Fish & Shellfish Immunology 68 (2017) 404–410

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Administration of a polyphenol-enriched feed to farmed sea bass (*Dicentrarchus labrax* L.): Kidney melanomacrophages response

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ARTICLE INFO

Article history:
Received 19 May 2017
Received in revised form
13 July 2017
Accepted 21 July 2017
Available online 22 July 2017

Keywords:
Polyphenols
Sea bass
Tyrosinase
Melanomacrophage centres
Immune response

ABSTRACT

The reinforcement of the defense mechanism of fish, through the administration of immunostimulants, is considered as a promising alternative to vaccines. Natural immunostimulants such as polyphenols, flavanoids, pigments and essential oils can modulate the innate immune response. In lower vertebrates, melano-macrophage centres, i.e. clusters of pigment-containing cells forming the extracutaneous pigment system, are wide-spread in the stroma of the haemopoietic tissue, mainly in kidney and spleen. In fishes, melano-macrophage centres play an important role in the immune response against antigenic stimulants and pathogens.

In the present study, we evaluated the effect of a polyphenol-enriched diet on the health status of European sea bass (Dicentrarchus labrax L.). Farmed sea bass were administered a feed containing a phytocomplex, rich in catechins and epigallocatechins, which was obtained from the seeds of Canosina Nero di Troia *Vitis vinifera* and mixed with conventional feed at two different concentrations. The effects of such a diet were investigated in juvenile and commercial size samples, i.e. undergoing a short- and long-term period of diet, respectively, focusing on their extracutaneous pigmentary system and, in more detail, on the enzymatic activities leading to melanin biosynthesis. Our results show that prolonged dietary treatments with higher concentration of polyphenols might modulate tyrosinase activity and gene expression in commercial size fishes. An increase of melano-macrophage activity is correlated to a stimulation of cytoprotective functions against antigenic stimulants and pathogens, as an expression of a robust and protective adaptive immune response.

1. Introduction

In recent years, the relationship between nutrition and health has aroused increasing interest [1]. It is well known that human and animal health are strongly correlated [2] and currently attention is focused mainly on human diet. The consumption of fish from acquaculture plants has been increasing, therefore the health status of farmed fish needs to be strongly monitored in order to avoid any side effects for consumers [3,4].

The European sea bass (*Dicentrarchus labrax* L.) is the most important commercial fish widely farmed in the Mediterranean Sea. The infectious diseases, which are closely associated to its breeding conditions, represent a relevant problem not only for the aquaculture industry, but also for human health. Some dietary approaches have been afforded to improve the health and growth of farmed fishes as well as the economic and environmental sustainability of acquaculture industries. Antibiotics and chemotherapeutics are commonly used to prevent and treat fish infections and diseases, however, they may generate resistant pathogens, bioaccumulation, and environmental pollution. A functional diet-based approach can improve the fish health status and represents a natural alternative to conventional treatments, moreover it can be cost effective compared to the use of drugs, hormones, and antibiotics [5,6]. For instance, the administration of probiotics and prebiotics to fish seems to favor the growth of a protective gut

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http://dx.doi.org/10.1016/j.fsi.2017.07.043
1050-4648/

microbiota [7,8], which, for its immunomodulating activities, may represent a very promising strategy for the biological control of aquaculture. Lot of studies have examined the effect of different diets and vitamin intake not only on cancer prevention but also on oxidative stress disorders. Many compounds of food and beverages have been identified as potential chemopreventive agents [9-11]; for example, phytochemicals, such as polyphenols, may function as potential protective agents against various diseases, such as cancer, cardiovascular, neurodegenerative and oxidative stress disorders in humans [12-14]. Polyphenols, largely present in fruits, vegetables and beverages such as wine and tea [15], have been shown to scavenge oxygen- and nitrogen-derived free radicals, modulating antioxidant enzymes and cellular redox transcription factors [16]. The most common polyphenols are phenolic acids, flavonoids, catechins, stilbenes, proanthocyanidins, ellagitannins and anthocyanins. The biological properties of polyphenols depend on their molecular structure; moreover their antioxidant activity is due, *in vitro* and *in vivo*, to their interactions with metal ions [17,18]. Metal ions are the main source of reactive oxygen species (ROS) which play an important role in the generation of oxidative stress, DNA damage and cell death. It is well documented that catechol and gallol are effective metal ion chelators [17,18], and they are chemopreventive agents. Besides, some polyphenolic compounds were found to inhibit tumor cell proliferation by inducing apoptosis events, hence suggesting their potential use in the management of chemoresistant tumors [19,20].

Lower vertebrates present a visceral pigment cell system, consisting of clusters of pigment-containing cells that form the melano-macrophage centres (MMC). Unlike the cutaneous one, such visceral pigmented system is not derived from the neural tube and is localized in liver, spleen, kidney and other body districts [21]. In the immune response of fish against antigenic stimulants and pathogens, it has been demonstrated that MMCs play an important role [22-24]. In fishes, the presence of melanin pigments has been associated to protective functions against pathological and stress conditions [22]. Pigment accumulations are caused by nonspecific immune mechanisms [23,25,26]. Several authors have suggested the involvement of the MCCs of kidney, liver and spleen as a possible monitor of the well-being of fish populations [22,27,28]. Recently, Magrone et al. [29] demonstrated that the administration of a polyphenol-enriched feed to farmed sea bass resulted in a lower level of intestinal proinflammatory cytokines and in a higher production of spleen IFN-γ, as an expression of a robust and protective adaptive immune response. Moreover, spleen MMCs number and area increased, thus indicating that a protective response was induced by such dietary regimen.

It was demonstrated that the MCCs of kidney, liver and spleen not only express the tyrosinase gene [30,31] and the tyrosinase related proteins (TRP1 and TRP2) genes [32], but also exhibit tyrosinase enzymatic activity [26,31-34]. Tyrosinase (EC 1.14.18.1) is the main enzyme involved in melanin biosynthesis. This copper-containing enzyme catalyzes the hydroxylation of L-tyrosine to L-3,4- dihydroxyphenylalanine (L-dopa) (monophenolase or cresolase activity, EC 1.14.18.1) and the subsequent oxidation of *o*-diphenols to o-quinones (diphenolase or catechol oxidase activity, EC 1.10.3.1) [35]. In a previous research, we highlighted a possible correlation between fish kidney disease and the level of dopa-oxidase activity, which was ascribable to the presence of tyrosinase in melanosomal protein extracts of kidney MCCs, thus suggesting such parameter as a possible indicator for fish kidney disease [28].

Data in literature report the involvement of peroxidases in melanin-synthesizing systems taking place in cells other than melanocytes, such as in the neuromelanin synthesis system or in the ink gland of *Sepia officinalis* [36,37]. A peroxidase/H_2O_2 system was demonstrated to be involved in the terminal stages of melanogenesis, acting solely or collaboratively with tyrosinase in the oxidative polymerizations of pigment precursors [38]. In addition, a peroxidase activity was highlighted in the kidney tissue of European sea bass [28].

In aquaculture, the use of immunostimulants for the prevention of diseases is a promising, new development. In this context, the aim of the present study was to evaluate the effect of a polyphenol-enriched diet on the health status of the European sea bass. Farmed sea-basses were administered a feed containing a phytocomplex, rich in catechins and epigallocatechins, which was obtained from the grape seeds. For the first time, the kidney melanomacrophages activity, marker of a non specific immune response, was monitored. The dopa-oxidase and peroxidase activities in kidney melanosomal proteins as well as the expression of tyrosinase genes were investigated.

2. Materials and methods

2.1. Chemicals and reagents

3-Methyl-2-benzothiazolinonehydrazone hydro-chloride (MBTH), catalase, L-3-(3,4-dihydroxyphenyl) alanine (L-dopa), PMSF (phenylmethylsulfonyl fluoride), and kojic acid were purchased from Sigma Aldrich Co. (St. Louis, Mo.), other chemicals were of analytical grade.

2.2. Polyphenol extracts and dietary regimen of farmed fishes

Polyphenol extracts were obtained from Canosina red grape *Nero di Troia*, an autochthonous *Vitis vinifera* grape cultivar which is cultivated in Apulia (South Italy). The extracts were prepared and analysed as previously described [29]. The preparation of fish feed and the administration of the diet were conducted as previously described [29].

2.3. Source, sample collection and preparation

The experiment was performed using about one hundred sexually juvenile samples and about one hundred adult, commercial size samples of European sea bass (*D. labrax*). Fishes, reared in captivity in a farm near Lesina lake (Foggia, Italy), were randomly collected from the aquaculture plants. Sample collection took place during the winter period at 5 months (juvenile samples) and 16 months (commercial size samples) from the beginning of the experiment. The animals were kept under climatic conditions, with water temperature following the seasonal variations. Fishes were treated according to the Council Directive 86/609 EEC for the protection of animals used for experimental and other scientific purposes and the Ethical Justification for the Use and Treatment of Fishes in Research, as described in Magrone et al. [29]. Fish diet consisted in conventional feed mixed with two different concentrations of grape extracts, daily administered.

The experiment was conducted on:

- (T1) Fishes fed with a mix composed of 50 g of grape extracts in 5 kg of cornstarch, namely, 10 g of mix (100 mg of extract) for 1 kg of conventional pellet (100 mg/kg).
- (T2) Fishes fed a mix composed of 100 g of grape extracts in 5 kg of cornstarch, namely, 10 g of mix (200 mg of extract) for 1 kg of conventional pellet (200 mg/kg).
- Controls, i.e. fishes fed a conventional feed.

Fishes were farmed into three different tanks (300 m^3 each), corresponding to the three different dietary conditions. Tissue