues, the total amount of cholesterol (CHO) was lower in wild fish. In contrast, when considering ovaries, TPL content was higher in wild fish than in cultured fish, due to the lower content of sphingomyelin (SM), phosphatidylcholine (PC) and phosphatidylinositol (PI) in cultured fish, while there were no differences in TNL content between groups, and CHO was slightly lower in cultured animals than in wild animals.

The relative fatty acid composition of TL from muscle, liver and ovary is shown in Table 3. In these organs, total level of saturated fatty acids was higher in wild fish compared to cultured ones due to the higher proportions of 16:0 and 17:0, although 14:0 percentages were higher in cultured fish. Among monounsaturated, a higher level of 18:1*n*-9 was found in wild fish muscle and ovary, while liver showed