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Effect of dietary lipids on plasma fatty acid profiles and prostaglandin and leptin production in gilthead seabream (*Sparus aurata*)

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Abstract

The aim of this study was to investigate the effects of different levels of substitution of fish oil by vegetable oils rich in oleic, linoleic and linolenic acids on gilthead seabream plasma and leukocyte fatty acid compositions and prostaglandin (PG) and leptin production. Juvenile seabream of 24 g initial body mass were fed four iso-energetic and iso-proteic experimental diets for 281 days. Fatty acid composition of plasma lipids was markedly affected by the inclusion of vegetable oils (VO). ARA (arachidonate), EPA (eicosapentaenoate) and DHA (docosahexaenoate) were preferentially incorporated into polar lipids of plasma, and DHGLA (di-homogammalinoleate) accumulated with increased vegetable oil inclusion. Dietary treatments resulted in alterations of DHGLA/ARA ratios, but not ARA/EPA. ARA-derived PGE_2 production in plasma was not affected by vegetable oils, in agreement with similar eicosanoid precursor ratio (ARA/EPA) in leukocytes total lipids and plasma phospholipids among fish fed with the different dietary treatments. Feeding vegetable oils leads to a decrease in plasma EPA which in turn reduced plasma PGE_3 concentration. Moreover, PGE_3 was the major prostaglandin produced in plasma of fish fed fish oil based diet. Such findings point out the importance of EPA as a precursor of prostaglandins in marine fish, at least for the correct function of the blood cells, and correlates well with the predominant role of this fatty acid in immune regulation in this species. A negative correlation was found between plasma PGE_2 and leptin plasma concentration, suggesting that circulating levels of leptin may act as a metabolic signal modulating PGE_2 release. The present study has shown that increased inclusion of vegetable oils in diet for gilthead seabream may profoundly affect the fatty acid composition of plasma and leukocytes, specially HUFA (highly unsaturated fatty acids), and consequently the production of PGE_3, which can be a major PG in plasma. Alteration in the amount and type of PG produced can be at least partially responsible for the changes in the immune system and health parameters of fish fed diets with high inclusion of VO.

Keywords: Leptin; Leukocytes; Seabream; Plasma; Prostaglandins; Polyunsaturated fatty acids

1. Introduction

Supply of fish oil (FO) and fish meal for aquaculture feeds has shown a reduction due to stagnation in capture fisheries as a consequence of over fishing and natural events such as El Niño. FO production has suffered a decrease and fluctuations which results in higher prices and uncertainty of its availability. Some scientists expect that supplies of FO for aquaculture production will become critical between 2005 and 2010 (Bell and Sargent, 2003). Thus, there is a strong need for diversification of feed ingredients used in aquaculture (Kaushik, 2000), and considerable research efforts have been directed towards the evaluation of other non-marine ingredients as a potential substitutes in fish diets (Hardy et al., 2001).

Previous experience has shown that it is possible to replace up to 60% of the FO by vegetable oils (VO) in diets for seabream without compromising growth, survival, fish feed utilisation or fillet organoleptic properties, when fish are fed either for a medium (3 months, Izquierdo et al., 2000, 2003) or long period (8 months) (Menoyo et al., 2004; Izquierdo et al.,

Abbreviations: ARA, arachidonic acid; DHGLA, di-homogammalinoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; HUFA, highly unsaturated fatty acid; LA, linoleic acid; LNA, linolenic acid; LO, linseed oil; NL, neutral lipid; OA, oleic acid; PL, polar lipid; PG, prostaglandin; RO, rapeseed oil; VO, vegetable oil.

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in press). However, a substitution increased up to 80%, significantly reduces growth and conversion indices (Izquierdo et al., in press) altering normal hepatocyte and enterocyte morphology (Caballero et al., 2003), and negatively affecting immune functions and post-stress plasma cortisol response (Montero et al., 2003). In addition, inclusion of soybean or rapeseed oil with of 60% substitution reduced macrophage phagocytic activity (Montero et al., 2003) whereas the reduction in the eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids in diet has been shown to significantly inhibit serum alternative complement pathway activity (Montero et al., 1998). The role of dietary highly unsaturated fatty acids (HUFAs) on immune regulation seems to be mediated, at least partly, by the eicosanoid production in target tissues. Eicosanoids are oxygenated derivatives of polyunsaturated fatty acids produced from membrane phospholipids by the action of phospholipases, cyclooxygenases and lipoxygenases (Rowley et al., 1995). In broad terms, eicosanoids are produced in response to stressful situations, both at a cellular and whole body level (Sargent et al., 1999). The principal substrate is arachidonic acid (ARA; 20:4n-6), generating 2-series prostanoids and 4-series leukotrienes, that have potent proinflammatory effects (Secombes, 1996). In addition, EPA, DHA and di-homogammalinolenic (DHGLA; 20:3n-6) are also important eicosanoid substrates in fish due to their high concentration in membrane phospholipids of aquatic organisms (Henderson and Sargent, 1985). Non-esterified ARA, through the action of cyclooxygenase enzymes, yields 2-series prostanoids (prostaglandins (PG) and thromboxanes) and, through the action of lipoxygenase enzymes produce 4-series leukotrienes and lipoxines (Lall, 2000). Alternatively, the metabolic derivatives produced from non-esterified EPA are 3-series prostanoids and 5-series leukotreines and lipoxines whereas the 1-series prostanoids derived from DHGLA. These compounds are known to play essential roles in the regulation of many physiological and immunological processes in the body (Balfry and Higgs, 2001). The effect of dietary VO on prostaglandins production (in different fish organs) has been previously documented in freshwater and some Nordic marine fish species (Bell et al., 1991, 1992, 1993, 1994b; Henderson et al., 1996), but no studies have been conducted until now on eicosanoid production in seabream, or their alteration by dietary lipids.

Leptin is known as a multifunctional hormone that plays numerous important roles in homeostasis, immune function and reproduction. Its major role pertains to the regulation of energy balance by decreasing food intake and increasing energy expenditure (Van Dijk, 2001). The level of secreted leptin is proportional to body fat level and, through its action on hypothalamic centers, leptin suppresses food intake and increases energy expenditure (Frederich et al., 1995). The reduction in energy availability leads to impairments in humoral immunity and hence leptin has been proposed as a neuroendocrine signal between body fat and immunity regulating humoral immune responses (Demas and Sakaria, 2005). Direct actions of leptin on immune cells seem to affect disease resistance through on hematopoiesis, proinflammatory response and other immune cell functions (Gainsford and Alexander, 1999; Fantuzzi and Faggioni, 2000). Leptin stimulates macrophages and neutrophils, its production is increased during inflammation and delays apoptosis of human mature neutrophils "in vitro" (Bruno et al., 2005). Leptins could also affect human Natural Killer Cell lines function (Zhao et al., 2003). Moreover, recently, Zerani et al. (2005) demonstrated the role of leptin as a metabolic signal of PG release in rabbit. However few studies have been conduct with leptin production in fish (Volkoff et al., 2003), and no one of them deal with their relation with dietary fat or prostaglandin production.

The aim of the present study was to investigate the effect of different levels of substitution of fish oil by vegetable oils (rich in oleic (OA), linoleic (LA) and linolenic (LNA) acids) on seabream plasma and leukocyte fatty acid compositions and effects on prostanoids and leptin production.

2. Materials and methods

2.1. Fish and husbandry

Two thousand four hundred juvenile seabream (*Sparus aurata*), obtained from a local fish farm (ADSA, Las Palmas, Spain) of 24g initial weight were maintained at the Instituto Canario de Ciencias Marinas (ICCM) (Canary Islands, Spain). Fish were distributed randomly into 16×1000 L polyethylene circular tanks (150 fish/tank, each diet assayed in quadruplicate) supplied with continuous seawater (36‰) flow and aeration. Fish were fed under natural photoperiod (approximately 12:12 L/D). Water temperature and dissolved oxygen during the experimental period ranged between 21.9–22.4 °C and 5.5–7.2 ppm, respectively. After 2 weeks of acclimation, the experimental diets were hand-fed until apparent satiation three times a day at 9:00, 12:00 and 15:00 h, 6 days per week. Fifteen fish were sampled for biochemical parameters at the beginning and after 98 days of the experiment period.

2.2. Diets

Four iso-energetic and iso-proteic experimental diets were formulated with a constant lipid content of ~22%. Two different VO blends, included at a 60% substitution of dietary FO, were used for seabream. The diets contained rapeseed, linseed and palm oils in ratio 15:60:25 in diet 60 LO and 40:40:20 in diet 60 RO. A 100 LO diet with 100% substitution of fish oil by the blend in diet 60 LO was also included. The diets were prepared by Nutreco ARC, Stavanger, Norway. The fatty acid compositions of the 5 mm diets were analyzed and were shown in Table 1.

2.3. Sampling procedure

After 281days, fish were individually sampled from each tank for final sample collection. Blood was collected from the caudal vein in heparinised syringes from 6 fish from 3 tanks per diet (18 fish per diet) and transferred to an eppendorf tube