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Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil

Rachid Ganga [a,*], Daniel Montero [a], John Gordon Bell [b], Eyad Atalah [a], Eneko Ganuza [a], Orestes Vega-Orellana [a], Lluis Tort [c], Laura Acerete [c], Juan Manuel Afonso [a], Tibiabin Benitez-Sanatana [a], Agustin Fernández Vaquero [d], Marisol Izquierdo [a]

[a] Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria, Instituto Canario de Ciencias Marinas, Transmontaña s/n, 35416 Arucas, Las Palmas, Canary Islands, Spain
[b] Institute of Aquaculture, University of Stirling FK9 4LA, Scotland, UK
[c] Departamento of Cell Biology, Physiology and Immunology, Universitat Autonóma de Barcelona, Bellaterra 08193, Spain
[d] Biomar Iberia/ProAqua Nutrition, S.A., A-62, Km, 99, ES-34210 Dueñas, Spain

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ABSTRACT

The physiological response to stressors in fish, including hormonal profiles and associated tissue responsiveness, is less documented. The aim of this study was to evaluate feeding gilthead sea bream (*Sparus aurata*) with diets containing linseed oil (LO) and soybean oil (SO) as substitutes to fish oil (FO) and their effect on fatty acid profile of head kidney and the consequent effect on stress response to a crowding challenge. Fish were fed 8 experimental diets with different levels of substitution 0% (FO), 70% (70LO, 70SO, 20LO50SO and 50LO20SO) and 100% (100LO, 100SO and 50LO50SO) over a period of 8 months. At the end of the feeding trial, samples of head kidney were collected for biochemical analysis and the fish were challenged by a crowding test. During the challenge, samples of plasma for cortisol analysis were collected at 0 h, 2 h, 5 h, 24 h, 48 h and 1 week in order to study acute and chronic stress responses. Results showed that fish fed vegetable oils (VO) had significantly decreased ARA, EPA, DHA and n-3 HUFA, while LA, LNA and total C18 PUFAs were significantly increased. The basal cortisol levels were significantly increased in fish fed 70LO, 100LO, 50LO20SO and 50LO50SO. The physiological response to crowding was significantly affected by the diet. After 2 h of crowding, all the treatments showed higher cortisol, with fish fed 100LO had significantly the highest response registering 131.38 pg/ml. After 5 h and 24 h, plasma cortisol was reduced in all treatments except in 50LO20SO. After 48 h of crowding, the plasma cortisol was increased in all treatments with the maximum value seen in fish fed 100LO (72.12 pg/ml). These levels were decreased in fish fed FO, 70LO, 100LO and 50L050SO after 1 week of crowding, but remained higher in fish fed 70SO, 100SO, 20LO50SO and 50LO20SO. In conclusion, fish fed LO diets showed the same response pattern as the control but with higher intensity regaining the basal levels after 1 week as the control, while fish fed SO had a slow response but changed the pattern characterized by a lower response at the beginning and longer recuperation without regaining the control value even after 1 week.

Introduction

Finfish aquaculture has traditionally used diets containing large amounts of fish meal (FM) and fish oil (FO), primarily as a cost-effective source of highly digestible animal protein and lipids. Moreover, FO is an important dietary source of omega-3 fatty acids, and in particular the long chain polyunstaurated fatty acids (PUFA), eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3) (Ackman, 1989; Henderson and Tocher, 1987). The steady increase in global production volume in aquaculture by 8–10% a year (Tacon, 2004; Tacon et al., 2006) has resulted in increasing prices and limited availability of these ingredients in the market. Consequently, it is expected that the demand for FO by aquaculture will probably exceed available resources over the next decade (Tacon, 2004; Bostock et al., 2010). Therefore, recently much interest has focused on researching alternatives to FO and FM in aquafeeds, and many studies have reported the successful use of vegetable oils (VO) and vegetable proteins as good substitutes without compromising fish growth or feed utilization (Torstensen et al., 2000; Bell et al., 2001, 2002; Caballero et al., 2002; Montero et al., 2003, 2005; Izquierdo et al., 2003, 2005).

VOs are devoid of n−3 highly unsaturated fatty acids (HUFA), including EPA and DHA while they contain high levels of C18 PUFA, linoleic acid (LA; 18:2n−6), linolenic acid (LNA; 18:3n−3) and monounsaturated fatty acids (MUFA; mainly oleic acid OA, 18:1n−9).

* Corresponding author.
E-mail address: rachid.ganga@gmail.com (R. Ganga).

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Thus, including linseed oil (LO) and/or soybean oil (SO) in diets for sea bream have demonstrated to reduce n−3 HUFA content in fish tissues, while the levels of C18 PUFA were significantly increased (Montero et al., 2003; Izquierdo et al., 2005; Wassef et al., 2009; Castro et al., 2010), confirming that these components cannot be synthesized de *novo*, and subsequently must be obtained from the diet (Ghioni et al., 1999). Moreover, reducing HUFA content could have many physiological and metabolic impacts in fish, by inducing structural modifications, such as integrity, fluidity and permeability of the cells (Yaqoob, 1998) that in turn modulate the functional properties of the membranes (Wills, 1985; Christon et al., 1992). Particularly in fish, HUFA were reported to modulate stress response (Montero et al., 1998, 2003; Tago et al., 1999) and some studies have suggested that these effects could be mediated partly by eicosanoids (Koven et al., 2001a,b; Van Anholt et al., 2004a,b; Ganga et al., 2006; Ganga et al., 2011) that are produced from C20 HUFA (Bell et al., 1991) in stressful situations.

Nowadays, high densities are commonly used in intensive aquaculture (Turnbull et al., 2005) to improve business profitability. This practice is considered as potential source of stress, causing negative effects on fish growth rate and welfare (Lefrançois et al., 2001; Ellis et al., 2002; Van de Nieuwegiessen et al., 2008, 2009), survival and feeding rate (Rowland et al., 2006). In teleosts, the stress response comprises of a number of physiological processes, which are largely regulated by the hypothalamus–pituitary–interrenal (HPI) axis. Cortisol is considered a key response to stress in fish and its production is under the control of the HPI axis activation (Wendelaar Bonga, 1997; Mommsen et al., 1999). Several studies have reported a relationship between the stress response in fish under crowded conditions and increased plasma cortisol levels (Montero et al., 1999; Ruane and Komen, 2003; Trenzado et al., 2006; Van de Nieuwegiessen et al., 2008, 2009). The magnitude and duration of cortisol response depends on the type, intensity and duration of the stressor as well as the feeding history of the animal (Wendelaar Bonga, 1997; Iwama et al., 2006). Moreover, under conditions of acute stress, cortisol elevation usually lasts hours, but with chronic stress, such as prolonged confinement, values may remain high for days or weeks (Pickering and Pottinger, 1989; Iwama et al., 2006).

The aim of the present study was to contribute to the understanding of how feeding sea bream with vegetable lipids from LO and SO in different proportions alters the dietary n−3/n−6 PUFAs ratio, on the fatty acids metabolism and their incorporation in head kidney tissue and their consequent effect on stress response caused by crowding. This was studied by measuring the plasma basal and post-stress cortisol levels.

Materials and methods

Fish and diets

One thousand two hundred juveniles of gilthead sea bream (*Sparus aurata*) (45 g initial body weight corresponding to 200 days) were provided by a local farm (ADSA S.A. Gran Canaria, Spain). The experiment was carried out at inland facilities of Instituto Canario de Ciencias Marinas (Las Palmas, Canary Islands, Spain). Fish were distributed in 24 fiberglass tanks of 500 l (50 fish/tank, each diet assayed in triplicate) supplied with continuously running seawater, constant aeration and natural photoperiod (12 h:12 h L:D). Along the experimental period, water temperature and dissolved oxygen ranged between 20–24.2 °C and 5.8–7.4 ppm, respectively. Experimental diets were provided by Proaqua (Dueñas, Spain) as extruded 3 and 4.5 mm pellet. Eight isoenergetic and isonitrogenous diets formulated containing different ingredients (Table 1) with lipid content of approximately 17%. Anchovy oil was the only added lipids source in the control diet (FO). While in the other diets, FO was replaced by linseed (LO) or soybean oil (SO) oils at 70% (70LO and 70SO, 20LO50SO, 50LO20SO) or 100% (100LO, 100SO and 50LO50SO). Fish were hand fed the experimental until apparent satiation (3 times/day, 6 days/week) for 240 days, 3 mm pellet was used up to 80 g of fish weight and then 4 mm pellet was used to the end of the feeding trial.

Table 1
Ingredients of the experimental diets used.

	% of dry weight
Oils (Fish oil[a]/linseed/soybean)	16.32
South-American fish meal	47.26
Wheat	7.00
Soybean meal 47%[b]	25.00
Sunflower meal	3.67
Vitamins premix[c]	0.27
Minerals premix[d]	0.48

[a] South-American, anchovy oil.
[b] Soybean meal with 47% as a dry protein, "no GMO".
[c] Vitamin premix contained: A — Retinol 11200 IU/kg, D3 — Cholecalciferol 112 IU/kg, E — Tocopherol 280 mg/kg, C — Ascorbic acid 336 mg/kg, B1 — Thiamin 9 mg/kg, B2 — Riboflavin 15.7 mg/kg, B3 — Nicotinic acid/Niacin 179.2 mg/kg, B5 — Panthothenic acid 31.4 mg/kg, B6 — Pyridoxin 13.4 mg/kg, B8 — Biotin 0.5 mg/kg, B9 — Folic acid 4.5 mg/kg, B12 — Cyanocobalamin 0.036 mg/kg, K — Menadion 6.7 mg/kg, Inositol 44.8 mg/kg.
[d] Mineral premix: I 4.5 mg/kg, Zn 44.8 mg/kg, Fe 67.2 mg/kg, Cu 3.6 mg/kg, Mn 14.6 mg/kg, Mg 136.1 mg/kg, Co 0.2 mg/kg and Se 0.06 mg/kg.

Biochemical analysis

Lipid peroxidation products were determined as thiobarbituric acid reactive substances (TBARS) and they showed no significant difference between the diets, ranging between 8.56 and 3.85 mmol of malonaldehyde (MDA)/kg of wet diet. No dietary difference was observed on TBARS concentration ($P<0.05$).

Extraction of total lipids from diets and fish head kidney (HK) was performed by the method of Folch et al. (1957) using a mixture of chloroform:methanol (2:1)(v:v) containing 0.01% BHT, as an antioxidant, followed by phase partition with KCl (0.88%). Vigorous vortex mixing followed by centrifugation to assist separation of chloroform and aqueous layers extracted the lipids from diet and head kidney samples. The lower layer was filtered through Whatman filter paper and dried under nitrogen; total lipids were weighed following desiccation.

Fatty acid methyl esters were produced from aliquots of total lipids extracted from different samples by acid-catalyzed transmethylation performed overnight at 50 °C as described by Christie (1982). Fatty acids methyl esters were separated and quantified by gas-chromatography (GC; Thermo Finnigan) with He as a carrier gas using a fused silica, carbowax 20 M (30 m × 0.32 mm × 0.27 m) column (Supleco, Bellefonte, USA). The initial temperature of the column was set to 170 °C for 10 min, and then it was raised to 220 °C at 2.5 °C/min and finally maintained at 215 °C for a further 5 min. The temperature of the injection port was 250 °C. The peaks in the chromatogram were identified by comparison to a well-characterised external standard (EPA 28, Nippai, Ltd Tokyo; Japan). Individual methyl esters were identified by comparison with known standards and published data.

The control diet (FO), formulated with 100% FO, contained approximately 37% total saturates, mainly 16:0, almost 29% total monounsaturated fatty acids with approximately one-third as 18:1n−9, 6% n−6 fatty acids, predominantly 18:2n−6, and 23% n−3 fatty acids predominantly n-3HUFA, mainly EPA (Table 2). Inclusion of different VO levels resulted in increased percentages of 18:1n−9, 18:2n−6 and 18:3n−3 with concomitant decreased proportions of n−3 HUFA, total PUFAs and long chain monoenes. Thus, the content on 18:2n−6 increased from 4% in FO to 16% and 38% of total fatty acids in 100LO and 100SO diets respectively, in parallel with increasing LA inclusion. 18:3n−3 was also increased from 0.5% in FO diet to 32% and 38% in 70LO and 100LO diets respectively. The 20LO50SO and particularly 50LO20SO diet showed higher contents of n−3, n−6 and n−9 fatty acids. The diet 50LO50SO had 18.97%, 27.15% and 22.95% of 18:1n−9, 18:2n−6 and 18:3n−3 respectively, and registered the lowest content on n−3 HUFA, with only 4.71%.