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MOLECULAR REGULATION OF THE IMMUNE FUNCTION IN THE GILLS OF GILTHEAD SEA BREAM (*SPARUS AURATA*) FED WITH IMMUNOSTIMULANT DIETS.

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ABSTRACT

Over the past 10 years, different immunostimulants have been tested in more than 18 fish species including: Carp, Yellow croaker, Turbot, Atlantic salmon and Seabream, amongst others. The compounds tested are varied including bacterial components, polysaccharides, animal, plant and algae extract, nutritional factors, and even hormones and cytokines and some synthetics such as Levamisole. However even although a lot of interest and studies have been carried out, commercially available immunostimulant diets mainly contain β-glucans. The majority of the studies reported are based upon cellular response assays such as phagocyte activity and ROS and simple blood measurements such as total serum IgM content. All studies have shown positive results, but little is known about the underlying molecular response to dietary administration of immunostimulants. In order to evaluate the transcriptomic response in gills we analyzed and evaluated gene expression profiles associated with exposure to immunostimulant diets over time, using both a molecular and cellular approach.

Experimentally, 360 healthy Gilthead Seabream (*Sparus aurata*) of average body weight of 38±7.3 g were separated in 27 tanks and fed with two Skretting immunostimulant diets (Diet A and Diet B) and a control diet (Diet C). Each diet were fed at a feeding rate of 3% of body weight twice daily for 28 days with a period of 14 days of pre-acclimation. Gills samples were taken at 2, 7, 14 and 28 days post diet. All samples were divided for microarray analysis (specific *Sparus aurata* 44K microarray, Agilent custom design) and *in situ* hybridization (ISH) analysis. A diet dependent and a loop analysis were carried out, with control diet as a reference point. Microarray results shown a differential expression of genes associated to immunological processes such as inflammation, T and B cell response amongst others but the intensity and magnitude of the modulation of these responses was not high. ISH analysis showed localization of immunological transcripts in a specific cellular type in the primary lamellae of gilthead seabream gills.

1. INTRODUCTION

1.1 FISH IMMUNE RESPONSE: A BRIEF OVERVIEW.

The host's ability to discriminate self from non-self mounting an appropriate response to a potentially harmful antigen is the key feature of the immune system. In this aspect, the majority of multicellular organisms are able to maintain their integrity through innate immune system based on phagocytosis, complement and secretion of soluble antimicrobial molecules, being a nonspecific and quick-response mechanism to not depend on the specific surface structures recognition. This is possible by the presence of cells involved in innate immune responses as epithelial cells (Press et al., 1994), macrophages (Frøystad et al., 1998), dendritic cells (DC) (Granja et al., 2015), and nonspecific cytotoxic cells (Press et al., 1994). One of the key components of the innate immune system corresponds to the physical barrier. The fish scales, mucosal surfaces of the skin, gills and skin act as first barrier against infection (Shepard, 1994). In this ambit, the mucus plays a fundamental role in the defense against the pathogen, as well as its efficient capture of the pathogen contains key immune components such as lectins, pentraxins, lysozyme, complement proteins, peptides and antibacterial IgM (Alexander and Ingram, 1992; Fast et al., 2002).

One of the central activation pathways of innate immune response is the recognition of pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRR), in which the interaction between them leads to induce the immune response (Kopp and Medzhitov, 2003). The identification and characterization of TLR, including TLR1 (Yniv Palti et al., 2010), TLR3 (Rodriguez et al., 2005), TLR5 (Tsoi et al., 2006), TLR7 (Y Palti et al., 2010), TLR8 (Skjæveland et al., 2009) and the presence of conserved signal cascades (Purcell et al., 2007) lead to the presumption of a conservative function in fish in regard to higher vertebrates.

Based on previous reports both in comparative genomics and cell biology studies, it is possible to indicate that fish possess a specific immune response of both cellular and humoral type. Accordingly, most primary and secondary lymphoid organs

present in higher organisms are also found in fish, except lymph nodes and bone marrow (Press, 1999). Thus, the head kidney is the main immune organ that performs functions as haematopoiesis (Abdel-Aziz et al., 2010; Kondera, 2011), phagocytosis (Dannevig, 1994), antigen presentation (Kaattari and Irwin, 1985) and immunoglobulins production (Meloni and Scapigliati, 2000), among others. In addition, it has been assigned a function as endocrine gland to release corticosteroids (Leblond et al., 2001).

Teleost fish have been designated as the first group of animals in the phylogeny in which the antibody production has been reported (Andersson et al., 1995; Magnadottir et al., 2005), suggesting that teleosts are the first to develop specific immunity (Secombes et al., 1983). In general, it was thought that the immunoglobulin repertoire was limited to only IgM in tetrameric form and an approximate size of 800 kDa (Castillo Sanchez et al., 1993; Hordvik et al., 1999). However, in the last years has shown the existence of other isotypes in the immunoglobulin heavy chain such as IgD (Hordvik, 2002), which shows a high relationship with mammalian IgD (Hordvik et al., 1999); and IgT/IgZ (Hansen et al., 2005; Zhang et al., 2011), the specialized immunoglobulin at mucosal level and proposed as the homologous IgA described in mammals (Zhang et al., 2010).

The master antibody-producing cells are B lymphocytes, also responsible of the antigen presentation and activation of T cells. In higher organisms has been shown that B cells are not able to carry out phagocytosis (Aderem and Underhill, 1999; Vidard et al., 1996) but is performed by professional phagocytes cells such as monocytes, macrophages and polymorphonuclear cells (Rabinovitch, 1995). However, it has been shown that B cells have phagocytic and microbicidal capacity (Li et al., 2006), indicating that fish immune system might have some outstanding features regarding higher organisms.

To date, the reports support the antigen processing and presentation by MHC class I and MHC class II in fish. The expression of MHC class I in lymphocytes, macrophages and neutrophils (Dijkstra et al., 2003), CD8 (Moore et al., 2005), and tapasin glycoprotein involved in the stabilization and control of peptide molecule loaded

(Jorgensen et al., 2007) suggests that antigen presentation in the context MHCI is performed. On the other hand, the antigen presentation in the context of MHC class II is carried out by antigen presenting cells (APC) and whose main objective is the activation of CD4+ T lymphocytes. Iliev et al. (Iliev et al., 2010) reported that salmon leukocytes secrete vesicles containing MHCII and the exosomes containing these molecules are released by APC. Also, the existence of two CD4 (CD4-1, CD4-2) in fish has been documented (Laing et al., 2006; Moore et al., 2009). The peptides associated with MHC molecules in the plasma membrane form the MHC-peptide complex which is recognized by the T cell receptor (TCR), whose alpha chain has been reported in fish in terms of structure and organization (Hordvik et al., 2004). The MHC-TCR complex is stabilized by CD4/8 and CD3, which is one of the responsible of the intracellular communication mediated TCR leading to subsequent cell activation. Different subunits of CD3 have also been described in fish: CD3ζ, CD3γδ, and CD3ε, and whose expression has been found more abundantly expressed in the thymus (Liu et al., 2008).

In summary, the antecedents indicate that fish possess innate and specific immune response and these mechanisms probably have similar characteristics to those described in mammals.

1.1.1 MOST FREQUENTLY EVALUATED IMMUNOLOGICAL PARAMETERS IN FISH FED WITH SUPPLEMENTED DIETS FROM THE IMMUNE RESPONSE PERSPECTIVE.

The immunostimulant effect of dietary supplements in fish has been focused mainly on the evaluation of non-specific immune parameters and, therefore, on the consequences of these treatment on the innate immune system. The innate immune system has both cellular and humoral components by which it carries out its protective function. The major components of the innate immune system at cellular level are leucocytes, mainly monocytes, macrophages and granulocytes (Magnadóttir, 2006; Secombes and Fletcher, 1992). Among granulocytes, neutrophils are the most abundant cell-type and its presence has been described in Salmoniformes, Cypriniformes and Perciformes (Flerova and Balabanova, 2013). Neutrophils and macrophages are the responsible to produce bioactive molecules

responsible of pathogen recognition and destruction, cellular communication and activation, initiation of an adaptive immune response and later, resolution of an inflammatory response and tissue repair. Thus, these cell types are the responsible at cellular level of phagocytosis (Silva and Correia-Neves, 2012), one of the main mediators of innate immunity to pathogens such as bacteria, viruses, and parasites. For this reason, these immune cell types are also called phagocytes. This microbe/killing mechanism triggers rich antimicrobial processes that use a wide variety of mechanisms such as cellular activation, production of oxidative radicals, and the production of other mediators of the inflammatory response (cytokines), among others.

Two of the most important antimicrobial systems of phagocytic cells are the NADPH phagocyte oxidase and inducible nitric oxide synthase (iNOS) pathways, which are responsible for the generation of superoxide (O_2^-) and nitric oxide (NO) radicals, respectively. NADPH oxidase, a multi-subunit complex capable of one-electron reduction of molecular oxygen into superoxide anion (O_2^-), also referred to as reactive oxygen species ROS, which is spontaneously converted to H_2O_2 and enzymatically by superoxide dismutase (SOD). Compared to neutrophils, the size of the respiratory burst is much reduced in macrophages (Iles and Forman, 2002). Since O_2^- is the first product to be released from the respiratory burst, the measurement of O_2^- has been accepted as a direct and accurate way of measuring respiratory burst activity (Secombes and Olivier, 1997): the reduction of ferricytochrome c to determine extracellular O_2^-, and the reduction of the nitroblue tetrazolium (NBT) redox dye to determine intracellular O_2^- (Dügenci et al., 2003). On the other hand, inducible nitric oxide synthase (iNOS) is the main responsible of the nitrogen oxide (NO) and its derivatives, which are collectively known as reactive nitrogen species (RNS)

Other antimicrobial molecule produce by phagocytes is nitric oxide (NO), also called reactive nitrogen species (RNS). Unlike to ROS, macrophages generally produce considerably more RNS than neutrophils (Nathan and Shiloh, 2000). iNOS is activated by interferon-gamma (IFN-γ) or by tumor necrosis factor (TNF) (Green et

al., 1993). Also, NO has been demonstrated to activate NF-κB in peripheral blood mononuclear cells, an important transcription factor in iNOS gene expression in response to inflammation (Kaibori et al., 1999). The ROS and RNS antibacterial activity has been widely discussed (Fang, 2004).

Although less studied, myeloperoxidase (MPO) is a lysosomal protein stored in azurophilic granules also involved in antimicrobial mechanisms and is also produced by phagocytes, most abundantly expressed in neutrophils although is also present in circulating mammal monocytes but is lost as these mature into macrophages (Locksley et al., 1987). It possess antimicrobial activity via hypohalous acids action (Klebanoff, 2005) and is released to the extracellular space during degranulation (Spitznagel et al., 1983).

Among the immune cell parameters, red blood cells (RBC) count is one parameter frequently used to evaluate possible undesired collateral effect provoked by immunostimulant administered as dietary supplemented fed. However, RBC has cited special attention in the last years. It has been reported the participation of erythrocytes by the expression of immune-related genes in rainbow trout (Morera et al., 2011).

In addition to the cellular response, humoral elements also participate in the innate immune response including lysozyme or complement system (Magnadóttir, 2006; Secombes and Fletcher, 1992). IgM is the most common immunoglobulin in serum and mucus and the key player in systemic immune responses (Parra et al., 2015) and, for this reason, the total immunoglobulin and total protein level (an indirect antibody level measurement) are frequents among the immune parameters evaluated in fed with immunostimulant supplemented diets. IgM also participates in the opsonization of pathogens, facilitating their phagocytosis. In this ambit, the complement is a vital component of innate immunity and represents one of the major effectors mechanisms of the innate immune system (Dunkelberger and Song, 2010). It begins with the identification of pathogenic surfaces and lead to the generation of potent proinflammatory mediators (anaphylatoxins), opsonization (coating) of the pathogenic surface through various complement opsonins (such as

C3b), and targeted lysis of the pathogenic surface through the assembly of membrane-penetrating pores known as the membrane attack complex (MAC). The complement system can be activated through three major pathways: classical (antigen:antibody immune complexes), lectin (PAMP recognition by lectins), and alternative pathway (spontaneous hydrolysis/pathogenic surfaces) (Dunkelberger and Song, 2010).

Various lytic enzymes, acting either singly or in a cascade, are also important in the defense against pathogens. Without any doubt, lysozyme is one of the most analyzed lytic enzyme to evaluate the improvement of the innate immunity by the immunostimulant dietary supplements. Lysozyme is bactericidal, hydrolyzing β-[1,4] linked glycoside bonds of both Gram positive and negative bacterial cell wall peptidoglycans resulting in lysis (Magnadóttir, 2006). As the innate components described above, it is also present in the fish mucosa (Parra et al., 2015).

Finally, at gene expression level the expression of mainly pro-inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory or immunosuppressive (IL-10, TGF-β) cytokines have been evaluated in fish fed with immunostimulant supplemented diets. Thus, the limited available information of the gene expression modulation does not allow to understand the possible pathways and immunological functions stimulated by the administration of β-glucan supplemented feed in a global context.

1.1.2 FISH MUCOSAL IMMUNITY AS TARGET OF IMMUNOSTIMULANT DIETS.

As it has been describe above, one of the main goal of the immunostimulant diets is to confer resistant to pathogens potentiating the immune system. Thus, the studies have focused in the immune response at systemic level. However, one key point in the mechanism of fish resistance against pathogens is primary centered in the portals of entry, i.e., the surfaces that are in contact with the external environment: gills, nose, gastrointestinal tract, and skin. The non-self stimuli will be recognized at first in these mucosal tissues and as consequence will produce local alterations that may also produce messenger substances (hormones, cytokines, peptides) that will

activate the overall physiological response (Parra et al., 2015) promoting the immune response at systemic level.

As immunological sites, the mucosal tissues are capable to mount a robust immune response against pathogens (Gomez et al., 2013; Salinas et al., 2011). In teleosts, four mucosal-associated lymphoid tissues (MALT), responsible of the immune response at mucosal site have been described: nose-associated lymphoid tissue (NALT), skin-associated lymphoid tissue (SALT), gill-associated lymphoid tissue (GIALT), and gut-associated lymphoid tissue (GALT) (Salinas, 2015). These lymphoid tissues have four main characteristics: (1) the lack of organized lymphoid structures, such as lymphoid nodes or germinal centers, that lead to a disperse location of leukocytes; (2) the presence of secretory Igs in the mucus, which are transported into the lumen through a polymeric Ig receptor (pIgR); (3) the presence of a specialized mucosal immunoglobulin class, IgT/Z; and 4) the presence of commensal bacteria, some of them coated by Igs (Parra et al., 2015).

Regarding dietary supplemented immunostimulants in fish, few studies in carp (Falco et al., 2014, 2012; Pionnier et al., 2014) have evaluated the β-glucan supplemented diet effect on MALT, specifically in GALT.

At immunological level, GALT has as resident cells granulocytes, macrophages, lymphocytes, and plasma cells (lamina propria leukocytes, LPLs), and T and B cells among epithelial cells (intraepithelial lymphocytes, IELs). These immune cells together with epithelial cells, goblet cells, and neuroendocrine cells produce and regulate gut immune responses (Parra et al., 2015).

Taking in account that the main portals of entry, and therefore the first fish immunological barrier, are SALT and GIALT it exists the urgency in generate knowledge that allow to understand the real influence of the immunosupplement diets in fish. The skin is the largest mucosal tissue in teleost. The presences of mucus-secreting cells in the epidermis of fish confer to teleost skin as mucosal tissue. The innate immune response is represented by lysozyme, complement components, lectins, and proteolytic enzymes (Nigam et al., 2012), while secreted

IgM and IgT have also been detected (Maki et al., 2003; Xu et al., 2013). On the other hand, GIALT takes special relevance due its continuous exposition to a high number of pathogens and antigens as an aquatic organism. Lymphocyte cell aggregation in the interbrachial lymphoid tissue (ILT) (Haugarvoll et al., 2008) mainly T cells and some scattered B cells (Koppang et al., 2010) are present.

Thus, in the future will be possible to choose specific immunostimulants administered as dietary supplements depending of the nature and MALT target as portal of entry to each specific pathogen, immunopotentiating MALT-specific effect according to the necessities that fish at mucosal level demand to enhance the immune response.

1.2 IMMUNOSTIMULANTS DIETARY SUPPLEMENTS IN TELEOST: A REVIEW.

Aquaculture sector has showed a rapid growth in the last 30 years with also a dramatic increase of disease problems in fish farms as result of the rapid expansion and high stocking density. In order to maintain fish health and to improve fish performance, the aquaculture has used immunostimulants as dietary additives in fish farms to improve weight gain, feed efficiency, and/or disease resistance in cultured fish.

An immunostimulant is a natural or chemical substance that stimulates the immune system by specific (vaccines or antigens) or non-specific (irrespective of antigenic specificity) route. In Aquaculture, the non-specific immunostimulants have been widely used, probably due to the limited knowledge of the immune response in fish. In this chapter we will focus on the recent studies on: (1) plant, herbs and algae; (2) prebiotics and probiotics; and (3) PAMPs, as immunostimulants administered by diets in fish.

1.2.1 PLANT, HERBS AND ALGAE EXTRACTS AS IMMUNOSTIMULANT DIETARY SUPPLEMENT IN FISH.

Different efforts have been made in order to evaluate the immunostimulant effect of algae, herbs and plant extract in different fish species. The immunostimulants

presented here will be introduced according to the fish Order in which their effect have been evaluated (Tables I and II). This in order to have a vision of all the different supplements diets used to date in fish that share common physiological and genetic characteristics.

1.2.1.1 *Anguilliformes*. In japanese eel was evaluated the immunostimulant effect of korean mistletoe, a semi-parasitic woody perennial commonly found growing in deciduous trees which possess activity as immunoadjuvant, induction of cytokines, and stimulate the natural killer (NK) cell activity (Hajto 1986; Kuttan et al. 1992; Mannel et al. 1991; Mertzer et al. 1985; Mueller and Anderer 1990), mainly reported to be derived from lectins (Yoon et al. 1999; Yoon et al. 2003).An increase in lysozyme and phagocytic activity in doses of 0.1, 0.5 and 1% (Choi et al. 2008), and in total survival in eels challenged against *A. hydrophila* was registered, thus probably could be implicated in potentiating the defense mechanism against bacterial infections.

1.2.1.2 *Cypriniformes*. The immunostimulant effect of several Chinese herbs have been evaluated: *Astragalus* root *(Astragalus radix*, *AR)*, a pla*nt that* con*t*ains polyssacharides, alkaloid and volatile oil that modulate the functions of the immune cells including T cells, B cells, NK cells and macrophage (X et al., 2003,Liu 2002); *Ganoderma lucidum (GL)*, a mushroom whose polysaccharides have been reported to be effective in modulating immune response inhibiting tumor growth, preventing oxidate damage and is capable to activate B lymphocytes (Yin et al. 2009; Zhang et al. 2002)(You and Lin 2002); Angelica root (*Angelicae sinensis, AS*), whose polyssacharide possess biological activities such as haematopoiesis, immunomodulation, antitumor, antioxidant, radioprotection and hypoglycemic activity (Jin et al., 2012); Herba Epimedii, the aerial parts of species of many Epimedium species (Berberidaceae) with immunostimulating effects (Kim et al., 2001); Rehmannia glutinosa (RG) (also known as Di-Huang in China) which belongs to the family of Scrophulariaceae; and *Ficus carica* polysaccharide (FCP), obtained from a plant which belongs to the largest genus of the Moraceae family with anti-inflammatory, antitumor and antioxidant properties (Baek et al., 2012; Chao et al.,

2006; Yu et al., 2006). These herbs showed an increase in plasma lysozyme activity and leukocyte phagocytic activity in carp (*Cyprinus carpio*) (Wang et al., 2015; Yin et al., 2009) and in Chinese sucker (*Beaufortia kweichowensis*) (Zhang et al. 2009). In Jian carp (Cyprinus carpio var. Jian) fed with AS the number of NBT-positive cells (blood), and lysozyme and complement activity (serum) was also registered (Jian and Wu, 2004). At gene expression level, an up-regulation of IL-1β, TNF-α and iNOS and a down-regulation of IL-10 and TGF-β has been detected in carp (Wang et al., 2015) while in FCP-fed grass carp (*Ctenopharyngodon idella*) the up-regulation of IL-1 and TNF-α with HSP70 down-regulation has been registered (Yang et al., 2015). An increase in respiratory burst activity but also in phagocytic activity of isolated blood cells and plasma lysozyme activity was observed when fish were immunostimulated with AR+GL and vaccinated against *A. hydrophila*/ *A. salmonicida* (Yin et al. 2009). A high survival rate in carp challenged with *A. hydrophila* in RG-treated fish (Wang et al., 2015), and high resistance to *Flavobacterium columnare* in grass carp fed with FCP (Yang et al., 2015) was observed, indicating a potential value of the immune response of these immunostimulants in aquaculture.

The immunostimulant effect of some Indian plants has been also evaluated as immunostimulant in fish. The Indian medicinal plant *Eclipta alba* (L.), a herb belonging to Asteraceae, has been reported anti-inflammatory and anti-microbial properties (Leal et al., 2000; Wiart et al., 2004). In tilapia, the Eclipta alba immunostimulant effect an increase of the non-specific humoral (lysozyme, antiprotease and complement) and cellular response (myeloperoxidase content, production of reactive oxygen and nitrogen species), with an improved cumulative mortality against *A. hydrophila* (Christybapita et al., 2007). A higher protection against *A. hydrophila* in *Labeo rohita* fed with *Ocimum sanctum* (Tulsi, "Queen of plants") has been reported accompanied with an enhanced non-specific immune (super oxide anion production, lysozyme activity, total protein,immunoglobulin) and haemato-immune parameters (total RBC/WBC counts, haemoglobin content) (Das et al., 2013). The effect of azadirachtin, a high-value carotenoid from an Indian plant (*Azadirachta indica*) responsible of its antibacterial property (Mistry et al., 2014), has been evaluated in goldfish (*Carassius auratus*) registering high Nitroblue

tetrazolium (NBT) activity, serum lysozyme, erythrocyte and leukocyte counts (Kumar et al., 2013). The dietary effect of andrographolide, the main medicinal compound of *Andrographis paniculata* native to India and Sri Lanka with antimicrobial, antioxidant, anti-inflammatory, and immunomodulator properties (Chao et al., 2010; Gao et al., 2009; Levita et al., 2010; Xu et al., 2006) had a stimulatory effect on non-specific immune parameters in *Labeo rohita* (Basha et al., 2013), a similar effect also observed with *Rauvolfia tetraphylla* supplemented diet (Yogeshwari et al., 2015), a plant of the family *Apocynaceae* distributed in tropical countries including India. The effect of guava (*Psidium guajava L.*) leaves, colloquially known as the "poor man's apple of the tropics" and widely distributed throughout Asia, including India, have reported anti-microbial and anti-oxidant activities (Chen and Yen, 2007; Metwally et al., 2010) has shown not only better growth and immune parameters in immunostimulated groups, but also changes in the immune-related genes of *Labeo rohita*: up-regulation of IL-1β and TNF-α, and down-regulation of IL-10, TGF-β, inducible nitric oxide synthase (iNOS), cyclooxigenase-2 (COX-2)and transcription nuclear factor-κB (NF-κB) (Giri et al., 2015). Also, a better resistance against *A. hydrophila* (Basha et al., 2013; Giri et al., 2015; Kumar et al., 2013) and *Aphanomyces invadans* (Yogeshwari et al., 2015) was reported.

The evaluation of changes in the modulation of genes associated with the immune system has not been a routine practice in evaluating the immunostimulant effects in diets. Moreover, three recent studies have evaluated the gene expression profile in fish fed with immunostimulant diets making an effort to complement the general and systemic information provided in these types of studies such as growth, non-specific humoral and cellular innate immune parameters, and cumulative mortality against pathogens. Based on the limited existing information, it is interesting the up-regulation of IL-1β and TNF-α (Giri et al., 2015; Wang et al., 2015; Yang et al., 2015) and the down-regulation of IL-10 and TGF-β (Giri et al., 2015; Wang et al., 2015) has been observed, proposing the expression of these genes as potential candidates of the immune modulation in fish fed with different immunostimulant diets. Further

studies evaluating the transcriptomic response of fish fed with immunostimulant diets are needed to confirm this hypothesis.

The algae-derived has centered the attention as potential immunostimulant and in the last years has been evaluated its effect as immunostimulant in fish diets. In Atlantic cod (*Gadus morhua*) has been reported the effect of alginate, a polyssacharide found in brown algae cell wall composed by M- and G-blocks and alternating both blocks (Haug et al. 1967), observing an increase in the specific growth rate (SGR) (Vollstad et al. 2006). However, when the same alginate treatments were evaluated in Perciformes like spotted wolffish (*Anarhichas minor*) the SGR increase only in lower dose (0.01%) (Vollstad et al. 2006) indicating that the immunostimulant effect may be specie-specific. Another algae-derived evaluated as immunostimulant in fish is astaxanthin, a high-value carotenoid produced from microalgae with anti-inflammatory activity, antioxidant benefits, and enhances the IL-1 and TNF-α release (Guerin et al., 2003; Higuera-Ciapara et al., 2006; Lorenz and Cysewski, 2000).. In carp fed with astaxanthin-supplementation diet formulation an increase in red and white blood cells, hemoglobin, haematocrit, and a better survival curve was also registered against *A. hydrophila* (Jagruthi et al., 2014).

A traditional medicine herb and one of the most used in both eastern and western traditions is *Mentha piperita* (also known as peppermint), a perennial herbs belonging to the Lamiaceae family with antioxidant, antiviral and antibacterial properties, among others (McKay and Blumberg, 2006). Although an increase in the haematological and both mucosal and systemic parameters were reported, a decrease in the number of lymphocytes was observed in fry Caspian white fish (*Rutilus frisii kutum*) fed with peppermint supplemented diets (Adel et al., 2015). Another plant used as immunostimulant in fish diets is the stinging nettle (*Urtica dioica*), a herbaceous perennial flowering plant native to Europe, Asia, northern Africa, and western North America with reported immunostimulatory, anti-inflammatory, antioxidant, antiviral, antibacterial, and antifungal activities (Gülçin et al., 2004; Hadizadeh et al., 2009; Uncini Manganelli et al., 2005).Together with the increase in haematological and immunological parameters, it was noted the plasma cortisol and glucose decreased with increasing *U. dioica* in the diet of juveniles and

adults Victoria Labeo (*Labeo victorianus*) after challenge with *A. hydrophila* (Ngugi et al., 2015). The cortisol and glucose response against the immunostimulant administration has not been extensively explored. Based on the different changes in the diet composition over the last years (i.e. vegetal protein source instead animal protein), the cortisol and glucose measurement to evaluate the effects of dietary administration of new immunostimulant seems to be important to be analyzed not only for the effect on the stress response but also for the consequences at systemic level in the response against pathogens since the tight regulation between endocrine and immune system (Tort, 2011). New efforts are necessary to evaluate the dietary immunostimulant administration at the endocrine system and their implications in the immune-related gene expression and serum immune parameters.

Coffee is one of the most popular drinks in the world with *Coffea arabica* (coffee bean, Rubiaceae family) representing the 75-80 percent of the world's coffee production. The caffeine has been reported to improve the defense against different stressors (Lacorte et al., 2013). In carp, coffee bean dietary administration showed that roasted coffee bean did not improve fish growth and feed utilization but improve some immune parameters (Abdel-Tawwab et al., 2015). This opens the possibility of the use of non-conventional immunostimulants in fish diet.

1.2.1.3 *Perciformes*. The influence of the traditional Chinese medicine has also tested in *Perciformes*. The effect of *Astragalus* root and in combination with *Angelica* root was evaluated in large yellow croaker (*Pseudosciaena crocea*) with a significantly enhance on respiratory burst activity of phagocytic cells, phagocytosis and lysozyme activities in plasma (Jian and Wu, 2003). In *Cypriniformes*, a similar effect in common carp fed with *Astragalus* and *Ganoderma* was reported (Yin et al., 2009). Similar non-specific immune parameters enhanced including superoxide dismutase (SOD), peroxidase (POD) activity and a reduced mortality following *A. hydrophila* challenge were obtained in tilapia (*Oreochromis niloticus*) supplemented with a Chinese herbal mixture composed of *Astragalus*, *Angelica*, hawthorn, Licorice

TABLE I: Effect of different plant and algal extracts used as immunostimulant diets in the immune response in fish.

Ssp.	BW (g)	IS	Dosis	Administration (Sampling)	Immunological effects	Ref
A. japonica	200	Korean mistletoe (*Viscum album* Coloratum)	0.10%	14d (14d)	lysozyme activity (↑14d), phagocytic activity (↑14d)	Choi et al., 2008
			0.50%		respiratory burst activity (↑14d), lysozyme activity (↑14d), phagocytic activity (↑14d)	
			1%		respiratory burst activity (↑14d), lysozyme activity (↑14d), phagocytic activity (↑14d)	
C. carpio	62.8	Chinese herb: *Astragalus radix* (plant)	1% Astragalus	5 wk (1,2,3,4,5waf)	Respiratory burst activity (↑3waf), phagocytic activity of isolated blood cells (↑3waf,4waf,5waf), plasma lysozyme activity (↑2waf,3waf,4waf)	Yin et al., 2009
		and Ganoderma lucidum (mushroom)	1% Ganoderma		Respiratory burst activity (↑1waf), phagocytic activity of isolated blood cells (↑2waf,3waf,4waf), plasma lysozyme activity (↑2waf,4waf)	
			0.5% Astragalus + 0.5% Ganoderma		phagocytic activity of isolated blood cells (↑3, 4waf), plasma lysozyme activity (↑2waf,3waf)	
		Vaccinated against *A. hydrophila*/*A. salmonicida*	1% Astragalus		Respiratory burst activity (↑2waf,5waf,↓3waf), phagocytic activity of isolated blood cells (↓3waf,↑5waf), plasma lysozyme activity (↑3waf)	
			1% Ganoderma		Respiratory burst activity (↓3waf), phagocytic activity of isolated blood cells (↑1waf,5waf, ↓2waf,3waf), plasma lysozyme activity (↑2waf)	
			0.5% Astragalus + 0.5% Ganoderma		Respiratory burst activity (↑5waf), phagocytic activity of isolated blood cells (↑1waf,5waf, ↓2waf), plasma lysozyme activity (↑5waf)	
M. asiaticus	58.2	Propolis and Herba Epimedii (ratio of 3:1 (w/w))	0.10%	5 wk (1,2,3,4,5waf)	lysozyme activity (↑4waf,5waf)	Zhang et al., 2009
			0.50%		Respiratory burst activity of phagocytic cells (↑3waf,4waf), phagocytic activity (↑4waf,5waf), lisozyme activity (↑2waf,4waf)	
			1.00%		phagocytic activity (↑3waf), lysozyme activity (↑1waf)	
G. morhua L	0.5-1	high-M alginate (*Durvillaea antarctica*)	0.01%	59d (every 10th days from day 0 to day 60)	SGR (↑)	Vollstad et al., 2006
			0.06%		SGR (↑)	
			0.10%		SGR (↑)	
O. niloticus	0.8	Echinacea (Echinacea purpurea) extract	1.0 ppt (begins in summer)	E1 2 mo control diet + 1 mo Echinacea (3mo)	Total leucocytes count (↑3mo), lymphocytes (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7 mo)	Aly & Mohamed, 2010
				E2 1 mo control diet + 2 mo Echinacea (3mo)	neutrophil adherence (↑3mo), Ht (↑3mo), total leucocytes count (↑3mo), lymphocytes (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				E3 3 mo Echinacea (3mo)	neutrophil adherence (↑3mo), Ht (↑3mo), neutrophils (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
		Garlic	1.0 ppt (begins in summer)	G1 2 mo control diet + 1 mo garlic (3mo)	neutrophils (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				G2 1 mo control diet + 2 mo garlic (3mo)	neutrophil adherence (↑3mo), neutrophils (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				G3 3 month garlic (3mo)	neutrophil adherence (↑3mo), Ht (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dosis	Administration (Sampling)	Immunological effects	Ref
O. niloticus	8	crude propolis and its ethanolic-extract	1% propolis-ethanolic-extract	28d (28d)	Mean weight (↓0d,↑7d,14d,28d), average daily gain (↑28d), SGR (↑28d), FCR (↓28d), FER (↑28d), HCV (↑28d), small lymphocytes (↑28d), monocytes (↑28d), neutrophils (↓28d), serum lysozyme content (↑28d), serum bactericidal activity (↓28d)	Azza M.M. Abd-El-Rhman, 2009
			1% ethanol containing crude propolis		Mean weight (↑28d), average daily gain (↑28d), SGR (↑28d), FCR (↓28d), FER (↑28d), HCV (↑28d), small lymphocytes (↑28d), serum lysozyme content (↑28d), serum bactericidal activity (↓28d)	
O. mosambicus	25 y 50	*Eclipta alba* aqueous extract	0.01%	3wk (1,2,3waf)	serum lysozyme activity (↑1waf,2waf,3waf), serum natural haemolytic complement activity (↑1waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf,2waf)	Christybapita et al., 2007
			0.10%		serum lysozyme activity (↑1waf,2waf,3waf), serum natural haemolytic complement activity (↑2waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf,2waf), reactive nitrogen species production by peripheral blood leucocytes (↑2waf)	
			1%		serum lysozyme activity (↑1waf,2waf,3waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf), reactive nitrogen species production by peripheral blood leucocytes (↑2waf)	
A. minor O.	fry	high-M alginate (*Durvillaea antarctica*)	0.01%	55d (every 10th days from day 0 to day 55)	SGR (↑)	Vollstad et al., 2006
			0.06%		NSD	
			0.10%		NSD	
H. hippoglossus L.	Fish larvae	high-M alginate (*Durvillaea antarctica*)	50-150 ng per larva/day	7-9, 20-22, 41-43, 85-87d (7,20,41,85d)	NSD (dry weight in larvae)	Skjermo & Bergh 2004
S. senegalensis	Fish larvae	microalgae (*Tetraselmis chuii*)	NS	12-81dph (64dph)	Survival (↑81dph), Number of CFU per fish in gut (↓64dph)	Makridis et al., 2009
		microalgae (*Chlorella minutissima*)			Survival (↑81dph), Number of CFU per fish in gut (↓64dph)	
S. senegalensis	80	red algae (*Porphyridium cruentum*) lyophilized cells	1%	4wk (2,3,4wk)	NSD	Diaz-Rosales et al., 2008
S. maximus L.	Fish larvae	FMI (Ascophyllum nodosum)	0.5 g FMI wet weight capsules/l	2-13dph (13dph)	protein synthesis (↑13d), protein degradation (↑13d), efficiency of retention of synthetised protein (↓13d)	Conciecao et al., 2001
O. mykiss	14	Garlic	0.50%	14 days (14,21daf)	Haematological parameters [RBC (↑21d), WBC (↓28d), monocytes (↑14d,28d, ↓21d), lymphocytes (↑21d), neutrophils (↓28d), thrombocytes (↑21d)], electrolyte indices [Calcium (↑14d)], respiratory burst of blood leucocytes (↑14d,21d,28d), lysozyme activity (↑14d,21d)	Nya & Austin, 2011
			1.00%		Haematological parameters [RBC (↑14d), WBC (↑14d,28d), monocytes (↑14d,28d), lymphocytes (↑21d), neutrophils (↓21d,28d)], electrolyte indices [Calcium (↑14d,21d)], respiratory burst of blood leucocytes (↑21d,28d), lysozyme activity (↑14d, 21d)	
O. mykiss	89.2	tetra (Cotinus coggyria)	0.50%	3wk (3,6,9wk)	non-specific immune parameters [extracellular superoxide anion production (↑6wk,9wk), intracellular superoxide anion production (↑6wk,9wk), phagocytic activity (↑6wk, 9wk), lysozyme activity (↑6wk,9wk), total protein level (↑6wk,9wk)]	Bilen et al., 2011
			1.00%		non-specific immune parameters [extracellular superoxide anion production (↑6wk,9wk), intracellular superoxide anion production (↑6wk,9wk), phagocytic activity (↑6wk,9wk), lysozyme activity (↑6wk,9wk), total protein level (↑6wk,9wk)]	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dosis	Administration (Sampling)	Immunological effects	Ref
O. mykiss	14	ginger (*Zingiber officinale* Roscoe)	0.05%	14d (14d)	Growth parameters (SGR↑, FCR↓, PER↑), average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑), phagocytic activity (phagocytic ratio↑), superoxide anion production by blood leucocytes↑, lysozyme activity (↑15, 30, 60 min), serum bactericidal activity↓, anti-protease activity↑, serum alternative haemolytic complement activity↑, biochemical indices (globulin↑)	Nya & Austin, 2009
			0.10%		Growth parameters (% weight gain↑, SGR↑, FCR↓, PER↑), average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑), phagocytic activity (phagocytic ratio↑), superoxide anion production by blood leucocytes↑, lysozyme activity (↑15, 30, 60 min), serum bactericidal activity↑, anti-protease activity↑, serum alternative haemolytic complement activity↑, biochemical indices (total protein↑, globulin↑)	
			0.50%		Growth parameters (% weight gain↑, SGR↑, FCR↓, PER↑), average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑), phagocytic activity (phagocytic ratio↑), superoxide anion production by blood leucocytes↑, lysozyme activity (↑15, 30, 60 min), serum bactericidal activity↑, anti-protease activity↑, serum alternative haemolytic complement activity↑, biochemical indices (total protein↑, globulin↑)	
			1.00%		Growth parameters (% weight gain↑, SGR↑, FCR↓, PER↑), average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↓, and neutrophils proportion↑), phagocytic activity (phagocytic ratio↑), superoxide anion production by blood leucocytes↑, lysozyme activity (↑15, 30, 60 min), serum bactericidal activity↑, anti-protease activity↑, serum alternative haemolytic complement activity↑, biochemical indices (total protein↑, globulin↑)	
O. mykiss	41	mistletoe (*Viscum album*)	0.10%	3wk (3wk)	Plasma protein concentration (↑3wk)	Dugenci et al., 2003
			1.00%		Plasma protein concentration (↑3wk)	
		nettle (Urtica dioica)	0.10%		Plasma protein concentration (↑3wk)	
			1.00%		Plasma protein concentration (↑3wk)	
		ginger (Zingiber officinale)	0.10%		NSD	
			1.00%		Extracellular oxidative radical production (↑3wk), phagocytosis of blood leukocytes (↑3wk), plasma protein concentration (↑3wk)	

root and honeysuckle (Tang et al., 2014). Also, the up-regulation of IL-1 and TNF-α was reported (Tang et al., 2014), confirming them as candidates genes of the immune modulation in fish fed with different immunostimulant diets as was mentioned above and, at the same time, the need to evaluate the supplementary diet effect at transcriptomic level in fish to provide information of other actors involved in the immune response.

The North American plant species has also been evaluated as immunostimulant in fish diets. The historical and traditional use of *Echinacea purpurea*, a flowering plant that belongs to Asteraceae family was noted among the native Americans. *Echinacea* activates macrophages and stimulates the phagocytic-function (See et al., 1997). The effect of Echinacea extract was evaluated in Nile tilapia (*Oreochromis niloticus*)

shown a higher effect in body gain, SGR, monocytes, neutrophil adherence, and survival rate against *A. hydrophila* (Aly and Mohamed, 2010).

The dihydroquercetin obtained from deodar (*Cedrus deodara,* family *Pinaceae*), a traditional plant used in the Hindu medicine native to the Indian subcontinent with a broad spectrum of action (Chandur et al., 2011), was evaluated in gilthead seabream (*Sparus aurata*) detecting a cellular (phagocytosis and respiratory burst activities) and humoral (seric complement activity, antiprotease, total protein, peroxidase, bactericidal activity and IgM level) increase with the highest parameters with the lowest doses (Awad et al., 2015). *Rhizophora apiculata* (Family of *Rhizophoraceae*) is one of the widely distributed mangrove tree species in tropical countries, like India, with a reported antimicrobial and antiviral activity (Bandaranayake, 2002; Premanathan et al., 1999). The survival rate was high in clownfish (*Amphiprion sebae*) infected with *Vibrio alginolyticus* (Dhayanithi et al., 2015b) and, interestingly, the same survival rate (although with different immunostimulant doses) was observed when fish were dietary supplemented with *Avicennia marina* (Dhayanithi et al., 2015a), another mangrove tree widely distributed along tropical and subtropical coastlines with antioxidant, antibacterial and antiviral activity (Abeysinghe, 2010; Khafagi et al., 2003; Lincy et al., 2013). Another tree mainly cultivated in subtropical regions is the sweet orange peel (*Citrus sinensis*), a plant member of the *Citrus* family) with antimicrobial and antifungic properties (Chee et al., 2009; Sharma and Tripathi, 2008).In tilapia (*Oreochromis mossambicus)* fed with essential oil an increase in weight gain, specific growth rate (SGR) and serum biochemical and haemato-immunological parameters and survival against *Streptococcus iniae* infection, with a decrease compared with control only in feed conversion rate (FCR), albumin (ALB), and mean cell hemoglobin (MCH) (Acar et al., 2015).

Aloe barbadensis, also called *Aloe vera* (family *Xanthorrhoeaceae*) is a plant frequently used in herbal medicine with several properties such as antiviral and immunomodulator, among others (Kim et al., 1999; Vázquez et al., 1996). In a study in Nile tilapia fed with *Aloe vera* supplemented diet and propolis no significant

differences were found (Dotta et al., 2014). However, an increase on growth performance but few and slight changes in red and white blood cell count (RBC, WBC), hemoglobin and haematocrit, and no changes in glucose and cortisol were observed in tilapia (GIFT) challenged with *S. iniae* (Gabriel et al., 2015). These differences may be related with the differences in the *A. vera* concentration used: in both works the fish were fed with 0.5%, 1%, and 2% of supplemented diet but in the case of Nile tilapia the *A. vera* was equally mixed with propolis, although is clear no big favorable health status changes were observed in tilapia (GIFT) fed with *A. vera* supplemented diet. Similarly, no differences were observed in Nile tilapia fed with propolis supplemented diet, although in 1% propolis-ethanolic-extract increased the monocytes count and decrease neutrophils at 28 days after treatment (Abd-El-Rhman 2009).

Green tea (*Camellia sinensis* L., GT) is a medicinal herb with non-oxidized and unfermented leaves, which have anti-inflammatory, antioxidative, antiproliferative, antibacterial, and antiviral properties (Crespy and Williamson, 2004; Isogai et al., 2001; Weber et al., 2003). In Nile tilapia fed with GT experimental diet for 12 weeks a higher growth performance, haemato-immune parameters and cumulative survival against *A. hydrophila* was observed (Abdel-Tawwab et al., 2010), while in yellowtail (*Seriola quinqueradiata*) fed with green tea polyphenols supplemented diet no significant differences were observed (Ishihara et al., 2002).

Other immunostimulant use as additive of fish farmed diets is the marine diatom *Navicula* sp., a boated-shaped algae belonging to the family *Naviculaceae* rich in antioxidant carotenoids and vitamins (Patil et al., 2007). Silage microalgae *Navicula* sp enriched with *Lactobacillus sakei* enhanced the immunity in gilthead seabream (Reyes-Becerril et al., 2013). This effect was evaluated in separate diets in a different fish species, Pacific red snapper (*Lutjanus peru*), showing a better growth rate, humoral immune response and antioxidant capabilities in fish fed supplemented with *Navicula* + *L. sakei* (a probiotic) or *L. sakei* alone (Reyes-Becerril et al., 2014).

1.2.1.4 *Pleuronectiformes*.

The efforts have focused on the evaluation of derivates of algae mainly administered to fish by artemia and rotifers (Conceição et al. 2001; Makridis et al. 2009; Skjermo and Bergh 2004; Skjermo et al. 1995). In this fish order the only work whose administration strategy is not by artemia and rotifers was did in *Senegalese sole* using as immunostimulant red algae (*Porphyridium cruentum*) lyophilized cells with commercial diet routinely used in fish farms; no statistical difference was found when was evaluated the respiratory burst activity of phagocytes (Díaz-Rosales et al., 2008). The same result was observed when fish larvae were immunostimulated with high-M alginate with artemia feeding rate in halibut (Skjermo and Bergh 2004) and turbot (Skjermo et al. 1995). However, in the work of Conceição et al. (2001) has been observed that turbot larvae fed with rotifers enriched with alginate capsules containing FMI had three fold higher protein turnover compared to control group. This will probably imply a higher larval viability and survival in case of environmental/disease stress (L.E.C. Conceição 2001). The rich alginate compounds showed an improved survival rate against *Vibrio anguillarum* both in juvenile turbot (Skjermo et al., 1995) and halibut larvae (Skjermo and Bergh, 2004). The high-M alginate has a stimulatory effect on human monocytes inducing the expression of TNF-α (Espevik et al. 1993; Otterlei et al. 1991). This cytokine production would be induced by the membrane CD14 together with either TLR2 and TLR4/MD-2, according to observed in human and mice (Flo et al. 2002) being more strong the TNF-α induction depending of molecular weight of high-M alginate (Otterlei et al. 1993).Thus, the TNF-α production may be involved in the better survival against *V. anguillarum* alginate-dependent.

1.2.1.5 *Salmoniformes*.

The efforts have been focused mainly in evaluate the immunostimulant effect in rainbow trout (*Oncorhynchus mykiss*). As has been mentioned before, several studies have used medicinal plants to evaluate its efficacy as dietary supplement. In rainbow trout fed with a diet containing 1% aqueous extract of powdered ginger roots for three weeks exhibited a significant non-specific immune response increase such as extracellular respiratory burst activity and phagocytosis of blood

leukocytes, processes considered to be one of the most important mechanisms involved in the bactericidal activity of macrophages; and an increase in plasma protein levels (Dügenci et al., 2003), indicating that humoral factors may enhance phagocytosis in fish (Chung and Secombes, 1987). Also, a proliferation in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities were observed in rainbow trout challenge with *A. hydrophila* (Nya and Austin, 2009). Ginger (*Zingiber officinale*) has reported anti-inflammatory and anti-oxidative activity and its effective control of a range of bacterial, fungal and parasitic conditions (Agarwal et al., 2001; Chrubasik et al., 2005; Endo et al., 1990; Grzanna et al., 2005; Kim et al., 2007). A higher survival rate was also observed in rainbow trout dietary supplemented with garlic (Nya and Austin, 2011).

Among the medicinal plants, stinging nettle (Quercetin) and black cumin seed oil (*Nigella sativa*) have been also evaluated. While black cumin has antibacterial, antioxidant and anti-inflammatory effects (El-Saleh et al., 2004; Hanafy and Hatem, 1991; Zedlitz et al., 2002), stinging nettle possess antimicrobial activity with effectiveness against a wide range of microorganisms (Gülçin et al., 2004). These supplements shown an increase in lysozyme, myeloperoxidase and antiprotease activities, and total serum protein and IgM levels (Awad et al., 2013). Tetra (*Cotinus coggyria*) is a medicinal plant with antimicrobial and antibacterial effects (Dülger et al., 2009) that in rainbow trout fed with 1% increased the extracellular and intracellular respiratory burst activity, phagocytic and lysozyme activity, and total protein level (Bilen et al., 2011). However, not all the medicinal plant-derivates has an effect in fish. Dietary *Aloe vera* inclusion had no effect on growth, non-specific immune parameters, the expression of several immune-related genes, and the immune response to formalin-killed atypical *Aeromonas salmonicida* in steelhead rainbow trout (*Oncorhynchus mykiss*, Walbaum) (Zanuzzo et al., 2015). The authors suggest that prolonged feeding with *A. vera* may have this effect undesired effect in salmonids, but no significant differences were also found in Nile tilapia fed for 2 weeks (Dotta et al., 2014) and few and slight changes were observed in haemato-immune parameters (RBC, WBC) in tilapia (GIFT) challenged with *S. iniae* prior fed

for 8 weeks (Gabriel et al., 2015). Thus, further studies are needed to evaluate the real impact and effectiveness of *A. vera* as immunostimulant dietary supplement in fish.

Green tea (GT) has been also evaluated in rainbow trout. The decaffeinated GT extract on rainbow trout showed a higher lysozyme and peroxidase content (Sheikhzadeh et al., 2011). However when rainbow trout were fed with Epigallocatechin-3-gallate (EGCG) supplemented diet, a very potent antioxidant derived from GT, no significant differences were observed (Thawonsuwan et al., 2010). These antecedents are consistent with the results observed in Nile tilapia fed with GT with changes in immune parameters (Abdel-Tawwab et al., 2010) but no significant differences were observed with green tea polyphenols supplemented diet (Ishihara et al., 2002). The GT effect and the potential immune effects in fish fed with supplementary diets should be addressed.

Spirulina platensis, which belongs to cyanobacteria (blue-green algae) family that can up-regulate IL-1β and TNF-α, increase the phagocytic activity and superoxide anion production in leucocytes of carp (Watanuki et al., 2006). In rainbow trout fed with *Spirulina* supplemented diet, an increase in haemato-immune parameters (RBC, WBC, total protein) and an decrease in cortisol and glucose was observed (Yeganeh et al., 2015). The cortisol and glucose decrease in fish fed with supplemented diet has been previously reported (Ngugi et al., 2015), confirming the urgency in to evaluate the effects and mechanisms of dietary administration of new immunostimulant on the stress response. *S. platensis* does not have cellulose cell wall and therefore fish can digest it (Karkos et al., 2008); however, some non-digestible components such as dietary fiber have been introduced as supplement diet and evaluate their effect in fish as is the case of Vitacel, a pure raw fibers composed of cellulose and hemicelluloses mainly. Vitacel has shown increased plasma lysozyme activity and the number of neutrophil and eosinophil in giant sturgeon (*Huso huso*) (Heidarieh et al., 2011). In rainbow trout, dietary administration of Vitacel increased the serum lysozyme, ACH50, bactericidal activity, and decreased the cumulative mortality after challenge with *A. hydrophila*.

Importantly, the HSP70 gene expression was down-regulated (Yarahmadi et al., 2014). The down-regulation of HSP70 has been reported in fish fed with supplemented diet (Yang et al., 2015), thus the effect of immunostimulant diets on the expression of stress-related genes response should be studied to elucidate the mechanisms developed by these supplements in fish.

The medicinal mushroom *Lentinula edodes* extract as a trout supplement diet showed an increase in the number of total leukocytes (percentage of monocytes and neutrophils was higher but lymphocytes was lower), phagocytic, lysozyme activity, and serum IgM levels. When fish were challenged against *Lactococcus garvieae* a higher survival was observed in fish fed with *L. edodes* extract (Baba et al., 2015).

In synthesis, the researchers have mostly carried out different efforts to assess the effect of dietary immunostimulants in fish from derivatives of algae, herbs and plant extract in a non-specific manner based on (1) traditions and folkways transferred by generations; and (2) their biological properties mainly evaluated in vitro or, in some cases, in experimental animals. The results indicate that there are few studies in which is possible to observe a clear and direct dose-dependent immunostimulatory effect of the dietary supplement in the different fish species presented in this review. Also, it seems clear that all the studies are focused on to evaluate the innate immune response evaluating almost the same non-specific (both humoral and cellular) and haemato-immune parameters and, hence, the ability of the immunostimulant to confer non-specific immune protection against fish pathogens. This limits the knowledge regarding the scope of treatment. Another critical limitation is the arbitrary use of dose and timing of administration making difficult the comparison and integration of results. Therefore, it is a priority to generate a consensus on this matter. Finally, more efforts are needed using high-throughput screening tools to elucidate the transcriptome and proteome response to assess the scopes of the dietary supplementation of immunostimulants in fish in order to establish in the future dietary supplemented immunostimulant according to the specific fish requirements.

TABLE II: Effect of different plant and algal extracts used as immunostimulant diets in the immune response different fish species challenged with a pathogen.

Spp.	BW (g)	IS	Dosis	Administration	Challenge	Challenge route	Ddosis	Time of challenge (dpd)	Time of evaluation	Effect	Ref
A. japonica	200	Korean mistletoe (*Viscum album* Coloratum)	0.10%	14d	*Aeromonas hydrophila* (ATCC 49140)	i.p. injection	$3x10^5$ CFU	14d	0-14 dac	33.3% total survival rates	Choi et al., 2008
			0.50%							66.6% total survival rates	
			1%							80% total survival rates	
C. carpio	62.8	Chinese herb: *Astragalus radix* (plant)	1% Astragalus	5wk	*Aeromonas hydrophila* strain OB 212	i.p. injection	$1x10^6$ cells/fish	5wk	0-6 dac (monitoring at 4h intervals)	↓cumulative mortality (60%)	Yin et al., 2009
		and Ganoderma lucidum (mushroom)	1% Ganoderma							↓cumulative mortality (58%)	
			0.5% Astragalus + 0.5% Ganoderma							↓cumulative mortality (60%)	
		Vaccinated against *A. hydrophila*/*A. salmonicida*	1% Astragalus							↓cumulative mortality compared with control group (% NS)	
			1% Ganoderma							↓cumulative mortality compared with control group (% NS)	
			0.5% Astragalus + 0.5% Ganoderma							↓cumulative mortality (38%) compared with control group	
M. asiaticus	58.2	Propolis and Herba Epimedii (ratio of 3:1 (w/w)), also called TCM	0.10%	5wk	*Aeromonas hydrophila* (Shering-Plough, Essex UK)	i.p. injection	$5x10^7$ bacteria	5wk	1 wac	NSD	Zhang et al., 2009
			0.50%							cumulative mortality (↓0.5% TCM)	
			1.00%							NSD	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	Dosis	Administration	Challenge	Challenge route	Ddosis	Time of challenge (dpd)	Time of evaluation	Effect	Ref
C. catla	16	Aegle marmelos	5 g/kg feed	30d	*Pseudomonas aeruginosa*	water exposure	$19.5x10^4$ cells/ml	30d	from 5 dac every 5 days until 15 dac	phagocytic ratio (↑5dac, 10 dac, 15 dac)	Pratheepa et al., 2011
			10 g/kg feed							phagocytic ratio (↑5dac, 10 dac, 15 dac)	
			15 g/kg feed							phagocytic ratio (↑5dac, 10 dac, 15 dac)	
			20 g/kg feed							phagocytic ratio (↑5dac, 10 dac, 15 dac)	
			25 g/kg feed							phagocytic ratio (↑5dac, 10 dac, 15 dac)	
			30 g/kg feed							phagocytic ratio (↑5dac, 10 dac, 15 dac)	
C. catla	150	Achyranthes aspera seed	0.5 g	4wk	*Chicken red blood cells (c-RBC)*	i.p. injection	500 µl of cRBC suspension in PBS (20%(v/v))	4wk	7 dac	Antigen-specific antibody response (↑), α1-antiprotease inhibitors level (↑), total protease inhibitors level (↑), RNA/DNA ratio of spleen (↑)	Rao Y et al., 2005
									14 dac	α1-antiprotease inhibitors level (↑), RNA/DNA ratio of spleen (↑)	
									21 dac	Antigen-specific antibody response (↑), globulin level (↑), α1-antiprotease inhibitors level (↑), total protease inhibitors level (↑), RNA/DNA ratio of kidney (↑)	
									28 dac	α1-antiprotease inhibitors level (↑) total protease inhibitors level (↑), RNA/DNA ratio of kidney (↑)	
O. niloticus	8	crude propolis and its ethanolic-extract	1% propolis-ethanolic-extract	28d	*Aeromonas hydrophila*	i.p. injection	$1x10^7$ cells	28d	0-15 dac	42% mortality (RLP=50.59%), compared with 85% mortality (RLP=0%) in control	Azza M.M. Abd-El-Rhman, 2009
			1% ethanol containing crude propolis							45% mortality, compared with 85% mortality in control	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	Dosis	Administration	Challenge	Challenge route	Ddosis	Time of challenge (dpd)	Time of evaluation	Effect	Ref
O. niloticus	0.8	Echinacea (Echinacea purpurea) extract	1.0 ppt (begins in summer)	E1 (2mo with balanced diet and 1mo with Echinacea)	*Aeromonas hydrophila*	i.p.	$1x10^8$ bacteria/ml	3mo,7mo	7 dac	70% mortality (RLP=26.32) 3 month. 65% mortality (RLP=27.78) 7 month	Aly & Mohamed, 2010
				E2 (1mo with balanced diet and 2mo with Echinacea)						65% mortality (RLP=31.58) 3 month. 85% mortality (RLP=5.56) 7 month	
				E3 (3mo with Echinacea)						50% mortality (RLP=47.37) 3 month. 50% mortality (RLP=44.44) 7 month.	
		Garlic	1.0 ppt (begins in summer)	G1 (2mo with balanced diet and 1mo with garlic)						80% mortality (RLP=15.79) 3 month. 65% mortality (RLP=27.78) 7 month	
				G2 (1mo with balanced diet and 2mo with garlic)						65% mortality (RLP=31.58) 3 month. 75% mortality (RLP=16.67) 7 month	
				G3 (3mo with garlic)						60% mortality (RLP=36.84) 3 month. 50% mortality (RLP=44.44) 7 month.	
A. minor O.	fry	high-M alginate (*Durvillaea antarctica*)	alginate in feed (0.02 and 0.06%) or in bath (0.01%)	55d	*Aeromonas salmonicida*	bath (1h)	$2.5x10^8$ cells/ml	79d	0-48 dac	NSD	Vollstad et al., 2006
O. mosambicus	25	Eclipta alba aqueous extract	0.0001	3wk	*Aeromonas hydrophila* (AHO21)	injected	$1x10^8$ cells/fish	1wk	0-15 dac	Percentage mortality (↓)	Christybapita et al., 2007
			0.001							Percentage mortality (↓)	
			0.01							Percentage mortality (↓)	
H.hippoglossus L.	Fish larvae	high-M alginate (*Durvillaea antarctica*)	50-150 ng per larva/day	7-9d, 20-22d, 41-43d, 85-87d	*Vibrio anguillarum* serotype O2, strain HI-610	exposure (1h)	$5x10^5$ CFU/ml	90d	0-15 dac	the mortality at the highest dose was 45±1% in the control and 28±8% in the stimulated group, corresponding to 38% reduction in mortality	Skjermo & Bergh 2004
							$1.4x10^7$ CFU/ml	90d	0-15 dac	NSD	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	Dosis	Administration	Challenge	Challenge route	Ddosis	Time of challenge (dpd)	Time of evaluation	Effect	Ref
S. senegalensis	80	red algae (*Porphyridium cruentum*) lyophilized cells	0.01	4wk	*Photobacterium damselae subsp. piscicida strain Lgh41/01*	i.p. injection	6x108 bacteria/ml	2wk	3 and 4 wk	respiratory burst activity of phagocytes from head kidney (↑4wk)	Diaz-Rosales et al., 2008
S. maximus L	Fish larvae	alginate with high mannuronic acid (*Ascophyllum nodosum*)	NS	1d	*Vibrio anguillarum*	exposure (30 min)	$1x10^5$ cells/ml	2d	1 wac	Average reduction in the mortality of 39% in the immunostimulated fish respect to control	Skjermo et al., 1995
O. mykiss	14	Garlic	0.005	14d	*Aeromonas hydrophila* AE 57	i.p. injection	$1x10^6$ cells/ml	24 h after stopping feeding trials	0-14 dac	14 days (RPS=86%), 21 days (RPS=75%), and 28 days (RPS=68%)	Nya & Austin, 2011
			0.01							14 days (RPS=80%), 21 days (RPS=55%), and 28 days (RPS=46%)	
O. mykiss	14	ginger (*Zingiber officinale* Roscoe)	0.0005	14d	*Aeromonas hydrophila* AE 57	i.p. injection	$1x10^7$ cells/ml	14d	0-14 dac	4% mortalities (RPS=94%)	Nya & Austin, 2009
			0.001							NS	
			0.005							0% mortalities (RPS=100%)	
			0.01							16% mortalities (RPS=75%)	

1.2.2 PROBIOTICS AND PREBIOTICS.

In the last years, probiotics have been incorporated in aquaculture practices to improve the general health status of fish. Probiotics application could be via artificial (Chiu et al. 2010) or alive feeding (Picchietti et al. 2009), by immersion in water (Ringø 1999) or by injection (Abbass et al. 2010). Until date the most studied probiotics were bacteria, mainly those belonging to the lactic acid group (Ringø et al. 2010). Other bacteria that received considerable attention include Bacillus, Pseudomonas, Aeromonas and Vibrio (Merrifield et al. 2010; Nayak 2010). More recently, yeast and microalgae have also been the focus of some studies (Oliva-Teles 2012).

Several definitions were suggested along the decades for probiotics (Fuller 1989; Reid et al. 2003). In the aquaculture context, they are found as alive, dead or components of microorganisms that provide protection through several ways, as by establishing an inadequate environment for pathogen proliferation, or competing with potential pathogens, or reducing gut pH and adhesion sites, or releasing inhibitory compounds with bactericidal or bacteriostatic effects on other microbial populations or by improving the immune response (Merrifield et al. 2010).

The majority of studies concerning the probiotic effects on fish focused on their capacity to stimulate growth and protect against disease (Capkin and Altinok 2009; Nayak 2010). However, recently more attention has been paid to the immunmodulatory effects of probiotics on fish and, some complete reviews were published (Dimitroglou et al. 2011; Merrifield et al. 2010; Nayak 2010; Oliva-Teles 2012). Despite the amount of available studies dealing with this question, the mechanisms by which probiotics induce changes in immune function are still poorly understood. In the present review, we will make a brief reference to the studies already reviewed by the above cited authors and review the studies published during the last two years that focus on the immune effects of probiotics administered via diet to farmed fish.

The available literature indicates that several probiotics either individually or in combination can enhance both systemic and local immunity in fish (Harikrishnan et al. 2011b; Nayak 2010). It was demonstrated that probiotics interact with immune cells such as monocytes, macrophages, neutrophils and natural killer cells to improve innate immune responses (Merrifield et al. 2010) and that some probiotics induce the proliferation of erythrocytes, granulocytes, macrophages and lymphocytes (Irianto and Austin 2002; Kumar et al. 2008). It was shown that probiotics like *Lactobacillus rhamosus* (Panigrahi et al. 2004) and *Clostridium butyricum* (Pan et al. 2008) induced an increase in the immunoglobulins levels in fish and, Arijo et al. (2008) demonstrated that the administration of probiotics resulted in the expression of immunoglobulins that protect against *Vibrio harveyi* challenge.

Probiotics administered via diet stimulate different components of the immune system, such as the phagocytic and respiratory burst activities, lysozyme, complement, peroxidase and anti-protease activities (Nayak 2010; Reyes-Becerril et al. 2008). However, many of these same treatments caused reduced activity in different experiments and some incoherency was found. Concerning cytokines, previous results revealed that probiotics such as *Carnobacterium maltaromaticum*, *L. rhamnosus* and *Bacillus subtilis* induced an up-regulation on the transcription of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-∝ (TNF∝) and transforming growth factor-β (TGF-β) in different fish species (Kim and Austin 2006; Panigrahi and Azad 2007). However, other probiotics like *Lactobacillus delbrueckiix* induced a down-regulation in cyclooxygenase 2 (Cox-2) and TGF-β transcription in *Dicentrarchus labrax* (Picchietti et al. 2009). Reyes-Becerril et al. (2008) fed *Sparus aurata* with yeast and the treatment also strongly regulated the transcription of immune related genes. Studies regarding the effect of probiotics on gut immunity are still scarce and few results indicated that they can stimulate the gut immune system of fish with a marked increase in the number of Ig^+ cells and acidophilic granulocytes (Nayak 2010).

Recent studies evaluated the effects of probiotics alone and in combination with other probiotics or other substances suspected to have an immunostimulatory effect (Harikrishnan et al. 2011a; Harikrishnan et al. 2011b). These authors found that *Paralichthys olivaceus* fed a diet supplemented with *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* had increased superoxide anion production and lysozyme activity. Furthermore, fish infected with *Uronema marinum* and fed with *L. plantarum* supplemented diet had higher survival rate than with other enriched diets (Harikrishnan et al. 2011a). The same authors evaluated the effects of a diet enriched with the herb *Scutellaria baicalensis* and/or the probiotic *Lactobacillus sakei* in *Oplegnathus fasciatus* challenged by *Edwardsiella tarda*. These authors found increased white and red blood cell count in fish fed the mixed diet and probiotics enriched diet. In the groups fed the mixed diet they also found increased number of lymphocytes, monocytes, neutrophils and eosinophils, as well as higher complement and antiprotease activities, reactive oxygen species and reactive nitrogen species production. Furthermore, the lysozyme activity was enhanced in all treated groups (Harikrishnan et al. 2011b). Pérez-Sanchez and collaborators (2011) investigated the effect of lactic acid bacteria, including *L. plantarum*, on the expression of immune-related genes in the head kidney and intestine of *Oncorhynchus mykiss* and in the protection against infection by *Lactococcus garvieae*. These authors found increased mRNA levels of IL-1β, interleukin-10 (IL-10) and TNFα in the *L. plantarum* fed group. Moreover, the mRNA levels of IL-10, interleukin-8 (IL-8) and IgT were significantly higher in the *L. plantarum* group after *L. garvieae* infection. The findings of Harikrishnan et al. (Harikrishnan et al. 2011a; 2011b) and Pérez-Sanchez et al. (2011) indicated that the administration of probiotics alone or in mixed diets stimulated the immune response of fish, protecting against challenge by different pathogens.

The widespread use of probiotics in aquaculture practices and the belief in their positive effects on fish health laid to the appearance of new functional foods, like the prebiotics. Prebiotics are non-digestible feed ingredients that promote growth of beneficial gut microbes and depress the proliferation of harmful microbes or enhance intestinal immunity (Oliva-Teles 2012; Tacchi et al. 2011). Instead of

introducing favourable bacteria via the diet the aim of prebiotics is to stimulate selected favourable indigenous microbial populations (Dimitroglou et al. 2011).

Prebiotics mainly consist of oligosaccharides, such as mannan oligosaccharides and fructooligosaccharides, probably the most well studied in fish. However, some information regarding applications of galactooligosaccharides, xylooligosaccharides, arabinoxylooligosaccharides and isomaltooligosaccharides is also available (Oliva-Teles 2012; Tacchi et al. 2011). While the effects of probiotics on fish immune system are relatively well documented, that of prebiotics is more limited and was recently reviewed by Tachi et al. (2011) and Oliva-Teles (2012). Some of the effects of prebiotics on fish immune function include increased cytokine expression (Russell et al. 2009) and activation of the complement cascade (Tsutsui et al. 2006).

1.2.3 IMMUNOSTIMULANT DIETS USING PAMPS.

On the last 10 years using PAMPs as immunostimulant has been published several articles in different species (Table III). One of the most used PAMPs in the Cypriniforms order are β-glucans, a heterogeneous group of glucose polymers also named β-1,3/1,6-glucans. In mammals, although various receptors e.g. complement receptor C3 and TLR1/6 have been described (Dalmo and Bøgwald, 2008), dectin-1 is considered as the main β-glucan receptor (Brown and Gordon, 2003). However, dectin-1 has not been identified in fish and it has been suggested that β-glucan could be detected by toll like receptors (Pietretti et al., 2013).

Other immunostimulant fish diets are commercial supplements compounds by β-glucans (Biosaf, DVAQUA, Ecoactiva, Ergosan, Fibosel, Macrogard, and VitaStim), chitin, lipopolysaccharides (LPS), mannan-oligosaccharides (MOS), peptidoglycan (PGN), and yeast extract (Table III), which have been also included in this revision.

1.2.3.1 Cypriniformes. In Falco (Falco et al., 2012) the effect of Macrogard in the expression of selected inflammatory genes has been studied (tnfα1, tnfα2, il1b, il6fam and il10) in gut and head kidney after 14 days of feeding. Only tnfα2 was significantly down regulated in gut and head kidney, and il10 was down regulated in gut. The gene expression has been also analyzed analyzing the effect of baker's yeast

extract (Biswas et al., 2012), a supplement containing as mayor component nucleotides and β-glucan, in immune parameters in carp in 10 days of feeding the experimental diets. The expression of cytokines as IL-1β, TNF-α, IL12p35, IL-12p40 and IFN-γ2 was significantly increased in head kidney after 1 day of feeding the diet and the expression of the CXC-chemokine was increased after 1, 5 and 7 days of feeding the diet; also was observed a reduction in the IL-10 gene expression in all days studied. The superoxide anion production and the phagocytic activity in head kidney leucocytes showed that at 3 days after treatment the superoxide anion production and the phagocytic index was higher for the fish fed with the IS diet, and the phagocytic activity was increased at 1 and 3 days of treatment. By contrast, in carp fed with β-glucan (Macrogard) for 25 days was observed that most of the selected cytokines analyzed (ilb1, il10, tnfa1, tnfa2 and cxca) were down-regulated, but the expression of mx was increased in liver and mid-gut (Falco et al., 2014). The posterior injection with poly (I:C) did not affect the expression of the cytokines, but was found also a up-regulation of mx in liver, head kidney, spleen and mid-gut (Falco et al., 2014). Also a down-regulation of complement-related genes at 7 and 25 days was observed in liver and head kidney carp fed with β-glucan fed fish, although a high serum CRP level at 7 days of administration of the diet and a increased in alternative complement activity at 25 days of feeding was also detected (Pionnier et al., 2014). In mid gut at 7 days, bf/c2, c3 and map2 were up-regulated, and crp2 and c3 were also up-regulated at 25 days of feeding. They also analyzed a subsequent LPS or poly(I:C) injection, and the results shown a regulation on the CRP and complement related genes profiles, with a greatest effect in fish fed with β-glucan; however on CRP levels, and complement activity in the serum, the effect was less than control fish, this suggesting that the β-glucan immunostimulation was sufficient enough to reduce the effects of LPS and poly(I:C) injection (Pionnier et al., 2014).

In carp has been reported the use of β-glucan on a basal diet for 1 week and have not been significant differences compared to fish fed the control diet (Selvaraj et al., 2005). In Rohu fingerlings four different diets with 100, 250 or 500 mg of β-glucan kg−1 diet for 56 days were administrated, immune parameters including the

leucocyte count, phagocytic ratio, phagocytic index, lysozyme activity, complement activity and serum bactericidal activity rose to their highest levels on 42 days after feeding with the diet containing 250 mg of β-glucan kg−1 diet (Misra et al., 2006)(Misra 2006), while in Sahoo (Sahoo and Mukherjee, 2001) was found that the fish fed for 1 week with β-glucan at a doses of 0.1% showed an increase in bacterial agglutination, haemagglutination and haemolysin titre, bactericidal activity, serum phagocytic ratio, serum phagocytic index and serum leucocrit compared with the control diet. A 60 days feeding trial using microbial levan at 0.25%, 0.5% 0.75%, 1.0% and 1.25% was conducted in juvenile Rohu, among the treatment groups the albumin/globulin ratio decrease with a small decrease in lower levan-supplemented groups and the haemoglobin content, total leucocyte count and serum total protein were increased with a dietary supplementation of levan at 1% or more. As the levan supplementation was increased, there was a gradual increase in serum lysozyme activity and respiratory burst activity with the highest activity in the 0.75% and 1.0% levan groups (Gupta et al., 2008).

On carp was also publish the effect of a combined diet of β-glucan and LPS (0.1% β-glucan + 0.025% LPS, 0.5% β-glucan + 0.125% LPS, 1% β-glucan + 0.25% LPS) administered at days 1, 7 and 14, at day 16, observing an increase on the bactericidal activity compared with control diet on the 3 different doses being greater at higher doses of β-glucan and LPS (Selvaraj et al., 2006). However, administration of a diet only with LPS at doses of 1, 2.5 y 5 mg did not show any immunostimulant effect compared the control diet(Selvaraj et al., 2009).

1.2.3.2 Perciformes. In Perciformes studies as been publish using different PAMPs as immunostimulants, being the most frequently used those derived from yeast cultures, by adding the whole yeast on basal diet (Cuesta et al., 2004; He et al., 2009; Ortuño et al., 2002; Rodríguez, 2003) and also extracts such as MOS (Torrecillas et al., 2007), chitin (Cuesta et al., 2004; Esteban et al., 2001), and β-glucan either extracted directly (Ai et al., 2007; El-Boshy et al., 2010) or using those commercially

TABLE III: Effect of different PAMPs immunostimulant diets in the immune response in fish.

Ssp.	BW (g)	IS	Dosis	Administration (Sampling)	Immunological effects	Ref
C. carpio	40	β-glucan Macrogard (*S. cerevisiae*)	10 mg/Kg of BW	25d (25d)	Expression of il1β (↓25d in MG); il10 (↓25d in spleen, HK and MG); tnfα1 (↓25d in MG); tnfα2 (↓25d in MG); cxca (↓25d in spleen and HK); mx (↑25d in liver and MG)	Falco et al., 2012
			Macrogard + PBS inyection AAD	25d (24hpi)	Expression of mx (↑25+1d in liver and HK)	
			Macrogard + poly(I:C) inyection AAD	25d (24hpi)	Expression of mx (↑25+1d in liver, HK, spleen and MG)	
C. carpio	40	β-glucan Macrogard (*S. cerevisiae*)	10 mg/Kg of BW	25d (1,3,7,25d)	Expression of crp1 (↓7d,25d in liver and HK, ↓25d in MG); crp2 (↓7d in liver, HK and MG, ↑25d in MG); c1rs (↓7d,25d in liver); bf/c2 (↓7d in liver, ↑7d in MG); c3 (↓7d,25d in liver, ↓7d in HK, ↑7d,25d in MG); masp2 (↓25d in liver, ↑25d in HK, ↑7d in MG)	Pionnier et al., 2014
			C + 4mg/kg LPS inyection AAD	25d (1,3,7dpi)	Expression of crp2 (↑7d, in liver, ↓1d in HK); c1rs (↓1d in liver, ↑7d in HK and MG); bf/c2 (↑7d in HK); c3 (↑1d in liver, ↑3d in liver and MG); masp2 (↑7d in HK and MG)	
			C + 5 mg/kg poly(I:C) inyection AAD	25d (1,3,7dpi)	Expression of crp1 (↓1d in liver, ↑1d in HK and MG); crp2 (↑1d,7d in liver and HK); c1rs (↓1d in liver, ↑3d in liver, ↑7d in HK and MG); c3 (↑1d in MG, ↑3d in liver, ↑7d in HK); masp2 (↓1d in liver, ↑3d in liver, ↑7d in HK)	
			10 mg/Kg Macrogard + PBS inyection AAD	25d (1,3,7dpi)	Expression of crp1 (↑1d,3d,7d in liver, ↓1d ↑3d in HK, ↑3d in MG); crp2(↑1d in liver and MG, ↓1d in HK, ↓3d in liver and HK, ↑7d in HK and MG); c1rs (↑7d in liver, ↓1d,3d in HK); bf/c2 (↓1d,3d in liver, ↑1d,7d, ↓3d in HK, ↑7d in MG); masp2 (↓1d in liver, HK and MG, ↓3d in liver and MG, ↑7d in liver and HK)	
			10 mg/Kg Macrogard + 4mg/kg LPS inyection AAD	25d (1,3,7dpi)	Expression of crp1 (↑1d in liver, ↑3d in liver and HK); crp2(↓3d in liver, ↑1d,7d in HK); c1rs (↓3d in HK); bf/c2 (↑1d in HK and MG, ↓3d in HK and MG, ↑7d in liver and HK); c3 (↑3d in liver, ↓3d in MG, ↑7d in HK); masp2 (↓3d in HK and MG, ↑7d in HK)	
			10 mg/Kg Macrogard + 5 mg/kg poly(I:C) inyection AAD	25d (1,3,7dpi)	Expression of crp1 (↑1d in liver, ↑3d in liver and HK); crp2 (↑1d in liver, HK and MG, ↓3d in liver and HK, ↑7d in HK); c1rs (↑1d,7d in liver, ↓3d in liver); bf/c2 (↑1d,7d in liver, HK and MG, ↓3d in liver, HK and MG); c3 (↑1d in liver, ↓3d in MG, ↑7d in HK); masp2 (↑1d in liver and HK, ↑7d in liver, HK and MG, ↓3d in liver, HK and MG)	
C. carpio	100	baker´s yeast extract CW-I (TableMark)	5mg/Fish	3d (1,3,5,7,10d AAD)	Expression of il1β (↑1d, ↓3d,10d in HK); TNF-α (↑1d, ↓3d,5d,7d,10d in HK); Il-12p35 (↑1d, ↓3d,5d,7d,10d in HK); Il-12p40 (↑1d, ↓3d,5d,7d,10d in HK); CXC-chemokine (↑1d,5d,7d in HK); IFN-γ2 (↑1d,5d in HK); IL-10 (↓1d,3d,5d,7d,10d in HK). Superoxide anion (↑3d in phagocytic cells); Phagocytic activity (↑1d,3d in kidney cells); Phagocytic index (↑3d in kidney cells)	Biswas et al., 2012
C. carpio	78	MacroGard	6 mg/Kg of BW	14d (14d)	Expression of tnfα2 (↓14d in gut and HK); il10 (↓14d in gut)	Falco et al., 2012
C. carpio	28	LPS *(A. hydrophila)*	1mg	1,7,14d (16d)	NE	Selvaraj et al., 2009
			2.5mg	1,7,14d (16d)	NE	
			5mg	1,7,14d (16d)	NE	
C. carpio	28	LPS (*A. hydrophila)*	0.1% β-glucan + 0.025% LPS	1,7,14d (16d)	HK-macrophage bactericial activity (↑16d)	Selvaraj et al., 2006
		β-glucan (*S. cerevisiae*)	0.5% β-glucan + 0.125% LPS	1,7,14d (16d)	HK-macrophage bactericial activity (↑16d); HK-macrophage oxigen burst activity (↑16d)	
			1% β-glucan + 0.25% LPS	1,7,14d (16d)	HK-macrophage bactericial activity (↑16d); HK-macrophage oxigen burst activity (↑16d)	
C. carpio	28	β-glucan (*S. cerevisiae*)	1%	1,3,5d (7d)	NE	Selvaraj et al., 2005
			2%	1,3,5d (7d)	NE	
			4%	1,3,5d (7d)	NE	

TABLE III: (*...continuation)*

Ssp	BW (g)	IS	Dosis	Administration Sampling	Immunological effects	Ref
L. rohita	4.5	microbial levan	0.25%	60d (60d)	Albumin/Globulin ratio (↓60d)	Gupta et al., 2008
			0.50%	60d (60d)	Serum lyzozyme activity (↑60d)	
			0.75%	60d (60d)	Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d)	
			1.00%	60d (60d)	Haemoglobin content (↑60d); Serum total protein content (↑60d); Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d); Blood phagocytes respiratory burst activity (↑60d)	
			1.25%	60d (60d)	Haemoglobin content (↑60d); Serum total protein content (↑60d); Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d); Blood phagocytes respiratory burst activity (↑60d)	
L. rohita	35	β-glucan Sigma (*S. cerevisiae*)	100mg/Kg feed	56d (14,28,42,56d)	Total serum protein content (↑42d); WBC count (↑42d); Blood leucocytes cells respiratory burst (↑42,56d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,42d,56d); Serum lysozyme activity (↑42d,56d); Haemolytic complement activity (↑14d,28d,42d,56d); Serum bactericidal activity (↑14d,28d,42d,56d)	Misra et al., 2006
			250mg/Kg feed	56d (14,28,42,56d)	Total serum protein content (↑28d,42d); WBC count (↑42d); Blood leucocytes cells respiratory burst (↑14d,28d,42d,56d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,28d,42d, ↓56d); Blood leucocytes-lymphokine production index (↑14d,42d); Serum lysozyme activity (↑28d,42d,56d); Haemolytic complement activity (↑14d,28d,42d,56d); Serum bactericidal activity (↑14d,28d,42d,56d)	
			500mg/Kg feed	56d (14,28,42,56d)	Total serum protein content (↑28d,42d); WBC count (↓14d,56d, ↑28d,42d); Blood leucocytes-respiratory burst(↑14d,28d,42d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,28d,42d,56d,); Blood leucocytes-lymphokine production index (↓42d); Serum lysozyme activity (↑28d,42d,56d); Haemolytic complement activity (↑14d,28d,42d); Serum bactericidal activity (↑14d,28d,42d,56d)	
L. rohita Hamilton	39	β-glucan yeast (Sigma)	0.1%	53-60d (60d)	Bacterial agglutination titre (↑60d); Haemagglutination titre (↑60d); Haemolysin titre (↑60d); Bactericidal activity (↑60d); Serum phagocytic ratio (↑60d); Serum phagocytic index (↑60d); Serum leucocrit (↑60d)	Sahoo and Mukherjee, 2001
L. japonicus	18	Yeast cell walls (S. cerevisiae)	250 mg/Kg	72d (72d)	NSD	Yu et al., 2014
			500 mg/Kg	72d (72d)	NSD	
			1000 mg/Kg	72d (72d)	NSD	
			2000 mg/Kg	72d (72d)	NSD	
			20000 mg/Kg	72d (72d)	NSD	
S. aurata L.	100-200	chitin (sigma)	25g/Kg	6wk (2,4,6wk)	Total serum IgM content (↑6wk)	Cuesta et al., 2004
			50g/Kg	6wk (2,4,6wk)	Total serum IgM content (↑2wk,4wk,6wk)	
			100g/Kg	6wk (2,4,6wk)	Total serum IgM content (↑6wk)	
		Yeast cells (*S. cerevisiae)*	1g/Kg	4wk (1,2,4wk)	Total serum IgM content (↑2wk)	
			5g/Kg	4wk (1,2,4wk)	Total serum IgM content (↑2wk)	
			10g/Kg	4wk (1,2,4wk)	Total serum IgM content (↑2wk)	
		levamisole synthetic (Sigma)	0.075g/Kg	10d (0,1,2,3,4,6wk)	Total serum IgM content (↑3wk)	
			0.15g/Kg	10d (0,1,2,3,4,6wk)	Total serum IgM content (↑2wk,3wk4wk)	
			0.3g/Kg	10d (0,1,2,3,4,6wk)	Total serum IgM content (↑2wk)	

TABLE III: (*...continuation)*

Ssp	BW (g)	IS	Dosis	Administration Sampling	Immunological effects	Ref
S. aurata L.	0.3, 5, 10	β-glucan Macrogard *(S. cerevisiae)*	1g/kg feed	2 wk (1,2,3wk)	HK-Macrophage phagocytic activity (↑1wk)	Couso et al., 2003
			10g/kg feed	2 wk (1,2,3wk)	Spleen-Macrophage respiratory burst activity (↑1wk,2wk); Spleen-Macrophage phagocytic activity (↑1wk)	
		β-glucan Fibosel *(S. cerevisiae)*	1g/kg feed	2 wk (1,2,3wk)	Spleen-Macrophage respiratory burst activity (↑2wk); Spleen-Macrophage phagocytic activity (↑1wk)	
			10g/kg feed	2 wk (1,2,3wk)	Spleen-Macrophage respiratory burst activity (↑1wk,2wk)	
		β-glucan VitaStim *(S. cerevisiae)*	1g/kg feed	2 wk (1,2,3wk)	Spleen-Macrophage respiratory burst activity (↑2wk)	
			10g/kg feed	2 wk (1,2,3wk)	NSD	
S. aurata L.	175	Whole yeast *(S. cerevisiae)*	10g/kg feed	6wk (2,4,6wk)	HK-Leucocyte respiratory burst activity (↑4wk); HK-Leucocyte Natural cytotoxic activity (↑4wk,6wk)	Rodríguez et al., 2003
		Whole yeast (*S. cerevisiae)* fks-1		6wk (2,4,6wk)	Natural complement activity (↓6wk); Serum peroxidase content (↓6 wk); Serum lysozyme activity (↑2wk,4wk); HK-Leucocyte respiratory burst activity (↑4wk); HK-leucocyte natural cytotoxic activity (↑4wk,6wk); Leucocyte phagocytic ability (↑2wk,4wk,6wk); Leucocyte phagocytic capacity (↑4wk)	
S. aurata L.	150	whole yeast *(S. cerevisiae)*	1g/kg feed	4wk (1,2,4wk)	HK-leucocyte natural cytotoxic activity (↑4wk)	Ortuño et al., 2002
			5g/kg feed	4wk (1,2,4wk)	HK-leucocyte phagocytic ability (↑4wk); Leucocyte phagocytic capacity (↑4wk)	
			10g/kg feed	4wk (1,2,4wk)	HK-Leucocyte respiratory burst activity (↑2wk); Leucocyte phagocytic ability (↑4wk); HK-leucocyte phagocytic capacity (↑4wk); Leucocyte myoloperoxidase content (↑2wk)	
S. aurata L.	125	chitin (Sigma)	25mg/Kg	6wk (2,4,6wk)	Natural haemolytic complement activity (↑2wk); HK-leucocyte respiratory burst activity (↑4wk); HK-leucocyte natural cytotoxic activity (↑2wk)	Esteban et al., 2001
			50mg/Kg	6wk (2,4,6wk)	Natural haemolytic complement activity (↑2wk); HK-leucocyte respiratory burst activity (↑4wk); HK-leucocyte natural cytotoxic activity (↑2wk)	
			100mg/Kg	6wk (2,4,6wk)	Natural haemolytic complement activity (↑2wk); HK-leucocyte respiratory burst activity (↑4wk); HK-leucocyte natural cytotoxic activity (↑2wk)	
P. auratus	180	β-glucan EcoActiva *(S. cerevisiae)*	winter 0.1% v/w	84d (0,3,7,14,28,56,84d)	HK-Macrophage respiratory burst activity (↑3d,7d,14d,28d,56d)	Cook et al., 2003
			summer 0.1% v/w	84d (0,3,7,14,28,56,84d)	HK-Macrophage respiratory burst activity (↑28d)	
O. niloticus	80-100	β-glucan Biosaf (*S. cerevisiae)*	10g/kg feed	21d (21d)	Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d)	El-Boshy et al., 2010
		β-glucan (extracted) *(S. cerevisiae)*	0.1%	21d (21d)	Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d); Lymphocyte transformation index (↑21d)	
		laminaria (*L. japonica)*	0.1%	21d (21d)	Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d)	
O. niloticus ♀ *x O. aureus* ♂	0.2	chito-oligosaccharides (*Panaeus vannamei)*	control + (0.1% commercial chitosan-oligosaccharides)	35d (35d)	Intestine expression of TNF-α (↓35d); TGF-β (↑35d); HSP70 (↓35d)	Qin et al., 2014
			0.80%	35d (35d)	Intestine expression of TNF-α (↓35d); HSP70 (↓35d)	
			1.60%	35d (35d)	Intestine expression of TNF-α (↓35d); TGF-β (↑35d); HSP70 (↓35d)	
			2.40%	35d (35d)	Intestine expression of TNF-α (↓35d); HSP70 (↓35d)	

TABLE III: (*...continuation)*

Ssp	BW (g)	IS	Dosis	Administration Sampling	Immunological effects	Ref
O. niloticus ♀ *x O. aureus* ♂	51	DVAQUA	0.125g/Kd diet	8wk (8wk)	Serum lysozyme activity (↑8wk); serum C3 content (↑8wk); serum C4 content (↑8wk); HK-macrophage phagocytic activity (↑8wk); HK-macrophage respiratory burst activity (↑8wk)	He et al., 2009
			0.25g/Kd diet	8wk (8wk)	Serum lysozyme activity (↑8wk); serum C3 content (↑8wk); serum C4 content (↑8wk); HK-macrophage phagocytic activity (↑8wk); HK-macrophage respiratory burst activity (↑8wk)	
			0.50g/Kd diet	8wk (8wk)	Serum lysozyme activity (↑8wk); serum C3 content (↑8wk); serum C4 content (↑8wk); HK-macrophage phagocytic activity (↑8wk)	
			1.00g/Kd diet	8wk (8wk)	Serum lysozyme activity (↑8wk); serum C3 content (↑8wk); serum C4 content (↑8wk); HK-macrophage phagocytic activity (↑8wk); HK-macrophage respiratory burst activity (↑8wk)	
			2.00g/Kd diet	8wk (8wk)	Serum lysozyme activity (↑8wk); serum C3 content (↑8wk); serum C4 content (↑8wk); HK-macrophage phagocytic activity (↑8wk)	
D. labrax	34	MOS	2%	9wk (9wk)	NE	Torrecillas et al., 2007
			4%	9wk (9wk)	HK-macrophages phagocytic activity (↑9wk)	
D. labrax	80	β-glucan Macrogard (*S. cerevisiae*)	0.1%	15d (5,30,45d AAD)	Serum complement activity (↑15d); Serum lysozyme activity (↑30d); gills-HSP70 content (↑30d); liver-HSP70 content (↑30d)	Bagni et al., 2005
			0.1%	4 cycles: 15 d every 60d (45d AEC I, II, III, IV)	NE	
		β-glucan Ergosan (*S. cerevisiae*)	0.5%	15d/15,30,45d AAD	Serum complement activity (↑15d,30d); Serum lysozyme activity (↑30d); gills-HSP70 content (↑30d); liver-HSP70 content (↑30d)	
			0.5%	4 cycles: 15 d every 60d (45d AEC I, II, III, IV)	Serum lysozyme activity (↑45d/IV)	
D. labrax	414	β-glucan Macrogard *(S. cerevisiae)*	2%	2wk every 3 months (40wk)	Serum complement activity (↑40wk); Plasma lysozyme activity (↑40wk)	Bagni et al., 2000
P. crocea	10	β-glucan (*S. cerevisiae*)	0.09%	8wk (8wk)	Serum lysozyme content (↑8wk); HK-macrophages phagocytosis activity (↑8wk); HK-macrophages respiratory burst activity (↑8wk)	Ai et al., 2007
			0.18%	8wk (8wk)	Serum lysozyme content (↑8wk)	
T. maccoyii	18.6 K	β-glucan Sanictum (*S. cerevisiae*)	5.2mg/Kg feed at 35% FR Inyected in baitfishes	every 2nd day for 12 wk (0,8wk, and harvest)	Serum lysozyme content (↑8wk)	Kirchhoff et al., 2011
O. mykiss	100	peptidoglican (PG)	10 mg/Kg feed	28d (21,28d)	Expression of omDB-3 (↑21d,28d); omDB-4 (↑21d,28d); omCATH-1 (↑21d,28d); omCATH-2 (↑21d,28d); omLEAP-2a (↑21d,28d)	Casadei et al., 2015
			14d PG + 7-14d control diet	14d + 7-14 d control diet (21, 28d)	Expression of omDB-3 (↑21d,28d); omDB-4 (↑21d,28d); omCATH-2 (↑21d); omLEAP-2a (↑21d,↓28d)	
O. mykiss	4 Kg	β-glucan Macrogard (S. cerevisiae)	0.10%	3mo (3mo)	Total serum Ig content (↑3mo); Serum IgM content (↑3mo)	Ghaedi et al., 2015
			0.20%	3mo (3mo)	ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	
	0.18		C+C (L1)	2mo (2mo)	NSD	
			C + 0,1% (L2)	2mo (2mo)	ACH50 (↑2mo)	
			C + 0,2% (L3)	2mo (2mo)	ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	
			0,1% + C (L4)	2mo (2mo)	NSD	
			0,1% + 0,1% (L5)	2mo (2mo)	NSD	
			0,2% + C (L6)	2mo (2mo)	NSD	
			0,2% + 0,2% (L7)	2mo (2mo)	ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	

TABLE III: (*...continuation)*

Ssp	BW (g)	IS	Dosis	Administration Sampling	Immunological effects	Ref
O. mykiss	150	Peptidoglican (PG)	5 mg PG/Kg	14d (1,7,14d)	Expression of omDB-1 (↑1d,7d in skin, ↓7d in liver); omDB-2 (↑7d in gills, ↓1d in gut); omDB-3 (↑1d,7d,14d in skin, ↑7d, ↓14d in gills, ↑1d in gut); omDB-4 (↑14d in skin, ↓7d in gills, ↑1d in gut, ↓14d in gut and liver); CATH-1 (↑1d in gut, ↑7d in skin, ↑14d in skin, gut and liver, ↑14d in gills); CATH-2 (↑1d in skin, gills and liver, ↑7d in skin, ↑14d in skin, gills and gut); Hepcidin (↑1d in liver, ↑7d in gills); LEAP-2a (↑14d in skin and gut, ↓14d in gills and liver)	Casadei et al., 2013
			10 mg PG/Kg	14d (1,7,14d)	Expression of omDB-1 (↑14d in skin); omDB-2 (↑7d in gills and gut); omDB-3 (↑7d,14d in skin, ↑1d in gills, ↓1d in gut, ↓14d in gills, gut and liver); omDB-4 (↑14d in skin, ↑7d in liver, ↓14d in gut and liver); CATH-1 (↑1d in liver, ↑14d in skin and gut, ↓14d in gills); CATH-2 (↑7d in skin, ↑14d in skin, gills, gut and liver); Hepcidin (↑7d in gills, ↑1d,7d,14d in liver); LEAP-2a (↑1d in liver, ↑7 in skin, ↑14d in skin and gut, ↓14d in gills and liver)	
			50 mg PG/Kg	14d (1,7,14d)	Expression of omDB-1 (↑7d,14d in skin); omDB-2 (↑1d in skin, ↑7d in gills, gut and liver); omDB-3 (↑1d,7d,14d in skin, ↑1d,7d in gills, ↓14d in gills, gut and liver); omDB-4 (↑1d,7d,14d in skin, ↑7d in gills and liver, ↓14d in gut and liver); CATH-1 (↑1d in skin, gills and gut, ↑7d in skin, ↑14d in skin, gut and liver, ↓14d in gills); CATH-2 (↑1d in skin and gills, ↑7d in skin, ↑14d in skin, gills and gut); Hepcidin (↑1d in skin, ↑7d in gills and liver, ↑14d in liver); LEAP-2a (↑1d,7d in skin, ↑14d in skin and gut, ↓14d in gills and liver)	
			100 mg PG/Kg	14d (1,7,14d)	Expression of omDB-1 (↑1d,14d in skin); omDB-2 (↑7d in gills and gut); omDB-3 (↑1d skin, gills, gut and liver, ↑7d in skin and gills, ↑14d in skin, ↓14d in gills and liver); omDB-4 (↑7d in gills and liver, ↑14d in skin, ↓14d in gut and liver); CATH-1 (↑1d in gut and liver, ↑7d in skin and gills, ↓14d in gills, ↑14d in skin, gut and liver); CATH-2 (↑7d, in skin, gills and liver, ↑1d,↑14d in skin, gills and gut); Hepcidin (↑7d in gills, ↑1d,14d in liver); LEAP-2a (↑1d in skin, ↑7d in skin and gills, ↓14d in gills and liver, ↑14d in skin and gut)	
O. mykiss	4.2	IP-PA1 (lipopolysaccharide *P. agglomerans*)	10 µg LPSs /Kg of body weight	93d (93d)	NSD	Skallli et al., 2013
			20 µg LPSs /Kg of body weight	93d (93d)	Blood bactericidal activity (↑93d); Blood lysozyme activity (↑93d); Blood hemolytic activity (↑93d); NBT (↑93d)	
O. mykiss	8.8	β-glucan Macrogard *(S. cerevisiae)*	0.2%	21d (7,14,21d)	NSD	Kunttu et al., 2009
			0.6%	21d (7,14,21d)	Blood-respiratory burst activity (↑21d)	
			1.8%	21d (7,14,21d)	Blood-respiratory burst activity (↑21d)	
O. mykiss	14.3	β-glucan barley (*H. vulgare*)	12.2g/Kg feed	9wk (0,3,9wk)	NSD	Sealey et al., 2008
			16.7g/Kg feed	9wk (0,3,9wk)	NSD	
			26.4g/Kg feed	9wk (0,3,9wk)	NSD	
		Wheat diet (control) + 2g/Kg Macrogard	4.5g/Kg feed	9wk (0,3,9wk)	NSD	

TABLE III: (*...continuation)*

Ssp	BW (g)	IS	Dosis	Administration Sampling	Immunological effects	Ref
S. salar	fry	LPS (*A. salmonicida)*	0.1%	62d (62d)	NSD	Guttvik et al., 2002
C.batrachus	49	Lactoferrin (bovine)	100mg/Kg feed	7d (31d)	Blood phagocytes-respiratory burst activity (↑31d); Leucocyte phagocytic activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	Kumari and Sahoo, 2006
		β-glucan yeast (sigma)	0.1% in feed	7d (31d)	Blood-phagocytes respiratory burst activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	
		levamisole synthetic (sigma)	50mg/Kg feed	10d (31d)	Blood-phagocytes respiratory burst activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	
		vitamin C CRNA Roche	500mg/Kf feed	30d (31d)	Leucocyte phagocytic activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	

NS: no specified, AAD: After administration diet, dpi: days post injection, NSD: No significant differences, MG: Mid-gut: HK: Head-Kidney, IS: Immunostimulant, AEC: After each cycle.

available as Biosaf (El-Boshy et al., 2010), VitaStim (Couso et al., 2003), Sanictum (Kirchhoff et al., 2011), EcoActiva (Cook et al., 2003), Fibosel (Couso et al., 2003), and MacroGard (Bagni et al., 2005, 2000; Couso et al., 2003).

Using as immunostimulant diet the whole yeast on doses of 1, 5 y 10 g/Kg of feed for 4 weeks, the main effects was observed with the highest doses, finding an increase of respiratory burst activity, phagocytic ability, natural cytotoxic activity and myeloperoxidase content in head kidney seabream leucocytes (Ortuño et al., 2002). It has also been report an increases in the serum IgM content after 2 week of treatment (Cuesta et al., 2004). On the other hand, Rodriguez et. al (Rodríguez, 2003) evaluated the effect of a modified strain of *S. cerevisiae* (fsk-1) which has a lower glucan composition and higher chitin on is cell wall, and they found that doses of 10 g/kg of feed increase levels of lysozyme activity in serum, the leucocyte phagocytic ability and leucocyte phagocytic capacity and a decrease in natural complement activity and peroxidase content in serum after 6 weeks of administration of the diet (Rodríguez, 2003).

The commercial product DVAQUA (product of fermentation of *S. cerevisiae*) was used in doses of 0.125, 0.25, 0.50, 1.0 and 2.0 g/kg of feed in hybrid tilapia (*Oreochromis niloticus* ♀ *x O. aureus* ♂) for 8 weeks and found for all doses an increase in serum lysozyme activity, serum C3 and C4 content and macrophage

phagocytic activity isolated from head kidney at the end of the period of administration of the diet; and also an increase in head kidney macrophage respiratory burst activity at 0.125, 0.25 and 1.0 g/Kg of DVAQUA (He et al., 2009).

Also has been used as immunostimulant derivates of yeast as chitin (poly [1 → 4]-β-N-acetyl-D-glucosamine), which is an insoluble polysaccharide present in the exoskeleton of shellfish, insects and in the cell walls of fungi. The synthetic compound (Sigma) was used to test the immunostimulatory action on seabream with doses of 25, 50 and 100 mg/kg of feed for 6 weeks. All doses showed an increase at 2 weeks of administration in natural haemolytic complement activity and HK-leukocyte natural cytotoxic activity and an increased HK-leukocyte respiratory burst activity at 4 weeks of administration compared to the control diet (Esteban et al., 2001), also with a doses of 25 mg/kg was observed an increase in the total content of IgM en serum of fishes feed for 6 weeks with the diet (Cuesta et al., 2004). Other derivatives of yeast has been used as IS diets is MOS (mannan oligosaccharides) extracted from *S. cerevisiae*, which when is administered in doses of 4% in the diet for 9 weeks and observed an increase in phagocytic macrophages HK-activity in Sea bass (Torrecillas et al., 2007).

The yeast component more used as immunostimulants has been the β-glucan, extracted directly or from commercial extracts. Ai et al (Ai et al., 2007) used a β-glucan extract added to the diet of large yellow croaker in doses of 0.09% and 0.18% for 8 weeks and it was observed that the lower doses produce an increase in lysozyme content in serum, and phagocytic and respiratory burst activity on macrophages isolated from head kidney at the end of the administration period. In addition, Nile tilapia was fed with doses of 0.1% obtaining an increase, in addition to the after mentioned parameters in serum bactericidal activity, serum nitric oxide and the lymphocyte transformation index after 21 days of treatment (El-Boshy et al., 2010). Also in tilapia fed with a composition of 10g/kg of feed of Biosaf (commercial extract) was detected an increase in the same parameters except lymphocyte transformation index which had no significant differences with the control diet (El-Boshy et al., 2010).

The effect of EcoActiva, other commercial β-glucan, was administrated in diet to Snapper at doses of 0.1% on winter and summer season during 84 days. It was observed that when the diet was administrated during winter season was an increase on the respiratory burst activity of head kidney macrophages even at 56 days from the administration of the diet, however when was administrated in summer season this parameter increase only at day 28 from the administration of the diet (Cook et al., 2003).

On Sea bream also has been used the commercial extract Fibosel and VitaStim (1 g/Kg of feed, 10 g/Kg of feed) observing an increase of respiratory burst activity on spleen macrophages at lower doses with both supplements and with the higher doses of Fibosel whereas no effect was observed in higher doses of VitaStim. Also with the lower doses of Fibosel was observed an increase of phagocytic activity in spleen macrophages (Couso et al., 2003).

Sea bass fed with macrogard 2% for 2 weeks every 3 months showed an increase in serum complement activity and plasma lysozyme activity at the end of 3 cycles (Bagni et al., 2000) when this fish was feed with Macrogard at 0.1% for 15 days, was found an increase in serum complement activity, serum lysozyme activity and gills HSP70 content at 30 days from the end of the diet, but when this diet was long term administrated (4 cycles of 15 days every 60 days) no effect was found compared with control diet (Bagni et al., 2005). On seabream fed with Macrogard for 2 weeks (1 g/Kg of feed and 10 g/Kg of feed) after one week of treatment an increase in head kidney macrophage phagocytic activity with the lower doses and an increase of spleen macrophage respiratory burst and phagocytic activity for the highest doses was detected(Couso et al., 2003).

1.2.3.3 Pleuronectiformes. The immunostimulatory effect of MacroGard also has been analyzed in Pleutonectiformes. In turbot fed with 2 g/Kg for a period of 5 weeks and was observed an increase in total white blood cells after 8 and 21 days from the end of the diet and an increased in the phagocytic activity of head kidney leukocytes after 1 day of the end of the diet (Ogier de Baulny et al., 1996).

1.2.3.4 Salmoniformes. In Salmoniformes, as in Perciformes, most of the reports have focused on evaluate the immunostimulant effect of β-glucan. However the capacity of this diet to stimulate the immune system activity has not achieved the results observed in Perciformes.

In 1994, Siwicki described the effect of different β-glucan feed as Macrogard 0.2g/100g feed, *C. utilis* 2.7g/100g feed, *S. cerevisiae* 2.7g/100g feed and a deacylated chitin 0.5g/100g feed) on rainbow trout administered for 7 days, which was observed in all diets an increased respiratory burst activity of blood, blood, phagocytic index, myeloperoxidase activity blood, serum total Ig and Blood potential killing activity after 1 week from the end of the diet (Siwicki et al., 1994). However, Kunttu et al. (Kunttu et al., 2009) administered MacroGard in doses of 0.2, 0.6 and 1.8%, finding only an increase in blood-respiratory burst activity in the two higher doses after 21 days of diet administration. In another study in rainbow trout was also administered MacroGard at a dose of 4.5 g/kg of feed and a β-glucan extracted from *H. vulgare* to 12.2, 16.7 and 26.4 g/kg of food for 9 weeks. None of these treatments were able to immunostimulate fish compared with control diet (Sealey et al., 2008).

In 2015 an interesting study was realized by Ghaedi et al in which they fed 4 Kg female broodstock for 3 month prior spawning with 0.1% and 0.2% of Macrogard and the control diet, and then for 2 month the descending fry in the same dosis of the immunostimulant diet. Brood fish fed with 0.2% β-glucan diet showed the highest levels of ACH50 and Lysozyme activity. The total serum Ig and IgM content was significantly higher in both treatments than the control diet. The descendent fry showed an increase in the levels of ACH50, lysozyme, Total Ig and IgM in descending fish from control feed and 0.2% Macrogard feed broodstock after 2 month with 0.2% of Macrogard, showing that feeding the immunostimulant diet in the broodstock did not promote the immunity in the fry.

In Atlantic salmon fry was evaluated the immunostimulant effect of LPS extracted from *A. salmonicida* at a concentration of 0.1% for 62 days, but neither was able to increase the immunoglobulins level (Guttvik, 2002).

1.2.3.5 Siluriformes.

Finally, in Siluriforms was also evaluated the effect of yeast derivates, on diets with β-glucan and synthetic levamisole (Sigma). The results showed the increase of blood-phagocytes respiratory burst activity and leucocyte myeloperoxidase content at 31 days post diet in both treatments (Kumari and Sahoo, 2006).

1.2.4 FISH IMMUNE RESPONSE INDUCED BY Β-GLUCANS.

Previous reports suggest that β-glucans modulate the expression of pro-inflammatory cytokines and chemokines. In carp head kidney macrophages has been observed that the β-glucan injection induced the expression of IL-1β (Selvaraj et al., 2005). Also, in rainbow trout (*Oncorhynchus mykiss*), the increase of IL-1 and also IL-6 was detected in head kidney, spleen, and liver after β-glucan injection (Løvoll et al., 2007). On the other hand, the up-regulation of pro-inflammatory (IL1, IL-6, TNF-α) and immunosuppresor cytokines (IL-10) and down-regulation of TGF-β was observed in rainbow trout head kidney leukocytes stimulated with β-glucan (Chettri et al., 2011). However, a diet supplemented with β-glucan (MacroGard) reduced the gene expression levels in gut of some pro-inflammatory-related cytokines (IL-1, IL-6, TNF-α1, TNF-α2) in common carp (Falco et al., 2014, 2012).

Thus, it seems that some controversy exists between in vitro experiments and the stimulatory effect of β-glucan administered as dietary supplement in fish. Moreover, the limited available information of the gene expression modulation does not allow to understand the possible pathways and immunological functions stimulated by the administration of β-glucan supplemented feed in a global context.

2. AIMS AND OVERVIEW

Based on the antecedents described above, the aims of this study were to evaluate the transcriptomic response in the gills of Gilthead sea bream (*Sparus aurata*) fed with β-glucan supplemented diet. Accordingly, the following tasks were proposed:

i) Determine the immunological and physiological effect of two different β-glucan supplemented diets from Gilthead seabream (*Sparus aurata*) serum samples.

ii) Evaluate the transcriptomic response in in the gills of Gilthead sea bream (*Sparus aurata*) fed with two different β-glucan supplemented diets in order to determine the possible immunological pathways modulated by the immunostimulant administration.

iii) Investigate the localization in the tissue of interest of the immunorelated genes differencially expressed in the microarray.

3. MATERIALS AND METHODS

3.1 FISH.

Juvenile Gilthead seabream (*Sparus aurata*) of 38.6±7.3g mean weight, were obtained from Institut de Recerca I Tecnologia Agroalimentàries (Sant Carles de la Ràpita, Spain). Fish were acclimatized to laboratory conditions for 14 days before the start of the experiment, maintained in a closed seawater recirculation system at 23°C, in a 12-h light/12-h dark cycle. Water quality indicators, such as dissolved oxygen, pH, nitrite, and ammonia, were analyzed periodically.

3.2 FEEDING.

Two different experimental diets, Diet A and Diet B (Skretting), and a control diet (Diet C, Skretting) were used in this study, using β-glucan as the main immunostimulant. For diet A was used the commercial diet Protec (5mg, 3mm, Skretting) which contains in its composition β-glucans, nucleotides, elevated levels of vitamins and minerals. These components were added in the diet in the fabrication process before extrusion of the pellet. Diet B also contains as main immunostimulant β-glucans but, unlike diet A, the component was added by top-coated to the commercial Nutra parr (Skretting) diet. Nutra parr with no additives was used as control diet (diet C).

The specimens were divided randomly into three groups; 120 fish per group distributed in 9 tanks (total 27 tanks, N=360), each group receiving one of the above mentioned diets. Fish were fed for 28 days at a rate of (3% body weight) thrice daily. Mortality in each group was recorded for the entire experiment.

3.3 BLOOD COLLECTION.

At 7, 14 and 28 of administration of the diet, 30 fish of every diet were randomly selected and sacrified by anesthetic overdoses (MS-222) and blood was taken from the caudal vein. The total erythrocytes were counted in a Neubauer camera; plasma

was separated by centrifugation at 720 *x g* per 5 minutes at 4 °C. Plasma aliquots were kept at -80 °C.

3.4 Plasma Physiological Measurements.

3.4.1 Plasma lysozyme activity was measured by a turbidimetric assay adapted to 96-well microplates (Sitjà-Bobadilla et al., 2005). Briefly, lyophilized *Micrococcus lysodeikticus* (Sigma, cat.#M3770) resuspended to 0.3mg/ml in 0.05M sodium phosphate buffer at pH 6.2 was used as a substrate for lysozyme (Sigma, cat.#L6876). Triplicates of plasma were added to 200µl of bacterial suspension and the reduction of the absorbance at 450 nm was measured after 0.5 and 5min.

3.4.2 Plasma Glucose content was measured in triplicates using the commercial kit Glucose LG (Spinreact, cat#41011) under manufacturer's instructions, with a detection limit of 1mg/dl. The reading was done at 490nm in VICTOR3.

3.4.3 Plasma Lactate activity was measured in triplicate using the commercial kit Lactate LO-POD (Spinreact, cat#1001330) under manufacturer's instructions adapted for 96 wells plate. The reading was done at 490nm in VICTOR3. The sensibility of the method is 1mg/dL and the linear limit is 150mg/dL.

3.4.4 Plasma Prostaglandin (PGE_2) levels were measured using the commercial kit Prostaglandin E2 EIA Kit – Monoclonal (Cayman, cat#514010.480) according manufacturer's instructions.

3.4.5 Cortisol was measured in the plasma by radioimmunoassay (Rotllant et al., 2000). The antibody for the assay was purchased from M.P. Biomedicals LLC and used in a final dilution of 1:4500. Antibody cross-reactivity with cortisol is 100%. The radioactivity was quantified using a liquid scintillation counter. The lower detection limit of the cortisol assay was 0.16ng/ml.

3.5 TISSUE SAMPLING.

Gills samples were taken at 2, 7, 14 and 28 days of administration of the Immunostimulant diets. All samples were divided and one part was frozen and kept at -80°C for RNA extraction.

3.6 RNA EXTRACTION.

100mg of frozen gills were used for RNA extraction using 1ml of TriReagent (Sigma, cat#T9424) following manufacturer's instructions. Total RNA concentration was determined by NanoDrop-1000 spectrophotometer (Thermo Scientific) and the integrity was measured by Agilent 2100 Bioanalyzer (Agilent Technologies). Samples with RIN values greater than 7 were chosen for microarray analysis. For diet A and B RNA from 3 different fish of the same treatment were pooled and 3 different pools of each group were used for microarray analysis (n=9). Control fish RNA from 4 different fish in every sampling point was pooled and 3 different pools were used for the microarray (n=16).

3.7 GILLS MICROARRAYS HYBRIDIZATIONS.

Hybridizations were performed using the Aquagenomic *Sparus aurata* oligonucleotide-microarray (SAQ) enriched in immune-related genes was used in this study. A full description is available in Gene Expression Onmibus (GEO) public repository (GSE28610). Briefly, a total of 43,398 oligonucleotide probes were used to construct a high-density seabream microarray based on the Agilent 4 × 44 K design format. 7,285 transcripts with annotated sequences were spotted in triplicate onto the slide (total probes 21,855), as well as 8,377 ESTs without annotation, 183 enriched sequences (GenBank) with 15 replicated probes (total probes 2,745), and finally 1,417 internal control probes of Agilent (N = 43,398). The platform developed used all available public ESTs stored and annotated in the Aquagenomic Consortium seabream library (10K).

Microarray analyses were conducted in pools samples obtained at 2, 7, 14 and 28 days of feeding (dof). For immunostimulant diets A and B, three pooled samples (n=3 fish per pool) were employed, whereas for diet C (reference sample) three pooled samples (n=4 fish per sampling point) were analyzed.

One-color microarray was applied in order to analyze the gene expression pattern in fish fed with three different diets. Briefly, Cyanine-3 (Cy3) labeled cRNA was prepared from 200ng of total RNA using the LowInput Quick Amp Labelling kit (Agilent, cat#) according to the manufacturer's instructions, followed by RNAeasy column purification (Qiagen, cat#). Dye incorporation and cRNA yield were checked with the NanoDrop ND-1000 Spectrophotometer. Then 1.65µg of Cy3-labelled cRNA (specific activity >6.0pmol Cy3/µg cRNA) were fragmented at 60°C for 30min in a reaction volume of 55µl containing 25x Agilent fragmentation buffer and 10x Agilent blocking agent following the manufacturer's instructions. On completion of the fragmentation reaction, 55 µl of 2x GEx hybridization buffer were added to the fragmentation mixture and hybridized to *Sparus aurata* custom array for 17h at 65°C in a rotating hybridization oven. After hybridization, microarrays were washed 1 min at room temperature (RT) with GE Wash Buffer 1 (Agilent, cat#), 1min at 37°C with GE Wash buffer 2 (Agilent, cat#), 45s at RT with Acetonitrile (Sigma, cat#), and 30s at RT with stabilization and drying solution (Agilent, cat#).

3.8 MICROARRAYS SCANNER, EXTRACTION AND DATA ANALYSIS.

Slides were scanned immediately after washing on the Agilent DNA Microarray Scanner (G2505B) using one color scan setting for 4x44k array slides (Scan Area: 61x21.6mm; Scan resolution: 5µm; Dye channel was set to Green and Green PMT was set to 100%). Spot intensities and other quality control features were extracted with Feature Extraction software version 10.4.0.0 (Agilent). Quality reports were checked for each array.

Extracted raw data were imported and analyzed with Genespring 12.5 GX software (Agilent technologies). Standard analytical methods were used to analyze the data. The percentile normalization (75%) was carried out and data was filtered by

expression. All samples were analyzed at gene-level by two different analytical approaches: relative to time (loop analysis), and a relative analysis to compare the immunostimulant diets with control diet (reference design). An unpaired t-test ($p < 0.01$) was used to identify significant differences between groups.

3.9 MICROARRAY VALIDATION BY ABSOLUTE QUANTIFICATION.

Specific primers for CD3ζ, MHC IIα, CD209, C/EBPB and PU.1 transcripts were designed. Plasmids were obtained using the pGEM-T easy vector (Promega, cat#A1380) and transformed into JM109 competent cells (Promega, cat #A1380). The plasmid was sequenced to check identity. A total of 1µg of RNA of all samples included in the microarray analysis were used to synthesize cDNA with iScript™ cDNA Synthesis Kit (BioRad, cat#170-8891). The cDNA was used as a template for absolute quantification in real-time RT-PCR (qRT-PCR) expression analysis, using the prepared plasmids as standard curve. The copy number of each transcript was analyzed using the MyIQ real-time PCR system (Bio-Rad, CA). Standard curves (Ct-Threshold cycle versus log copy number) of each transcript were done with serial dilutions of DNA plasmid purifications from $1x10^0$ to $1x10^9$ copies. Each sample was tested in triplicate in a 384-well plate (Bio-Rad, CA). The reaction mix (10µL final volume) consisted of 5µL of iQ SYBR Green supermix (Bio-Rad), 0.5µL of each primer (500nM final concentration), 1.5µL of H2O, and 2.5µL of a 1/40 dilution of the cDNA sample. The running condition consisted of one step at 95°C for 3min, followed by 40 cycles of 10s at 95°C and 30 s at 60°C, following a melting curve dissociation analysis, from 65 °C to 95°C with increments of 0.5°C. Data were analyzed by one-way analysis of variance (ANOVA) followed by the post hoc multiple comparison (Bonferroni's analysis, $p<0.05$).

3.10 PARAFFIN EMBEDDING.

Gills samples, previously fixed in 4% of paraformaldehyde (PFA), were embedded in paraffin. The inclusion protocol was performed in an automatic inclusor (Leica TP1020) as follows; increasing ethanol concentrations (70% for 30min; 80%, and 96% two times for 20min each; 100% two times for 30min, and 100% for 40min),

then ethanol 100%/xilol for 30 min, xilol two times for 40min, and paraffin two times for 1h were added to the samples. The blocks were made with paraffin Histo-comp fusion temperature 56-58°C. The blocks were maintained at RT until sectioning.

3.11 *IN SITU* HYBRIDIZATION.

In situ hybridization was performed to characterize the mRNA distribution of some interesting differentially expressed genes related with immune response and obtaining in the microarray analysis. CCAAT/Enhancer Binding Protein (C/EBP) Beta (C/EBPB), transcription factor PU.1, transmembrane receptor CD209, major histocompatibility complex class II alpha (MHC IIα), T-cell receptor CD3-ς, and tumor necrosis factor receptor (TNFRSF1A) were monitored in gills of seabream fed with immunostimulant diets. Hybridization of each gene was performed in several fish. Probes were amplified with specific primers (Table V) from cDNA synthesized from fish fed with the immunostimulant diet. The probes were cloned into vector pGEMT-Easy (Promega, cat#A1360) cloned into *E. coli* DH5α purified with NucleoSping Plasmid QuickPure (Macherey-Nagel, cat#74065) and the insert was sequenced in order to check identity and orientation. Riboprobes) Antisense (AS) and Sense (S were synthesized with the linearized plasmid using Riboprobe in vitro Transcription Systems (Promega) according to manufacturer's instruction, with 0.35mM digoxigenin-UTP (Roche). The RNA probes were kept at -80ºC until use.

TABLE IV: List of primers used for ISH probes.

Gen	*Primer*	*Primer Rev*	*Length (pb)*	*T° Annealing (°C)*
CD3-z	Fwd	CCACCAAGGACACCTACGAC	**277**	**60**
	Rev	TTAACGCAGAACGTCCACGA		
MHCII-α	Fwd	CCCAACACCCTCATCTGCTT	**318**	**60**
	Rev	GACAACTCCAATCAGGCCCA		
CD209	Fwd	GGGAAGTGGTGTCCTGATGG	**314**	**60**
	Rev	CATGATGCGTACTGGCTCCT		
C/EBPB	Fwd	GGAGACAAGTCAGTGCGACA	**314**	**60**
	Rev	ACCTGCTTTTACCTGACGGG		
TNFRSF1A	Fwd	TTTTAGCTGCTGTTGCTGCG	**300**	**60**
	Rev	GACGGAAGAACCTCCTGTGG		
PU.1	Fwd	CCCCGCTTGAGGTTTCAGAT	**347**	**60**
	Rev	CACTGCCTCACTGAACTGGT		

Sections of 5µm were deparafined washing 2 times with xilene for 10min, followed in decreasing concentrations of ethanol (100% two times, 70% and 50%, 2min each) and washed in 1x KPBS for 2min. Hybridization was performed as followed, 5 minutes with 10µg/ml Proteinase K, (100mM Tris-HCl pH=8, 50mM EDTA) and the tissue was post-fixate with 4% PF pH=7.5 in 1x KPBS, sections were rinsed again 2 times in 1x KPBS for 2min. Slides were treated with 0.1M of TEA buffer (Triethanolamine pH=8.0) for 3min and then incubated for 10min in 0.25% acetic anhydride in 0.1M of TEA buffer followed by a wash for 1min with 2x SSC. The tissue was dehydrated in increasing concentrations of ethanol 1min each (50%, 70%, 90% and two times in 100%). The slides were hybridized with 100ng each probe S and AS in hybridization buffer (10mM Tris-HCl pH=7.5, 1mM EDTA, 50% formamide, 10% dextran sulphate, 0.2% tween 20 and 0.1% block solution) for 16h at 65°C in *in situ* humid chamber. The slides were washed two times for 30min with 2x SSC at RT and treated with 50% of deionized formamide in 2x SSC at 65°C per 30min followed by two washes for 10min in 2x SSX at 37°C. The tissue was treated with 0.02mg/ml RNase A in RNase buffer (10mM Tris-HCl pH=7.5, 500mM NaCl, 1mM EDTA) for 30min at 37°C and then washed with RNase buffer per 30min at 65°C. Non specific sites were blocked with 2% of block solution (0.05% Triton x-100, in 2x SSC) for 3h and then rinsed in maleat buffer 2 times per 5min and sections were incubated for immunohistochemical detection with antideoxigenin-alcaline phosphatase FAB-fragment (1:2000) (in 1x maleate buffer, 1% block solution, 0.3% triton x-100) overnight in humid chamber. Sections were rinsed 2 times per 10min with 1x maleate buffer, and washed with visualization buffer per 10min (100mM Tris-HCl pH=9.5, 5mM MgCl, 100mM NaCl) following by the revelation with NBT (nitroblue tetrazolium chloride, Sigma) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate, 4 toluidine salt, Sigma) in visualization buffer, slides were incubated in dark until developed of the color, the reaction was stopped with 10mM Tris-HCl pH=7.5, 1mM EDTA, 150mM NaCl. Sections were mounted with DAPI and laid overnight. The pictures were taken with Eclipse 80 at 20x or 40x.

4. RESULTS

4.1 PLASMA PHYSIOLOGICAL MEASUREMENTS.

The mortality was recorded each day from the beginning until the end of the experiment, and no significant differences in mortality were found between the fish feed with the control diet and the immunostimulant diets A and B (Figure 1A). The erythrocyte count was analyzed at 7, 14 and 28 days of administration of the diets. There were no significant differences through all the time sampling points and between the groups in this study (Figure 1B).

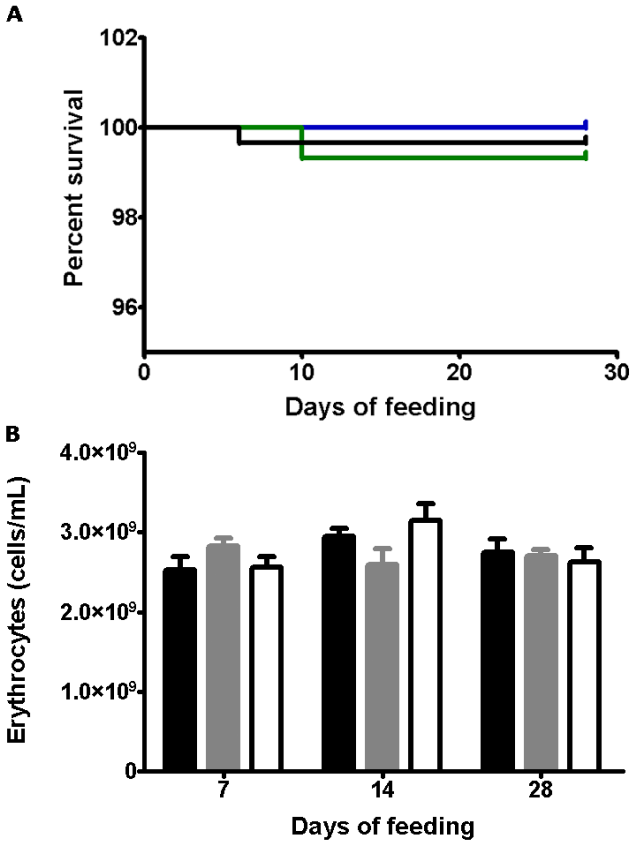

FIGURE 1: **(A)** Mortality rate after 28 days of feeding with the control diet (black line) and IS diets A (blue line) and B (green line). **(B)** Erythrocytes count after 7, 14 and 28 days of administration of IS diets. Control diet (black bars), diet A (grey bars), diet B (white bars). Data are expressed as means ±SEM. No significant differences are observed after two way ANOVA and Bonferroni post-test ($p< 0.05$).

Serum lysozyme activity, cortisol, glucose, lactate, and prostaglandin plasma content were measured. A significant increase was found in lysozyme activity at 7 and 14 days when diet A was compared with control diet. Also was observed an increase in the lysozyme level in diet B compared with control diet at 14 days of feeding. At 28 days there were no significant differences between the IS diets and control diet (Figure 2).

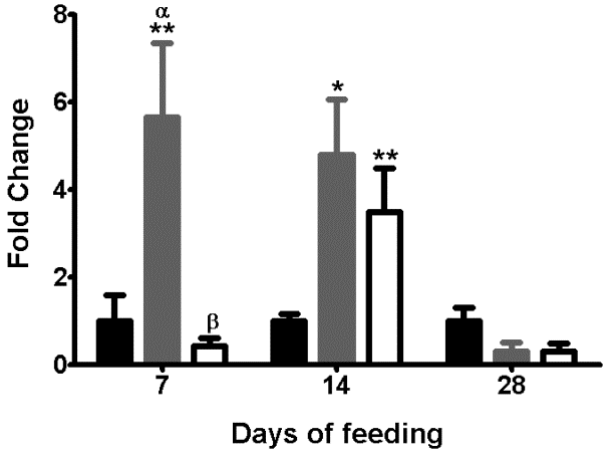

FIGURE 2: Serum lysozyme activity of *Sparus aurata* after 7, 14 and 28 days of administration of IS diets. Black bars represent control diet , grey bars represent diet A and white bars represent diet B. Data are expressed as means ±SEM. Asterisks represent significant differences compared with the control group. (*= $p< 0.05$, **=$p<0.01$, ***=$p<0.001$). Different letters represent significant differences between IS diets A and B. *P* values from two way ANOVA and Bonferroni post-test.

The cortisol results (Figure 3) showed at 7 and 14 days a significant lower level of cortisol in the fish that were feed with both immunostimulant diets. This reduction in the cortisol level was observed in all sampling time-point, although at 28 days of administration of the IS diets only diet A shows a significant reduction compared with the control group

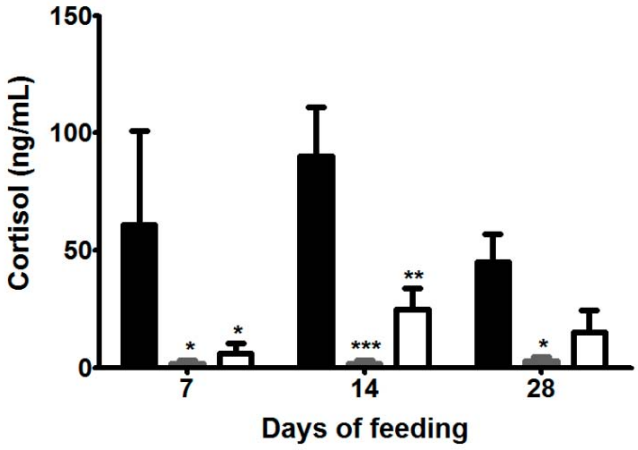

FIGURE 3: Cortisol plasma levels of *Sparus aurata* after 7, 14 and 28 days of administration of IS diets. Control diet (black bars), diet A (grey bars), diet B (white bars). Data are expressed as means ±SEM. Asterisk represents significant differences compared with the control group (*=p< 0.05, **=p<0.01, ***=p<0.001). Different letters represent significant differences between IS diets A and B. *P* values from two way ANOVA and Bonferroni post-test.

The glucose content showed no significant differences in both IS diets at 7 or 28 days of administration of the diet; however, a small significant increase at 14 days was found in diet A compared with the control diet (Figure 4A). For plasma lactate content an increased trend is observed among all days analyzed, noting also a significant decrease in both IS diets levels compared with the control diet at 28 days of feeding (Figure 4B).

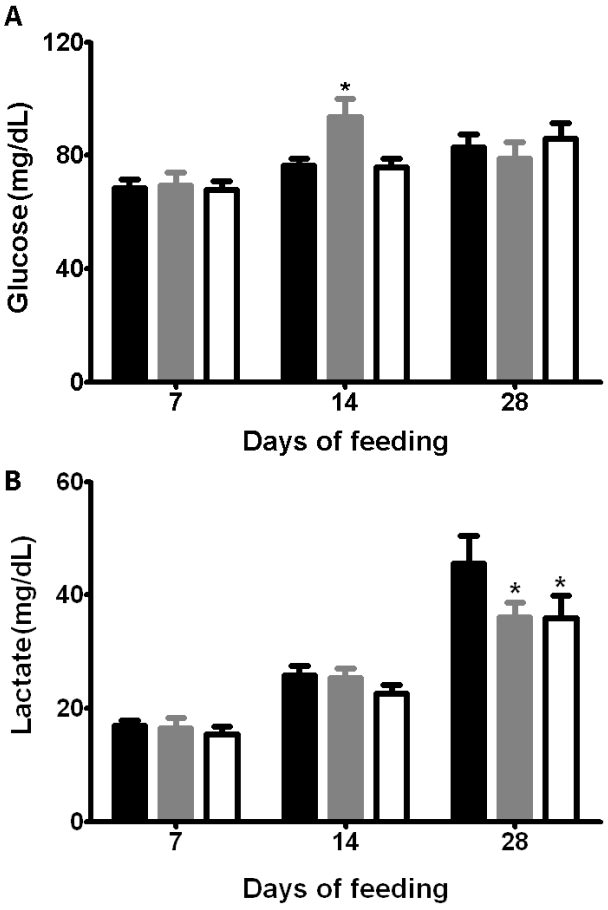

FIGURE 4: Plasma glucose **(A)** and lactate level **(B)** in *Sparus aurata* after 7, 14 and 28 days of administration of IS diets. Control diet (black bars), diet A (grey bars), diet B (white bars). Data are expressed as means ±SEM. Asterisk represents significant differences compared with the control group. (*=p< 0.05, **=p<0.01, ***=p<0.001). Different letters represent significant differences between IS diets A and B. *P* values from two way ANOVA and Bonferroni post-test.

The serum prostaglandin content showed an increase only at 14 dof in diet A compared both with control diet and diet B (Figure 5).

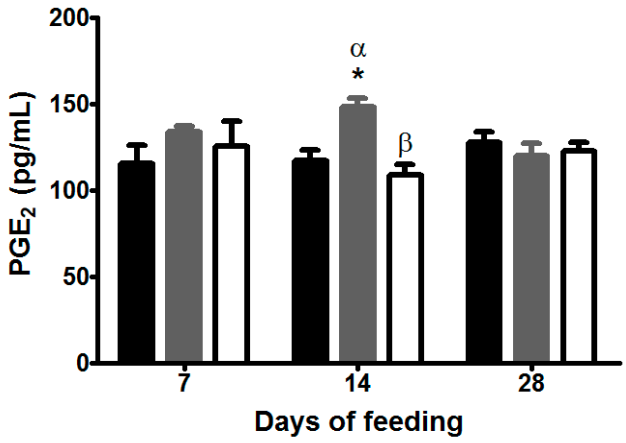

FIGURE 5: Plasma Prostaglandin (PEG2) content of *Sparus aurata* after 7, 14 and 28 days of administration of IS diets. Control diet (black bars), diet A (grey bars), diet B (white bars). Data are expressed as means ±SEM. Asterisk represents significant differences compared with the control group (*=p< 0.05, **=p<0.01, ***=p<0.001).. Different letters represent significant differences between IS diets A and B. *P* values from two way ANOVA and Bonferroni post-test

4.2 TRANSCRIPTOME RESPONSE TO IMMUNOSTIMULANT DIETS.

To understand the effect of the immunostimulant diets in the seabream at transcriptomic level, an oligunucleotide-specific microarray was carried out. Two analysis were conducted, a control reference analysis (Figures 6 and 7) and a loop analysis (Figure 8). The control reference analysis showed 462 genes for diet A and 337 genes for diet B were differentially expressed compared to the control diet (Figure 6A), with 179 common genes between both IS diets. For the diet A, 164 transcripts showed a absolute fold change (AFC) between 1 and 1.5, 154 transcripts with a AFC between 1.5 and 2, 138 transcripts with a AFC between 2 and 3, and 5 transcripts with a AFC higher than 4 (Figure 6A). Diet B shows a similar profile than diet A, except for the transcript with AFC higher than 3, in which were differentially expressed 15 transcripts. Only the 1% (Diet A) and 4% (Diet B) of them has a fold change highest than 3.

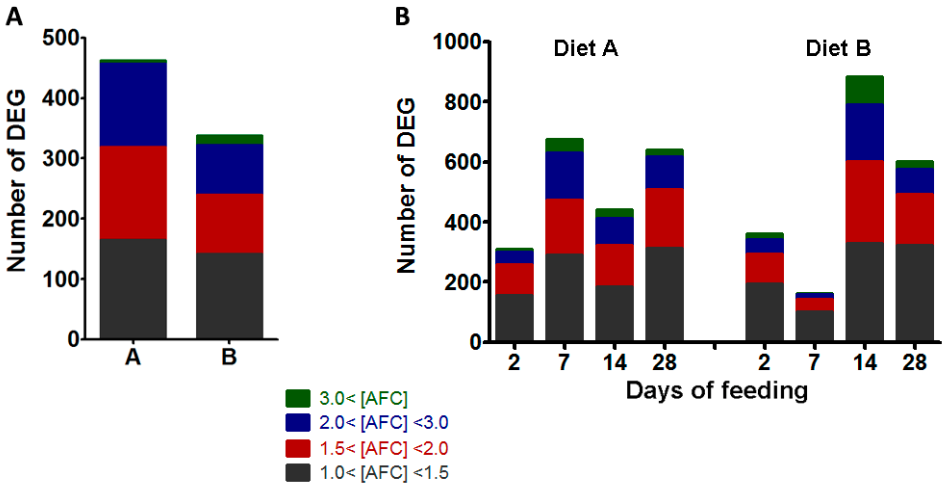

FIGURE 6: Magnitude of gills transcriptome response of *Sparus aurata* fed with immunostimulant diets in a control reference analysis. Number of differential expressed genes (DEGs) with an AFC interval of 1.0< [AFC] <1.5 (grey), 1.5< [AFC] <2.0 (red), 2.0< [AFC] <3.0 (blue), 3.0< [AFC] (green) are represented. **(A)** Total numbers of DEGs in diet A and diet B using as reference the control diet (p< 0.01). The number of DEGs for each IS diet are shown **(B)** DEGs in diets A and B in a time-dependent analysis using the control diet as reference (p< 0.01). The number of DEGs for each time-point analyzed for diet A (left) and diet B (right) are indicated.

The time-dependent analysis of diets A and B using the control diet as reference point showed that the regulation of the response it is higher at 7 days for diet A and at 14 days for diet B (Figure 6B). Also at 7 days in diet B was observed the lowest quantity of DEGs, with only 159 transcripts. For both diets, most of the transcripts that were differentially expressed had an absolute fold change (AFC) value under 1.5 (Figure 6B).

When the IS diet A was compared with diet B (A vs B) 130 DEGs were obtained (Figure 7). When we look into the time-dependent analysis between both diets, the highest difference was observed after of administration of the diet with 514 DEGs and the most of them had an AFC less than 2, indicating the transcripts that are differentially expressed between both diets had a low magnitude (Figure 7).

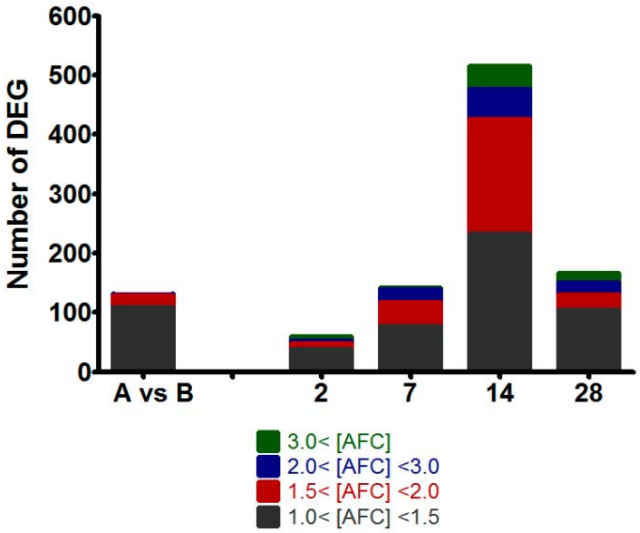

FIGURE 7: Time-dependent analysis between diets A and B of *Sparus aurata* fed with immunostimulant diets after 2, 7, 14 and 28 days of feeding. The number of DEGs between diet A and B are shown on the left, and the time-dependent analysis between diets A and B is shown on the right. The AFC interval of 1.0< [AFC] <1.5 (grey), 1.5< [AFC] <2.0 (red), 2.0< [AFC] <3.0 (blue), 3.0< [AFC] (green) are represented. DEGs with p< 0.01 are shown.

In order to observe the transient changes in both IS diets using the control diet as a starting point, a loop analysis was performed comparing each time-point with the previous one in a temporal scale. The loop design analysis of gills transcriptome response showed on day 2 of feeding a high total number of DEGs, both in diet A (307) and diet B (358) (Figure 8). This result is mainly because on day 2 both IS diets were compared with the control diet. The total number of DEGs became larger from day 7 onward for both IS diets, up- and down-regulated genes, noting that there was more down- than up-regulated genes with exception of diet B at 28 days of feeding in which were found 390 up-regulated and 294 down-regulated genes (Figure 8).

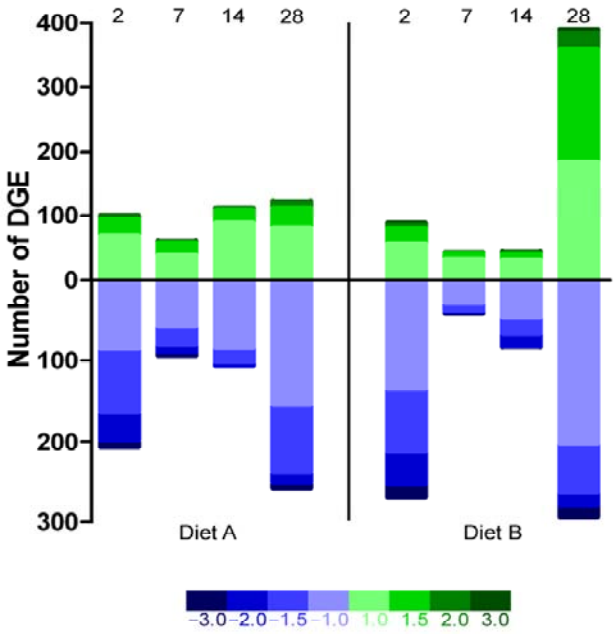

FIGURE 8: Number of differentially expressed genes (DEGs) on gills transcriptome response of *Sparus aurata* fed with immunostimulant diets after 2, 7, 14 and 28 days obtained from the loop design analysis. Green bars are up-regulated genes and blue bars are down-regulated genes. DEGs with p< 0.01 are shown. The fold change is represented by color scale (bottom).

4.3 CLASSIFICATION OF MICROARRAY DEGS INVOLVED IN THE *SPARUS AURATA* IMMUNE SYSTEM.

To unveil the effects of the IS diets on sea bream immune system at expression level, the DEGs involved in the immune response in fish fed with each diet were listed based on the loop analysis. The list of differentially expressed immune-related genes with a p value <0.01 and with a cut-off fold change higher than 1.0, is shown on the Table G. For diet A, most of the immune-related genes were mainly expressed among days 2, 7 and 14 of feeding. In the case of diet B, the immune-related genes were expressed on day 2, 14 and 28 but not immune-related relevant genes were

expressed on day 7 of feeding. The complete list of the microarray analysis is provided in the Supplementary Tables S1 to S8.

The loop analysis shown the regulation in the expression of genes involved in Inflammatory response, B and T cell response, transcription factors and dendritic cell response, apoptosis related process, among others. For diet A, on day 2 of feeding were up-regulated genes like perforin 1 (Prf1), programmed cell death 7 (PDCD7), Interferon induced 35kD protein (IFP35), and caspase 1 (Casp1) while on diet B only IFI35 and hepcidin were also up-regulated. At day 7 was found immune-related DEGs only in diet A such as Mast cell preprotein (CMA1), T cell receptor (TCRβ), CD82 and C type lectin domain family 4 member E (CLEC4E, also named Mincle). On day 14, for diet A were up-regulated several genes involved in innate and adaptive immunity such as interleukin enhancer binding factor 2 (ILF2), Granulocyte colony stimulating factor receptor (CSF3R) and B cell receptor associated protein 31 (BCAP31) among others. On day 14 for diet B were found up-regulated genes like T cell receptor gamma chain (TCRγ), CLEC4E, lysozyme and CCAAT/ enhancer binding protein beta (C/EBPB). At 28 days we found up-regulated Neurolina and Gamma interferon inducible lysosomal thiol reductase (IFI30) for diet A, while Tumor necrosis factor receptor 1 (TNFRSF1A), C/EBPB, chemokine receptor 7b (CXCCR7) were also up-regulated on diet B.

Several immune-related genes were down-regulated in fish fed with the IS diets like FYN binding protein (FYB) and Tyrosine protein kinase BTK (BTK) for diet A, and genes like Complement component C7 (C7), MHC-II or CMA1 for diet B at 2 days. On day7 was down-regulated only cathepsin L (CTSL1) in diet A. At 14 days adrenomedullin 1 (ADM1) and CD40 for diet A, and CD38 for diet B were also down-regulated. At 28 days was down-regulated only C7 on diet A, and on diet B several genes were involved with T cell function as TCRβ, CD3ζ, CD8α, and transcription factors such as PU.1 and Suppressor of cytokine signaling 1 (SOCS1).

TABLE G: Differential expressed genes involved in the immune response of in fish fed with each IS diet. The DEG classified based on the loop analysis with a cut-off fold change higher than 1.0 on day 2, 7, 14, and 28 of feeding are shown. The immune-related genes p-value and the fold change (FC) for up-(green) and down-regulated genes (blue) are represented

Accession Number	Gene	Annotation	Diet A		Diet B	
			p-value	FC	p-value	FC
Day 2						
gb:AM972222.1	PRF1	Perforin1 precursor	7,18E-05	2,65		
gb:AAT68065.1	PDCD7	Programmed cell death 7	7,75E-03	2,02		
gb:ACI34366.1	IFI35	Interferon induced 35 kDa protein homolo	6,29E-04	1,57	1,74E-03	1,36
gb:ABB05055.1	CASP1	Caspase1	2,46E-03	1,47		
gb:AM952446.1	CD59	CD59	6,98E-03	1,35		
gb:FP332189.1	HAMP	Hepcidin			5,05E-03	1,27
gb:FP338632.1	CLEC	Ctype lectin receptor			5,04E-03	-1,19
gb:AAH98511.1	TRIM8	Tripartite motif containing 8	4,94E-03	-1,26		
gb:ACI33377.1	FYB	FYN binding protein	2,46E-03	-1,27		
gb:AM979060.1	MAP3K8,	Mitogen activated protein kinase kinase kin	6,26E-03	-1,28		
gb:ACI33181.1	BTK	Tyrosine protein kinase BTK	1,29E-03	-1,34		
gb:AM970253.1	CD209	CD209 antigenlike protein A			6,20E-03	-1,44
gb:ACI32892.1	C7	Complement component C7			3,60E-03	-1,58
gb:AM965522.1	MHC2	MHC class II alpha chain			4,38E-03	-1,90
gb:P23946.1	CMA1,	Mast cell preproprotein	6,15E-03	-2,15	8,41E-03	-2,06
Day 7						
gb:P23946.1	CMA1,	Mast cell preproprotein	5,17E-03	1,55		
gb:AM971036.1	TCRB	Tcell receptor beta chain (tcrb gene)	8,36E-03	1,41		
gb:AM962749.1	CLEC4E	Ctype lectin domain family 4 member E	6,16E-03	1,37		
gb:AM976088.1	CD82	CD82 antigen	7,93E-03	1,36		
gb:AM954188.1	CTSL1	Cathepsin L	7,55E-04	-1,46		
Day 14						
gb:AM968967.1	TCR	T cell receptor gamma chain			1,68E-03	1,89
gb:AM951455.1	ILF2	Interleukin enhancer binding factor 2	4,77E-03	1,60		
gb:AM950807.1	CSF3R	Granulocyte colony stimulating factor	1,16E-03	1,51		
gb:AM966093.1	TRPM4	Transient receptor potential cation	5,38E-03	1,49		
embl:CAQ15712.1	IGSF2_3	Member 3 (IGSF3)	8,33E-03	1,45		
gb:AM962749.1	CLEC4E	Ctype lectin domain family 4 member E			9,18E-04	1,44
gb:AM959877.1	BCAP31	B cell receptor associated protein 31	4,70E-03	1,43		
gb:AM954714.1	C/EBPB	CCAAT/enhancer-binding protein beta 2			7,95E-03	1,39
gb:AM955284.1	Lysozyme	Lysozyme			7,68E-03	1,30
gb:AM976088.1	CD82	CD82 antigen			3,52E-03	1,25
gb:AAV52829.1	p38	Mitogen activated protein kinase p38a (MA	1,41E-03	-1,22		
ddbj:BAD02341.1	ADM1	Adrenomedullin1	9,01E-03	-1,27		
dg:AM976484.1	CD40	Tumor necrosis factor receptor	2,54E-03	-1,50		
sp:Q64244	CD38	CD38 antigen (ADP-ribosyl cyclase 1)			2,30E-03	-2,19

TABLE G: (*continuation...*)

Accession Number	Gene	Annotation	Diet A		Diet B	
			p-value	FC	p-value	FC
		Day 28				
gb:AM975118.1	TNFRSF1A	Tumor necrosis factor receptor1			3,53E-04	1,76
gb:AM964255.1		Class I helical cytokine receptor number 29			3,39E-03	1,74
gb:AM954714.1	C/EBPB	CCAAT/enhancerbinding protein beta 2			1,58E-03	1,64
gb:AM961799.1	CXCR7	Chemokine (CXC motif) receptor 7b			1,60E-03	1,58
gb:AM963610.1	IFI30, GILT	Gamma interferon inducible lysosomal	5,67E-04	1,58		
gb:ABC50098.1	ALCAM	Neurolina	3,73E-03	1,54		
gb:ABC70999.1	CASP9	Caspase9			1,41E-03	1,32
embl:CAL90974.2	ITGB2	CD18 protein			6,45E-03	-1,33
gb:AM954103.1	CASP3	Caspase3 (CASP3)			6,46E-03	-1,37
gb:AM974862.1	PU.1	Transcription factor PU.1			2,88E-03	-1,41
gb:AM971036.1	TCRB	T cell receptor beta chain			8,04E-04	-1,43
gb:AM976122.1	CD247	T cell surface glycoprotein CD3 zeta chain			1,47E-03	-1,46
ddbj:BAD02341.1	ADM1	Adrenomedullin1			8,81E-04	-1,57
gb:AM970739.1	CD8	CD8 alpha			9,36E-03	-1,60
gb:ACI32892.1	C7	Complement component C7	4,51E-03	-1,73		
gb:AAH77158.1	SOCS1	Suppressor of cytokine signaling 1			2,06E-03	-2,19

4.4 TEMPORAL MODULATION OF THE IMMUNO-RELATED GENES IN THE IS DIETS.

In order to associate the immune-related DEGs obtained from the loop analysis to functional processes, the genes were grouped in inflammatory response (Figure 9), T cell-mediated immune response (Figure 10), and apoptosis (Figure 11). The temporal modulation was represented by the normalized expression for each immune-related gene in all days of feeding evaluated in this study.

Several genes involved in inflammatory response were differentially expressed based on the loop analysis. It was noted that some genes had a similar expression curve on the days of feeding analyzed such as TNFRSF1A and C/EBPB, CLEC4E and CMA, SOCS1 and CD18, and CSF3R and Hepcidin (Figure 9).

Among these genes, TNFRSF1A was up-regulated after 14 days of administration of diet A, with a higher increase at 28 dof, also is significantly up-regulated at 28 dof in diet B. Similar curve is observed on C/EBPB for both immunostimulant diets, with a significant up-regulation in the loop analysis at 14 and 28 days of feeding on diet B,

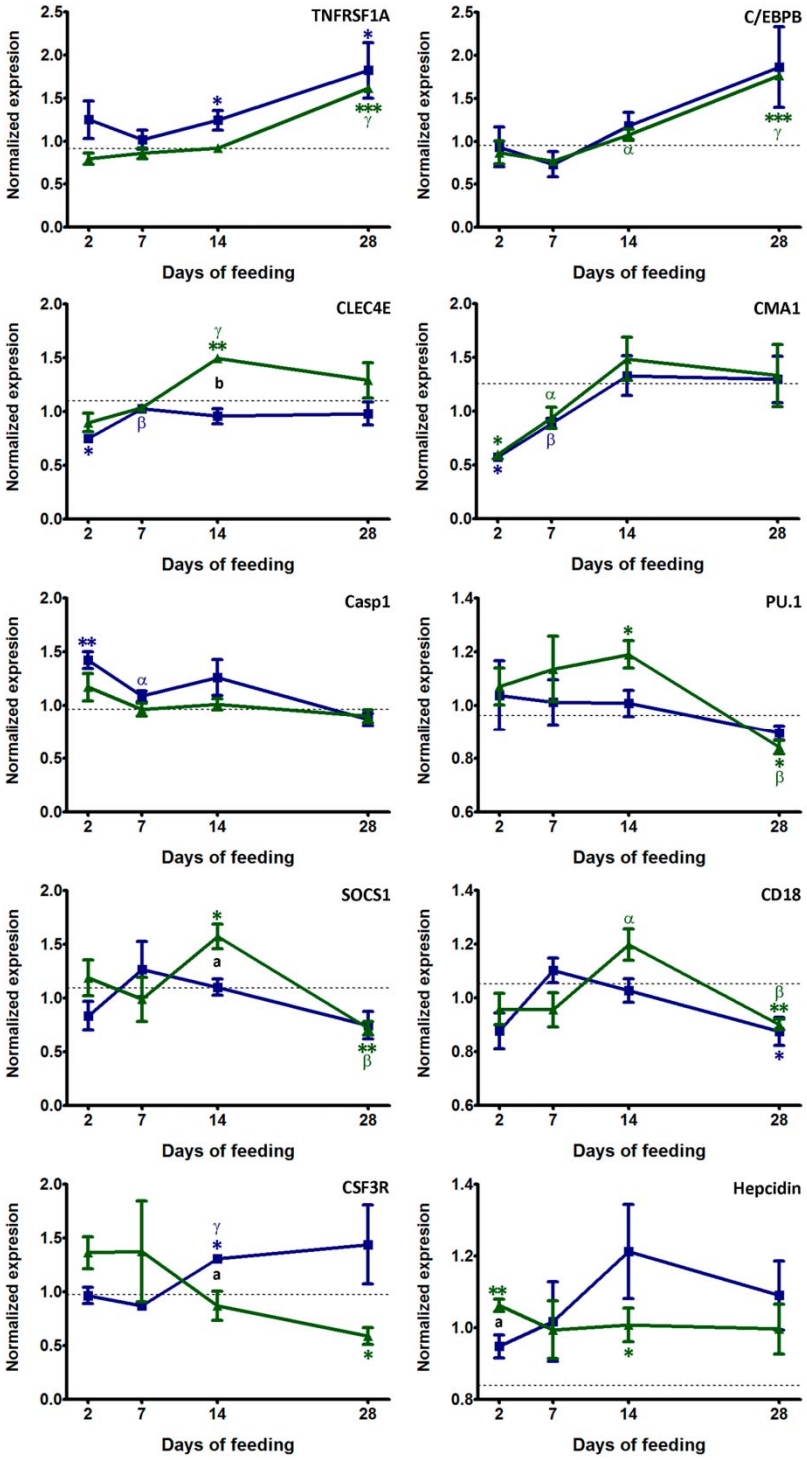
TNFRSF1A
C/EBPB
CLEC4E
CMA1
Casp1
PU.1
SOCS1
CD18
CSF3R
Hepcidin
Normalized expresion
Days of feeding
2
7
14
28

FIGURE 9 (PAGE 66): DEGs involved in **INFLAMMATORY RESPONSE** based on the loop analysis The temporal modulation of TNFRSF1A, C/EBPB, CLEC4E, CMA-1, Caspase 1, Transcription factor PU.1, SOCS1, CD18, CSF3R and Hepcidin on days 2, 7, 14 and 28 of feeding of diet A (square, blue line), diet B (triangle, green line), and pooled fish fed with control diet (discontinuous line) is represented.. Asterisk represent significant differences compared to control group (*=p< 0.05, **=p<0.01, ***=p<0.001), latin letters represent significant differences between diet A and B (a=p< 0.05, b=p<0.01, c=p<0.001), and greek letters represent significant differences in the loop analysis (α=p< 0.05, β=p<0.01, γ=p<0.001).

also at 28 dof was found an up-regulation compared to control diet. For diet A, despite the curves were very similar with diet B, there were no significant differences among the loop analysis or compared to control diet. In the case of CLEC4E and CMA1 on day 2 of feeding a down-regulation was found on diet A and both IS diets, respectively. Moreover, this trend in the expression of CLEC4E on diet A was maintained during the days evaluated in this study. Both in CLEC4E and CMA1 the expression increased on day 14 and 28 although this change was significant only for diet B on day 14. Casp 1 showed an up-regulation only at the beginning of the experiment (2 dof) for diet A, while on diet B the levels remain unchanged the whole experiment. The transcription factor PU.1 and SOCS1 are both up-regulated on diet B at 14 days of feeding with a significant reduction at 28 days of feeding, which was also observed in CD18 in both IS diets. Significant differences were found between diet A and diet B at 14 dof in the expression of SOCS1.

Similar expression is found in CSF3R, at 14 dof it is observed an increased in the expression of this gene for diet A both compared to control diet and at 7 dof; in contrast, for diet B only a down-regulation at 28 dof compared to control diet was noted. For hepcidin gene, a trend in up-regulation was observed in all days compared to control diet, but this regulation was significant only at 2 and 14 dof in diet B.

Regarding T cell-mediated immune response, on diet A an increased in CD209, CD40 and CD82 was observed mainly at 7 days of feeding (Figure 10). By contrast, on diet B most of genes (CD209, TCRβ, TCRγ, CD8, CD3ζ and CD82) were up-regulated at 14 days of feeding and then the levels returned to the basal level on day 28. Also was

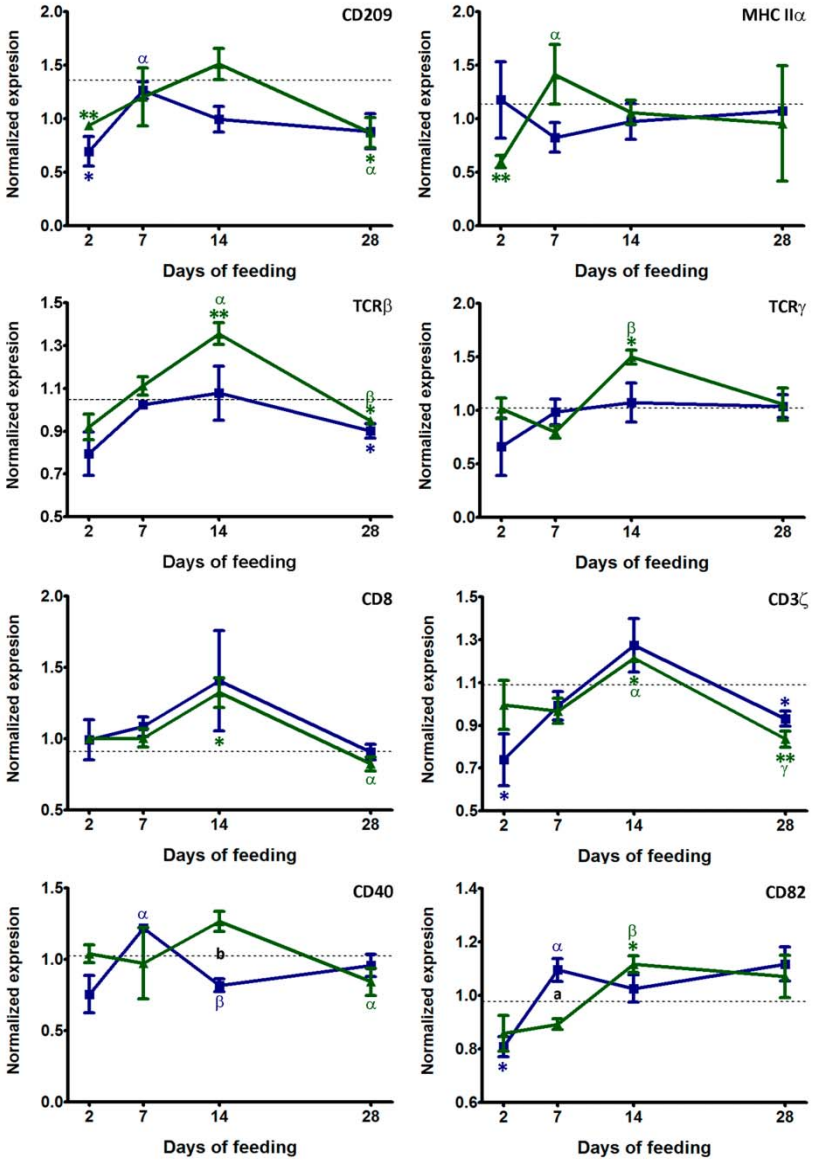

FIGURE 10: DEGs involved in **T CELL-MEDIATED IMMUNE RESPONSE** based on the loop analysis. The temporal modulation of CD209, MHC IIα, TCRβ, TCRγ, CD8α, CD3ζ, CD40 and CD82 on days 2, 7, 14 and 28 of feeding of diet A (square, blue line), diet B (triangle, green line), and pooled fish fed with control diet (discontinuous line) is represented. Asterisk represent significant differences compared to control group (*=p< 0.05, **=p<0.01, ***=p<0.001), latin letters represent significant differences between diet A and B (a=p< 0.05, b=p<0.01, c=p<0.001), and greek letters represent significant differences in the loop analysis (α=p< 0.05, β=p<0.01, γ=p<0.001).

noted for MHC IIα a trend toward down-regulation in regard to the control diet on both IS diets, whose decrease was significant for diet B at 2 days of feeding. However a significant slight increase in the loop analysis was found at day 7 on diet B (Figure 10).

The expression of Perforin 1, BCAP31 and IFI35, involved in apoptosis-related processes (Figure 11), shown a similar profile with an up-regulation at the beginning of the immunostimulant diet administration and then fell to control diet expression level whit a posterior return to basal levels. In the case of Perforin 1 a significant increased was observed for diet A at 2 dof and then return to basal level, but for diet B no significant differences were observed in all days tested. For BCAP31 also an increased expression trend was observed at the beginning of the feeding, but only in the loop analysis a significant up-regulation was observed

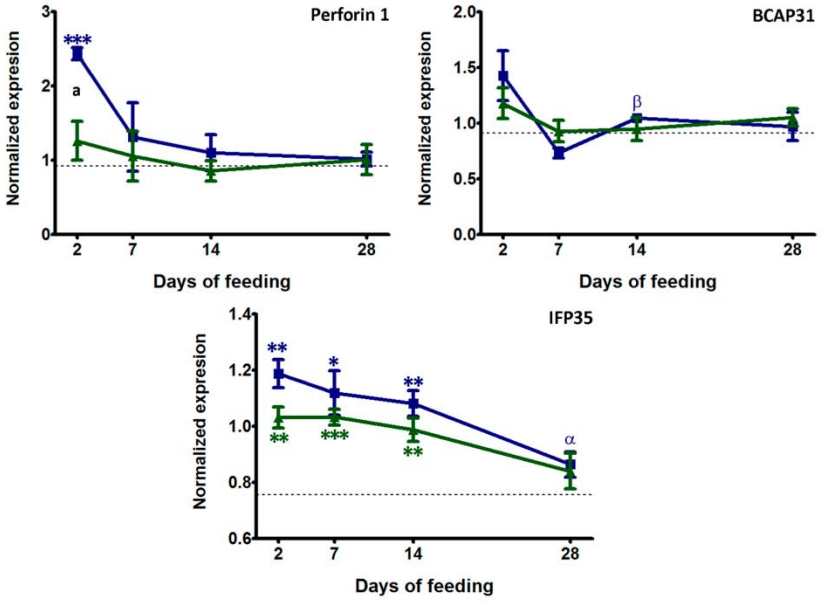

FIGURE 11: DEGs associated with **APOPTOSIS** based on the loop analysis. The temporal modulation of Perforin 1, BCAP31, and IFI35 on days 2, 7, 14 and 28 of feeding of diet A (square, blue line), diet B (triangle, green line), and pooled fish fed with control diet (discontinuous line) is represented. Asterisk represent significant differences compared to control group (*=p< 0.05, **=p<0.01, ***=p<0.001), latin letters represent significant differences between diet A and B (a=p< 0.05, b=p<0.01, c=p<0.001), and greek letters represent significant differences in the loop analysis (α=p< 0.05, β=p<0.01, γ=p<0.001).

at 14 dof compared to 7dof. For IFI35 a slight significant decrease in the expression level was noted until 14 days of feeding on both IS diets and then the values fell to control diet expression level.

4.5 MICROARRAY VALIDATION BY ABSOLUTE QUANTIFICATION QPCR.

To validate the microarray results, absolute qPCR quantification was performed. Five different transcripts were analyzed, Perforin 1, TCRβ, CLEC4E, C/EBPB and PU.1. Fold-change regulation values of the microarrays and the qPCR are summarized in Table 5, supporting and corroborating the results obtained in the transcriptomic analysis.

TABLE V: Microarray validation analysis by absolute qPCR quantification based on mRNA fold change (FC).

				Microarray		QPCR	
Gene	**Annotation**	**Diet**	**Condition**	**FC**	**Regulation**	**FC**	**Regulation**
Perforin 1	Perforin 1 precursor	A	2d vs C	2.65	Up	1.93	Up
TCRβ	T cell receptor beta chain	A	7d vs 2d	1.40	Up	2.19	Up
CLEC4E	C type lectin domain family 4 member E	A	7d vs 2d	1.36	Up	1.17	Up
		B	14d vs 7d	1.44	Up	1.65	Up
C/EBPB	CCAAT/enhancer-binding protein beta	B	14d vs 7d	1.40	Up	1.56	Up
PU.1	PU.1	B	28d vs 14d	-1.14	Down	-3.07	Down

4.6 *IN SITU* HYBRIDIZATION ANALYSIS.

In situ hybridization was performed in gills of seabream, in order to characterize the mRNA distribution of some interesting differentially expressed genes involved in the immune response to β-glucan suplemented immunostimulant diets.

Gill tissue sections were analyzed with gene-specific designed probes for CD3ζ, MHC IIα, CD209, C/EBPB, TNFRSF1A and PU.1. PU.1 did not give a clear signal, and therefore was discarded for further analysis. In all the analyzed genes the specificity of each probe was checked showing the antisense probe (target gene) a very strong brown signal localized in the secondary lamellae (Figures 12 and 13), whereas no signal in the sense probe (S, control) was found in any genes tested. For CD209 (Figure 12 A and B) and C/EBPB (Figure 12C and D) a strong signal was found in the

secondary lamellae cells. The morphology of this cell type is granulated and about 8 µm of diameter. For TNFRSF1A (Figure 12E and F) a strong staining in the same cellular type was detected, but only in a few cells in the gill filament. For CD3-ζ and MHC IIα the strong signal was observed in the same cell type but only on few positive cells (Figure 13A, B and C and Figure 13 D, E and F, respectively). Importantly, in an oblique tissue section a greater number of CD3-ζ and MHC IIα positive cells were observed located in the secondary lamellae (Figure 13 C and F respectively).

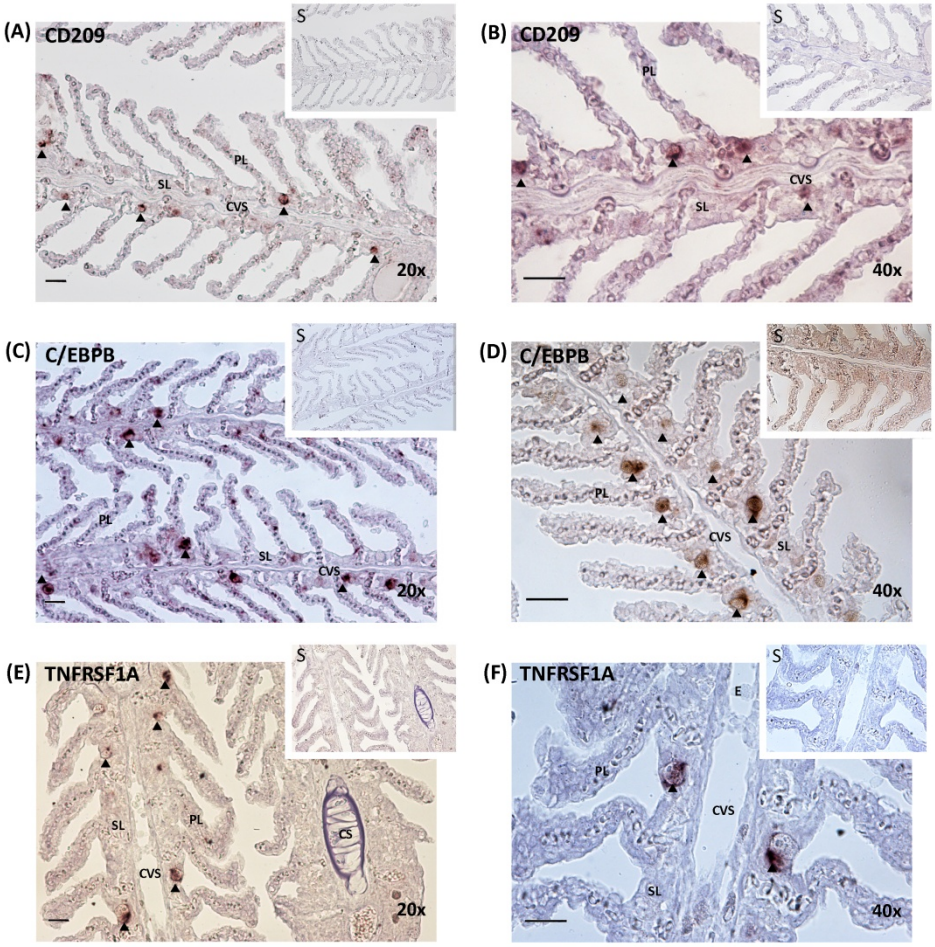

FIGURE 12: *In situ* hybridization using antisense riboprobes of CD209 (A and B), C/EBPB (C and D) and TNFRSF1A (E and F) in saggital sections in gills of *Sparus aurata*. The arrowhead shows the positive reactions for each probe (brown staining). S: sense probe (control, top-right); PL: Primary lamellae; SL: Secondary lamellae; CVS: Central venous sinus; E: Erythrocytes. Microscope augment: 20x/40x. Bars: 20µm.

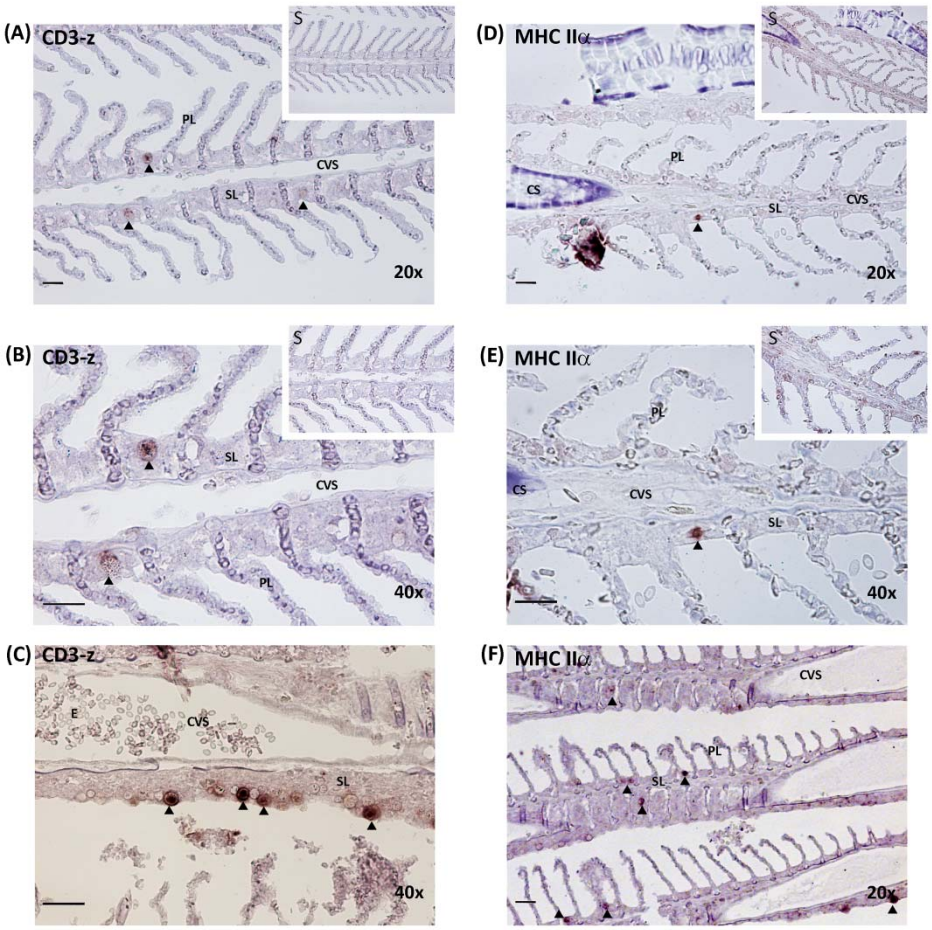

FIGURE 13: *In situ* hybridization using antisense riboprobes of CD3ζ (A, B and C) and MHC IIα (D, E and F) in saggital sections in gills of *Sparus aurata*. The arrowhead shows the positive reactions for each probe (brown staining). S: sense probe (control, top-right); PL: Primary lamellae; SL: Secondary lamellae; CVS: Central venous sinus; E: Erythrocytes. Bars: 20µm.

5. DISCUSSION

The present work describes the effect of the oral administration in Seabream (*Sparus aurata*) of two different diets supplemented with β-glucan. For this, 360 fish were feed the control and IS diets for a 28 days period. Non-specific immune parameters in plasma were measured, and also a high-throughput screening of the genes involved in this process on gills were conducted using microarrays.

The majority of studies centered on evaluate the immunostimulant dietary supplement effect in fish have been mainly focused to its suitable application in aquaculture. However, very few immunostimulants are currently used in this industry. In this line, β-glucan is the most used immunostimulant dietary supplement among the aquaculture feeds producers. In nature, β-glucans are widespread and are found in plants, algae, bacteria, yeast and mushrooms, with differences in their molecular weights and degree of branching depending of their source (Dalmo and Bøgwald, 2008). Currently, there are several commercial supplements compounds by β-glucans which are used as additives to the basal diets and whose immunostimulant effect in fish has been studied. Among them, there is Macrogard which includes β-1,3/1,6 glucans from *S. cerevisiae* (Bagni et al., 2005, 2000; Couso et al., 2003; Falco et al., 2014, 2012; Ghaedi et al., 2015; Kunttu et al., 2009; Pionnier et al., 2014; Sealey et al., 2008); Fibosel which contains yeast β-glucan (Couso et al., 2003); VitaStim which contains the mycelia of the fungus *Schizophyllum commune* (Couso et al., 2003); Ecoactiva which contains a mixture of β-(1,3)-linked-glucan and mannan from *S. cerevisiae* (Cook et al., 2003); Biosaf which contains a live yeast concentrate (El-Boshy et al., 2010); Ergosan that consist in an extract of *Laminaria digitata* and *Ascophyllum nodosum* (Bagni et al., 2005); Sanictum, composed from β-glucan (Kirchhoff et al., 2011); and DVAQUA, a *S. cerevisiae* fermentation product (He et al., 2009).

As alternative to these commercial additives for basal diets, the aquaculture feeds producers have also developed commercial diets which include the immunostimulant together with all the components needed in a basal diet and to prepare fish to stressful or challenging periods. To date, exist three main products

available for its use in gilthead seabream: EFICO Plus 805 (Biomar), which contains nucleotides, mannan-oligosaccharides (MOS), β-glucans, and anti-oxidant vitamins; Ewos have immunostimulant formulations (Ewos Functional feeds), including natural extracts in the form of nucleotides, glucans and prebiotics; and Protec (Skretting). Skretting and Ewos include yeast β-glucan in their fish feed, while BioMar includes bioactive alginate (Dalmo and Bøgwald, 2008). The yeast β-glucan is the major constituent of its cell membrane that consists of glucose and mannose and whose immunstimulant effect has been previously reported (Ai et al., 2007; Biswas et al., 2012; Cook, 2001; Cuesta et al., 2004; Ortuño et al., 2002; Rodríguez, 2003; Selvaraj et al., 2006, 2005; Yu et al., 2014). In our study, two immunostimulant diets using β-glucan as the main immunostimulant were used. For diet A was used the commercial diet Protec (5mg, 3mm, Skretting) which contains in its composition β-glucans, nucleotides, elevated levels of vitamins and minerals. These components were added in the diet in the fabrication process before extrusion of the pellet. Diet B also contains as main immunostimulant β-glucans but, unlike diet A, the component was added by top-coated to the commercial Nutra parr (Skretting) diet. Nutra parr with no additives was used as control diet (diet C). The addition of β-glucan in the diet before the extrusion (Ai et al., 2007; Bagni et al., 2005; Couso et al., 2003; He et al., 2009; Sahoo and Mukherjee, 2001; Sealey et al., 2008; Yu et al., 2014) or using a top-coated strategy (Bagni et al., 2005; Cook, 2001; El-Boshy et al., 2010),with immunostimulant effect in both cases, has been previously reported in fish.

Feeding this immunostimulant diets shows no effect in the percent of survival or Erythrocytes counts.

Lysozyme activity is one of the most studied parameter in innate response in fish (Tort et al., 2003). It is an enzyme which hydrolyses N-acetylmuramic acid and N-acetylglucosamine that form part of the peptidoglycan layer of bacterial cell walls (Ellis, 1999), resulting in the lysis of the bacteria (Tort et al., 2003). Lysozyme is also known to be an opsonin and activate the complement system and phagocytes (Magnadóttir, 2006). Lysozyme has been identified in fish mucus, serum and tissues

rich in leucocytes. Histochemically has been found in monocytes and neutrophils, which are probably the source of the serum lysozyme (Ellis, 1999). In this study, we found that at 7 days of feeding a significant increased in the serum lysozyme activity in fish fed with diet A, and a significant increased in the lysozyme activity at 14 dof with both immunostimulant diets. Other studies has been carried out in seabream fed with β-glucans with no significant differences found between fish fed with β-glucans and the control diets (Rodríguez, 2003; Verlhac et al., 1998). Otherwise, in other fish species has been reported an increased in lysozyme activity in fish fed with β-glucans such as European seabass after 30 days of feeding (Bagni et al., 2005), and at 40wks in a cyclic administration for 2 wks every 3 month. In other fish Order the increase in serum lysozyme activity has been also reported as in Rohu (*Labeo rohita*) at 28, 42 and 56 days of feeding (Misra et al., 2006), Nile tilapia after 21 days of feeding (El-Boshy et al., 2010) and after 8wks (He et al., 2009), and also . in trout after 3 months of administration (Ghaedi et al., 2015; Skalli et al., 2013). The increase in serum lysozyme activity has been also reported when β-glucan was administered by other routes than oral (REF). Thus, the antecedents in other fish species support the augmented serum lysozyme activity, whose increase was significant with only 7 days of feeding in the case of diet A.

Cortisol is the central corticosteroid metabolite in teleosts (Barton and Iwama, 1991), and plasma circulating levels is the most common indicator of stress in fish (Wendelaar Bonga, 1997). Its secretion from interrenal tissue by activation of the hypothalamic–pituitary–interrenal (HPI) axis is the main indicator of the primary stress response (Barton, 2002; Mommsen et al., 1999; Reid et al., 1998). As the main hormone produced in stress situations in fish, studies in vivo and in vitro testing the effect of cortisol over the immune system have been widely used as model to understand the stress process in teleosts. The consequences of cortisol on the immune response have been shown mainly in the systemic compartment (blood, head kidney, spleen, liver) affecting directly the fish immune system due to immunosuppression of several genes related to antigen presentation, as well as down-regulating B- and T-cell activation, inflammatory responses, and antiviral responses (Krasnov et al., 2012), and thus exposing fish to an increased

susceptibility to disease (Iguchi et al., 2003; Tort, 2011). At mucosal level, few studies have been published regarding the effect of stressors on gills in fish, which have focused on salmonid smoltification and their adaptation to sea water (McCormick, 2001; Olson, 2002; Shrimpton and McCormick, 1999; Wong and Chan, 2001). Moreover, few studies have been focused on evaluate the effects of diets, and particularly immunostimulant diets, on the fish stress response and their consequences at immunological level. In the present study, a significant high plasma level of cortisol was observed in the control diet (diet C) while a lower cortisol level was observed in fish fed with the immunostimulant diets, more markedly in diet A. This result indicate that the IS diets have no effect on the fish stress response whereas the control diet induce the cortisol release at systemic level. Considering that fish used in this study were kept under laboratory conditions and sacrificed by anesthetic overdoses (MS-222), the intrinsic experimental effect on fish stress response should have been minimal. Thus, only the fish nutritional state may have had significant effects on cortisol level. The studies focused on the effect of the IS diets on cortisol are quite minimal. Despite this, the lower cortisol level in fish fed with IS diets is in agreement with previous data in which tilapia (*Oreochromis nilotocus L.*) fed also with a IS diet containing β-1,3 glucan had lower serum cortisol values than basal diet (Cain et al., 2003) supporting that supplementation of β-1,3 glucan in the feed may serve as a potential stress reducer.

The primary response can lead to secondary stress responses if the stress duration persists, as many of the steroids can have longer-term effects than the initial catecholamine release. These secondary responses, as a result of circulating cortisol and catecholamines, can result in physiological changes including plasma lactate and glucose increase (Ellis et al., 2007). Importantly, a time-dependent accumulation trend in all diets was detected in the lactate but not in glucose content, with the lesser values at 7 and the greater values at 28 dof. It has been reported when insufficient oxygen is available to support aerobic ATP production, fish may resort to anaerobic metabolism resulting in the accumulation of lactate (Dunn and Hochachka, 1986; van Raaij et al., 1996; Zhou et al., 2000), suggesting an anaerobic machinery activation due to IS administration diets in seabream. Normal level of

glucose was observed in the control diet compared with the previous glucose levels observed in teleosts (Yeganeh et al., 2015). However, the marked differences observed for cortisol were not observed in glucose and lactate content when were compared the control with both immunostimulant diets. In this perspective, the plasma glucose and lactate results seem unexpected based on gluconeogenesis and lipolysis effects produced by cortisol (Brown et al., 1984; Sheridan, 1986). However, in some studies the plasma cortisol was not accompanied by plasma glucose increase (Pacheco and Santos, 2001; Tamm et al., 1988). The effect of IS diets on cortisol and glucose has been poorly described. Eslamloo et al. (Eslamloo et al., 2012) evaluated the dietary bovine lactoferrin effect on Siberian sturgeon (*Acipenser baeri*) with no changes in plasma cortisol and glucose, while a decreased in cortisol and glucose with increasing percentage of *S. platensis* inclusion has been reported (Yeganeh et al., 2015). This antecedent together with our result using β-glucan as immunostimulant opens the possibility that the IS nutritional composition of the diet may be involved in the cortisol and glucose secretion at systemic level and this regulation is not directly associated with the HPI axis cortisol/glucose release. Taken together, the results suggest that sea bream fed β-1,3 glucan supplemented diet may have a greater ability to fight against the stress conditions although more studies are needed to understand the mechanisms involved upon plasma cortisol, glucose and lactate regulation.

Prostaglandin E2 (PGE2) is one of the most abundant metabolites of arachidonic acid, generated through an enzymatic cascade controlled by cyclooxgenase (COX) enzymes (Chizzolini and Brembilla, 2009). PGE2 has a role in inflammation increasing vascular permeability, fever generation and T-cell adaptive immune response (Boniface et al., 2009; Solomon et al., 1968; Yao et al., 2009). In fish, prostaglandins are found in different cells and tissues, including macrophages (Pettitt et al., 1991), red blood cells (Cagen et al., 1983) and oocytes (Stacey and Goetz, 1982). Although in fish there is no previous reports whether β-glucan could promotes the PGE2 production, the antecedents in mammals indicate that β-glucan induces the production of PGE2 (Gagliardi et al., 2010; Smeekens et al., 2010). In the present study is shown that β-glucan present in diet A induces the serum PGE2

increase after 14 days of administration of the diet in gilthead seabrem. This is the first report of β-glucan-mediated PGE2 production in fish fed with supplemented diet.

The role of prostaglandin in glucose metabolism by controlling glycogen metabolism in liver has also been reported (Okumura et al., 1993; Püschel and Christ, 1994). Busby et al. (2002) found that PGE2 induces glycogenolysis and glyconeogenesis in rockfish (*Sebastes caurinus*) hepatocytes, activating the plasma membrane adenyl cyclase and hepatocyte glycogen phosporylase leading to increases in hepatocyte glucose output. Importantly, in this study was observed the same increase in the serum PGE2 and glucose levels at 14 dof in seabream fed with diet A, suggesting a relation between PGE2 and glucose metabolism in vivo after administration of β-glucans as dietary supplement. Further studies are needed to verify this hypothesis and the mechanisms involved in this process.

In order to characterize the gills transcriptomic response in Gilthead seabream fed with β-glucan supplemented diet, a microarrays analysis was carried out. GIALT (gill-associated lymphoid tissue) is one of the most relevant portal of entry due is directly in contact with the water external environment and, together with skin, the first fish immunological barrier. Curiously, to date no previous reports have been published in regard to the gene expression modulated of β-glucan administered as dietary supplement. Hence, this is the first report in characterize the gills transcriptomic response in seabream fed with β-glucan supplemented diet at fish mucosal level.

In a general overview of the gills transcriptomic response, it was observed that the changes with the administration of both IS diet in terms of the intensity of the response (expressed as AFC) was not so high. Most of the DEGs had a fold change smaller than 2.0 and only a few DEGs had a fold change greater than 3. Thus, the administration of β-glucan does not highly manipulate the response in gills of the seabream, suggesting that this immunostimulant may prevent an exacerbated and potentially dangerous response to the host.

To understand the effect of this dietary immunostimulant diets in the seabream two analysis were conducted according to the MIAME guidelines manual (Brazma et al., 2001): a reference design, that allowed us to evaluate the accumulative changes in feeding the immunostimulant diets; and a loop design, comparing each sampling point with its previous one that allowed us to determine the transient changes based on the modulation of the response in a time dependent manner. In the reference analysis was observed a greater number of DEGs with the administration of diet A. At day 2 of feeding, a similar number of DEGs were observed in both diets; however differences in the number of DEGs were detected at 7 and 14 dof, turning to similar levels in both diets at 28 dof. Thus, based on the number of DEGs, it seems that diet A induced an early and most marked immunostimulant effect while a later response compared to diet A was observed in diet B in gills of seabream fed with β-glucan supplemented diets. Despite this, results interesting the cyclic number of DEGs observed during the study. In diet A from the basal number of DEGs at 2 dof an increase was observed at 7 dof and then this trend was repeated at 14 and 28 dof. In contrast, in diet B from the basal number of DEGs a decrease was observed at 7 dof and then the same trend, although with an increased magnitude, was observed at 14 and 28 dof. On the other hand, the loop analysis showed a similar number of DEGs during the study but in diet B a drop in the number of genes was noted at 7 and 14 dof compared to 2 dof, with a remarkable increase at 28 dof.

Some of the genes with a marked regulation (up- or down-regulated) correspond to transcripts with an unknown function (Supplementary tables). Therefore, further studies are necessary to determine the nature of these genes and their role and implications in fish immune response and their implications in the effect of β-glucans administered as dietary supplement.

Taking together, these results suggest that the immunostimulant dietary inclusion (extruded versus top-coated strategy) has a direct effect on gene expression modulation in gills fed with β-glucan supplemented diets.

According to functional classification, several immune-related genes modulated by the administration of the IS diets were related with the T-cell mediated immune

response. In this study, CD209 was up-regulated in the loop analysis in fish fed with diet A at 7dof, and a tendency to up-regulation was also observed in fish fed with diet B at 14dof. CD209, a C-type lectin receptor (CLR), is a transmembrane protein categorized as pathogen-recognition receptor present in the surface of dendritic cell and macrophages. CD209 is directly associated with MHC class II since CD209 is thought mediate the endocytosis of the pathogens degraded in lysosomal compartments and then the pathogen-derived antigen are presented in a context of MHC class II to resting T cells to initiate the adaptative immune response. As in the case of CD209, also MHC IIα was up-regulated in the loop analysis at 7dof in fish fed with diet B, but no modulation was observed in diet A. The antigen presentation by the APCs via MHC class II leads to the activation of T cells (Bromley et al., 2001) mediated by T cell receptors (TCR). Each TCR is composed of ligand-binding subunits, the alpha and beta chains, and signaling subunits, namely the CD3 epsilon, gamma, delta and zeta chains. Small population of T cells contains TCRs that consist of gamma and delta chains instead alpha and beta (Nel, 2002). In this study was found the up-regulation of TCRβ and TCRγ chain in the loop analysis at 14 days compared to 7 dof in diet B, an also a significant up-regulation was observed at 14 days compared to control diet. Importantly, the same modulation was observed for the signaling subunit CD3 zeta, which was up-regulated in diet B at 14 dof. The TCR-MHC class II complex is stabilized by several co-stimulatory molecules i.e. CD40, a gene member of the TNF-receptor superfamily expressed in a wide variety of cells including APC (B cells, macrophages, and dendritic cells), endothelial cells, and fibroblasts. The binding with is counter receptor CD40L in T cells, leads to both humoral and cellular immune responses (Pype et al., 2000). In our study, the up-regulation of CD40 in the loop analysis at 7dof compared with the 2 dof followed with a down-regulation at 14dof was regulated in fish fed with the immunostimulant diet A. By contrast, in fish fed with the diet B, a down-regulation was found at 28dof compared with the same diet at 14dof.

The co-stimulation, activation and mobilization of T cells is a key process for the development of the Immune response. A molecule involved in this process is CD82, that it is a member of tetraspan family (tetraspanin), is a multifunctional molecule

that is involved in cell activation, co-stimulation, and cell spreading of T cells (Iwata et al., 2002). In this study the expression of CD82 was up-regulated in the loop analysis at 7dof in the diet A, and at 14dof for the diet B.

The interactions of these components lead to the activation of the humoral or cellular immune response. In the cell-mediated immune response , one of the actors is the citotoxic T linfocyte (or CTL) $CD8^{+}$. CD8 is a surface glycoprotein that mediates efficient cell-cell interactions within the immune system. This antigen, act as a co-receptor, that helps T cell to the recognition of antigens presented for the APC in the context of MHC class I molecules (Cresswell et al., 2005). The functional co-receptor is either a homodimer composed of two α chains, or a heterodimer composed of one α and one β chain. Our result shows the up-regulation of CD8 β chain at 14 dof with the diet B.

CTLs may kill target cells by one of at least three distinct pathways. One of this mechanisms required direct cell-cell contact between the efector and the target cell, and it is mediated by the release from the CTL of perforin and granzymes into the intercellular space (Andersen et al., 2006). The uptake of the granular material by the target cell causes cell death in a caspase-dependent and -independent manner (Trapani and Smyth, 2002). In our study was detected the up-regulation of Perforin 1 at 2 days of feeding with diet A. Perforin has previous been described in Japanese flounder (Jee et al., 2004), trout (Athanasopoulou et al., 2009) and ginbuna (Toda et al., 2011). Jee et al. (Jee et al., 2004) demonstrated the lytic activity of Japanese flounder recombinant perforin, and also inhibitors of perforin suppressed cytotoxic activity of T cell clones (Zhou et al., 2001). Also, Toda et al. (Toda et al., 2011) has demonstrated that the citotoxicity of CD8+ cell is dependent of perforin 1, suggesting that fish perforin has a mechanism of killing similar of those described in mammals, and that the cytotoxic mechanism of CTLs is highly conserved through vertebrates. In our work, others molecules that are involved in apoptosis were differentially expressed. BCAP31 was also up-regulated in the loop analysis at 14 dof with diet A. BCAP31, member of the B-cell receptor associated protein 31 superfamily, is a multi-pass transmembrane protein of the endoplasmic reticulum

that is involved in the anterograde transport of membrane proteins from the endoplasmic reticulum to the Golgi and in caspase 8-mediated apoptosis. IFP35 was also up-regulated in fish fed with both immunostimulant diets from the beginning until 14dof. There is some evidence that IFI35 associated with N-myc interac- tor (Nmi) interacts with STATs and is involved in the apoptosis process (Chen and Naumovski, 2002). Also, IFP35 is known to be induced by interferon gamma, a cytokine involved in the cellular-mediated immunity, reinforcing the possibility that a cellular-mediated immune response may be stimulated in seabream fed with β-glucan supplemented diets.

From the gills transcriptomic response, some immune-related genes were selected to find out in the gills architecture, the spatial localization of the cells which differentially express those mRNAs. In this study, the ISH showed a strong signal in the same cell type in five (CD209, C/EBPB, TNFRSF1A, CD3ζ, MHC class IIα) of the six (PU.1) selected genes. This cell type is located in the secondary lamelae in the interlamellar space, and morphologicaly is a granulate cell with a diameter of 6μm. Based on localization and morphology these cells appear to be chloride cells. The chloride cells (CCs) tend to be concentrated in the afferent region of the filament epithelium and have an intimate association with the arteriovenous circulation, although in the interlamellar region mitocondrial rich cells (MRCs) are also associated with the basal channels of the lamellar arterioarterial circulation (Laurent, 1984; Wilson and Laurent, 2002). In teleost fish, CCs are cells presented mostly in the secondary lamelae but also can be found in the primary lamelae, and it is characterized for a very granulated cytoplasm, due to the presence of a rich population of mitochondria and an extensive tubular system (Uchida et al., 2000). The term ‘‘chloride cell’’ relates to the function of the mitocondrial rich cells (MRC) in Cl elimination. In seawater teleosts, the MRCs have quite convincingly been shown to be sites of active Cl elimination and hence the name is fitting (Marshall et al., 2002; Wilson and Laurent, 2002). However, as far as our knowledge, no studies have been carried out to investigate the role of this cell type in the immune response. On the other hand, a study published in 2014 shows the abundance and distribution of T cells in gills of European sea bass using a specific T cell mAb DLT15

in a immunohistochemistry analysis (Nuñez Ortiz et al., 2014). The presence of these T cells in the epithelium of sea bass gills are in the same localization than our in situ hybridization-positive cells, opening the possibility of the presence of T cells in the lamellae of seabream fed with β-glucan supplemented diets. Further studies are needed in order to confirm the presence of chloride cells, T cells and other possible cell types involved in the expression of these immune-related genes differentially expressed in gills seabream fed with β-glucan dietary supplemented.

6. CONCLUSION

This study describes the effect of the oral administration in Seabream (*Sparus aurata*) of two different diets supplemented with β-glucan. At physiological level, β-glucan produced the decrease in the cortisol level in both immunostimulant diets. Although the serum glucose level was not associated with cortisol, it seems to be related with non-specific immune parameter as prostaglandin and whose interaction at metabolic level has been demonstrated in mammals. The first transcriptomic response in teleost to evaluate the β-glucan effect showed the modulation of genes related with inflammatory response, T cell response and apoptosis. Based on these results, β-glucan could stimulate the antigen presentation and cell-mediated immune response, mainly through T cell-mediated cytotoxicity. The *in situ* hybridization-positive cells found in the interlamelar space of the secondary lamelae opens the possibility to the presence of chloride or T cells. The results indicate that β-glucan administered in diet produces an immunostimulant effect in gills of seabream. Further analyses are needed to confirm the hypothesis and scope considered in this study.

7. REFERENCES

Abdel-Aziz, E.S.H., Abdu, S.B.S., Ali, T.E.S., Fouad, H.F., 2010. Haemopoiesis in the head kidney of tilapia, Oreochromis niloticus (Teleostei: Cichlidae): A morphological (optical and ultrastructural) study. Fish Physiol. Biochem. 36, 323–336. doi:10.1007/s10695-008-9297-z

Abdel-Tawwab, M., Ahmad, M.H., Seden, M.E. a, Sakr, S.F.M., 2010. Use of Green Tea, Camellia sinensis L., in Practical Diet for Growth and Protection of Nile Tilapia, Oreochromis niloticus (L.), against Aeromonas hydrophila Infection. J. World Aquac. Soc. 41, 203–213. doi:10.1111/j.1749-7345.2010.00360.x

Abdel-Tawwab, M., Sharafeldin, K.M., Mosaad, M.N.M., Ismaiel, N.E.M., 2015. Coffee bean in common carp, Cyprinus carpio L. diets: Effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity. Aquaculture 448, 207–213. doi:10.1016/j.aquaculture.2015.06.010

Abeysinghe, P., 2010. Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenica bacteria. Indian J. Pharm. Sci. 72, 167–172.

Acar, Ü., Kesbiç, O.S., Yılmaz, S., Gültepe, N., Türker, A., 2015. Evaluation of the effects of essential oil extracted from sweet orange peel (Citrus sinensis) on growth rate of tilapia (Oreochromis mossambicus) and possible disease resistance against Streptococcus iniae. Aquaculture 437, 282–286. doi:10.1016/j.aquaculture.2014.12.015

Adel, M., Abedian Amiri, A., Zorriehzahra, J., Nematolahi, A., Esteban, M.Á., 2015. Effects of dietary peppermint (Mentha piperita) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (Rutilus frisii kutum). Fish Shellfish Immunol. 45, 841–847. doi:10.1016/j.fsi.2015.06.010

Aderem, A., Underhill, D.M., 1999. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 17, 593–623.

Agarwal, M., Walia, S., Dhingra, S., Khambay, B.P.S., 2001. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/ derived from Zingiber officinale Roscoe (ginger) rhizomes. Pest Manag. Sci. 57, 289–300. doi:10.1002/ps.263

Ai, Q., Mai, K., Zhang, L., Tan, B., Zhang, W., Xu, W., Li, H., 2007. Effects of dietary beta-1, 3 glucan on innate immune response of large yellow croaker, Pseudosciaena crocea. Fish Shellfish Immunol. 22, 394–402. doi:10.1016/j.fsi.2006.06.011

Alexander, J.B., Ingram, G. a., 1992. Noncellular nonspecific defence mechanisms of fish. Annu. Rev. Fish Dis. 2, 249–279. doi:10.1016/0959-8030(92)90066-7

Aly, S.M., Mohamed, M.F., 2010. Echinacea purpurea and Allium sativum as immunostimulants in fish culture using Nile tilapia (Oreochromis niloticus). J. Anim. Physiol. Anim. Nutr. (Berl). 94. doi:10.1111/j.1439-0396.2009.00971.x

Andersen, M.H., Schrama, D., Thor Straten, P., Becker, J.C., 2006. Cytotoxic T cells. J Invest Dermatol 126, 32–41. doi:10.1038/sj.jid5700001

Andersson, E., Peixoto, B., Tormanen, V., Matsunaga, T., 1995. Evolution of the immunoglobulin M constant region genes of salmonid fish, rainbow trout (Oncorhynchus mykiss) and Arctic charr (Salvelinus alpinus): implications concerning divergence time of species. Immunogenetics 41, 312–315.

Athanasopoulou, S., Marioli, D., Mikrou, A., Papanastasiou, A.D., Zarkadis, I.K., 2009. Cloning and characterization of the trout perforin. Fish Shellfish Immunol. 26, 908–12. doi:10.1016/j.fsi.2009.03.021

Awad, E., Austin, D., Lyndon, A.R., 2013. Effect of black cumin seed oil (Nigella sativa) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, Oncorhynchus mykiss (Walbaum). Aquaculture 388-391, 193–197. doi:10.1016/j.aquaculture.2013.01.008

Awad, E., Awaad, A.S., Esteban, M.A., 2015. Effects of dihydroquercetin obtained from deodar (Cedrus deodara) on immune status of gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol. 43, 43–50. doi:10.1016/j.fsi.2014.12.009

Baba, E., Uluköy, G., Öntaş, C., 2015. Effects of Feed Supplemented with Lentinula edodes Mushroom Extract on The Immune Response of Rainbow Trout, Oncorhynchus mykiss, and Disease Resistance Against Lactococcus garvieae. Aquaculture 448, 476–482. doi:10.1016/j.aquaculture.2015.04.031

Baek, G.-H., Jang, Y.-S., Jeong, S.-I., Cha, J., Joo, M., Shin, S.-W., Ha, K.-T., Jeong, H.-S., 2012. Rehmannia glutinosa Suppresses Inflammatory Responses Elicited by Advanced Glycation End Products. Inflammation 35, 1232–1241. doi:10.1007/s10753-012-9433-x

Bagni, M., Archetti, L., Amadori, M., Marino, G., 2000. Effect of long-term oral administration of an immunostimulant diet on innate immunity in sea bass (Dicentrarchus labrax). J. Vet. Med. B. Infect. Dis. Vet. Public Health 47, 745–51.

Bagni, M., Romano, N., Finoia, M.G., Abelli, L., Scapigliati, G., Tiscar, P.G., Sarti, M., Marino, G., 2005. Short- and long-term effects of a dietary yeast beta-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (Dicentrarchus labrax). Fish Shellfish Immunol. 18, 311–25. doi:10.1016/j.fsi.2004.08.003

Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manag. 10, 421–452. doi:10.1023/A:1021397624349

Barton, B. a, 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525. doi:10.1093/icb/42.3.517

Barton, B. a., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26. doi:10.1016/0959-8030(91)90019-G

Basha, K.A., Raman, R.P., Prasad, K.P., Kumar, K., Nilavan, E., Kumar, S., 2013. Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against Aeromonas hydrophila in Labeo rohita (Hamilton). Fish Shellfish Immunol. 35, 1433–41. doi:10.1016/j.fsi.2013.08.005

Bilen, S., Bulut, M., Bilen, A.M., 2011. Immunostimulant effects of Cotinus coggyria on rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 30, 451–5. doi:10.1016/j.fsi.2010.12.013

Biswas, G., Korenaga, H., Takayama, H., Kono, T., Shimokawa, H., Sakai, M., 2012. Cytokine responses in the common carp, Cyprinus carpio L. treated with baker's yeast extract. Aquaculture 356-357, 169–175. doi:10.1016/j.aquaculture.2012.05.019

Boniface, K., Bak-Jensen, K.S., Li, Y., Blumenschein, W.M., McGeachy, M.J., McClanahan, T.K., McKenzie, B.S., Kastelein, R. a, Cua, D.J., de Waal Malefyt, R., 2009. Prostaglandin E2

regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. J. Exp. Med. 206, 535–548. doi:10.1084/jem.20082293

Brazma, a, Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C. a, Causton, H.C., Gaasterland, T., Glenisson, P., Holstege, F.C., Kim, I.F., Markowitz, V., Matese, J.C., Parkinson, H., Robinson, a, Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J., Vingron, M., 2001. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat. Genet. 29, 365–371. doi:10.1038/ng1201-365

Bromley, S.K., Burack, W.R., Johnson, K.G., Somersalo, K., Sims, T.N., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., Dustin, M.L., 2001. The immunological synapse. Annu. Rev. Immunol. 19, 375.

Brown, G.D., Gordon, S., 2003. Fungal beta-glucans and mammalian immunity. Immunity 19, 311–315. doi:http://dx.doi.org/10.1016/S1074-7613(03)00233-4

Brown, S., Eales, J., Evans, R., TJ., H., 1984. Interrenal, thyroidal, and carbohydrate responses to rainbow trout (Salmo gairdneri) to environmental acidification. Can. J. Fish. Aquat. Sci. 41, 36–45.

Busby, E.R., Cooper, G. a, Mommsen, T.P., 2002. Novel role for prostaglandin E2 in fish hepatocytes: regulation of glucose metabolism. J. Endocrinol. 174, 137–146.

Cagen, L.M., Qureshi, Z., Nishimura, H., 1983. Synthesis of prostaglandin E2 and prostaglandin F2 alpha by toadfish red blood cells. Biochem. Biophys. Res. Commun. 110, 250–255.

Cain, K.D., Grabowski, L., Reilly, J., Lytwyn, M., 2003. Immunomodulatory effects of a bacterial-derived??-1,3 glucan administered to tilapia (Oreochromis nilotocus L.) in a Spirulina-based diet. Aquac. Res. 34, 1241–1244. doi:10.1046/j.1365-2109.2003.00934.x

Castillo Sanchez, C., Dominguez, J., Kaattari, S.L., Villena, A.J., 1993. Ontogeny of IgM and IgM-bearing cells in rainbow trout. Dev Comp Immunol. 17, 419–424.

Chandur, U., Shashidhar, S., Chandrasekar, S., Rao, M.N., 2011. Research Journal of Pharmaceutical , Biological and Chemical Sciences Studies of preliminary phytochemical and Anti-arthritic activity of heart wood of. Res. J. Pharm. Biol. Chem. Sci. 2, 654–660.

Chao, J.C.J., Chiang, S.W., Wang, C.C., Tsai, Y.H., Wu, M.S., 2006. Hot water-extracted Lycium barbarum and Rehmannia glutinosa inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. World J. Gastroenterol. 12, 4478–4484.

Chao, W.W., Kuo, Y.H., Lin, B.I.F., 2010. Anti-inflammatory activity of new compounds from andrographis paniculata by nf-κb transactivation inhibition. J. Agric. Food Chem. 58, 2505–2512. doi:10.1021/jf903629j

Chee, H.Y., Kim, H., Lee, M.H., 2009. In vitro Antifungal Activity of Limonene against Trichophyton rubrum. Mycobiology 37, 243–6. doi:10.4489/MYCO.2009.37.3.243

Chen, H.Y., Yen, G.C., 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves. Food Chem. 101, 686–694. doi:10.1016/j.foodchem.2006.02.047

Chen, J., Naumovski, L., 2002. Intracellular redistribution of interferon-inducible proteins Nmi and IFP 35 in apoptotic cells. J. Interferon Cytokine Res. 22, 237–243. doi:10.1089/107999002753536211

Chettri, J.K., Raida, M.K., Holten-Andersen, L., Kania, P.W., Buchmann, K., 2011. PAMP induced expression of immune relevant genes in head kidney leukocytes of rainbow trout

(Oncorhynchus mykiss). Dev. Comp. Immunol. 35, 476–82. doi:10.1016/j.dci.2010.12.001

Chizzolini, C., Brembilla, N.C., 2009. Prostaglandin E2: igniting the fire. Immunol. Cell Biol. 87, 510–511. doi:10.1038/icb.2009.56

Christybapita, D., Divyagnaneswari, M., Dinakaran Michael, R., 2007. Oral administration of Eclipta alba leaf aqueous extract enhances the non-specific immune responses and disease resistance of Oreochromis mossambicus. Fish Shellfish Immunol. 23, 840–852. doi:10.1016/j.fsi.2007.03.010

Chrubasik, S., Pittler, M.H., Roufogalis, B.D., 2005. Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles. Phytomedicine 12, 684–701. doi:10.1016/j.phymed.2004.07.009

Chung, S., Secombes, C.J., 1987. Activation of rainbow trout macrophages. ournal Fish Biol. 31, 51–56.

Cook, M., 2001. The efficacy of a commercial β-glucan preparation, EcoActiva™, on stimulating respiratory burst activity of head-kidney macrophages from pink snapper (Pagrus auratus), Sparidae. Fish Shellfish Immunol. 11, 661–672. doi:10.1006/fsim.2001.0343

Cook, M.T., Hayball, P.J., Hutchinson, W., Nowak, B.F., Hayball, J.D., 2003. Administration of a commercial immunostimulant preparation, EcoActiva (TM) as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (Pagrus auratus, Sparidae (Bloch and Schneider)) in winter. Fish Shellfish Immunol. 14, 333–345. doi:10.1006/fsim.2002.0441

Couso, N., Castro, R., Magariños, B., Obach, A., Lamas, J., 2003. Effect of oral administration of glucans on the resistance of gilthead seabream to pasteurellosis. Aquaculture 219, 99–109. doi:10.1016/S0044-8486(03)00019-X

Crespy, V., Williamson, G., 2004. A review of the health effects of green tea catechins in in vivo animal models. J. Nutr. 134, 3431S–3440S.

Cresswell, P., Ackerman, A.L., Giodini, A., Peaper, D.R., Wearsch, P.A., 2005. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunol. Rev. 207, 145–157.

Cuesta, a, Meseguer, J., Esteban, M. a, 2004. Total serum immunoglobulin M levels are affected by immunomodulators in seabream (Sparus aurata L.). Vet. Immunol. Immunopathol. 101, 203–10. doi:10.1016/j.vetimm.2004.04.021

Dalmo, R.A., Bøgwald, J., 2008. Beta-glucans as conductors of immune symphonies. Fish Shellfish Immunol. 25, 384–96. doi:10.1016/j.fsi.2008.04.008

Dannevig, B., 1994. Receptor-mediated endocytosis and phagocytosis by rainbow trout head kidney sinusoidal cells. Fish Shellfish Immunol. doi:10.1006/fsim.1994.1002

Das, R., Prakash Raman, R., Saha, H., Singh, R., 2013. Effect of Ocimum sanctum Linn. (Tulsi) extract on the immunity and survival of Labeo rohita (Hamilton) infected with Aeromonas hydrophila. Aquac. Res. 1111–1121. doi:10.1111/are.12264

Dhayanithi, N.B., Ajith Kumar, T.T., Arockiaraj, J., Balasundaram, C., Harikrishnan, R., 2015a. Dietary supplementation of Avicennia marina extract on immune protection and disease resistance in Amphiprion sebae against Vibrio alginolyticus. Fish Shellfish Immunol. 45, 52–8. doi:10.1016/j.fsi.2015.02.018

Dhayanithi, N.B., Ajithkumar, T.T., Arockiaraj, J., Balasundaram, C., Ramasamy, H., 2015b. Immune protection by Rhizophora apiculata in clownfish against Vibrio alginolyticus. Aquaculture 446, 1–6. doi:10.1016/j.aquaculture.2015.04.013

Díaz-Rosales, P., Chabrillón, M., Abdala, R.T., Figueroa, F.L., Balebona, M.C., Moriñigo, M. a., 2008. Effect of dietary administration of Porphyridium cruentum on the respiratory burst activity of sole, Solea senegalensis (Kaup), phagocytes. J. Fish Dis. 31, 489–495. doi:10.1111/j.1365-2761.2008.00923.x

Dijkstra, J.M., Kollner, B., Aoyagi, K., Sawamoto, Y., Kuroda, A., Ototake, M., Nakanishi, T., Fischer, U., 2003. The rainbow trout classical MHC class I molecule Onmy-UBA*501 is expressed in similar cell types as mammalian classical MHC class I molecules. Fish Shellfish Immunol 14, 1–23.

Dotta, G., de Andrade, J.I.A., Tavares Gonçalves, E.L., Brum, A., Mattos, J.J., Maraschin, M., Martins, M.L., 2014. Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and Aloe barbadensis. Fish Shellfish Immunol. 39, 280–4. doi:10.1016/j.fsi.2014.05.020

Dügenci, S.K., Dugenci, S.K., Arda, N., Candan, A., 2003. Some medicinal plants as immunostimulant for fish. J. Ethnopharmacol. 88, 99–106. doi:10.1016/S0378-8741(03)00182-X

Dülger, B., Hacıoglu, N., Bilen, S., 2009. Antimicrobial activity of Cotinus coggyria from Turkey. Asian J. Chem. 21, 4139–4140.

Dunkelberger, J.R., Song, W.-C., 2010. Complement and its role in innate and adaptive immune responses. Cell Res. 20, 34–50. doi:10.1038/cr.2009.139

Dunn, J., Hochachka, P., 1986. Metabolic Responses of Trout (Salmo Gairdneri) To Acute Environmental Hypoxia. J. Exp. Biol. 242, 229–242.

El-Boshy, M.E., El-Ashram, A.M., Abdelhamid, F.M., Gadalla, H. a, 2010. Immunomodulatory effect of dietary Saccharomyces cerevisiae, beta-glucan and laminaran in mercuric chloride treated Nile tilapia (Oreochromis niloticus) and experimentally infected with Aeromonas hydrophila. Fish Shellfish Immunol. 28, 802–8. doi:10.1016/j.fsi.2010.01.017

Ellis, A., 1999. Immunity to bacteria in fish. Fish Shellfish Immunol. 9, 291–308.

Ellis, T., James, J.D., Sundh, H., Fridell, F., Sundell, K., Scott, a. P., 2007. Non-invasive measurement of cortisol and melatonin in tanks stocked with seawater Atlantic salmon. Aquaculture 272, 698–706. doi:10.1016/j.aquaculture.2007.07.219

El-Saleh, S.C., Al-Sagair, O. a., Al-Khalaf, M.I., 2004. Thymoquinone and Nigella sativa oil protection against methionine-induced hyperhomocysteinemia in rats. Int. J. Cardiol. 93, 19–23. doi:10.1016/S0167-5273(03)00108-6

Endo, K., Kanno, E., Oshima, Y., 1990. Structures of antifungal diarylheptenones, gingerenones A, B, C and isogingerenone B, isolated from the rhizomes of Zingiber officinale. Phytochemistry 29, 797–799. doi:10.1016/0031-9422(90)80021-8

Eslamloo, K., Falahatkar, B., Yokoyama, S., 2012. Effects of dietary bovine lactoferrin on growth, physiological performance, iron metabolism and non-specific immune responses of Siberian sturgeon Acipenser baeri. Fish Shellfish Immunol. 32, 976–85. doi:10.1016/j.fsi.2012.02.007

Esteban, M., Cuesta, A., Ortuno, J., Meseguer, J., 2001. Immunomodulatory effects of dietary intake of chitin on gilthead seabream (Sparus aurata L.) innate immune system. Fish Shellfish Immunol. 11, 303–315. doi:10.1006/fsim.2000.0315

Falco, A., Frost, P., Miest, J., Pionnier, N., Irnazarow, I., Hoole, D., 2012. Reduced inflammatory response to Aeromonas salmonicida infection in common carp (Cyprinus carpio L.) fed with β-glucan supplements. Fish Shellfish Immunol. 32, 1051–7. doi:10.1016/j.fsi.2012.02.028

Falco, A., Miest, J.J., Pionnier, N., Pietretti, D., Forlenza, M., Wiegertjes, G.F., Hoole, D., 2014. β-Glucan-supplemented diets increase poly(I:C)-induced gene expression of Mx, possibly via Tlr3-mediated recognition mechanism in common carp (Cyprinus carpio). Fish Shellfish Immunol. 36, 494–502. doi:10.1016/j.fsi.2013.12.005

Fang, F.C., 2004. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat. Rev. Microbiol. 2, 820–832. doi:10.1038/nrmicro1004

Fast, M.D., Sims, D.E., Burka, J.F., Mustafa, A., Ross, N.W., 2002. Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. Comp Biochem Physiol A Mol Integr Physiol 132, 645–657.

Flerova, E. a., Balabanova, L. V., 2013. Ultrastructure of granulocytes of teleost fish (Salmoniformes, Cypriniformes, Perciformes). J. Evol. Biochem. Physiol. 49, 223–233. doi:10.1134/S0022093013020126

Frøystad, M.K., Rode, M., Berg, T., Gjøen, T., 1998. A role for scavenger receptors in phagocytosis of protein-coated particles in rainbow trout head kidney macrophages. Dev. Comp. Immunol. 22, 533–549. doi:10.1016/S0145-305X(98)00032-9

Gabriel, N.N., Qiang, J., He, J., Ma, X.Y., Kpundeh, M.D., Xu, P., 2015. Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). Fish Shellfish Immunol. 44, 504–14. doi:10.1016/j.fsi.2015.03.002

Gagliardi, M.C., Teloni, R., Mariotti, S., Bromuro, C., Chiani, P., Romagnoli, G., Giannoni, F., Torosantucci, A., Nisini, R., 2010. Endogenous PGE2 promotes the induction of human Th17 responses by fungal ß-glucan. J. Leukoc. Biol. 88, 947–954. doi:10.1189/jlb.0310139

Gao, X., Wang, W., Wei, S., Li, W., 2009. Review of pharmacological effects of Glycyrrhiza radix and its bioactive compounds. Zhongguo Zhong Yao Za Zhi 34, 2695–2700. doi:10.1002/ptr

Ghaedi, G., Keyvanshokooh, S., Mohammadi Azarm, H., Akhlaghi, M., 2015. Effects of dietary β-glucan on maternal immunity and fry quality of rainbow trout (Oncorhynchus mykiss). Aquaculture 441, 78–83. doi:10.1016/j.aquaculture.2015.02.023

Giri, S.S., Sen, S.S., Chi, C., Kim, H.J., Yun, S., Park, S.C., Sukumaran, V., 2015. Effect of guava leaves on the growth performance and cytokine gene expression of Labeo rohita and its susceptibility to Aeromonas hydrophila infection. Fish Shellfish Immunol. doi:10.1016/j.fsi.2015.05.051

Gomez, D., Sunyer, J.O., Salinas, I., 2013. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35, 1729–39. doi:10.1016/j.fsi.2013.09.032

Granja, A.G., Leal, E., Pignatelli, J., Castro, R., Abos, B., Kato, G., Fischer, U., Tafalla, C., 2015. Identification of Teleost Skin CD8 + Dendritic-like Cells, Representing a Potential Common Ancestor for Mammalian Cross-Presenting Dendritic Cells. J. Immunol. doi:10.4049/jimmunol.1500322

Green, S.J., Nacy, C. a., Schreiber, R.D., Granger, D.L., Crawford, R.M., Meltzer, M.S., Fortier, a. H., 1993. Neutralization of gamma interferon and tumor necrosis factor alpha blocks in

vivo synthesis of nitrogen oxides from L-arginine and protection against Francisella tularensis infection in Mycobacterium bovis BCG-treated mice. Infect. Immun. 61, 689–698.

Grzanna, R., Lindmark, L., Frondoza, C.G., 2005. Ginger--an herbal medicinal product with broad anti-inflammatory actions. J. Med. Food 8, 125–132. doi:10.1089/jmf.2005.8.125

Guerin, M., Huntley, M.E., Olaizola, M., 2003. Haematococcus astaxanthin: Applications for human health and nutrition. Trends Biotechnol. 21, 210–216. doi:10.1016/S0167-7799(03)00078-7

Gülçin, I., Küfrevioğlu, Ö. I., Oktay, M., Büyükokuroğlu, M.E., 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J. Ethnopharmacol. 90, 205–215. doi:10.1016/j.jep.2003.09.028

Gupta, S.K., Pal, a K., Sahu, N.P., Dalvi, R., Kumar, V., Mukherjee, S.C., 2008. Microbial levan in the diet of Labeo rohita Hamilton juveniles: effect on non-specific immunity and histopathological changes after challenge with Aeromonas hydrophila. J. Fish Dis. 31, 649–57. doi:10.1111/j.1365-2761.2008.00939.x

Guttvik, a, 2002. Oral administration of lipopolysaccharide to Atlantic salmon (Salmo salar L.) fry. Uptake, distribution, influence on growth and immune stimulation. Aquaculture 214, 35–53. doi:10.1016/S0044-8486(02)00358-7

Hadizadeh, I., Peivastegan, B., Hamzehzarghani, H., 2009. Antifungal Activity of Essential Oils from Some Medicinal Plants of Iran against Alternaria alternate Department of Plant Protection , Collage of Agriculture , Shahid Chamran University , Postal code: Ahvaz , Iran Department of Agronomy , Collage of Agri. Am. J. Appl. Sci. 6, 857–861.

Hanafy, M.S., Hatem, M.E., 1991. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J. Ethnopharmacol. 34, 275–278.

Hansen, J.D., Landis, E.D., Phillips, R.B., 2005. Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: Implications for a distinctive B cell developmental pathway in teleost fish. Proc Natl Acad Sci U S A 102, 6919–6924.

Haugarvoll, E., Bjerkås, I., Nowak, B.F., Hordvik, I., Koppang, E.O., 2008. Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon. J. Anat. 213, 202–209. doi:10.1111/j.1469-7580.2008.00943.x

He, S., Zhou, Z., Liu, Y., Shi, P., Yao, B., Ringø, E., Yoon, I., 2009. Effects of dietary Saccharomyces cerevisiae fermentation product (DVAQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (Oreochromis niloticus ♀×O. aureus ♂) cultured in cages. Aquaculture 294, 99–107. doi:10.1016/j.aquaculture.2009.04.043

Heidarieh, M., Soltani, M., Tamimi, A., Toluei, M., 2011. Comparative effect of raw fiber (Vitacel) and alginic acid (Ergosan) on growth performance, immunocompe- tent cell population and plasma lysozyme content of giant sturgeon (Huso huso). Turkish J. Fish. Aquat. Sci. 11, 445–450. doi:10.4194/1303-2712-v11

Higuera-Ciapara, I., Félix-Valenzuela, L., Goycoolea, F.M., 2006. Astaxanthin: a review of its chemistry and applications. Crit. Rev. Food Sci. Nutr. 46, 185–196. doi:10.1080/10408690590957188

Hordvik, I., 2002. Identification of a novel immunoglobulin delta transcript and comparative analysis of the genes encoding IgD in Atlantic salmon and Atlantic halibut. Mol Immunol 39, 85–91.

Hordvik, I., Thevarajan, J., Samdal, I., Bastani, N., Krossoy, B., 1999. Molecular cloning and phylogenetic analysis of the Atlantic salmon immunoglobulin D gene. Scand J Immunol 50, 202–210.

Hordvik, I., Torvund, J., Moore, L., Endresen, C., 2004. Structure and organization of the T cell receptor alpha chain genes in Atlantic salmon. Mol Immunol 41, 553–559.

Iguchi, K., Ogawa, K., Nagae, M., Ito, F., 2003. The influence of rearing density on stress response and disease susceptibility of ayu (Plecoglossus altivelis). Aquaculture 220, 515–523. doi:10.1016/S0044-8486(02)00626-9

Iles, K.E., Forman, H.J., 2002. Macrophage signaling and respiratory burst. Immunol. Res. 26, 95–105. doi:10.1385/IR:26:1-3:095

Iliev, D.B., Jorgensen, S.M., Rode, M., Krasnov, A., Harneshaug, I., Jorgensen, J.B., 2010. CpG-induced secretion of MHCIIbeta and exosomes from salmon (Salmo salar) APCs. Dev Comp Immunol 34, 29–41.

Ishihara, N., Araki, T., Tamaru, Y., Nishimura, A., Aoi, N., Chu, D., Juneja, L.R., Morishita, T., Science, M.P., 2002. Influence of Green Tea Polyphenols on Feed Performance, Growth Performance, and Fish Body Component in Yellowtail (Seriola quinqueradiata). Jpn. J. Food Chem. 9, 7–14.

Isogai, E., Isogai, H., Hirose, K., Hayashi, S., Oguma, K., 2001. In vivo synergy between green tea extract and levofloxacin against enterohemorrhagic Escherichia coli O157 infection. Curr. Microbiol. 42, 248–251. doi:10.1007/s002840010212

Iwata, S., Kobayashi, H., Miyake-Nishijima, R., Sasaki, T., Souta-Kuribara, A., Nori, M., Hosono, O., Kawasaki, H., Tanaka, H., Morimoto, C., 2002. Distinctive signaling pathways through CD82 and beta1 integrins in human T cells. Eur. J. Immunol. 32, 1328–1337. doi:10.1002/1521-4141(200205)32:5<1328::AID-IMMU1328>3.0.CO;2-6

Jagruthi, C., Yogeshwari, G., Anbazahan, S.M., Mari, L.S.S., Arockiaraj, J., Mariappan, P., Sudhakar, G.R.L., Balasundaram, C., Harikrishnan, R., 2014. Effect of dietary astaxanthin against Aeromonas hydrophila infection in common carp, Cyprinus carpio. Fish Shellfish Immunol. 41, 674–80. doi:10.1016/j.fsi.2014.10.010

Jee, Y.H., Ohira, T., Hirono, I., Aoki, T., 2004. A pore-forming protein, perforin, from a non-mammalian organism, Japanese flounder, Paralichthys olivaceus. Immunogenetics 56, 360–367. doi:10.1007/s00251-004-0688-8

Jian, J., Wu, Z., 2003. Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, Pseudosciaena crocea (Richardson). Aquaculture 218, 1–9. doi:10.1016/S0044-8486(02)00192-8

Jian, J., Wu, Z., 2004. Influences of traditional Chinese medicine on non-specific immunity of Jian Carp (Cyprinus carpio var. Jian). Fish Shellfish Immunol. 16, 185–191. doi:10.1016/S1050-4648(03)00062-7

Jin, M., Zhao, K., Huang, Q., Xu, C., Shang, P., 2012. Isolation, structure and bioactivities of the polysaccharides from Angelica sinensis (Oliv.) Diels: A review. Carbohydr. Polym. 89, 713–722. doi:10.1016/j.carbpol.2012.04.049

Jorgensen, S.M., Grimholt, U., Gjoen, T., 2007. Cloning and expression analysis of an Atlantic salmon (Salmo salar L.) tapasin gene. Dev Comp Immunol 31, 708–719.

Kaattari, S.L., Irwin, M.J., 1985. Salmonid spleen and anterior kidney harbor populations of lymphocytes with different B cell repertoires. Dev. Comp. Immunol. 9, 433–444.

Kaibori, M., Sakitani, K., Oda, M., Kamiyama, Y., Masu, Y., Nishizawa, M., Ito, S., Okumura, T., 1999. Immunosuppressant FK506 inhibits inducible nitric oxide synthase gene

expression at a step of NF-kappaB activation in rat hepatocytes. J. Hepatol. 30, 1138–1145.

Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji, N., Assimakopoulos, D. a., 2008. Spirulina in clinical practice: Evidence-based human applications. Evidence-based Complement. Altern. Med. 2011, 27–31. doi:10.1093/ecam/nen058

Khafagi, I., Alla, A., Salama, S., Fouda, M., 2003. Biological activities and phytochemical constituents of the gray mangrove Avicennia mariana (Forsk.) Vierh. Egypt. J. Biol. 5, 62–69.

Kim, J.H., Mun, Y.J., Im, S.J., Han, J.H., Lee, H.S., Woo, W.H., 2001. Effects of the aqueous extract of epimedii herba on the antibody responses in mice. Int. Immunopharmacol. 1, 935–944.

Kim, J.-K., Kim, Y., Na, K.-M., Surh, Y.-J., Kim, T.-Y., 2007. [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression in vitro and in vivo. Free Radic. Res. 41, 603–614. doi:10.1080/10715760701209896

Kim, K.H., Hwang, Y.J., Bai, S.C., 1999. Resistance to Vibrio alginolyticus in juvenile rockfish (Sebastes schlegeli) fed diets containing different doses of aloe. Aquaculture 180, 13–21. doi:10.1016/S0044-8486(99)00143-X

Kirchhoff, N.T., D'Antignana, T., Leef, M.J., Hayward, C.J., Wilkinson, R.J., Nowak, B.F., 2011. Effects of immunostimulants on ranched southern bluefin tuna Thunnus maccoyii: immune response, health and performance. J. Fish Biol. 79, 331–55. doi:10.1111/j.1095-8649.2011.03019.x

Klebanoff, S.J., 2005. Myeloperoxidase: friend and foe. J. Leukoc. Biol. 77, 598–625. doi:10.1189/jlb.1204697.1

Kondera, E., 2011. Haematopoiesis in the head kidney of common carp (Cyprinus carpio L.): a morphological study. Fish Physiol. Biochem. 37, 355–362. doi:10.1007/s10695-010-9432-5

Kopp, E., Medzhitov, R., 2003. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol 15, 396–401.

Koppang, E.O., Fischer, U., Moore, L., Tranulis, M. a., Dijkstra, J.M., Köllner, B., Aune, L., Jirillo, E., Hordvik, I., 2010. Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue. J. Anat. 217, 728–739. doi:10.1111/j.1469-7580.2010.01305.x

Krasnov, A., Skugor, S., Todorcevic, M., Glover, K. a, Nilsen, F., 2012. Gene expression in Atlantic salmon skin in response to infection with the parasitic copepod Lepeophtheirus salmonis, cortisol implant, and their combination. BMC Genomics 13, 130. doi:10.1186/1471-2164-13-130

Kumar, S., Raman, R.P., Pandey, P.K., Mohanty, S., Kumar, A., Kumar, K., 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish Carassius auratus (Linn. 1758) and resistance against Aeromonas hydrophila. Fish Shellfish Immunol. 34, 564–73. doi:10.1016/j.fsi.2012.11.038

Kumari, J., Sahoo, P.K., 2006. Non-specific immune response of healthy and immunocompromised Asian catfish (Clarias batrachus) to several immunostimulants. Aquaculture 255, 133–141. doi:10.1016/j.aquaculture.2005.12.012

Kunttu, H.M.T., Valtonen, E.T., Suomalainen, L.-R., Vielma, J., Jokinen, I.E., 2009. The efficacy of two immunostimulants against Flavobacterium columnare infection in juvenile

rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 26, 850–7. doi:10.1016/j.fsi.2009.03.013

Lacorte, L.M., Seiva, F.R.F., Rinaldi, J.C., Delella, F.K., Moroz, A., Sarobo, C., Godinho, A.F., Fávaro, W.J., Fernandes, A. a H., Felisbino, S.L., 2013. Caffeine reduces cadmium accumulation in the organism and enhances the levels of antioxidant protein expression in the epididymis. Reprod. Toxicol. 35, 137–143. doi:10.1016/j.reprotox.2012.10.009

Laing, K.J., Zou, J.J., Purcell, M.K., Phillips, R., Secombes, C.J., Hansen, J.D., 2006. Evolution of the CD4 family: teleost fish possess two divergent forms of CD4 in addition to lymphocyte activation gene-3. J Immunol 177, 3939–3951.

Laurent, P., 1984. Gill internal morphology, in: Hoar, W., Randall, D. (Eds.), Fish Physiology. Academic Press, New York, pp. 73–183.

Leal, L.K. a M., Ferreira, a. a G., Bezerra, G. a., Matos, F.J. a, Viana, G.S.B., 2000. Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: A comparative study. J. Ethnopharmacol. 70, 151–159. doi:10.1016/S0378-8741(99)00165-8

Leblond, V.S., Bisson, M., Hontela, A., 2001. Inhibition of cortisol secretion in dispersed head kidney cells of rainbow trout (Oncorhynchus mykiss) by endosulfan, an organochlorine pesticide. Gen Comp Endocrinol 121, 48–56.

Levita, J., Nawawi, A., Mutholib, A., Ibrahim, S., 2010. Andrographolide inhibits COX-2 expression in human fibroblast cells due to its interaction with arginine and histidine in cyclooxygenase site. J. Appl. Sci. 10, 1481–1484.

Li, J., Barreda, D.R., Zhang, Y.A., Boshra, H., Gelman, A.E., Lapatra, S., Tort, L., Sunyer, J.O., 2006. B lymphocytes from early vertebrates have potent phagocytic and microbicidal abilities. Nat Immunol 7, 1116–1124.

Lincy, M.P., Paulpriya, K., Mohan, V.R., 2013. In vitro antioxidant activity of Avicennia marina (Forssk) Vierh pneumatophore (Avicenniaceae) 3, 106–114.

Liu, Y., Moore, L., Koppang, E.O., Hordvik, I., 2008. Characterization of the CD3zeta, CD3gammadelta and CD3epsilon subunits of the T cell receptor complex in Atlantic salmon. Dev Comp Immunol 32, 26–35.

Locksley, R.M., Nelson, C.S., Fankhauser, J.E., Klebanoff, S.J., 1987. Loss of granulemyeloperoxidase during in vitro culture of human monocytes correlates with decay in antiprotozoa activity. Am. J. Trop. Med. Hyg. 36, 541–548.

Lorenz, R.T., Cysewski, G.R., 2000. Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. Trends Biotechnol. 18, 160–167. doi:10.1016/S0167-7799(00)01433-5

Løvoll, M., Fischer, U., Mathisen, G.S., Bøgwald, J., Ototake, M., Dalmo, R. a, 2007. The C3 subtypes are differentially regulated after immunostimulation in rainbow trout, but head kidney macrophages do not contribute to C3 transcription. Vet. Immunol. Immunopathol. 117, 284–95. doi:10.1016/j.vetimm.2007.03.005

Magnadóttir, B., 2006. Innate immunity of fish (overview). Fish Shellfish Immunol. 20, 137–51. doi:10.1016/j.fsi.2004.09.006

Magnadottir, B., Lange, S., Gudmundsdottir, S., Bøgwald, J., Dalmo, R. a, 2005. Ontogeny of humoral immune parameters in fish. Fish Shellfish Immunol. 19, 429–39. doi:10.1016/j.fsi.2005.03.010

Maki, J.L., Dickerson, H.W., Maki, J.L., Dickerson, H.W., 2003. Systemic and Cutaneous Mucus Antibody Responses of Channel Catfish Immunized against the Protozoan Parasite Ichthyophthirius multifiliis Systemic and Cutaneous Mucus Antibody Responses of Channel Catfish Immunized against the Protozoan Parasite Ichthyopht 10, 876–881. doi:10.1128/CDLI.10.5.876

Marshall, W.S., Lynch, E.M., Cozzi, R.R.F., 2002. Redistribution of immunofluorescence of CFTR anion channel and NKCC cotransporter in chloride cells during adaptation of the killifish Fundulus heteroclitus to sea water. J. Exp. Biol. 205, 1265–1273.

McCormick, S.D., 2001. Endocrine Control of Osmoregulation in Teleost Fish1. Am. Zool. 41, 781–794. doi:10.1668/0003-1569(2001)041[0781:ECOOIT]2.0.CO;2

McKay, D.L., Blumberg, J.B., 2006. A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). Phyther. Res. 20, 619–633. doi:10.1002/ptr

Meloni, S., Scapigliati, G., 2000. Evaluation of immunoglobulins produced in vitro by head-kidney leucocytes of sea bass Dicentrarchus labrax by immunoenzymatic assay. Fish Shellfish Immunol 10, 95–99.

Metwally, A.M., Omar, A.A., Harraz, F.M., El Sohafy, S.M., 2010. Phytochemical investigation and antimicrobial activity of Psidium guajava L leaves. Pharmacogn. Mag. 6, 212–218.

Misra, C., Das, B., Mukherjee, S., Pattnaik, P., 2006. Effect of long term administration of dietary Î²-glucan on immunity, growth and survival of Labeo rohita fingerlings. Aquaculture 255, 82–94.

Mistry, K.S., Sanghvi, Z., Parmar, G., Shah, S., 2014. The antimicrobial activity of azadirachta indica, mimusops elengi, tinospora cardifolia, ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. Eur. J. Dent. 8, 172–177. doi:10.4103/1305-7456.130591

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268. doi:10.1023/A:1008924418720

Moore, L.J., Dijkstra, J.M., Koppang, E.O., Hordvik, I., 2009. CD4 homologues in Atlantic salmon. Fish Shellfish Immunol 26, 10–18.

Moore, L.J., Somamoto, T., Lie, K.K., Dijkstra, J.M., Hordvik, I., 2005. Characterisation of salmon and trout CD8alpha and CD8beta. Mol. Immunol. 42, 1225–34. doi:10.1016/j.molimm.2004.11.017

Morera, D., Roher, N., Ribas, L., Balasch, J.C., Donate, C., Callol, A., Boltana, S., Roberts, S., Goetz, G., Goetz, F.W., MacKenzie, S.A., 2011. RNA-Seq reveals an integrated immune response in nucleated erythrocytes. PLoS One 6, e26998.

Nathan, C., Shiloh, M.U., 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc. Natl. Acad. Sci. U. S. A. 97, 8841–8848. doi:10.1073/pnas.97.16.8841

Nel, A.E., 2002. T-cell activation through the antigen receptor. Part 1: Signaling components, signaling pathways, and signal integration at the T-cell antigen receptor synapse. J. Allergy Clin. Immunol. 109, 758–770. doi:10.1067/mai.2002.124259

Ngugi, C.C., Oyoo-Okoth, E., Mugo-Bundi, J., Orina, P.S., Chemoiwa, E.J., Aloo, P.A., 2015. Effects of dietary administration of stinging nettle (Urtica dioica) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (Labeo victorianus) challenged with Aeromonas hydrophila. Fish Shellfish Immunol. 44, 533–41. doi:10.1016/j.fsi.2015.03.025

Nigam, A.K., Kumari, U., Mittal, S., Mittal, A.K., 2012. Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches. Fish Physiol. Biochem. 38, 1245–1256. doi:10.1007/s10695-012-9613-5

Nuñez Ortiz, N., Gerdol, M., Stocchi, V., Marozzi, C., Randelli, E., Bernini, C., Buonocore, F., Picchietti, S., Papeschi, C., Sood, N., Pallavicini, A., Scapigliati, G., 2014. T cell transcripts and T cell activities in the gills of the teleost fish sea bass (Dicentrarchus labrax). Dev. Comp. Immunol. 47, 309–18. doi:10.1016/j.dci.2014.07.015

Nya, E.J., Austin, B., 2009. Use of dietary ginger, Zingiber officinale Roscoe, as an immunostimulant to control Aeromonas hydrophila infections in rainbow trout, Oncorhynchus mykiss (Walbaum). J. Fish Dis. 32, 971–977. doi:10.1111/j.1365-2761.2009.01101.x

Nya, E.J., Austin, B., 2011. Development of immunity in rainbow trout (Oncorhynchus mykiss, Walbaum) to Aeromonas hydrophila after the dietary application of garlic. Fish Shellfish Immunol. 30, 845–850. doi:10.1016/j.fsi.2011.01.008

Ogier de Baulny, M., Quentel, C., Fournier, V., Lamour, F., Le Gouvello, R., 1996. Effect of long-term oral administration of beta-glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot Scophthalmus maximus. Dis. Aquat. Organ. 26, 139–147. doi:10.3354/dao026139

Okumura, T., Kanemaki, T., Kitade, H., 1993. Stimulation of glucose incorporation into glycogen by E-series prostaglandins in cultured rat hepatocytes. Biochim. Biophys. Acta - Mol. Cell Res. 1176, 137–142.

Olson, K.R., 2002. Cell signaling and ion transport across the fish gill epithelium. J. Exp. Zool. 293, 336–347. doi:10.1002/jez.10128

Ortuño, J., Cuesta, A., Rodríguez, A., Esteban, M.A., Meseguer, J., 2002. Oral administration of yeast, Saccharomyces cerevisiae, enhances the cellular innate immune response of gilthead seabream (Sparus aurata L.). Vet. Immunol. Immunopathol. 85, 41–50.

Pacheco, M., Santos, M. a., 2001. Tissue distribution and temperature-dependence of Anguilla anguilla L. EROD activity following exposure to model inducers and relationship with plasma cortisol, lactate and glucose levels. Environ. Int. 26, 149–155. doi:10.1016/S0160-4120(00)00101-X

Palti, Y., Gahr, S.A., Purcell, M.K., Hadidi, S., Rexroad 3rd, C.E., Wiens, G.D., 2010. Identification, characterization and genetic mapping of TLR7, TLR8a1 and TLR8a2 genes in rainbow trout (Oncorhynchus mykiss). Dev Comp Immunol 34, 219–233.

Palti, Y., Rodriguez, M.F., Gahr, S. a, Purcell, M.K., Rexroad, C.E., Wiens, G.D., 2010. Identification, characterization and genetic mapping of TLR1 loci in rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 28, 918–926. doi:10.1016/j.fsi.2010.02.002

Parra, D., Reyes-Lopez, F.E., Tort, L., 2015. Mucosal Immunity and B Cells in Teleosts: Effect of Vaccination and Stress. Front. Immunol. 6, 1–12. doi:10.3389/fimmu.2015.00354

Patil, V., Källqvist, T., Olsen, E., Vogt, G., Gislerød, H.R., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquac. Int. 15, 1–9. doi:10.1007/s10499-006-9060-3

Pettitt, T.R., Barrow, S.E., Rowley, a. F., 1991. Thromboxane, prostaglandin and leukotriene generation by rainbow trout blood. Fish Shellfish Immunol. 1, 71–73. doi:10.1016/S1050-4648(06)80021-5

Pietretti, D., Vera-Jimenez, N.I., Hoole, D., Wiegertjes, G.F., 2013. Oxidative burst and nitric oxide responses in carp macrophages induced by zymosan, MacroGard?? and selective dectin-1 agonists suggest recognition by multiple pattern recognition receptors. Fish Shellfish Immunol. 35, 847–857. doi:10.1016/j.fsi.2013.06.022

Pionnier, N., Falco, A., Miest, J.J., Shrive, A.K., Hoole, D., 2014. Feeding common carp Cyprinus carpio with β-glucan supplemented diet stimulates C-reactive protein and complement immune acute phase responses following PAMPs injection. Fish Shellfish Immunol. 39, 285–95. doi:10.1016/j.fsi.2014.05.008

Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N., Nakashima, H., 1999. Antiviral properties of a mangrove plant, Rhizophora apiculata Blume, against human immunodeficiency virus. Antiviral Res. 44, 113–122. doi:10.1016/S0166-3542(99)00058-3

Press, C., 1999. The morphology of the immune system in teleost fishes. Fish Shellfish Immunol. 9, 309–318. doi:10.1006/fsim.1998.0181

Press, C.M., Dannevig, B.H., Landsverk, T., 1994. Immune and enzyme histochemical phenotypes of lymphoid and nonlymphoid cells within the spleen and head kidney of Atlantic salmon (Salmo salar L.). Fish Shellfish Immunol. doi:10.1006/fsim.1994.1007

Purcell, M.K., Smith, K.D., Hood, L., Winton, J.R., Jared, C., 2007. Conservation of Toll-Like Receptor Signaling Pathways in Teleost Fish. Comp Biochem Physiol Part D Genomics Proteomics 1, 77–88. doi:10.1016/j.cbd.2005.07.003

Püschel, G.P., Christ, B., 1994. Inhibition by PGE2 of glucagon-induced increase in phosphoenolpyruvate carboxykinase mRNA and acceleration of mRNA degradation in cultured rat hepatocytes. FEBS Lett. 351, 353–356. doi:10.1016/0014-5793(94)00877-9

Pype, S., Declercq, W., Ibrahimi, A., Michiels, C., Van Rietschoten, J.G.I., Dewulf, N., De Boer, M., Vandenabeele, P., Huylebroeck, D., Remacle, J.E., 2000. TTRAP, a novel protein that associates with CD40, tumor necrosis factor (TNF) receptor-75 and TNF receptor-associated factors (TRAFs), and that inhibits nuclear factor-κB activation. J. Biol. Chem. 275, 18586–18593. doi:10.1074/jbc.M000531200

Rabinovitch, M., 1995. Professional and non-professional phagocytes: an introduction. Trends Cell Biol 5, 85–87.

Reid, S.G., Bernier, N.J., Perry, S.F., 1998. The adrenergic stress response in fish: contraol of catecholamine storage and release. Comp. Biochem. Physiol. Part C 120, 1–27.

Reyes-Becerril, M., Angulo, C., Estrada, N., Murillo, Y., Ascencio-Valle, F., 2014. Dietary administration of microalgae alone or supplemented with Lactobacillus sakei affects immune response and intestinal morphology of Pacific red snapper (Lutjanus peru). Fish Shellfish Immunol. 40, 208–16. doi:10.1016/j.fsi.2014.06.032

Reyes-Becerril, M., Guardiola, F., Rojas, M., Ascencio-Valle, F., Esteban, M.Á., 2013. Dietary administration of microalgae Navicula sp. affects immune status and gene expression of gilthead seabream (Sparus aurata). Fish Shellfish Immunol. 35, 883–9. doi:10.1016/j.fsi.2013.06.026

Rodríguez, a, 2003. Immunostimulant properties of a cell wall-modified whole Saccharomyces cerevisiae strain administered by diet to seabream (Sparus aurata L.). Vet. Immunol. Immunopathol. 96, 183–192. doi:10.1016/S0165-2427(03)00164-8

Rodriguez, M.F., Wiens, G.D., Purcell, M.K., Palti, Y., 2005. Characterization of Toll-like receptor 3 gene in rainbow trout (Oncorhynchus mykiss). Immunogenetics 57, 510–519. doi:10.1007/s00251-005-0013-1

Rotllant, J., Arends, R.J., Mancera, J.M., Flik, G., Wendelaar Bonga, S.E., Tort, L., 2000. Inhibition of HPI axis response to stress in gilthead sea bream (Sparus aurata) with physiological plasma levels of cortisol. Fish Physiol. Biochem. 23, 13–22. doi:10.1023/A:1007848128968

Sahoo, P.K., Mukherjee, S.C., 2001. Effect of dietary beta-1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B1-induced immunocompromised rohu (Labeo rohita Hamilton). Fish Shellfish Immunol. 11, 683–95. doi:10.1006/fsim.2001.0345

Salinas, I., 2015. The Mucosal Immune System of Teleost Fish. Biology (Basel). 4, 525–539. doi:10.3390/biology4030525

Salinas, I., Zhang, Y.-A., Sunyer, J.O., 2011. Mucosal immunoglobulins and B cells of teleost fish. Dev. Comp. Immunol. 35, 1346–65. doi:10.1016/j.dci.2011.11.009

Sealey, W.M., Barrows, F.T., Hang, A., Johansen, K.A., Overturf, K., LaPatra, S.E., Hardy, R.W., 2008. Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout Oncorhynchus mykiss. Anim. Feed Sci. Technol. 141, 115–128. doi:10.1016/j.anifeedsci.2007.05.022

Secombes, C.J., Fletcher, T.C., 1992. The role of phagocytes in the protective mechanisms of fish. Annu. Rev. Fish Dis. 2, 53–71. doi:10.1016/0959-8030(92)90056-4

Secombes, C.J., Olivier, G., 1997. Host—Pathogen Interactions in Salmonids, Furunculosis. doi:DOI: 10.1016/B978-012093040-1/50014-2

Secombes, C.J., van Groningen, J.J., Egberts, E., 1983. Separation of lymphocyte subpopulations in carp Cyprinus carpio L. by monoclonal antibodies: immunohistochemical studies. Immunology 48, 165–175.

See, D., Broumand, N., Sahl, L., Tilles, J., 1997. In vitro effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. Immunopharmacology 35, 229–235.

Selvaraj, V., Sampath, K., Sekar, V., 2005. Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (Cyprinus carpio) infected with Aeromonas hydrophila. Fish Shellfish Immunol. 19, 293–306. doi:10.1016/j.fsi.2005.01.001

Selvaraj, V., Sampath, K., Sekar, V., 2006. Adjuvant and immunostimulatory effects of beta-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with Aeromonas hydrophila. Vet. Immunol. Immunopathol. 114, 15–24. doi:10.1016/j.vetimm.2006.06.011

Selvaraj, V., Sampath, K., Sekar, V., 2009. Administration of lipopolysaccharide increases specific and non-specific immune parameters and survival in carp (Cyprinus carpio) infected with Aeromonas hydrophila. Aquaculture 286, 176–183. doi:10.1016/j.aquaculture.2008.09.017

Sharma, N., Tripathi, A., 2008. Effects of Citrus sinensis (L.) Osbeck epicarp essential oil on growth and morphogenesis of Aspergillus niger (L.) Van Tieghem. Microbiol. Res. 163, 337–344. doi:10.1016/j.micres.2006.06.009

Sheikhzadeh, N., Nofouzi, K., Delazar, A., Oushani, A.K., 2011. Immunomodulatory effects of decaffeinated green tea (Camellia sinensis) on the immune system of rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 31, 1268–9. doi:10.1016/j.fsi.2011.09.010

Shepard, K., 1994. Functions for fish mucus. Rev Fish Biol Fish. 4, 401–429.

Sheridan, M., 1986. Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, Oncorhynchus kisutch, during smoltification. Gen. Comp. Endocrinol. 64, 220–238.

Shrimpton, J., McCormick, S.D., 1999. Responsiveness of gill Na+/K+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. J. Exp. Biol. 202 (Pt 8), 987–95.

Silva, M.T., Correia-Neves, M., 2012. Neutrophils and macrophages: The main partners of phagocyte cell systems. Front. Immunol. 3, 2008–2013. doi:10.3389/fimmu.2012.00174

Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Médale, F., Kaushik, S., Pérez-Sánchez, J., 2005. Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (Sparus aurata). Aquaculture 249, 387–400. doi:10.1016/j.aquaculture.2005.03.031

Siwicki, a K., Anderson, D.P., Rumsey, G.L., 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet. Immunol. Immunopathol. 41, 125–39.

Skalli, A., Castillo, M., Andree, K.B., Tort, L., Furones, D., Gisbert, E., 2013. The LPS derived from the cell walls of the Gram-negative bacteria Pantoea agglomerans stimulates growth and immune status of rainbow trout (Oncorhynchus mykiss) juveniles. Aquaculture 416-417, 272–279. doi:10.1016/j.aquaculture.2013.09.037

Skjæveland, I., Iliev, D.B., Strandskog, G., Jørgensen, J.B., 2009. Identification and characterization of TLR8 and MyD88 homologs in Atlantic salmon (Salmo salar). Dev. Comp. Immunol. 33, 1011–1017. doi:10.1016/j.dci.2009.04.007

Skjermo, J., Bergh, Ø., 2004. High-M alginate immunostimulation of Atlantic halibut (Hippoglossus hippoglossus L.) larvae using Artemia for delivery, increases resistance against vibriosis. Aquaculture 238, 107–113. doi:10.1016/j.aquaculture.2004.05.038

Skjermo, J., Defoort, T., Dehasque, M., Espevik, T., Olsen, Y., Skjak-brfek, G., Sorgeloos, P., Vadstein, O., 1995. Immunostimulation of juvenile turbot (Scophthalmusmaximus L.) using an alginate with high mannuronic acid content administered via the live food organism Artemia. Fish Shellfish Immunol. 5, 531–534. doi:10.1016/S1050-4648(95)80053-0

Smeekens, S.P., van de Veerdonk, F.L., van der Meer, J.W.M., Kullberg, B.J., Joosten, L. a B., Netea, M.G., 2010. The Candida Th17 response is dependent on mannan- and beta-glucan-induced prostaglandin E2. Int. Immunol. 22, 889–895. doi:10.1093/intimm/dxq442

Solomon, L.M., Juhlin, L., Kirschenbaum, B., 1968. Prostaglandin on cutaneous vasculature. J. Investig. dermatology 280–283.

Spitznagel, J.K., Martin, E., Kinkade, M., 1983. Differential distribution of distinct forms of myoloperoxidase in different azuropholic granule subpopulations from human neutrophils. Biochem. Biophys. Res. Commun. 114, 296–303.

Stacey, N., Goetz, F., 1982. Role of prostaglandins in fish reproduction. Can. J. Fish. Aquat. Sci. 39, 92–98.

Tamm, W., Fryer, J., Ali, I., Dallaire, M., Valentine, B., 1988. Growth inhibition, gluconeogenesis, and morphometric studies of the pituitary and interrenal cells of acid-stressed brook trout (Salvelinus fontinalis). Can. J. Fish. Aquat. Sci. 45, 1197–1211.

Tang, J., Cai, J., Liu, R., Wang, J., Lu, Y., Wu, Z., Jian, J., 2014. Immunostimulatory effects of artificial feed supplemented with a Chinese herbal mixture on Oreochromis niloticus against Aeromonas hydrophila. Fish Shellfish Immunol. 39, 401–6. doi:10.1016/j.fsi.2014.05.028

Thawonsuwan, J., Kiron, V., Satoh, S., Panigrahi, a., Verlhac, V., 2010. Epigallocatechin-3-gallate (EGCG) affects the antioxidant and immune defense of the rainbow trout, Oncorhynchus mykiss. Fish Physiol. Biochem. 36, 687–697. doi:10.1007/s10695-009-9344-4

Toda, H., Araki, K., Moritomo, T., Nakanishi, T., 2011. Perforin-dependent cytotoxic mechanism in killing by CD8 positive T cells in ginbuna crucian carp, Carassius auratus langsdorfii. Dev. Comp. Immunol. 35, 88–93. doi:10.1016/j.dci.2010.08.010

Torrecillas, S., Makol, a, Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., Izquierdo, M.S., 2007. Immune stimulation and improved infection resistance in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides. Fish Shellfish Immunol. 23, 969–81. doi:10.1016/j.fsi.2007.03.007

Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–75. doi:10.1016/j.dci.2011.07.002

Tort, L., Balasch, J.C., Mackenzie, S., 2003. Fish immune system. A crossroads between innate and adaptive responses. Immunologia 22, 277–286.

Trapani, J.A., Smyth, M.J., 2002. Functional significance of the perforin/granzyme cell death pathway. Nat. Rev. Immunol. 2, 735–747.

Tsoi, S., Park, K.C., Kay, H.H., O'Brien, T.J., Podor, E., Sun, G., Douglas, S.E., Brown, L.L., Johnson, S.C., 2006. Identification of a transcript encoding a soluble form of toll-like receptor 5 (TLR5) in Atlantic salmon during Aeromonas salmonicida infection. Vet Immunol Immunopathol 109, 183–187.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S., Hirano, T., 2000. Excellent Salinity Tolerance of Mozambique Tilapia (Oreochromis mossambicus): Elevated Chloride Cell Activity in the Branchial and Opercular Epithelia of the Fish Adapted to Concentrated Seawater. Zoolog. Sci. 17, 149–160. doi:10.2108/zsj.17.149

Uncini Manganelli, R.E., Zaccaro, L., Tomei, P.E., 2005. Antiviral activity in vitro of Urtica dioica L., Parietaria diffusa M. et K. and Sambucus nigra L. J. Ethnopharmacol. 98, 323–327. doi:10.1016/j.jep.2005.01.021

Van Raaij, M.T., Pit, D.S., Balm, P.H., Steffens, a B., van den Thillart, G.E., 1996. Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. Horm. Behav. 30, 85–92. doi:S0018506X96900124 [pii]

Vázquez, B., Avila, G., Segura, D., Escalante, B., 1996. Antiinflammatory activity of extracts from Aloe vera gel. J. Ethnopharmacol. 55, 69–75.

Verlhac, V., Obach, A., Gabaudan, J., 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (Oncorhynchus mykiss). Fish Shellfish … 409–424.

Vidard, L., Kovacsovics-Bankowski, M., Kraeft, S.K., Chen, L.B., Benacerraf, B., Rock, K.L., 1996. Analysis of MHC class II presentation of particulate antigens of B lymphocytes. J Immunol 156, 2809–2818.

Wang, J.-L., Meng, X., Lu, R., Wu, C., Luo, Y.-T., Yan, X., Li, X.-J., Kong, X.-H., Nie, G.-X., 2015. Effects of Rehmannia glutinosa on growth performance, immunological parameters and disease resistance to Aeromonas hydrophila in common carp (Cyprinus carpio L.). Aquaculture 435, 293–300. doi:10.1016/j.aquaculture.2014.10.004

Watanuki, H., Ota, K., Tassakka, A.C.M. a R., Kato, T., Sakai, M., 2006. Immunostimulant effects of dietary Spirulina platensis on carp, Cyprinus carpio. Aquaculture 258, 157–163. doi:10.1016/j.aquaculture.2006.05.003

Weber, J.M., Ruzindana-Umunyana, A., Imbeault, L., Sircar, S., 2003. Inhibition of adenovirus infection and adenain by green tea catechins. Antiviral Res. 58, 167–173. doi:10.1016/S0166-3542(02)00212-7

Wendelaar Bonga, S., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M., Narayana, a. K., Sulaiman, M., 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia 75, 68–73. doi:10.1016/j.fitote.2003.07.013

Wilson, J.M., Laurent, P., 2002. Fish gill morphology: inside out. J. Exp. Zool. 293, 192–213. doi:10.1002/jez.10124

Wong, C.K.C., Chan, D.K.O., 2001. Effects of cortisol on chloride cells in the gill epithelium of Japanese eel, Anguilla japonica. J. Endocrinol. 168, 185–192. doi:10.1677/joe.0.1680185

X, K., Y, H., D, S., 2003. Research progress of immunopharmacology of Astragalus polysaccharide. J Chin Vet 3, 34–37 [China].

Xu, Y., Marshall, R.L., Mukkur, T.K.S., 2006. An investigation on the antimicrobial activity of Andrographis paniculata extracts and andrographolide in vitro. Asian J. Plant Sci. doi:10.3923/ajps.2006.527.530

Xu, Z., Parra, D., Gómez, D., Salinas, I., Zhang, Y.-A., Jørgensen, L.V.G., Heinecke, R.D., Buchmann, K., LaPatra, S., Sunyer, J.O., 2013. Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. Proc. Natl. Acad. Sci. 110, 13097–13102. doi:10.1073/pnas.1304319110

Yang, X., Guo, J.L., Ye, J.Y., Zhang, Y.X., Wang, W., 2015. The effects of Ficus carica polysaccharide on immune response and expression of some immune-related genes in grass carp, Ctenopharyngodon idella. Fish Shellfish Immunol. 42, 132–7. doi:10.1016/j.fsi.2014.10.037

Yao, C., Sakata, D., Esaki, Y., Li, Y., Matsuoka, T., Kuroiwa, K., Sugimoto, Y., Narumiya, S., 2009. Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. Nat. Med. 15, 633–640. doi:10.1038/nm.1968

Yarahmadi, P., Kolangi Miandare, H., Farahmand, H., Mirvaghefi, A., Hoseinifar, S.H., 2014. Dietary fermentable fiber upregulated immune related genes expression, increased innate immune response and resistance of rainbow trout (Oncorhynchus mykiss) against Aeromonas hydrophila. Fish Shellfish Immunol. 41, 326–31. doi:10.1016/j.fsi.2014.09.007

Yeganeh, S., Teimouri, M., Amirkolaie, A.K., 2015. Dietary effects of Spirulina platensis on hematological and serum biochemical parameters of rainbow trout (Oncorhynchus mykiss). Res. Vet. Sci. 101, 84–88. doi:10.1016/j.rvsc.2015.06.002

Yin, G., Ardó, L., Thompson, K.D., Adams, a., Jeney, Z., Jeney, G., 2009. Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. Fish Shellfish Immunol. 26, 140–145. doi:10.1016/j.fsi.2008.08.015

Yogeshwari, G., Jagruthi, C., Anbazahan, S.M., Mari, L.S.S., Selvanathan, J., Arockiaraj, J., Dhayanithi, N.B., Ajithkumar, T.T., Balasundaram, C., Ramasamy, H., 2015. Herbal supplementation diet on immune response in Labeo rohita against Aphanomyces invadans. Aquaculture 437, 351–359. doi:10.1016/j.aquaculture.2014.12.024

Yu, H.H., Han, F., Xue, M., Wang, J., Tacon, P., Zheng, Y.H., Wu, X.F., Zhang, Y.J., 2014. Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (Lateolabrax japonicus). Aquaculture 432, 217–224. doi:10.1016/j.aquaculture.2014.04.043

Yu, H.H., Seo, S.J., Kim, Y.H., Lee, H.Y., Park, R.K., So, H.S., Jang, S.L., You, Y.O., 2006. Protective effect of Rehmannia glutinosa on the cisplatin-induced damage of HEI-OC1 auditory cells through scavenging free radicals. J. Ethnopharmacol. 107, 383–388. doi:10.1016/j.jep.2006.03.024

Zanuzzo, F.S., Urbinati, E.C., Rise, M.L., Hall, J.R., Nash, G.W., Gamperl, A.K., 2015. Aeromonas salmonicida induced immune gene expression in Aloe vera fed steelhead trout, Oncorhynchus mykiss (Walbaum). Aquaculture 435, 1–9. doi:10.1016/j.aquaculture.2014.09.010

Zedlitz, S., Kaufmann, R., Boehncke, W.-H., 2002. Allergic contact dermatitis from black cumin (Nigella sativa) oil-containing ointment. Contact Dermatitis 46, 188. doi:10.1034/j.1600-0536.2002.460318.x

Zhang, Y.A., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., Lapatra, S.E., Bartholomew, J., Sunyer, J.O., 2010. IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nat Immunol 11, 827–835.

Zhang, Y.-A., Salinas, I., Oriol Sunyer, J., 2011. Recent findings on the structure and function of teleost IgT. Fish Shellfish Immunol. 31, 627–34. doi:10.1016/j.fsi.2011.03.021

Zhou, B.S., Wu, R.S.S., Randall, D.J., Lam, P.K.S., Ip, Y.K., Chew, S.F., 2000. Metabolic adjustments in the common carp during prolonged hypoxia. J. Fish Biol. 57, 1160–1171. doi:10.1006/jfbi.2000.1382

Zhou, H., Stuge, T.B., Miller, N.W., Bengten, E., Naftel, J.P., Bernanke, J.M., Chinchar, V.G., Clem, L.W., Wilson, M., 2001. Heterogeneity of channel catfish CTL with respect to target recognition and cytotoxic mechanisms employed. J. Immunol. 167, 1325–1332. doi:10.4049/jimmunol.167.3.1325

8. ANNEX

TABLE S1: List of differential expressed genes (p<0.01) in gills of seabream fed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **CONTROL DIET (DIET C) AND DIET A 2 DAYS OF FEEDING**. The p-value and the absolute fold change (FCA) for up-(green) and down-regulated genes (blue) are represented.

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
Deoxycytidylate deaminase [Salmo salar]	4.93E-03	3.19	up
Perforin1 precursor [Salmo salar]	7.18E-05	2.65	up
unknown	3.11E-03	2.50	up
sulfide quinone reductaselike [Danio rerio]	7.03E-03	2.26	up
unknown	2.81E-03	2.13	up
programmed cell death 7 [Danio rerio]	7.75E-03	2.02	up
Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial precursor [Salmo salar]	3.10E-03	1.94	up
NADHcytochrome b5 reductase 2 [Salmo salar]	8.02E-03	1.84	up
DnaJlike subfamily B member 6 [Paralichthys olivaceus]	3.35E-03	1.84	up
PPPDE peptidase domain containing 2a [Danio rerio]	8.44E-03	1.81	up
unknown	1.96E-03	1.80	up
intraflagellar transport 81like [Bos taurus]	8.91E-03	1.78	up
unknown	2.44E-03	1.72	up
G patch domain containing 4 [Xenopus (Silurana) tropicalis]	8.09E-03	1.71	up
unknown	6.31E-03	1.65	up
isocitrate dehydrogenase 3 (NAD+) beta, isoform CRA_d [Homo sapiens]	7.07E-03	1.63	up
Zincbinding alcohol dehydrogenase domaincontaining protein 1 [Salmo salar]	7.00E-03	1.63	up
unknown	4.32E-03	1.63	up
YY1 transcription factor a [Danio rerio]	6.80E-03	1.62	up
3hydroxyisobutyrylCoA hydrolase, mitochondrial precursor [Salmo salar]	5.72E-03	1.62	up
Heparan sulfate 2Osulfotransferase 1 [Salmo salar]	8.70E-03	1.61	up
Epoxide hydrolase 2 [Salmo salar]	6.78E-03	1.61	up
unknown	8.71E-03	1.60	up
Interferoninduced 35 kDa protein homolog [Salmo salar]	6.29E-04	1.57	up
unknown	5.57E-03	1.56	up
unknown	7.23E-03	1.55	up
Nacetylglucosamine1phosphate transferase [Danio rerio]	5.32E-03	1.55	up
unknown	7.20E-04	1.55	up
member 6 [Xenopus (Silurana) tropicalis]	3.88E-03	1.54	up
delta9desaturase 1 [Takifugu rubripes]	5.00E-04	1.51	up
coiledcoil domain containing 94 [Danio rerio]	8.49E-03	1.51	up
kinesin family member 23 [Bos taurus]	7.72E-03	1.49	up
Protein nightcap	2.77E-03	1.49	up
DET1 and DDB1 associated 1 [Danio rerio]	8.31E-03	1.49	up
Peroxisomal 3,2transenoylCoA isomerase [Salmo salar]	6.56E-03	1.48	up
caspase1 [Dicentrarchus labrax]	2.46E-03	1.47	up
short coiledcoil protein [Danio rerio]	2.37E-03	1.43	up
unknown	3.30E-03	1.43	up
transforming, acidic coiledcoil containing protein 3 [Takifugu rubripes]	2.42E-04	1.43	up
Integrin beta2 precursor [Salmo salar]	6.31E-03	1.43	up
isoform CRA_e [Mus musculus]	4.31E-03	1.43	up
COX4 neighbor isoform 2 [Homo sapiens]	7.23E-03	1.42	up
unknown	6.71E-03	1.41	up
unknown	4.70E-03	1.40	up
unknown	4.44E-03	1.39	up
RecName: Full=UPF0709 protein C3orf34 homolog	4.32E-03	1.39	up
Chloride intracellular channel protein 4 [Esox lucius]	7.82E-03	1.39	up

TABLE S1: (*continued*...)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
member 9 [Xenopus laevis]	5.26E-03	1.39	up
unknown	3.36E-03	1.39	up
Transforming protein RhoA precursor [Salmo salar]	7.27E-03	1.38	up
unknown	5.34E-03	1.38	up
Salmo salar clone ssalrgf516125 E3 ubiquitinprotein ligase BRE1B putative mRNA, pseudogene cds	1.08E-03	1.38	up
unknown	7.39E-03	1.38	up
RecName: Full=Probable 2oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial; AltName: Full=Dehydrogenase E1 and transketolase domaincontaining protein 1; Flags: Precursor	8.60E-03	1.38	up
unknown	4.65E-03	1.38	up
unknown	7.88E-03	1.36	up
unknown	2.24E-03	1.36	up
proteinaseactivated receptor2a [Salmo salar]	5.50E-03	1.36	up
unknown	1.98E-03	1.35	up
CD59 [Pseudosciaena crocea]	6.98E-03	1.35	up
RAB1, member RAS oncogene family [Mus musculus]	7.67E-03	1.35	up
claudin 7a [Takifugu rubripes]	1.05E-03	1.34	up
Lithognathus mormyrus clone lmos9p04f08 mRNA sequence	5.41E-03	1.33	up
ubiquitin specific peptidase 10 [Xenopus (Silurana) tropicalis]	7.50E-03	1.32	up
LYR motifcontaining protein 4 [Esox lucius]	8.50E-03	1.32	up
BCL2like 12 [Bos taurus]	6.26E-03	1.31	up
unknown	1.69E-03	1.30	up
unknown	9.16E-03	1.30	up
unknown	4.03E-03	1.30	up
novel protein similar to vertebrate Xray repair complementing defective repair in Chinese hamster cells 5 (doublestrandbreak rejoining; Ku autoantigen, 80kDa) (XRCC5) [Danio rerio]	2.36E-03	1.30	up
mitochondrial precursor [Oncorhynchus mykiss]	1.51E-03	1.29	up
septin 8a [Danio rerio]	1.75E-03	1.28	up
unknown	7.19E-03	1.28	up
unknown	7.09E-03	1.27	up
unknown	4.35E-03	1.27	up
epidermal growth factor receptor pathway substrate 8like protein 1 isoform a [Homo sapiens]	9.24E-03	1.26	up
Lithognathus mormyrus clone lithmor139 mRNA sequence	3.23E-03	1.26	up
cyclin B3 [Oreochromis niloticus]	9.46E-03	1.25	up
Dynein, cytoplasmic 1, light intermediate chain 2 [Danio rerio]	7.93E-03	1.25	up
Livertype aldolase	1.43E-04	1.25	up
Lithognathus mormyrus clone lmos9p04f09 mRNA sequence	2.99E-03	1.24	up
unknown	1.45E-04	1.24	up
RecName: Full=Glutamine and serinerich protein 1	9.33E-04	1.23	up
DNA segment, Chr 1, ERATO Doi 622, expressed, isoform CRA_b [Mus musculus]	1.43E-03	1.23	up
alpha polypeptide [Danio rerio]	6.81E-03	1.23	up
Arfaptin1 [Salmo salar]	9.02E-03	1.22	up
unknown	8.55E-03	1.22	up
cathepsin L [Lates calcarifer]	4.67E-03	1.21	up
Factincapping protein subunit alpha1 [Salmo salar]	8.28E-03	1.20	up
CWC15 homolog [Salmo salar]	4.81E-03	1.19	up
unknown	6.71E-03	1.17	up
Ironresponsive elementbinding protein 2 [Salmo salar]	9.00E-03	1.17	up
unknown	6.29E-03	1.17	up
unknown	8.61E-03	1.16	up
unknown	8.49E-03	1.16	up

TABLE S1: (*continued...*)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.30E-03	1.16	up
component of oligomeric golgi complex 7 [Danio rerio]	6.09E-03	1.15	up
unknown	3.23E-03	1.12	up
Lithognathus mormyrus clone lmos3p03H03 mRNA sequence	3.82E-03	1.12	up
unknown	8.42E-03	1.10	up
unknown	4.14E-03	8.25	down
unknown	2.05E-03	3.73	down
unknown	6.71E-03	3.62	down
MGC84181 protein [Xenopus laevis]	9.19E-03	3.59	down
unknown	1.00E-04	3.54	down
unknown	2.57E-03	3.40	down
unknown	8.18E-03	3.20	down
Ubiquitinlike protein [Salmo salar]	8.14E-03	3.19	down
unknown	7.31E-03	3.16	down
unknown	3.98E-04	2.97	down
unknown	2.30E-03	2.90	down
unknown	6.31E-03	2.85	down
unknown	3.21E-03	2.74	down
unknown	2.02E-03	2.65	down
unknown	7.66E-05	2.63	down
Gzmb [Mus musculus]	2.34E-03	2.63	down
unknown	1.16E-03	2.62	down
unknown	9.53E-03	2.59	down
unknown	6.03E-04	2.58	down
unknown	5.08E-03	2.54	down
unknown	5.34E-03	2.51	down
unknown	9.26E-04	2.51	down
unknown	5.33E-03	2.47	down
unknown	4.31E-03	2.37	down
unknown	2.25E-03	2.36	down
novel protein (likely ortholog of H. sapiens KIAA1529) [Mus musculus]	7.35E-03	2.36	down
unknown	7.91E-03	2.34	down
Sushi repeatcontaining protein SRPX2 precursor [Salmo salar]	6.62E-03	2.28	down
unknown	1.44E-03	2.27	down
unknown	1.22E-03	2.23	down
unknown	1.27E-03	2.22	down
mast cell preproprotein [Homo sapiens]	6.15E-03	2.15	down
unknown	4.88E-03	2.14	down
unknown	2.27E-04	2.13	down
unknown	3.15E-03	2.13	down
unknown	4.67E-03	2.12	down
unknown	1.88E-05	2.11	down
unknown	5.64E-03	2.11	down
unknown	5.86E-03	2.10	down
unknown	9.57E-04	2.10	down
unknown	1.21E-03	2.09	down
unknown	8.25E-03	2.06	down
Efha2 protein [Mus musculus]	1.06E-03	2.05	down

TABLE S1: (*continued*...)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	8.66E-03	2.00	down
unknown	5.88E-03	1.99	down
unknown	3.11E-03	1.97	down
unknown	4.28E-03	1.97	down
unknown	3.81E-03	1.97	down
unknown	5.75E-03	1.96	down
unknown	3.72E-03	1.96	down
unknown	4.52E-03	1.96	down
unknown	1.06E-03	1.95	down
unknown	1.42E-03	1.94	down
unknown	7.20E-04	1.93	down
unknown	2.38E-03	1.92	down
unknown	4.54E-03	1.91	down
unknown	5.15E-03	1.91	down
unknown	2.03E-04	1.91	down
unknown	8.97E-03	1.89	down
unknown	2.53E-03	1.89	down
ribosomal protein L12 [Solea senegalensis]	4.57E-03	1.87	down
unknown	3.86E-03	1.87	down
unknown	7.83E-03	1.85	down
unknown	5.79E-03	1.85	down
unknown	1.62E-03	1.84	down
unknown	6.27E-04	1.83	down
viral Atype inclusion protein [Trichomonas vaginalis G3]	8.27E-03	1.83	down
unknown	3.54E-04	1.83	down
unknown	2.48E-03	1.83	down
unknown	4.21E-03	1.82	down
unknown	6.30E-03	1.82	down
unknown	9.83E-03	1.81	down
unknown	6.49E-04	1.79	down
unknown	6.06E-03	1.79	down
unknown	1.53E-03	1.78	down
unknown	2.61E-03	1.78	down
unknown	7.81E-03	1.76	down
unknown	3.85E-03	1.76	down
unknown	1.88E-03	1.75	down
unknown	1.29E-03	1.71	down
unknown	4.36E-03	1.70	down
unknown	3.10E-04	1.70	down
unknown	5.97E-04	1.70	down
unknown	2.07E-03	1.70	down
unknown	9.86E-04	1.66	down
unknown	8.45E-03	1.66	down
unknown	6.29E-03	1.65	down
Hippocalcinlike protein 1 [Salmo salar]	2.10E-03	1.65	down
Oryzias latipes PSMB10 and PSMB8 genes for proteasome subunit, beta type 10 and proteasome subunit, beta type 8, partial cds, haplotype: 24	6.55E-03	1.64	down
Oncorhynchus mykiss cyclin L1 mRNA, complete cds	4.34E-03	1.63	down
reverse transcriptase [Cyprinodon variegatus]	4.36E-03	1.63	down

TABLE S1: (*continued...*)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	8.82E-03	1.63	down
FBP32II precursor [Morone chrysops]	5.88E-04	1.63	down
unknown	1.34E-03	1.62	down
unknown	7.54E-03	1.62	down
unknown	5.03E-03	1.62	down
unknown	8.95E-03	1.62	down
unknown	4.42E-04	1.61	down
unknown	2.55E-03	1.60	down
unknown	6.99E-03	1.59	down
unknown	4.89E-03	1.59	down
unknown	2.05E-03	1.58	down
unknown	4.96E-03	1.58	down
unknown	7.66E-03	1.57	down
unknown	6.67E-03	1.57	down
unknown	6.43E-03	1.57	down
phosphoglucomutase 5 [Bos taurus]	2.28E-03	1.56	down
unknown	9.16E-03	1.55	down
unknown	4.27E-04	1.55	down
unknown	3.91E-03	1.54	down
unknown	2.82E-03	1.54	down
Fmsrelated tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) [Danio rerio]	7.56E-03	1.54	down
unknown	3.26E-03	1.54	down
proteintyrosine kinase [Mus musculus]	6.29E-03	1.53	down
unknown	9.59E-03	1.53	down
zinc finger protein 354C [Rattus norvegicus]	9.54E-03	1.52	down
unknown	1.39E-03	1.51	down
unknown	1.83E-03	1.51	down
unknown	7.70E-03	1.51	down
transposase [Oryzias latipes]	2.16E-03	1.51	down
unknown	1.77E-03	1.51	down
adrenomedullin1 [Takifugu rubripes]	7.73E-03	1.50	down
unknown	8.68E-03	1.50	down
unknown	3.65E-04	1.50	down
unknown	2.10E-03	1.48	down
unknown	2.94E-03	1.48	down
unknown	7.43E-03	1.48	down
Transposable element Tc1 transposase [Rana catesbeiana]	8.38E-03	1.47	down
unknown	2.76E-03	1.47	down
unknown	3.33E-03	1.46	down
unknown	5.07E-03	1.46	down
unknown	4.52E-03	1.46	down
unknown	1.48E-03	1.45	down
unknown	7.98E-03	1.45	down
unknown	2.95E-03	1.45	down
unknown	6.99E-03	1.44	down
unknown	8.73E-04	1.44	down
unknown	7.72E-03	1.43	down
unknown	9.35E-03	1.43	down

TABLE S1: (*continued*...)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	4.97E-03	1.42	down
im:7038599 [Danio rerio]	1.57E-03	1.42	down
unknown	2.49E-03	1.42	down
unknown	6.56E-03	1.42	down
unknown	8.34E-03	1.42	down
unknown	6.16E-03	1.42	down
unknown	7.58E-03	1.42	down
Lithognathus mormyrus clone lmos7p06f06 mRNA sequence	7.19E-03	1.41	down
unknown	1.00E-02	1.41	down
3Nacetylglucosaminyltransferase 5 [Danio rerio]	1.65E-03	1.41	down
unknown	1.58E-03	1.41	down
unknown	4.25E-03	1.41	down
unknown	6.66E-03	1.41	down
unknown	1.14E-03	1.40	down
Lithognathus mormyrus clone lmos2p03c10 mRNA sequence	2.79E-03	1.40	down
unknown	7.84E-03	1.39	down
unknown	4.46E-03	1.39	down
Tetraspanin4 [Salmo salar]	7.75E-03	1.38	down
unknown	9.19E-03	1.38	down
1a [Danio rerio]	6.05E-03	1.38	down
unknown	3.51E-03	1.37	down
Ras association domaincontaining protein 2 [Salmo salar]	4.63E-03	1.37	down
unknown	7.47E-03	1.36	down
Bos taurus TSC22 domain family, member 2 (TSC22D2), mRNA	3.49E-03	1.36	down
unknown	3.43E-04	1.35	down
unknown	5.00E-03	1.35	down
unknown	2.89E-03	1.35	down
Ctbp2 protein [Danio rerio]	8.84E-03	1.35	down
Tyrosineprotein kinase BTK [Salmo salar]	1.29E-03	1.34	down
unknown	6.76E-04	1.34	down
unknown	1.23E-03	1.34	down
Salmo salar clone ssalrgf531382 deltex3like putative mRNA, complete cds	7.55E-03	1.33	down
zinc finger, DHHC domain containing 7, isoform CRA_a [Rattus norvegicus]	4.58E-03	1.32	down
unknown	6.68E-03	1.32	down
unknown	2.93E-03	1.32	down
unknown	8.66E-04	1.31	down
unknown	8.70E-03	1.31	down
TANKbinding kinase 1 [Danio rerio]	4.64E-03	1.31	down
unknown	6.33E-03	1.30	down
unknown	7.07E-03	1.29	down
unknown	5.53E-03	1.29	down
unknown	4.30E-03	1.28	down
FXYD domain containing ion transport regulator 5b [Salmo salar]	1.26E-03	1.28	down
ZNF554 protein [Homo sapiens]	2.35E-03	1.28	down
Mitogenactivated protein kinase kinase kinase 8 [Salmo salar]	6.26E-03	1.28	down
unknown	5.91E-03	1.28	down
unknown	9.43E-03	1.27	down
FYNbinding protein [Salmo salar]	2.46E-03	1.27	down

TABLE S1: (*continued*...)

Control diet (all days) vs Diet A day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	6.21E-04	1.27	down
Mus musculus enhancer of polycomb homolog 1 (Drosophila) (Epc1), transcript variant 2, mRNA	3.88E-03	1.26	down
Lithognathus mormyrus clone lmos2p02d03 mRNA sequence	5.04E-03	1.26	down
tripartite motifcontaining 8 [Xenopus (Silurana) tropicalis]	4.94E-03	1.26	down
unknown	6.95E-03	1.26	down
unknown	3.71E-04	1.25	down
unknown	4.60E-03	1.25	down
unknown	1.04E-03	1.24	down
member 2A1 [Homo sapiens]	4.32E-03	1.24	down
DAP12 [Ictalurus punctatus]	8.50E-04	1.24	down
unknown	4.07E-03	1.23	down
Diplodus sargus igfII mRNA for preproinsulingrowth factor II, complete cds	8.04E-03	1.23	down
unknown	6.61E-04	1.22	down
unknown	9.57E-03	1.21	down
unknown	2.05E-03	1.21	down
unknown	2.14E-03	1.20	down
tyrosine phosphatase type IVA 2 [Salmo salar]	2.79E-03	1.20	down
unknown	3.21E-03	1.19	down
unknown	3.56E-03	1.17	down
unknown	4.09E-03	1.16	down

TABLE S2: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **DIET A 2 DOF AND DIET A 7 DOF.** The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Diet A day 2 vs Diet A day 7			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.48E-03	7.76	up
unknown	2.50E-03	2.50	up
AAD32909.1 [Dictyostelium discoideum]	8.97E-03	2.28	up
viral Atype inclusion protein [Trichomonas vaginalis G3]	1.72E-03	1.79	up
zinc finger, DHHC domain containing 7, isoform CRA_a [Rattus norvegicus]	9.22E-03	1.78	up
unknown	8.16E-03	1.75	up
unknown	8.19E-04	1.68	up
Thunnus orientalis LPL mRNA for lipoprotein lipase, complete cds	8.42E-03	1.67	up
unknown	7.11E-03	1.63	up
unknown	1.61E-03	1.61	up
unknown	9.19E-03	1.59	up
unknown	3.47E-03	1.58	up
unknown	3.74E-03	1.57	up
unknown	8.88E-03	1.57	up
unknown	4.22E-03	1.56	up
1]	4.63E-03	1.56	up
mast cell preproprotein [Homo sapiens]	5.17E-03	1.55	up
unknown	3.00E-03	1.53	up
unknown	6.08E-03	1.52	up
adrenomedullin1 [Takifugu rubripes]	5.98E-03	1.52	up
unknown	3.44E-03	1.52	up
unknown	2.71E-03	1.51	up
unknown	6.02E-03	1.50	up
unknown	6.70E-03	1.48	up
unknown	2.01E-03	1.43	up
Homo sapiens chromosome 15 clone RP11123C21 map 15q21.3, complete sequence	8.64E-03	1.42	up
unknown	2.74E-03	1.42	up
Sparus aurata mRNA for Tcell receptor beta chain (tcrb gene), clone 3	8.36E-03	1.41	up
Lithognathus mormyrus clone lithmor242 mRNA sequence	6.95E-03	1.39	up
unknown	9.84E-03	1.39	up
unknown	9.32E-03	1.38	up
unknown	4.47E-04	1.38	up
unknown	5.72E-03	1.38	up
inositol polyphosphate4phosphatase, type II [Mus musculus]	8.79E-03	1.38	up
unknown	4.10E-03	1.37	up
Ctype lectin domain family 4 member E [Esox lucius]	6.16E-03	1.37	up
CD82 antigen [Salmo salar]	7.93E-03	1.36	up
unknown	4.55E-03	1.34	up
unknown	3.29E-03	1.32	up
unknown	9.18E-03	1.32	up
Cold shock domaincontaining protein E1 [Salmo salar]	2.28E-03	1.32	up
unknown	6.49E-03	1.32	up
unknown	2.89E-03	1.31	up
unknown	8.13E-03	1.31	up
Lithognathus mormyrus clone lithmor89 mRNA sequence	7.03E-03	1.31	up
unknown	9.09E-04	1.31	up
unknown	7.29E-03	1.29	up

TABLE S2: (*continued*...)

Diet A day 2 vs Diet A day 7			
Description	**p-value**	**FCA**	**Regulation**
unknown	3.46E-04	1.28	up
3Nacetylglucosaminyltransferase 5 [Danio rerio]	8.42E-03	1.26	up
unknown	9.95E-03	1.26	up
unknown	8.30E-03	1.26	up
unknown	9.31E-03	1.26	up
unknown	9.09E-03	1.26	up
unknown	8.62E-03	1.25	up
unknown	5.23E-03	1.22	up
unknown	7.56E-03	1.21	up
unknown	3.05E-03	1.21	up
unknown	9.30E-03	1.21	up
PREDICTED: id:ibd5057 [Danio rerio]	9.21E-03	1.19	up
unknown	3.45E-03	1.18	up
unknown	7.11E-03	1.17	up
type II antifreeze protein [Lates calcarifer]	4.88E-03	12.44	down
unknown	9.73E-03	5.20	down
LFABP	3.58E-04	3.18	down
transmembrane 7 superfamily member 2 [Bos taurus]	7.46E-03	3.16	down
S100A1 [Salmo salar]	5.24E-03	3.03	down
unknown	6.44E-03	2.64	down
kinesin family member 23 [Bos taurus]	1.97E-03	2.48	down
unknown	7.26E-03	2.46	down
cytosolic [Salmo salar]	9.77E-03	2.43	down
unknown	7.99E-03	2.39	down
Lithognathus mormyrus clone lmos9p01c01 mRNA sequence	7.55E-03	2.32	down
unknown	8.56E-03	2.31	down
centromere protein P [Danio rerio]	7.20E-03	2.12	down
Betaureidopropionase [Salmo salar]	5.94E-03	2.01	down
unknown	2.69E-03	1.86	down
IQ motif containing GTPase activating protein 3 [Homo sapiens]	9.29E-03	1.78	down
unknown	2.25E-03	1.76	down
Nephtys incisa 28S ribosomal RNA gene, partial sequence	5.92E-03	1.76	down
unknown	3.04E-03	1.73	down
unknown	1.09E-04	1.72	down
cell growth regulator with EFhand domain 1 [Bos taurus]	4.92E-03	1.69	down
Cyclindependent kinase inhibitor 3 [Salmo salar]	4.37E-03	1.69	down
unknown	1.28E-03	1.69	down
3hydroxyisobutyrylCoA hydrolase, mitochondrial precursor [Salmo salar]	6.67E-03	1.68	down
unknown	9.31E-03	1.66	down
Protein nightcap	8.76E-03	1.66	down
unknown	1.68E-03	1.65	down
cyclin B3 [Oreochromis niloticus]	2.44E-03	1.58	down
unknown	5.39E-03	1.57	down
unknown	8.61E-04	1.57	down
unknown	4.07E-03	1.56	down
UPF0420 protein C16orf58 homolog	4.49E-03	1.56	down
Ssu72 RNA polymerase II CTD phosphataselike [Salmo salar]	8.41E-03	1.55	down
unknown	6.51E-03	1.55	down

TABLE S2: (*continued*...)

Diet A day 2 vs Diet A day 7			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.32E-03	1.55	down
RecName: Full=Probable 2oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial; AltName: Full=Dehydrogenase E1 and transketolase domaincontaining protein 1; Flags: Precursor	1.81E-03	1.54	down
unknown	6.16E-03	1.53	down
Lithognathus mormyrus clone lmos7p03b05 mRNA sequence	7.67E-03	1.50	down
unknown	6.04E-03	1.50	down
unknown	6.42E-03	1.48	down
cathepsin L [Lates calcarifer]	7.55E-04	1.46	down
unknown	6.95E-03	1.44	down
unknown	3.38E-05	1.44	down
unknown	5.50E-03	1.43	down
kinesin family member 18A [Xenopus (Silurana) tropicalis]	5.08E-03	1.43	down
unknown	9.47E-03	1.41	down
Dermal papilladerived protein 6 homolog [Salmo salar]	9.15E-03	1.41	down
unknown	5.49E-03	1.41	down
unknown	8.57E-03	1.40	down
unknown	3.48E-03	1.39	down
FAM36A [Oncorhynchus mykiss]	7.52E-03	1.39	down
unknown	2.58E-03	1.38	down
arylesterase 2 [Salmo salar]	6.57E-03	1.37	down
unknown	8.14E-03	1.37	down
Mediator of RNA polymerase II transcription subunit 20 [Salmo salar]	2.45E-03	1.36	down
unknown	4.65E-03	1.36	down
alpha polypeptide [Danio rerio]	2.19E-03	1.36	down
delta9desaturase 1 [Takifugu rubripes]	9.25E-03	1.36	down
unknown	7.86E-03	1.35	down
galectin 8 [Sparus aurata]	3.14E-03	1.35	down
unknown	8.10E-03	1.35	down
unknown	1.05E-03	1.34	down
unknown	1.90E-03	1.34	down
methyltransferase Mb3374 [Salmo salar]	4.14E-03	1.33	down
unknown	2.53E-03	1.31	down
component of oligomeric golgi complex 7 [Danio rerio]	9.09E-03	1.31	down
Lithognathus mormyrus clone lmos9p04f08 mRNA sequence	6.88E-03	1.30	down
selenoprotein W2a [Oreochromis mossambicus]	7.41E-04	1.29	down
unknown	8.18E-03	1.29	down
epidermal growth factor receptor pathway substrate 8like protein 1 isoform a [Homo sapiens]	6.72E-03	1.28	down
ATP synthasecoupling factor 6, mitochondrial precursor [Esox lucius]	5.81E-04	1.27	down
member 9 [Xenopus laevis]	7.23E-03	1.27	down
ryanodine receptor domain and SOCS box containing 1 [Danio rerio]	7.20E-03	1.27	down
unknown	7.04E-03	1.27	down
Antizyme inhibitor 1 [Salmo salar]	6.95E-03	1.26	down
unknown	3.77E-03	1.25	down
ezrin like [Danio rerio]	1.38E-03	1.23	down
Transmembrane protein 103 [Danio rerio]	4.29E-03	1.23	down
unknown	9.99E-03	1.22	down
C6orf64 homolog [Esox lucius]	2.12E-03	1.22	down
unknown	7.46E-03	1.21	down
unknown	7.95E-03	1.21	down

TABLE S2: (*continued*...)

Diet A day 2 vs Diet A day 7			
Description	**p-value**	**FCA**	**Regulation**
PREDICTED: hypothetical protein [Taeniopygia guttata]	7.88E-03	1.19	down
unknown	7.26E-03	1.18	down
Salmo salar clone HM5_1489 ubiquitinconjugating enzyme E2 variant 1 (ube2v1) mRNA, partial cds	9.76E-03	1.18	down
Plateletactivating factor acetylhydrolase IB subunit gamma [Salmo salar]	9.94E-03	1.18	down
unknown	6.84E-03	1.17	down
unknown	4.01E-03	1.17	down
calcium binding protein 39 [Danio rerio]	6.00E-03	1.17	down
unknown	6.37E-03	1.16	down
unknown	6.74E-03	1.16	down
Lithognathus mormyrus clone lmos8p02h02 mRNA sequence	1.13E-03	1.15	down
Ironresponsive elementbinding protein 2 [Salmo salar]	4.55E-03	1.14	down
similar to cullin 4A (predicted), isoform CRA_b [Rattus norvegicus]	4.85E-03	1.11	down

TABLE S3: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **DIET A 7DOF AND DIET A 14 DOF**. The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Diet A day 7 vs Diet A day 14			
Description	**p-value**	**FCA**	**Regulation**
Vibrio vulnificus YJ016 DNA, chromosome II, complete sequence	1.43E-04	8.95	up
unknown	3.16E-03	3.27	up
unknown	4.89E-03	2.18	up
unknown	3.90E-03	1.93	up
tryptophanyltRNA synthetase [Danio rerio]	6.65E-03	1.85	up
unknown	7.62E-03	1.79	up
cyclin B1 [Larimichthys crocea]	5.35E-03	1.78	up
unknown	3.99E-03	1.67	up
TraB domaincontaining protein [Salmo salar]	8.24E-03	1.67	up
unknown	9.14E-03	1.66	up
Retinol dehydrogenase 3 [Oncorhynchus mykiss]	6.73E-03	1.64	up
unknown	8.56E-03	1.64	up
unknown	6.42E-03	1.60	up
interleukin enhancer binding factor 2 [Epinephelus tauvina]	4.77E-03	1.60	up
unknown	8.83E-03	1.59	up
novel protein similar to vertebrate asparaginyltRNA synthetase (NARS) [Danio rerio]	5.75E-03	1.57	up
unknown	3.21E-03	1.55	up
cytochrome c oxidase subunit III [Aeoliscus strigatus]	9.80E-03	1.55	up
member 20 [Danio rerio]	8.46E-03	1.55	up
unknown	5.60E-03	1.54	up
Borealin [Salmo salar]	3.07E-03	1.52	up
Granulocyte colonystimulating factor receptor precursor [Salmo salar]	1.16E-03	1.51	up
centromere protein P [Danio rerio]	6.78E-03	1.50	up
PREDICTED: similar to transient receptor potential cation channel, subfamily M, member 4 [Danio rerio]	5.38E-03	1.49	up
shugoshin1 [Oryzias latipes]	8.14E-03	1.48	up
cell growth regulator with EFhand domain 1 [Bos taurus]	8.99E-04	1.48	up
lecithincholesterol acyltransferase [Xenopus (Silurana) tropicalis]	6.74E-03	1.47	up
unknown	7.58E-03	1.47	up
member 3 (IGSF3) [Danio rerio]	8.33E-03	1.45	up
unknown	9.01E-05	1.45	up
Mannose1phosphate guanyltransferase alphaA [Salmo salar]	2.98E-03	1.45	up
unknown	5.13E-03	1.45	up
mitochondrial 1 [Homo sapiens]	9.36E-03	1.44	up
unknown	9.96E-03	1.44	up
lysophosphatidic acid receptor 2 [Xenopus (Silurana) tropicalis]	1.65E-03	1.43	up
Bcell receptorassociated protein 31 [Salmo salar]	4.70E-03	1.43	up
unknown	4.11E-03	1.43	up
Xbox binding protein 1 [Takifugu rubripes]	8.06E-03	1.42	up
C8orf55 homolog precursor [Salmo salar]	2.69E-03	1.42	up
Translocationassociated membrane protein 2 [Salmo salar]	4.07E-04	1.41	up
Nephtys incisa 28S ribosomal RNA gene, partial sequence	2.43E-03	1.41	up
unknown	4.61E-03	1.41	up
unknown	3.02E-03	1.41	up
Translation initiation factor eIF2B subunit epsilon [Salmo salar]	7.55E-03	1.39	up
Protein zwilch homolog	2.60E-03	1.39	up
Replication protein A 70 kDa DNAbinding subunit [Salmo salar]	2.93E-03	1.38	up
PRP4 premRNA processing factor 4 homolog B [Danio rerio]	1.16E-03	1.38	up

TABLE S3: (*continued*...)

Diet A day 7 vs Diet A day 14			
Description	**p-value**	**FCA**	**Regulation**
unknown	9.18E-04	1.38	up
aldehyde dehydrogenase [Danio rerio]	4.72E-03	1.37	up
unknown	1.37E-03	1.37	up
Cellular apoptosis susceptibility protein	9.05E-03	1.37	up
unknown	4.43E-03	1.36	up
unknown	4.48E-03	1.36	up
unknown	2.14E-03	1.35	up
RGD1311345 protein [Rattus norvegicus]	4.25E-03	1.35	up
unknown	9.03E-03	1.35	up
member 3 [Xenopus (Silurana) tropicalis]	2.01E-03	1.34	up
unknown	2.61E-03	1.34	up
Dynactin subunit 5 [Salmo salar]	9.73E-03	1.34	up
CN130 protein [Salmo salar]	4.85E-04	1.32	up
Oleoyl[acylcarrierprotein] hydrolase	5.77E-03	1.32	up
regulator of cytokinesis 1 [Salmo salar]	5.17E-03	1.32	up
MKL/myocardinlike 2, isoform CRA_b [Homo sapiens]	8.84E-03	1.31	up
unknown	9.08E-03	1.31	up
transducin (beta)like 2 [Danio rerio]	7.69E-03	1.30	up
novel protein similar to H.sapiens C13orf31, chromosome 13 open reading frame 31 (C13orf31) [Danio rerio]	4.23E-03	1.30	up
novel protein similar to human and mouse cytochrome b561 domain containing 2 (CYB561D2) [Danio rerio]	7.24E-03	1.30	up
unknown	4.55E-04	1.30	up
Hnrnpu protein [Danio rerio]	1.58E-03	1.30	up
antizyme inhibitor [Danio rerio]	6.22E-03	1.29	up
soluble [Danio rerio]	7.90E-03	1.29	up
WW domain binding protein 11 [Xenopus (Silurana) tropicalis]	8.73E-03	1.29	up
ADPribosylation factorlike protein 4D [Salmo salar]	4.40E-03	1.28	up
Galactosidase, beta 1like [Danio rerio]	7.44E-03	1.27	up
unknown	8.87E-03	1.27	up
Chain A, Crystal Structure Of The GdpBound Conformation Of A G AlphaI1 Mutant With Enhanced Gtpase Activity Chain A, Crystal Structure Of The AmfBound Conformation Of A G AlphaI1 Mutant With Enhanced Gtpase Activity	9.55E-03	1.27	up
candidate 1 [Bos taurus]	8.67E-03	1.27	up
unknown	4.77E-04	1.27	up
unknown	2.90E-04	1.26	up
epidermal growth factorcontaining fibulinlike extracellular matrix protein 2, isoform CRA_c [Mus musculus]	4.05E-03	1.26	up
mesoderm specific transcript [Takifugu rubripes]	6.24E-03	1.26	up
unknown	8.52E-03	1.26	up
kinetochore associated 1 [Xenopus tropicalis]	1.26E-03	1.25	up
NogoB receptor [Salmo salar]	3.91E-03	1.25	up
Vesicleassociated membrane protein 3 [Salmo salar]	8.83E-03	1.25	up
2amino3ketobutyrate coenzyme A ligase, mitochondrial precursor [Salmo salar]	8.77E-03	1.24	up
Mitochondrial folate transporter/carrier [Salmo salar]	4.33E-03	1.24	up
Ornithodoros coriaceus clone OC72 hypothetical protein mRNA, complete cds	2.17E-03	1.23	up
unknown	4.97E-03	1.23	up
E2F transcription factor 3, isoform CRA_a [Homo sapiens]	9.89E-03	1.23	up
leucine rich repeat containing 57 [Danio rerio]	8.62E-03	1.23	up
mannosePdolichol utilization defect 1b [Danio rerio]	5.51E-03	1.23	up
mitochondrial ribosomal protein L19 [Danio rerio]	7.33E-03	1.22	up
glutathione Stransferase [Pleuronectes platessa]	2.29E-03	1.21	up
unknown	8.74E-03	1.21	up

TABLE S3: (*continued*...)

Diet A day 7 vs Diet A day 14			
Description	**p-value**	**FCA**	**Regulation**
novel protein similar to vertebrate fusebinding proteininteracting repressor (SIAHBP1) [Danio rerio]	6.40E-03	1.21	up
unknown	6.06E-03	1.19	up
unknown	7.95E-03	1.19	up
unknown	9.97E-03	1.19	up
unknown	3.79E-03	1.18	up
Nacylaminoacylpeptide hydrolase, isoform CRA_d [Homo sapiens]	9.48E-04	1.18	up
ubiquitinconjugating enzyme E2K [Salmo salar]	7.05E-03	1.18	up
novel protein similar to vertebrate translocated promoter region (to activated MET oncogene) (TPR) [Danio rerio]	5.54E-03	1.17	up
Salmo salar clone ssalrgf537103 Dihydropyrimidinaserelated protein 3 putative mRNA, partial cds	7.56E-03	1.16	up
3hydroxybutyrate dehydrogenase type 2 [Salmo salar]	8.24E-03	1.15	up
unknown	5.02E-03	1.15	up
TruB pseudouridine (psi) synthase homolog 1 variant [Homo sapiens]	7.98E-03	1.14	up
WD repeat domain 68 [Danio rerio]	2.88E-03	1.13	up
unknown	5.70E-03	1.12	up
unknown	1.54E-03	1.10	up
Extracellular matrix protein 1 precursor [Salmo salar]	2.74E-03	1.10	up
StARrelated lipid transfer protein 7 [Salmo salar]	2.59E-03	1.10	up
goosefish kalliklectin [Lophiomus setigerus]	7.53E-04	2.37	down
unknown	3.04E-03	2.36	down
AAD32909.1 [Dictyostelium discoideum]	5.08E-03	2.31	down
unknown	5.74E-03	2.24	down
unknown	5.47E-03	2.19	down
unknown	1.23E-04	1.97	down
unknown	3.45E-03	1.94	down
unknown	1.52E-03	1.92	down
unknown	1.89E-03	1.91	down
unknown	8.73E-03	1.74	down
unknown	3.86E-03	1.72	down
Samd9l protein [Xenopus tropicalis]	6.55E-03	1.71	down
unknown	4.31E-03	1.67	down
unknown	5.25E-03	1.64	down
absent in melanoma 1 [Mus musculus]	5.09E-03	1.63	down
GTPase, IMAP family member 7 [Oncorhynchus mykiss]	8.81E-03	1.61	down
ADPribosylation factorlike 16 [Danio rerio]	3.03E-04	1.58	down
unknown	8.83E-03	1.58	down
unknown	2.02E-03	1.57	down
Lithognathus mormyrus clone lithmor89 mRNA sequence	1.66E-03	1.54	down
unknown	8.79E-03	1.53	down
unknown	4.64E-03	1.52	down
unknown	1.68E-03	1.50	down
Tumor necrosis factor receptor superfamily member 5 precursor [Oncorhynchus mykiss]	2.54E-03	1.50	down
truncated type I keratin KA21 [Bos taurus]	9.82E-03	1.50	down
unknown	3.97E-03	1.49	down
unknown	1.75E-03	1.49	down
unknown	8.27E-04	1.48	down
Lithognathus mormyrus clone lmos2p05g04 mRNA sequence	6.62E-03	1.48	down
unknown	5.99E-03	1.48	down
DNA segment, Chr 7, Wayne State University 128, expressed [Mus musculus]	8.50E-03	1.47	down

TABLE S3: (*continued...*)

Diet A day 7 vs Diet A day 14			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.81E-04	1.46	down
unknown	4.31E-03	1.46	down
unknown	3.50E-03	1.44	down
unknown	6.49E-03	1.43	down
unknown	7.20E-03	1.43	down
unknown	5.80E-03	1.42	down
unknown	8.39E-03	1.42	down
unknown	2.14E-03	1.42	down
M2904]	1.52E-03	1.40	down
unknown	1.23E-03	1.39	down
RecName: Full=Ethanolamine kinase 1; Short=EKI 1	6.94E-03	1.38	down
unknown	6.72E-03	1.37	down
unknown	2.21E-03	1.37	down
unknown	5.77E-03	1.37	down
unknown	4.99E-03	1.36	down
unknown	9.58E-03	1.36	down
FLJ16636 protein, isoform CRA_b [Homo sapiens]	2.01E-03	1.35	down
unknown	3.25E-04	1.35	down
unknown	5.31E-03	1.34	down
unknown	5.30E-03	1.34	down
unknown	6.35E-03	1.34	down
unknown	3.49E-03	1.33	down
unknown	2.54E-03	1.32	down
unknown	4.68E-03	1.32	down
unknown	8.25E-03	1.32	down
unknown	9.35E-03	1.32	down
unknown	6.22E-03	1.32	down
unknown	6.98E-03	1.31	down
unknown	9.64E-03	1.31	down
PREDICTED: im:6912447 [Danio rerio]	4.16E-03	1.30	down
unknown	4.31E-03	1.30	down
unknown	8.66E-03	1.30	down
unknown	5.06E-04	1.30	down
unknown	3.98E-05	1.29	down
unknown	1.88E-03	1.29	down
unknown	4.82E-03	1.28	down
NADP+arginine ADPribosyltransferase 1 [Salmo salar]	1.39E-03	1.27	down
adrenomedullin1 [Takifugu rubripes]	9.01E-03	1.27	down
Lens epitheliumderived growth factor [Salmo salar]	4.40E-03	1.27	down
unknown	3.70E-03	1.27	down
Fugu rubripes neurofibromatosis type 1 (NF1), Akinase anchor protein (AKAP84), BAW protein (BAW), and WSB1 protein (WSB1) genes, complete cds	2.68E-03	1.26	down
unknown	7.42E-03	1.25	down
sallike 2 [Xenopus (Silurana) tropicalis]	3.15E-03	1.25	down
PEST proteolytic signalcontaining nuclear protein [Salmo salar]	1.17E-03	1.25	down
unknown	2.26E-03	1.25	down
similar to FLJ46154 protein (predicted) [Rattus norvegicus]	8.00E-03	1.24	down
unknown	3.10E-03	1.24	down
torsin A interacting protein 2 [Rattus norvegicus]	4.32E-04	1.24	down

TABLE S3: (*continued*...)

Diet A day 7 vs Diet A day 14			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.20E-03	1.24	down
unknown	2.16E-03	1.23	down
required for meiotic nuclear division 1 homolog [Danio rerio]	9.22E-03	1.23	down
unknown	3.96E-03	1.23	down
mitogen activated protein kinase p38a [Salmo salar]	1.41E-03	1.22	down
unknown	9.25E-03	1.20	down
Homo sapiens NFKB inhibitor interacting Raslike 2 (NKIRAS2), transcript variant 5, mRNA	3.52E-03	1.20	down
unknown	3.84E-03	1.20	down
FAM83H protein [Homo sapiens]	6.71E-03	1.20	down
PREDICTED: centrosomal protein 57kDa [Taeniopygia guttata]	7.13E-03	1.20	down
unknown	3.39E-03	1.20	down
unknown	3.11E-03	1.20	down
unknown	3.14E-03	1.19	down
unknown	4.41E-03	1.19	down
polypeptide 1 [Danio rerio]	7.02E-03	1.19	down
unknown	9.40E-03	1.18	down
unknown	4.45E-03	1.18	down
member 1b [Danio rerio]	5.14E-03	1.17	down
unknown	6.28E-03	1.17	down
FXYD domain containing ion transport regulator 8 [Salmo salar]	3.07E-03	1.17	down
unknown	8.68E-03	1.16	down
unknown	4.30E-03	1.15	down
unknown	9.48E-03	1.14	down
unknown	2.48E-03	1.14	down
unknown	7.96E-03	1.13	down
unknown	8.89E-03	1.13	down
YIPF4 [Esox lucius]	8.90E-03	1.11	down
unknown	9.53E-03	1.09	down

TABLE S4: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 **BETWEEN DIET A 14 DOF AND DIET A 28 DOF.** The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.55E-03	3.22	up
unknown	8.44E-03	2.79	up
AAD32909.1 [Dictyostelium discoideum]	2.07E-03	2.60	up
unknown	5.83E-03	2.55	up
unknown	8.43E-03	2.43	up
ceruloplasmin, isoform CRA_b [Mus musculus]	2.15E-03	2.31	up
unknown	2.70E-03	2.27	up
unknown	9.31E-03	2.17	up
Acetylcoenzyme A synthetase 2like, mitochondrial precursor [Salmo salar]	5.91E-03	2.13	up
novel protein similar to elongation factor RNA polymerase II (ell) [Danio rerio]	9.59E-03	2.04	up
unknown	9.41E-03	1.99	up
unknown	1.69E-03	1.91	up
unknown	5.44E-03	1.84	up
unknown	7.25E-03	1.83	up
unknown	8.35E-03	1.83	up
unknown	4.64E-03	1.77	up
unknown	2.43E-03	1.77	up
unknown	7.02E-03	1.75	up
transposase [Rana pipiens]	5.15E-03	1.75	up
Abnormal spindlelike microcephalyassociated protein homolog	6.14E-03	1.74	up
unknown	7.61E-03	1.72	up
unknown	8.57E-03	1.71	up
unknown	1.72E-03	1.68	up
unknown	3.40E-03	1.68	up
PREDICTED: similar to zinc finger protein [Hydra magnipapillata]	1.81E-03	1.64	up
unknown	3.57E-03	1.64	up
unknown	6.04E-03	1.63	up
unknown	2.67E-03	1.62	up
unknown	6.40E-03	1.62	up
unknown	6.96E-03	1.62	up
Lithognathus mormyrus clone lithmor89 mRNA sequence	5.08E-03	1.61	up
CI119 protein [Salmo salar]	5.41E-03	1.61	up
Sparus aurata thyroid hormone receptorbeta mRNA, complete cds	9.44E-03	1.61	up
unknown	5.69E-05	1.58	up
gammainterferoninduciblelysosomal thiol reductase [Pseudosciaena crocea]	5.67E-04	1.58	up
unknown	6.80E-03	1.56	up
Coiledcoil domaincontaining protein 127 [Salmo salar]	2.45E-03	1.56	up
ribosomal protein L18a [Pagrus major]	4.30E-03	1.54	up
neurolina [Takifugu rubripes]	3.73E-03	1.54	up
unknown	7.42E-04	1.54	up
unknown	8.56E-03	1.52	up
unknown	4.43E-03	1.50	up
unknown	3.30E-03	1.50	up
unknown	8.32E-03	1.49	up
unknown	5.90E-03	1.48	up
unknown	2.10E-03	1.47	up
Programmed cell death protein 2 [Salmo salar]	5.82E-03	1.46	up

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	9.06E-03	1.46	up
unknown	8.91E-03	1.46	up
unknown	7.05E-03	1.45	up
unknown	3.97E-04	1.44	up
unknown	6.74E-03	1.44	up
transposase [Oryzias latipes]	3.74E-03	1.44	up
unknown	7.99E-03	1.43	up
Lithognathus mormyrus clone lmos2p05g04 mRNA sequence	3.14E-03	1.42	up
unknown	4.53E-03	1.41	up
absent in melanoma 1 [Mus musculus]	2.84E-03	1.41	up
unknown	3.81E-04	1.40	up
unknown	6.08E-03	1.40	up
Oryzias latipes hox gene cluster, complete cds, contains hoxDb	1.00E-03	1.40	up
unknown	8.49E-03	1.39	up
unknown	4.91E-03	1.39	up
ADPribosylation factorlike 16 [Danio rerio]	8.28E-03	1.38	up
unknown	9.02E-04	1.37	up
unknown	6.32E-05	1.36	up
unknown	8.41E-03	1.36	up
unknown	1.49E-03	1.34	up
unknown	7.28E-03	1.34	up
unknown	5.29E-03	1.33	up
unknown	9.02E-03	1.33	up
unknown	1.89E-04	1.33	up
unknown	2.81E-04	1.32	up
unknown	9.84E-03	1.32	up
unknown	7.91E-03	1.32	up
Vesicleassociated membrane proteinassociated protein B/C [Salmo salar]	6.12E-03	1.32	up
ORF2encoded protein [Danio rerio]	2.09E-03	1.32	up
ataxin 2 [Mus musculus]	6.59E-03	1.32	up
unknown	8.56E-03	1.32	up
unknown	2.31E-03	1.32	up
unknown	6.12E-03	1.32	up
unknown	7.78E-05	1.31	up
Sodium and chloridedependent taurine transporter [Salmo salar]	6.41E-03	1.31	up
unknown	2.27E-03	1.30	up
unknown	4.22E-03	1.30	up
proline racemaselike [Bos taurus]	6.28E-04	1.30	up
unknown	3.18E-03	1.30	up
unknown	5.36E-03	1.29	up
unknown	1.98E-03	1.28	up
unknown	3.72E-03	1.28	up
unknown	8.79E-03	1.27	up
unknown	6.38E-03	1.27	up
unknown	4.90E-03	1.26	up
unknown	3.03E-03	1.26	up
unknown	9.02E-03	1.26	up
BolAlike protein 2 [Oncorhynchus mykiss]	8.91E-04	1.26	up

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.58E-03	1.25	up
clone A24K23, A4118 of Tetraodon nigroviridis	8.92E-03	1.25	up
unknown	7.47E-05	1.25	up
unknown	4.86E-03	1.25	up
tetratricopeptide repeat, ankyrin repeat and coiledcoil containing 1 isoform 1 [Homo sapiens]	8.77E-03	1.25	up
Angelman syndrome 2 [Danio rerio]	5.67E-03	1.24	up
unknown	8.12E-03	1.23	up
unknown	3.46E-03	1.23	up
unknown	5.99E-03	1.23	up
unknown	4.71E-03	1.23	up
unknown	8.00E-03	1.22	up
unknown	6.53E-03	1.22	up
unknown	6.48E-03	1.21	up
unknown	7.60E-03	1.21	up
unknown	5.47E-03	1.21	up
WD repeat, sterile alpha motif and Ubox domain containing 1, isoform CRA_c [Homo sapiens]	3.45E-03	1.21	up
tyrosine phosphatase type IVA 2 [Salmo salar]	2.05E-03	1.21	up
PEST proteolytic signalcontaining nuclear protein [Salmo salar]	4.65E-03	1.20	up
Chain A, Ap2 Clathrin Adaptor AlphaAppendage In Complex With Eps15 Dpf Peptide	2.46E-05	1.20	up
unknown	4.62E-03	1.18	up
unknown	7.16E-03	1.18	up
unknown	9.60E-03	1.18	up
Salmo salar clone ssalrgf540086 Chloride intracellular channel protein 4 putative mRNA, complete cds	6.21E-03	1.18	up
unknown	8.75E-03	1.17	up
Lithognathus mormyrus clone lithmor1019 mRNA sequence	5.43E-03	1.17	up
Immediate early response gene 5 protein [Salmo salar]	3.03E-03	1.16	up
unknown	9.68E-03	1.13	up
unknown	5.69E-03	7.94	down
secretory calciumbinding phosphoprotein 5 [Takifugu rubripes]	6.45E-06	6.34	down
Vibrio vulnificus YJ016 DNA, chromosome II, complete sequence	3.43E-03	4.29	down
olfactomedin 4like protein precursor [Ictalurus punctatus]	2.67E-04	4.02	down
immunoglobulin light chain L2 [Oncorhynchus mykiss]	7.08E-03	3.35	down
unknown	5.44E-03	3.32	down
Pvalb3a [Danio rerio]	4.79E-04	3.22	down
unknown	1.48E-03	2.99	down
unknown	6.03E-03	2.84	down
olfactomedin 4 [Xenopus (Silurana) tropicalis]	3.81E-03	2.71	down
unknown	1.07E-03	2.69	down
unknown	4.23E-03	2.44	down
aldehyde dehydrogenase 1 family, member L1 [Xenopus tropicalis]	2.92E-03	2.29	down
cathepsin [Paralabidochromis chilotes]	1.34E-04	2.21	down
unknown	7.56E-03	2.21	down
C1qlike1 [Siniperca chuatsi]	2.50E-03	2.14	down
lysyl oxidaselike 2b [Danio rerio]	8.71E-04	2.12	down
small inducible cytokine SCYA101 [Paralabidochromis chilotes]	2.19E-04	2.09	down
Cellular retinoic acidbinding protein [Salmo salar]	9.54E-05	2.07	down
cyclin B1 [Larimichthys crocea]	2.89E-03	2.07	down
unknown	5.05E-04	2.03	down

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	2.96E-03	1.97	down
Epinephelus coioides EPO gene, 3' flanking region	6.71E-03	1.97	down
unknown	6.84E-03	1.94	down
Lithognathus mormyrus clone lmos2p02a02 mRNA sequence	9.95E-03	1.93	down
lysosomal alphamannosidase [Bos taurus]	1.91E-03	1.93	down
unknown	3.58E-03	1.90	down
unknown	1.11E-03	1.87	down
Leucinerich repeatcontaining protein 33 precursor [Salmo salar]	6.63E-04	1.86	down
nucleolar and coiledbody phosphoprotein 1 [Xenopus (Silurana) tropicalis]	3.95E-03	1.85	down
cytochrome c1 [Danio rerio]	9.93E-04	1.84	down
unknown	6.14E-03	1.80	down
monooxygenase 1 [Danio rerio]	8.73E-03	1.80	down
RNA polymerase 12 [Rattus norvegicus]	1.44E-03	1.80	down
CD81 antigen [Salmo salar]	1.82E-03	1.79	down
Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial precursor [Salmo salar]	3.68E-03	1.79	down
Tetraspanin14 [Salmo salar]	1.23E-03	1.79	down
Fibulin1 precursor [Salmo salar]	8.12E-04	1.76	down
family with sequence similarity 54, member A, isoform CRA_b [Homo sapiens]	1.03E-03	1.76	down
Nucleolar protein 5 [Salmo salar]	3.23E-04	1.76	down
Protein zwilch homolog	2.85E-03	1.75	down
ubiquitinactivating enzyme E1C, isoform CRA_c [Mus musculus]	1.04E-03	1.75	down
unknown	6.44E-03	1.75	down
cathepsin D [Sparus aurata]	2.88E-03	1.75	down
ornithine decarboxylase antizyme AZS [Salmo salar]	6.28E-04	1.74	down
denticleless homolog [Danio rerio]	4.79E-03	1.73	down
cholesteryl ester hydrolase [Salmo salar]	4.13E-03	1.73	down
Complement component C7 [Salmo salar]	4.51E-03	1.73	down
U3 small nucleolar RNAassociated protein 15 homolog [Salmo salar]	2.96E-03	1.72	down
talin 1 [Danio rerio]	9.92E-03	1.71	down
unknown	8.59E-03	1.70	down
CDC28 protein kinase 1 [Oreochromis mossambicus]	9.85E-03	1.69	down
GrpE protein homolog 1, mitochondrial precursor [Salmo salar]	1.56E-03	1.68	down
CD9 protein [Oncorhynchus mykiss]	8.03E-03	1.68	down
Cfactor [Salmo salar]	4.28E-03	1.67	down
unknown	7.22E-03	1.67	down
rCG25895 [Rattus norvegicus]	2.28E-03	1.67	down
unknown	2.75E-04	1.66	down
smooth muscle cellspecific protein SM22 alpha [Epinephelus coioides]	1.03E-03	1.66	down
prohibitin 2 [Danio rerio]	4.19E-03	1.65	down
Lipocalin precursor [Esox lucius]	8.41E-03	1.65	down
beta 2C [Xenopus (Silurana) tropicalis]	8.47E-03	1.64	down
NADH dehydrogenase 1 alpha subcomplex subunit 4 [Esox lucius]	1.51E-03	1.64	down
SNFrelated matrixassociated actindependent regulator of chromatin subfamily B member 1	3.56E-03	1.64	down
centromere protein P [Danio rerio]	6.80E-03	1.64	down
unknown	1.51E-03	1.63	down
carbamoylphosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase [Danio rerio]	9.66E-03	1.63	down
Lithognathus mormyrus clone lmos8p08d01 mRNA sequence	4.42E-03	1.63	down
unknown	1.03E-03	1.62	down

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
highmobility group 20B isoform 1 [Danio rerio]	3.69E-04	1.62	down
Ezrinradixinmoesinbinding phosphoprotein 50 [Salmo salar]	1.74E-04	1.61	down
general transcription factor II B [Oreochromis mossambicus]	4.02E-03	1.61	down
Sept2 protein [Danio rerio]	2.28E-04	1.61	down
unknown	2.40E-03	1.61	down
lysocardiolipin acyltransferase [Danio rerio]	8.10E-03	1.60	down
unknown	6.93E-03	1.60	down
unknown	2.92E-04	1.60	down
Transmembrane protein 120A [Salmo salar]	7.44E-03	1.60	down
Integrin beta2 precursor [Salmo salar]	8.05E-03	1.59	down
Pterin4alphacarbinolamine dehydratase [Salmo salar]	6.68E-03	1.59	down
AP2 complex subunit sigma1 [Salmo salar]	2.16E-04	1.59	down
heterogeneous nuclear ribonucleoprotein G [Astatotilapia burtoni]	1.98E-04	1.59	down
C1rsA [Cyprinus carpio]	8.23E-03	1.58	down
unknown	3.19E-03	1.58	down
histone acetyltransferase 1 [Danio rerio]	1.01E-03	1.58	down
Diphosphoinositol polyphosphate phosphohydrolase 1 [Oncorhynchus mykiss]	5.79E-03	1.57	down
Carbonic anhydrase 12 [Salmo salar]	3.29E-03	1.57	down
unknown	1.67E-04	1.57	down
member 3 [Xenopus (Silurana) tropicalis]	1.56E-03	1.57	down
fructosebisphosphate aldolase [Epinephelus coioides]	6.76E-03	1.56	down
MOB1, Mps One Binder kinase activatorlike 1B (yeast), isoform CRA_b [Mus musculus]	3.82E-03	1.55	down
unknown	4.41E-03	1.54	down
exosomal core protein CSL4 [Bos taurus]	7.90E-03	1.54	down
RNAbinding motif protein 42	9.00E-05	1.54	down
unknown	7.87E-03	1.53	down
Tetratricopeptide repeat protein 31 [Salmo salar]	8.80E-04	1.53	down
unknown	1.49E-03	1.52	down
unknown	5.78E-04	1.52	down
unknown	7.35E-03	1.52	down
nucleolin [Oncorhynchus mykiss]	4.42E-03	1.52	down
mitochondrial ribosomal protein L19 [Danio rerio]	2.67E-03	1.51	down
Translation initiation factor eIF2B subunit epsilon [Salmo salar]	4.30E-03	1.51	down
Trifunctional enzyme subunit alpha, mitochondrial precursor [Salmo salar]	4.39E-04	1.51	down
ferritin lower subunit [Oreochromis mossambicus]	4.13E-03	1.50	down
unknown	1.40E-03	1.50	down
Metaxin2 [Salmo salar]	7.67E-03	1.50	down
DEAH (AspGluAlaHis) box polypeptide 15 [Danio rerio]	9.44E-03	1.50	down
CMPsialic acid transporter [Takifugu rubripes]	4.62E-03	1.50	down
alpha1 globin [Sparus aurata]	9.78E-03	1.50	down
peroxisome proliferatoractivated receptor gamma, coactivatorrelated 1 [Homo sapiens]	4.47E-03	1.48	down
unknown	9.88E-03	1.48	down
Dynactin subunit 5 [Salmo salar]	7.14E-03	1.47	down
Glycogen phosphorylase, muscle form [Salmo salar]	8.95E-03	1.47	down
NADH dehydrogenase 1 alpha subcomplex subunit 2 [Salmo salar]	5.55E-03	1.47	down
unknown	9.69E-03	1.46	down
RIKEN cDNA 5230400G24, isoform CRA_c [Mus musculus]	7.85E-03	1.46	down
unknown	9.41E-03	1.46	down

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	9.27E-03	1.46	down
reductase SDR family member 1 [Salmo salar]	4.84E-04	1.46	down
Superkiller viralicidic activity 2like 2 [Salmo salar]	8.88E-03	1.45	down
unknown	7.16E-03	1.45	down
PsTX20A [Siniperca chuatsi]	2.89E-03	1.45	down
Fbox only protein 42 [Salmo salar]	3.61E-03	1.45	down
unknown	4.41E-03	1.45	down
mitochondrial [Danio rerio]	9.79E-03	1.44	down
Glutamate receptorassociated protein 1 [Salmo salar]	4.42E-03	1.43	down
unknown	9.93E-03	1.43	down
unknown	2.36E-03	1.42	down
alpha subunit [Danio rerio]	3.86E-03	1.42	down
Retinal dehydrogenase 2 [Salmo salar]	1.19E-03	1.42	down
ATP synthase H+ transporting F0 complex subunit c [Epinephelus coioides]	1.62E-03	1.42	down
methylthioadenosine phosphorylase [Danio rerio]	7.34E-03	1.41	down
hydroxyprostaglandin dehydrogenase 15(NAD) [Salmo salar]	1.19E-03	1.41	down
unknown	5.78E-04	1.41	down
AP1 complex subunit mu2 [Salmo salar]	4.87E-03	1.41	down
Crystal Structure Of Seabream Antiquitin And Elucidation Of Its Substrate Specificity Chain A, Chain B, Chain C, Chain D, Chain E, Chain F, Chain G, Chain H	9.60E-03	1.41	down
28S ribosomal protein S24, mitochondrial precursor [Salmo salar]	3.90E-03	1.40	down
coproporphyrinogen oxidase [Danio rerio]	3.26E-03	1.40	down
Vacuolar proton translocating ATPase 116 kDa subunit a isoform 3 [Salmo salar]	9.81E-03	1.40	down
unknown	6.27E-03	1.40	down
Lithognathus mormyrus clone lmos8p01h10 mRNA sequence	9.83E-03	1.40	down
TPA_inf: HN1like protein [Takifugu rubripes]	5.19E-03	1.40	down
mitogenactivated protein kinase kinase 2 [Danio rerio]	8.02E-04	1.40	down
Acadvl protein [Danio rerio]	8.35E-03	1.39	down
unknown	3.03E-03	1.39	down
LysyltRNA synthetase [Salmo salar]	4.24E-03	1.39	down
Clathrin light chain A [Salmo salar]	3.24E-03	1.39	down
Ras association domaincontaining protein 1 [Salmo salar]	2.58E-05	1.39	down
Centromere protein O [Xenopus tropicalis]	9.92E-03	1.39	down
Lithognathus mormyrus clone lithmor139 mRNA sequence	5.05E-03	1.39	down
unknown	4.79E-03	1.38	down
RecName: Full=UPF0582 protein C13orf37 homolog	8.42E-03	1.38	down
unknown	1.97E-04	1.38	down
Glutamate dehydrogenase 1, mitochondrial precursor [Salmo salar]	3.84E-03	1.38	down
unknown	9.09E-03	1.37	down
unknown	9.38E-03	1.37	down
unknown	9.11E-03	1.37	down
Mitogenactivated proteinbinding proteininteracting protein [Esox lucius]	3.42E-04	1.37	down
CREG2 protein [Salmo salar]	1.54E-03	1.37	down
unknown	8.30E-03	1.37	down
Plasma glutamate carboxypeptidase [Salmo salar]	6.04E-03	1.37	down
nucleoporin 153 [Takifugu rubripes]	3.80E-03	1.36	down
acid lysosomal [Bos taurus]	5.43E-03	1.36	down
Kunitztype protease inhibitor 1 precursor [Salmo salar]	6.84E-03	1.36	down
unknown	4.42E-03	1.36	down

TABLE S4: (*continued...*)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	1.41E-03	1.36	down
39S ribosomal protein L15, mitochondrial precursor [Salmo salar]	6.88E-03	1.36	down
sorting nexin 10 [Takifugu rubripes]	8.47E-03	1.36	down
unknown	3.10E-04	1.35	down
unknown	1.23E-03	1.35	down
malate dehydrogenase [Sphyraena idiastes]	6.96E-03	1.35	down
coiledcoil domain containing 109B [Bos taurus]	3.00E-03	1.35	down
S100A5 [Salmo salar]	7.74E-03	1.35	down
unknown	3.52E-03	1.35	down
Aa2141 [Rattus norvegicus]	7.46E-03	1.34	down
reggie protein 2b [Takifugu rubripes]	2.94E-03	1.34	down
Nacetyltransferase ARD1 homolog (S. cerevisiae), isoform CRA_c [Mus musculus]	8.44E-03	1.34	down
FK506 binding protein 12rapamycin associated protein 1 [Danio rerio]	8.39E-03	1.33	down
StARrelated lipid transfer protein 7 [Salmo salar]	8.45E-03	1.33	down
Tetraspanin1 [Salmo salar]	5.40E-03	1.33	down
unknown	9.00E-03	1.33	down
unknown	8.84E-03	1.33	down
Sb:cb283 protein [Danio rerio]	9.80E-03	1.33	down
KIAA1632 protein [Homo sapiens]	3.16E-03	1.33	down
mitochondrial ATP synthase F0 complex subunit c isoform 3 [Takifugu rubripes]	1.65E-03	1.32	down
WD repeat domain 68 [Danio rerio]	3.64E-03	1.32	down
CHCH domaincontaining protein C22orf16, mitochondrial precursor [Salmo salar]	7.15E-03	1.32	down
isozyme B [Danio rerio]	4.15E-03	1.32	down
CDGSH iron sulfur domaincontaining protein 2 [Salmo salar]	7.71E-03	1.31	down
ATPase family AAA domain containing 4 [Takifugu rubripes]	9.11E-03	1.31	down
fatty acid binding protein H6isoform [Gobionotothen gibberifrons]	2.50E-03	1.31	down
Takifugu rubripes TPM42 mRNA for tropomyosin42, complete cds, spliced variant:exon 1b, 3, 4, 5, 6b, 7, 8 and 9d	9.26E-03	1.31	down
unknown	6.18E-04	1.31	down
Tetraspanin 5 [Mus musculus]	1.30E-03	1.31	down
unknown	9.77E-03	1.30	down
methylcrotonoylCoenzyme A carboxylase 2 (beta) [Danio rerio]	2.51E-03	1.30	down
dynamin 1like [Danio rerio]	2.98E-03	1.30	down
required for meiotic nuclear division 5 homolog B [Danio rerio]	3.97E-03	1.30	down
mitochondrial 1 [Homo sapiens]	1.17E-03	1.30	down
nucleoporin 155 [Danio rerio]	6.36E-03	1.30	down
TATA binding protein associated factor 9 [Sander vitreus]	8.17E-03	1.29	down
cell cycle associated protein 1 [synthetic construct]	5.92E-03	1.29	down
unknown	5.31E-03	1.29	down
unknown	3.85E-03	1.29	down
unknown	4.37E-03	1.29	down
tcomplex 11 (mouse) like 2 [Homo sapiens]	1.95E-03	1.27	down
Lysosomal thioesterase PPT2A precursor [Salmo salar]	1.97E-03	1.27	down
Galactosidase, beta 1like [Danio rerio]	1.27E-03	1.27	down
similar to RIKEN cDNA 8430437G11, isoform CRA_b [Rattus norvegicus]	4.31E-03	1.26	down
unknown	4.33E-03	1.26	down
unknown	8.84E-03	1.26	down
Sorting nexin9 [Salmo salar]	5.18E-03	1.26	down
ATPdependent RNA helicase DDX18 [Salmo salar]	6.62E-03	1.26	down

TABLE S4: (*continued*...)

Diet A day 14 vs Diet A day 28			
Description	**p-value**	**FCA**	**Regulation**
microfibrillarassociated protein 1 [Danio rerio]	7.76E-03	1.26	down
centrosomal protein 68, isoform CRA_b [Mus musculus]	2.56E-03	1.26	down
unknown	1.97E-03	1.25	down
unknown	8.42E-03	1.24	down
unknown	4.70E-03	1.24	down
adenine phosphoribosyl transferase [Scophthalmus maximus]	7.57E-03	1.24	down
glutaminerich 1 [Mus musculus]	1.06E-03	1.24	down
Vps8 protein [Mus musculus]	1.56E-03	1.24	down
unknown	1.54E-03	1.23	down
RecName: Full=Cryptochrome DASH; AltName: Full=Protein CRYDASH; Short=zCRYDASH	9.83E-04	1.23	down
unknown	9.80E-03	1.23	down
unknown	7.62E-04	1.23	down
novel protein with a Prominin domain [Danio rerio]	2.65E-03	1.22	down
novel protein similar to vertebrate dynactin 1 (p150, glued homolog, Drosophila) (DCTN1) [Danio rerio]	1.11E-03	1.22	down
Rras [Kryptolebias marmoratus]	1.07E-04	1.22	down
unknown	4.94E-03	1.21	down
RNA polymerase B transcription factor 3 [Scophthalmus maximus]	2.73E-03	1.21	down
TATA box binding protein, isoform CRA_b [Mus musculus]	6.80E-04	1.21	down
unknown	5.00E-03	1.21	down
unknown	7.65E-03	1.21	down
subunit 1 [Danio rerio]	6.56E-03	1.21	down
similar to RIKEN cDNA 3110043O21, isoform CRA_b [Rattus norvegicus]	2.28E-03	1.21	down
Lithognathus mormyrus clone lmos9p10f06 mRNA sequence	6.54E-03	1.21	down
receptorlike tyrosine kinase [Rattus norvegicus]	1.89E-03	1.21	down
copper chaperone [Scophthalmus maximus]	1.91E-03	1.20	down
Signal peptide peptidaselike 2A [Salmo salar]	7.08E-03	1.20	down
kinetochore associated 1 [Xenopus tropicalis]	1.83E-05	1.20	down
Calciumbinding mitochondrial carrier protein Aralar1 [Salmo salar]	7.06E-03	1.20	down
PRP4 premRNA processing factor 4 homolog B [Danio rerio]	1.35E-03	1.20	down
deoxyhypusine hydroxylase/monooxygenase [Danio rerio]	7.96E-03	1.20	down
Replication protein A 70 kDa DNAbinding subunit [Salmo salar]	9.31E-03	1.20	down
similar to thymineDNA glycosylase [Xenopus laevis]	7.53E-03	1.19	down
unknown	3.44E-03	1.19	down
CN130 protein [Salmo salar]	6.07E-03	1.19	down
unknown	7.48E-03	1.19	down
Zinc finger HIT domaincontaining protein 3 [Salmo salar]	6.43E-03	1.19	down
PREDICTED: zinc finger, CCHC domain containing 11 [Taeniopygia guttata]	8.03E-03	1.18	down
brain and reproductive organexpressed protein [Danio rerio]	2.09E-03	1.18	down
RAN binding protein 3 isoform RANBP3a [Homo sapiens]	7.24E-03	1.16	down
proteasome activator subunit 3 [Danio rerio]	6.30E-03	1.15	down
unknown	7.67E-03	1.15	down
Galactose1phosphate uridylyltransferase [Salmo salar]	3.98E-03	1.15	down
Phosphatidylinositol 4kinase type 2alpha [Salmo salar]	9.20E-03	1.15	down
Cisd3 protein [Mus musculus]	6.64E-03	1.12	down
Major facilitator superfamily domaincontaining protein 5	5.39E-03	1.05	down

TABLE S5: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **CONTROL DIET (DIET C) AND DIET B 2 DOF**. The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
basic leucine zipper transcription factor, ATFlike [Mus musculus]	9.17E-03	13.71	up
Nisch protein [Mus musculus]	7.74E-04	4.86	up
unknown	4.44E-03	4.13	up
Salmo salar clone ssalrgf524002 Cyclindependent kinase inhibitor 1B putative mRNA, complete cds	4.92E-03	3.41	up
Arrestin domain containing 1a [Danio rerio]	2.07E-03	2.82	up
RNA binding motif protein 22 [Xenopus (Silurana) tropicalis]	1.98E-03	2.68	up
acetylserotonin Omethyltransferaselike [Danio rerio]	9.19E-03	2.64	up
unknown	7.70E-04	2.09	up
PCAF [Danio rerio]	1.82E-03	2.08	up
chromosome 2 open reading frame 7 [Bos taurus]	1.64E-03	1.97	up
Lithognathus mormyrus clone lmos7p10f12 mRNA sequence	1.65E-03	1.90	up
unknown	8.43E-03	1.85	up
unknown	7.03E-03	1.83	up
unknown	4.14E-03	1.82	up
unknown	1.48E-03	1.80	up
eukaryotic translation termination factor 1, isoform CRA_d [Homo sapiens]	3.93E-03	1.78	up
member B3 [Danio rerio]	6.16E-03	1.76	up
unknown	2.18E-03	1.75	up
NADHcytochrome b5 reductase 2 [Salmo salar]	5.73E-03	1.72	up
hydroxypyruvate reductase [Salmo salar]	5.27E-03	1.71	up
subunit 25 [Danio rerio]	6.26E-03	1.69	up
unknown	8.76E-03	1.68	up
unknown	8.90E-03	1.66	up
unknown	9.94E-03	1.61	up
unknown	1.00E-03	1.58	up
RAB1, member RAS oncogene family [Mus musculus]	1.65E-03	1.56	up
DNA polymerase delta subunit 3 [Oreochromis mossambicus]	1.48E-03	1.56	up
unknown	1.13E-03	1.55	up
kinesin family member 23 [Bos taurus]	8.41E-03	1.54	up
Lithognathus mormyrus clone lmos9p01c01 mRNA sequence	6.61E-03	1.52	up
cerevisiae) [Homo sapiens]	4.95E-03	1.52	up
kinesin family member 15 [Homo sapiens]	2.47E-04	1.51	up
adenine phosphoribosyl transferase [Scophthalmus maximus]	7.74E-04	1.47	up
Deoxyribonuclease gamma precursor [Salmo salar]	3.85E-04	1.45	up
transducin (beta)like 3 [Xenopus (Silurana) tropicalis]	1.35E-03	1.45	up
low density lipoprotein receptor [Danio rerio]	7.57E-04	1.45	up
Nacetylglucosamine1phosphate transferase [Danio rerio]	9.87E-03	1.44	up
Livertype aldolase	5.20E-04	1.42	up
zinc finger protein 330 [Danio rerio]	5.33E-04	1.41	up
protein kinase C substrate 80KH [Danio rerio]	5.14E-03	1.40	up
Vigilin [Salmo salar]	4.60E-03	1.38	up
Origin recognition complex subunit 2 [Salmo salar]	3.65E-03	1.37	up
unknown	3.87E-03	1.37	up
Interferoninduced 35 kDa protein homolog [Salmo salar]	1.74E-03	1.36	up
Nucleoside diphosphate kinase 3 [Salmo salar]	2.64E-03	1.36	up
SERPINE1 mRNA binding protein 1 [Danio rerio]	5.18E-03	1.36	up
unknown	8.77E-03	1.36	up

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
type I keratin isoform 1 [Solea senegalensis]	4.61E-03	1.35	up
RAB1A, member RAS oncogene family, isoform CRA_f [Homo sapiens]	1.02E-03	1.35	up
unknown	7.93E-03	1.34	up
ADP ribosylation factor 79F [Argas monolakensis]	5.33E-04	1.33	up
DNA replication complex GINS protein PSF2 [Salmo salar]	8.59E-03	1.33	up
cleft lip and palate associated transmembrane protein 1 [Danio rerio]	9.94E-03	1.33	up
transforming, acidic coiledcoil containing protein 3 [Takifugu rubripes]	7.23E-04	1.33	up
Shmt1 protein [Danio rerio]	9.03E-03	1.32	up
Cytosolic sulfotransferase 3 [Salmo salar]	7.99E-03	1.32	up
Mitochondrial 39S ribosomal protein L23 [Esox lucius]	4.13E-03	1.32	up
unknown	8.64E-03	1.32	up
Hsc70interacting protein [Salmo salar]	1.73E-03	1.31	up
isocitrate dehydrogenase 3 (NAD+) beta, isoform CRA_d [Homo sapiens]	1.97E-03	1.31	up
Eukaryotic peptide chain release factor GTPbinding subunit ERF3B [Salmo salar]	1.28E-03	1.31	up
Setb protein [Danio rerio]	4.43E-03	1.30	up
G patch domain containing 4 [Xenopus (Silurana) tropicalis]	2.85E-03	1.29	up
Lithognathus mormyrus clone lmos8p01e08 mRNA sequence	3.08E-03	1.29	up
Betacateninlike protein 1 [Salmo salar]	6.73E-03	1.28	up
Sadenosylhomocysteine hydrolaselike protein [Pimephales promelas]	5.93E-03	1.28	up
TAP2a protein [Oncorhynchus mykiss]	7.82E-03	1.28	up
ubiquitin specific protease 14 [Danio rerio]	2.18E-03	1.27	up
hepcidin [Sparus aurata]	5.05E-03	1.27	up
heat shock protein 60 [Pseudosciaena crocea]	5.79E-05	1.25	up
RecName: Full=PH domaincontaining protein C10orf81	5.22E-03	1.25	up
Suppressor of actin mutations 1like protein B	5.92E-03	1.24	up
lysosomal accessory protein 1 [Danio rerio]	7.09E-03	1.23	up
unknown	8.33E-03	1.23	up
unknown	9.45E-03	1.23	up
subunit 5 (epsilon) [Danio rerio]	4.88E-04	1.22	up
Eukaryotic translation initiation factor 4E type 2 [Salmo salar]	1.71E-03	1.22	up
coiledcoil domain containing 94 [Danio rerio]	7.53E-03	1.22	up
chromosome 14 open reading frame 106 [Homo sapiens]	9.23E-03	1.21	up
Transmembrane protein 32 [Salmo salar]	9.41E-03	1.21	up
novel protein with a Prominin domain [Danio rerio]	7.68E-03	1.20	up
WD repeat domain 5 [Danio rerio]	2.98E-03	1.19	up
ATAD2 protein [Homo sapiens]	1.04E-03	1.19	up
unknown	8.13E-03	1.19	up
unknown	9.20E-03	1.18	up
Cell division cycle 5like protein [Salmo salar]	8.45E-03	1.17	up
wibg homolog [Salmo salar]	3.25E-03	1.16	up
RING finger protein 170 [Salmo salar]	2.36E-03	1.13	up
unknown	4.21E-03	1.12	up
unknown	7.68E-03	6.26	down
unknown	4.71E-03	5.73	down
unknown	7.37E-04	5.57	down
unknown	4.06E-03	4.24	down
unknown	5.67E-04	4.20	down
unknown	2.15E-03	4.05	down

TABLE S5: (*continued...*)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	3.50E-03	3.80	down
unknown	5.74E-03	3.63	down
unknown	8.35E-05	3.58	down
Nterminal EFhand calcium binding protein 1 [Mus musculus]	1.23E-03	3.44	down
unknown	2.46E-03	3.31	down
unknown	3.27E-03	3.25	down
asparaginelinked glycosylation 9 protein [Danio rerio]	1.24E-03	3.20	down
unknown	3.44E-03	3.17	down
unknown	9.87E-03	3.16	down
unknown	8.22E-03	3.08	down
hypothetical protein LOC560226 [Danio rerio]	1.46E-03	2.96	down
unknown	6.11E-03	2.94	down
unknown	9.78E-03	2.94	down
unknown	6.96E-03	2.87	down
unknown	8.67E-05	2.87	down
unknown	3.68E-03	2.83	down
unknown	8.09E-03	2.82	down
Kelch domain containing 1 [Mus musculus]	1.41E-03	2.76	down
unknown	1.11E-03	2.72	down
unknown	1.07E-03	2.70	down
StefinD1	1.95E-03	2.69	down
PREDICTED: similar to hCG39059 isoform 1 [Danio rerio]	6.24E-03	2.68	down
unknown	7.82E-03	2.64	down
unknown	8.00E-03	2.61	down
unknown	5.61E-04	2.56	down
unknown	1.79E-04	2.54	down
alkyldihydroxyacetone phosphate synthase [Takifugu rubripes]	7.10E-04	2.53	down
Gzmb [Mus musculus]	7.72E-03	2.50	down
unknown	8.27E-03	2.47	down
unknown	5.06E-03	2.43	down
unknown	1.30E-03	2.38	down
unknown	8.13E-04	2.37	down
unknown	1.30E-03	2.35	down
unknown	1.69E-03	2.33	down
unknown	8.55E-03	2.33	down
unknown	7.48E-03	2.31	down
Cytochrome bc1 complex subunit 8 [Salmo salar]	7.13E-03	2.29	down
unknown	1.04E-04	2.22	down
unknown	6.47E-05	2.19	down
unknown	8.59E-03	2.18	down
unknown	6.66E-03	2.15	down
unknown	4.74E-03	2.14	down
unknown	5.83E-03	2.14	down
unknown	2.85E-04	2.11	down
unknown	1.45E-03	2.09	down
unknown	4.33E-03	2.09	down
unknown	9.94E-03	2.07	down
mast cell preproprotein [Homo sapiens]	8.41E-03	2.06	down

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	1.95E-03	2.04	down
unknown	4.72E-03	2.03	down
unknown	8.63E-03	2.01	down
unknown	1.65E-03	2.00	down
unknown	8.67E-03	1.99	down
unknown	8.93E-03	1.98	down
unknown	5.75E-04	1.96	down
Takifugu rubripes TPM42 mRNA for tropomyosin42, complete cds, spliced variant:exon 1a, 2b, 3, 4, 5, 6b, 7, 8 and 9a	1.12E-03	1.96	down
unknown	9.77E-03	1.95	down
unknown	2.61E-03	1.94	down
unknown	1.41E-03	1.94	down
unknown	1.06E-04	1.93	down
unknown	5.67E-03	1.93	down
unknown	1.71E-03	1.90	down
MHC class II alpha chain [Oncorhynchus mykiss]	4.38E-03	1.90	down
unknown	3.75E-03	1.87	down
unknown	7.60E-04	1.87	down
Major facilitator superfamily domaincontaining protein 4 [Salmo salar]	9.10E-03	1.87	down
unknown	4.83E-03	1.87	down
deltalike 4 [Danio rerio]	9.61E-04	1.86	down
unknown	7.34E-03	1.85	down
RecName: Full=Protocadherin24; AltName: Full=Protocadherin LKC; Short=PCLKC; Flags: Precursor	4.79E-03	1.84	down
unknown	7.42E-03	1.83	down
MGC84181 protein [Xenopus laevis]	1.82E-03	1.83	down
unknown	7.54E-03	1.82	down
unknown	3.02E-03	1.81	down
unknown	9.97E-03	1.81	down
unknown	2.59E-03	1.80	down
unknown	4.21E-03	1.80	down
Sparus aurata methallothionein mRNA, complete cds	6.05E-04	1.79	down
HIG1 domain family member 2A [Salmo salar]	6.79E-03	1.78	down
type 9B [Danio rerio]	2.55E-03	1.78	down
unknown	9.90E-03	1.78	down
unknown	1.42E-04	1.78	down
unknown	3.39E-04	1.76	down
unknown	9.46E-04	1.76	down
unknown	4.52E-03	1.75	down
Ebox binding protein 2 [Ictalurus punctatus]	8.86E-04	1.74	down
PREDICTED: similar to ReO_6 [Danio rerio]	8.40E-03	1.74	down
Xbox binding 1 [Mus musculus]	6.51E-03	1.73	down
unknown	3.93E-03	1.71	down
axonemal [Salmo salar]	9.92E-03	1.71	down
unknown	4.79E-03	1.71	down
unknown	8.10E-03	1.70	down
unknown	5.68E-04	1.69	down
PREDICTED: similar to C18B2.5a [Danio rerio]	4.52E-03	1.68	down
unknown	8.36E-03	1.66	down
unknown	6.17E-04	1.66	down

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	8.98E-03	1.65	down
unknown	5.69E-03	1.65	down
unknown	2.76E-03	1.65	down
unknown	2.35E-03	1.63	down
unknown	3.94E-03	1.62	down
unknown	1.83E-03	1.62	down
Exocyst complex component 3like protein	4.47E-03	1.61	down
EFhand calcium binding domain 2 isoform a [Homo sapiens]	9.79E-03	1.60	down
Quo protein [Danio rerio]	8.22E-03	1.60	down
unknown	9.80E-03	1.60	down
unknown	2.48E-04	1.60	down
unknown	4.83E-03	1.59	down
unknown	2.45E-04	1.59	down
rCG23364, isoform CRA_b [Rattus norvegicus]	9.72E-03	1.59	down
unknown	1.08E-03	1.58	down
unknown	2.77E-03	1.58	down
unknown	7.95E-03	1.58	down
pollike protein [Biomphalaria glabrata]	8.22E-03	1.58	down
complement component C7 [Paralichthys olivaceus]	3.60E-03	1.58	down
unknown	4.32E-03	1.57	down
unknown	3.38E-04	1.56	down
unknown	1.16E-03	1.56	down
unknown	2.44E-03	1.55	down
ASC [Siniperca chuatsi]	4.15E-03	1.55	down
unknown	5.40E-03	1.54	down
unknown	7.48E-03	1.53	down
unknown	9.94E-04	1.53	down
unknown	2.87E-03	1.53	down
unknown	6.65E-03	1.52	down
unknown	7.55E-03	1.52	down
unknown	4.83E-03	1.51	down
unknown	4.43E-03	1.51	down
unknown	8.02E-03	1.50	down
unknown	5.63E-03	1.49	down
unknown	2.29E-03	1.49	down
unknown	2.26E-03	1.49	down
unknown	3.30E-04	1.49	down
unknown	9.38E-03	1.49	down
unknown	5.98E-03	1.48	down
unknown	1.11E-03	1.48	down
unknown	4.31E-03	1.47	down
unknown	5.73E-03	1.47	down
FAM49B [Salmo salar]	8.11E-03	1.46	down
unknown	9.24E-03	1.45	down
unknown	4.41E-04	1.45	down
unknown	3.73E-03	1.45	down
unknown	2.74E-03	1.45	down
unknown	8.11E-03	1.45	down

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	4.29E-03	1.44	down
CD209 antigenlike protein A [Salmo salar]	6.20E-03	1.44	down
Diplodus sargus igfII mRNA for preproinsulingrowth factor II, complete cds	7.11E-03	1.44	down
unknown	8.60E-03	1.44	down
unknown	7.19E-03	1.44	down
Lithognathus mormyrus clone lmos8p07a03 mRNA sequence	6.37E-03	1.44	down
unknown	6.99E-03	1.44	down
unknown	3.67E-03	1.43	down
ZPA domain containing protein [Oryzias latipes]	9.94E-03	1.43	down
reverse transcriptaselike protein [Paralichthys olivaceus]	2.68E-03	1.43	down
unknown	9.38E-04	1.42	down
unknown	9.84E-03	1.42	down
unknown	9.84E-03	1.42	down
unknown	5.48E-03	1.42	down
unknown	2.38E-03	1.42	down
unknown	6.07E-03	1.41	down
unknown	6.85E-03	1.41	down
Exostosin2 [Salmo salar]	4.22E-03	1.41	down
unknown	8.18E-03	1.41	down
unknown	8.83E-03	1.41	down
ectoADPribosyltransferase 5 precursorlike [Ictalurus punctatus]	5.03E-03	1.40	down
unknown	1.48E-03	1.40	down
unknown	1.15E-04	1.39	down
unknown	4.61E-03	1.39	down
unknown	5.10E-03	1.39	down
unknown	3.50E-03	1.39	down
unknown	5.73E-03	1.38	down
cytochrome P450 2N1 [Oryzias latipes]	2.25E-04	1.37	down
Mus musculus dystrophin, muscular dystrophy (Dmd), mRNA	9.30E-03	1.37	down
Four and a half LIM domains protein 1 [Salmo salar]	1.51E-03	1.37	down
unknown	4.97E-03	1.37	down
unknown	2.86E-03	1.36	down
unknown	1.12E-03	1.36	down
rCG32598 [Rattus norvegicus]	4.96E-03	1.35	down
unknown	1.21E-03	1.35	down
unknown	8.02E-03	1.35	down
PREDICTED: im:7163520 [Danio rerio]	7.88E-03	1.35	down
unknown	1.87E-03	1.35	down
unknown	8.14E-04	1.34	down
unknown	6.94E-03	1.34	down
Takifugu rubripes ZnT1 (ZnT1) mRNA, complete cds	5.78E-03	1.33	down
unknown	9.71E-03	1.33	down
unknown	2.53E-03	1.33	down
unknown	1.00E-03	1.33	down
unknown	5.51E-03	1.33	down
unknown	3.78E-03	1.32	down
unknown	1.06E-03	1.32	down
unknown	2.83E-03	1.32	down

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.73E-03	1.32	down
unknown	5.19E-03	1.32	down
unknown	6.53E-03	1.32	down
unknown	7.52E-03	1.31	down
unknown	4.24E-03	1.31	down
Tetraspanin4 [Salmo salar]	3.74E-03	1.31	down
unknown	8.78E-03	1.31	down
Musculoskeletal embryonic nuclear protein 1 [Esox lucius]	8.48E-03	1.31	down
unknown	7.65E-03	1.31	down
Fugu rubripes cosmid 259C6, complete sequence	9.19E-03	1.30	down
Heat shock protein 14 [Danio rerio]	4.89E-03	1.30	down
unknown	9.48E-04	1.30	down
unknown	8.58E-03	1.29	down
unknown	9.94E-03	1.29	down
unknown	7.38E-03	1.29	down
unknown	6.52E-03	1.29	down
Lithognathus mormyrus clone lithmor1043 mRNA sequence	3.49E-03	1.29	down
unknown	4.86E-03	1.28	down
unknown	4.79E-03	1.27	down
unknown	1.09E-03	1.27	down
unknown	6.58E-03	1.27	down
Lithognathus mormyrus clone lmos8p04f06 mRNA sequence	8.76E-03	1.26	down
unknown	4.54E-03	1.25	down
crossover junction endonuclease EME1like protein [Callithrix jacchus]	9.27E-03	1.25	down
unknown	7.05E-03	1.25	down
unknown	8.48E-03	1.25	down
unknown	8.57E-04	1.24	down
Lithognathus mormyrus clone lmos8p07e02 mRNA sequence	4.97E-03	1.24	down
unknown	3.72E-03	1.24	down
ADPribosylation factor 4 [Salmo salar]	3.34E-03	1.24	down
unknown	8.52E-03	1.24	down
Lithognathus mormyrus clone lithmor598 mRNA sequence	7.56E-03	1.24	down
tcomplex 11 (mouse) like 2 [Homo sapiens]	1.55E-03	1.24	down
unknown	6.78E-03	1.24	down
unknown	7.34E-03	1.23	down
unknown	6.18E-03	1.23	down
unknown	9.30E-03	1.23	down
unknown	1.42E-03	1.23	down
unknown	8.40E-03	1.23	down
unknown	8.84E-03	1.23	down
Lithognathus mormyrus clone lmos8p09f04 mRNA sequence	6.66E-03	1.21	down
unknown	7.51E-03	1.21	down
unknown	6.24E-03	1.21	down
finTRIM family protein [Danio rerio]	3.46E-03	1.21	down
unknown	3.92E-03	1.21	down
Paralichthys olivaceus ctrlr mRNA for calcitonin receptorlike receptor, complete cds	3.55E-03	1.20	down
unknown	6.59E-04	1.20	down
unknown	6.08E-03	1.20	down

TABLE S5: (*continued*...)

Control diet (all days) vs Diet B day 2			
Description	**p-value**	**FCA**	**Regulation**
unknown	6.77E-03	1.19	down
PremRNAsplicing regulator WTAP [Salmo salar]	9.78E-03	1.19	down
Ctype lectin receptor [Paralabidochromis chilotes]	5.04E-03	1.19	down
unknown	8.96E-03	1.19	down
Lithognathus mormyrus clone lmos7p01E07 mRNA sequence	5.68E-03	1.18	down
unknown	1.08E-03	1.17	down
unknown	3.19E-03	1.17	down
unknown	9.15E-03	1.16	down
unknown	8.37E-03	1.16	down
unknown	3.82E-03	1.16	down
unknown	7.69E-03	1.16	down
Lithognathus mormyrus clone lmos7p03a11 mRNA sequence	9.58E-03	1.16	down
unknown	9.31E-03	1.16	down
unknown	6.14E-03	1.15	down
unknown	3.43E-03	1.14	down
Ubiquitin thioesterase OTUB1 [Esox lucius]	5.87E-03	1.13	down
plakophilin 3 [Bos taurus]	9.44E-03	1.12	down
unknown	2.86E-03	1.12	down
unknown	7.66E-04	1.12	down
rCG62161, isoform CRA_a [Rattus norvegicus]	1.90E-03	1.10	down
member 6 [Danio rerio]	8.39E-03	1.10	down
unknown	8.03E-03	1.08	down
Chiloscyllium plagiosum sHRI protein 2 mRNA, complete cds	6.06E-03	1.08	down

TABLE S6: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **CONTROL DIET B 2 DOF AND DIET B 7 DOF**. The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented.

Diet B day 2 vs Diet B day 7			
Description	**p-value**	**FCA**	**Regulation**
StefinD1	5.27E-03	2.34	up
hepatic glucose6phosphate dehydrogenase [Rhabdosargus sarba]	5.46E-03	2.20	up
unknown	6.57E-03	1.84	up
Takifugu rubripes ETR mRNA for endothelin receptor type A, complete cds	5.14E-04	1.68	up
unknown	3.96E-03	1.61	up
unknown	1.41E-03	1.60	up
ectoADPribosyltransferase 5 precursorlike [Ictalurus punctatus]	5.74E-03	1.55	up
unknown	3.72E-03	1.55	up
unknown	8.50E-03	1.50	up
unknown	1.96E-03	1.50	up
unknown	1.90E-03	1.40	up
unknown	4.47E-03	1.40	up
unknown	4.81E-04	1.40	up
unknown	5.09E-03	1.39	up
unknown	4.57E-03	1.39	up
unknown	4.14E-03	1.39	up
unknown	9.30E-04	1.37	up
39S ribosomal protein L20, mitochondrial precursor [Salmo salar]	9.80E-03	1.35	up
unknown	1.31E-03	1.35	up
unknown	3.57E-04	1.34	up
unknown	4.03E-03	1.32	up
unknown	2.70E-04	1.31	up
unknown	7.64E-03	1.28	up
Lithognathus mormyrus clone lmos2p03e02 mRNA sequence	9.60E-03	1.28	up
Transmembrane emp24 domaincontaining protein 1 [Salmo salar]	5.29E-03	1.28	up
unknown	5.04E-03	1.27	up
unknown	8.29E-03	1.26	up
unknown	7.33E-03	1.20	up
Exostosin2 [Salmo salar]	9.68E-03	1.20	up
Fugu rubripes gene for Notch 2, partial cds	5.64E-03	1.20	up
Lithognathus mormyrus clone lithmor598 mRNA sequence	9.37E-03	1.19	up
unknown	6.40E-03	1.18	up
Annexin A3 [Salmo salar]	9.93E-03	1.18	up
unknown	2.57E-03	1.18	up
unknown	3.06E-03	1.17	up
acid lysosomal [Bos taurus]	5.45E-03	1.17	up
Lithognathus mormyrus clone lithmor242 mRNA sequence	9.12E-03	1.15	up
unknown	3.32E-03	1.13	up
reggie protein 2b [Takifugu rubripes]	1.22E-03	1.12	up
neural cell adhesion molecule 1 isoform 3 [Mus musculus]	2.34E-03	1.11	up
Chiloscyllium plagiosum sHRI protein 2 mRNA, complete cds	6.23E-04	1.11	up
unknown	6.62E-03	1.11	up
unknown	2.14E-03	1.08	up
dopamine receptor [Takifugu rubripes]	8.98E-03	7.70	down
unknown	1.28E-03	3.56	down
unknown	1.40E-03	2.00	down
unknown	4.96E-03	1.81	down

TABLE S6: (*continued*...)

Diet B day 2 vs Diet B day 7			
Description	**p-value**	**FCA**	**Regulation**
unknown	8.82E-03	1.78	down
unknown	6.85E-03	1.77	down
unknown	1.90E-03	1.76	down
Lithognathus mormyrus clone lmos9p01c01 mRNA sequence	2.21E-04	1.73	down
unknown	8.32E-03	1.72	down
unknown	8.61E-03	1.70	down
unknown	9.39E-03	1.68	down
unknown	4.48E-03	1.54	down
unknown	2.23E-03	1.53	down
unknown	6.14E-04	1.46	down
Cyclindependent kinases regulatory subunit 1 [Esox lucius]	8.35E-03	1.45	down
Cyclindependent kinase inhibitor 3 [Salmo salar]	7.76E-03	1.44	down
Ictalurus punctatus CC chemokine SCYA111 gene, complete cds	7.61E-03	1.42	down
Mitochondrial 39S ribosomal protein L23 [Esox lucius]	4.83E-03	1.41	down
Solute carrier family 22 member 4 [Salmo salar]	7.18E-03	1.41	down
transforming, acidic coiledcoil containing protein 3 [Takifugu rubripes]	3.58E-03	1.40	down
Fancd2 protein [Danio rerio]	3.99E-03	1.38	down
unknown	6.12E-03	1.38	down
unknown	9.88E-03	1.34	down
CDC28 protein kinase 1 [Oreochromis mossambicus]	4.83E-03	1.33	down
unknown	3.31E-03	1.33	down
antigen processing proteasomeassociated protein [Oryzias luzonensis]	4.20E-03	1.33	down
unknown	5.00E-03	1.33	down
ribosomal protein S272 [Solea senegalensis]	1.86E-03	1.32	down
Lithognathus mormyrus clone lmos9p10b01 mRNA sequence	8.01E-03	1.29	down
Chain A, Crystal Structure Of The Ubiquitin Conjugating Enzyme Ube2g2 Bound To The G2br Domain Of Ubiquitin Ligase Gp78 Chain B, Crystal Structure Of The Ubiquitin Conjugating Enzyme Ube2g2 Bound To The G2br Domain Of Ubiquitin Ligase Gp78	9.97E-03	1.28	down
Histone RNA hairpinbinding protein [Salmo salar]	4.38E-03	1.24	down
Peptidylprolyl cistrans isomerase H [Salmo salar]	2.01E-03	1.23	down
unknown	4.82E-03	1.22	down
unknown	3.54E-03	1.22	down
unknown	1.09E-03	1.22	down
unknown	2.21E-03	1.19	down
unknown	9.68E-05	1.18	down
ATAD2 protein [Homo sapiens]	9.45E-03	1.17	down
ubiquitin specific protease 14 [Danio rerio]	7.41E-03	1.15	down
Fbox protein 2 [Bos taurus]	5.73E-03	1.15	down
unknown	9.66E-03	1.09	down

TABLE S7: List of differential expressed genes (p<0.01) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **CONTROL DIET B 2 DOF AND DIET B 7 DOF**. The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Diet B day 7 vs Diet B day 14			
Description	**p-value**	**FCA**	**Regulation**
Vibrio vulnificus YJ016 DNA, chromosome II, complete sequence	6.29E-03	4.02	up
unknown	2.57E-03	2.20	up
unknown	7.98E-03	2.07	up
unknown	9.43E-04	2.02	up
unknown	1.91E-03	1.95	up
T cell receptor gamma chain VJC1 [Paralichthys olivaceus]	1.68E-03	1.89	up
unknown	2.88E-03	1.75	up
Lithognathus mormyrus clone lmos9p07d06 mRNA sequence	5.49E-03	1.73	up
unknown	7.31E-04	1.70	up
Regulator of Gprotein signaling 1 [Oncorhynchus mykiss]	7.97E-03	1.63	up
Lithognathus mormyrus clone lithmor906 mRNA sequence	2.84E-03	1.59	up
unknown	2.53E-04	1.58	up
Lithognathus mormyrus clone lmos8p05a11 mRNA sequence	7.68E-03	1.48	up
unknown	6.39E-04	1.48	up
Ctype lectin domain family 4 member E [Esox lucius]	9.18E-04	1.44	up
unknown	4.95E-03	1.42	up
CCAAT/enhancerbinding protein beta 2 [Epinephelus coioides]	7.95E-03	1.39	up
Musculoskeletal embryonic nuclear protein 1 [Esox lucius]	7.01E-03	1.37	up
lysozyme [Sparus aurata]	7.68E-03	1.30	up
unknown	8.55E-03	1.30	up
Il1rap protein [Rattus norvegicus]	2.85E-03	1.28	up
unknown	3.66E-03	1.27	up
unknown	4.10E-03	1.26	up
unknown	5.66E-03	1.26	up
unknown	5.28E-03	1.25	up
CD82 antigen [Salmo salar]	3.52E-03	1.25	up
unknown	5.97E-03	1.24	up
FBP32II precursor [Morone chrysops]	5.87E-03	1.22	up
receptorlike tyrosine kinase [Rattus norvegicus]	5.85E-03	1.22	up
unknown	3.36E-03	1.21	up
unknown	1.23E-03	1.20	up
unknown	1.82E-04	1.19	up
unknown	9.35E-03	1.19	up
unknown	3.38E-03	1.18	up
unknown	7.77E-03	1.18	up
unknown	6.91E-03	1.16	up
G proteincoupled receptor 183 [Danio rerio]	9.76E-03	1.16	up
unknown	1.16E-03	1.16	up
Cytoplasmic dynein 1 intermediate chain 2 [Salmo salar]	1.47E-03	1.14	up
Vesicleassociated membrane proteinassociated protein B/C [Salmo salar]	6.18E-03	1.14	up
unknown	5.78E-03	1.14	up
unknown	9.02E-03	1.13	up
unknown	2.17E-04	1.10	up
unknown	9.20E-03	1.06	up
hepatic glucose6phosphate dehydrogenase [Rhabdosargus sarba]	3.45E-03	3.25	down
unknown	4.28E-03	2.55	down
goosefish kalliklectin [Lophiomus setigerus]	9.47E-03	2.42	down

TABLE S7: (*...continued*)

Diet B day 7 vs Diet B day 14			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.28E-03	2.38	down
unknown	2.68E-04	2.38	down
unknown	3.36E-04	2.31	down
unknown	9.28E-04	2.25	down
CD38 antigen [Rattus norvegicus]	2.30E-03	2.19	down
unknown	9.61E-03	2.13	down
unknown	1.19E-03	2.12	down
unknown	9.79E-03	2.12	down
Lithognathus mormyrus clone lithmor771 mRNA sequence	8.15E-03	2.11	down
unknown	2.74E-03	2.09	down
unknown	8.20E-03	2.04	down
DNAdamageinducible transcript 4like [Salmo salar]	9.37E-03	2.04	down
unknown	3.27E-03	2.00	down
unknown	6.88E-03	1.98	down
DNAH5 variant protein [Homo sapiens]	2.78E-03	1.98	down
unknown	5.65E-03	1.93	down
allantoicase [Danio rerio]	6.82E-03	1.91	down
ablinteractor 1, isoform CRA_b [Mus musculus]	2.94E-03	1.87	down
unknown	6.99E-03	1.87	down
unknown	9.85E-03	1.87	down
unknown	8.86E-03	1.85	down
unknown	1.36E-03	1.83	down
Pirin [Salmo salar]	2.73E-03	1.81	down
threonineprotein phosphatase 2B catalytic subunit gamma isoform [Salmo salar]	1.98E-03	1.81	down
unknown	8.31E-03	1.80	down
glycogen phosphorylase [Oreochromis mossambicus]	7.59E-03	1.78	down
unknown	7.65E-03	1.75	down
Lithognathus mormyrus clone lmos8p07h02 mRNA sequence	7.42E-03	1.70	down
unknown	4.58E-03	1.65	down
unknown	5.29E-03	1.59	down
unknown	1.44E-03	1.58	down
Cystathionine gammalyase [Salmo salar]	1.41E-03	1.58	down
p22phox [Siniperca chuatsi]	4.82E-03	1.57	down
Pagrus major gstA2 gene for glutathione Stransferase, complete cds	4.38E-04	1.55	down
sodium bicarbonate cotransporter 1 [Zoarces viviparus]	1.49E-03	1.48	down
Bloodthirsty [Danio rerio]///bloodthirsty [Danio rerio]	2.49E-03	1.47	down
Probable palmitoyltransferase ZDHHC4 [Salmo salar]	1.59E-04	1.46	down
member 1 [Bos taurus]	6.80E-03	1.46	down
unknown	4.91E-03	1.45	down
unknown	1.11E-03	1.44	down
GTPase IMAP family member 4 [Salmo salar]	3.68E-03	1.38	down
unknown	7.41E-03	1.36	down
tetraspanin 13 [Danio rerio]	4.83E-03	1.34	down
actin binding proteinlike [Danio rerio]	1.05E-03	1.32	down
unknown	3.29E-03	1.32	down
Srp72 protein [Danio rerio]	9.29E-03	1.31	down
kielin [Xenopus laevis]	8.20E-03	1.30	down
unknown	9.54E-03	1.29	down

TABLE S7: (*...continued*)

Diet B day 7 vs Diet B day 14			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.47E-03	1.28	down
deoxyribosephosphate aldolaselike [Danio rerio]	4.73E-03	1.28	down
unknown	5.63E-03	1.28	down
translocase of outer mitochondrial membrane 40 homolog (yeast), isoform CRA_d [Homo sapiens]	7.83E-03	1.28	down
unknown	8.21E-03	1.27	down
unknown	1.94E-04	1.26	down
unknown	2.28E-03	1.26	down
unknown	1.57E-03	1.25	down
unknown	6.89E-03	1.25	down
Lithognathus mormyrus clone lmos9p01a02 mRNA sequence	6.22E-04	1.25	down
unknown	5.87E-03	1.23	down
Z [Salmo salar]	8.80E-03	1.23	down
6phosphofructo2kinase/fructose2,6biphosphatase 4 [Salmo salar]	3.79E-03	1.22	down
subunit 3 [Danio rerio]	4.93E-03	1.22	down
unknown	2.78E-04	1.21	down
KIAA0459 protein [Homo sapiens]	6.64E-03	1.20	down
Lithognathus mormyrus clone lmos8p09f10 mRNA sequence	1.40E-03	1.20	down
mitochondrial 1 [Homo sapiens]	5.73E-03	1.20	down
unknown	2.14E-03	1.19	down
unknown	1.41E-04	1.19	down
expressed sequence AV312086, isoform CRA_a [Mus musculus]	2.42E-03	1.17	down
unknown	4.55E-03	1.17	down
FAST kinase domaincontaining protein 1 [Salmo salar]	8.03E-03	1.17	down
novel protein similar to vertebrate ER degradation enhancer, mannosidase alphalike 3 (EDEM3) [Danio rerio]	4.91E-03	1.17	down
Cox15 protein [Danio rerio]	8.65E-03	1.16	down
Inositol monophosphatase [Salmo salar]	7.69E-03	1.15	down
diaphanous 2 [Danio rerio]	9.97E-03	1.15	down
vacuolar protein sorting 13 homolog B [synthetic construct]	9.56E-04	1.12	down
member Ab [Salmo salar]	5.94E-03	1.12	down
Influenza virus NS1Abinding protein homolog A [Salmo salar]	4.36E-03	1.10	down
unknown	8.95E-03	1.10	down
alpha globin regulatory element containinglike [Danio rerio]	6.01E-03	1.08	down

TABLE S8: List of differential expressed genes ($p<0.01$) in gills of seabream feed with immunostimulant diets. Loop analysis with a cut-off fold change higher than 1.0 between **CONTROL**

DIET B 14 DOF AND DIET B 28 DOF. The p-value and the absolute fold change (FCA) for up- (green) and down-regulated genes (blue) are represented

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	2.87E-03	3.80	up
unknown	4.72E-05	3.33	up
unknown	1.93E-03	3.23	up
unknown	4.43E-04	3.12	up
unknown	4.46E-03	3.09	up
kinetoplastid membrane protein 11 [Trypanosoma rangeli]	3.86E-03	3.06	up
unknown	6.19E-03	2.80	up
unknown	1.30E-03	2.77	up
unknown	4.55E-03	2.65	up
unknown	3.02E-03	2.60	up
unknown	8.90E-05	2.57	up
unknown	3.24E-03	2.50	up
unknown	8.38E-03	2.50	up
unknown	4.81E-03	2.45	up
rCG57161 [Rattus norvegicus]	8.01E-03	2.44	up
unknown	1.76E-03	2.42	up
unknown	1.07E-03	2.40	up
unknown	6.73E-03	2.39	up
52 kDa Ro protein [Salmo salar]	4.90E-03	2.35	up
unknown	9.75E-03	2.34	up
unknown	5.34E-04	2.31	up
glycogen phosphorylase [Oreochromis mossambicus]	4.04E-03	2.26	up
unknown	1.34E-03	2.25	up
unknown	3.46E-03	2.20	up
Rab GTPasebinding effector protein 2 [Salmo salar]	5.05E-03	2.17	up
unknown	3.90E-04	2.16	up
unknown	6.63E-03	2.10	up
unknown	1.10E-03	2.08	up
phosphatidylinositolspecific phospholipase C, X domain containing 2 [Rattus norvegicus]	7.92E-03	2.07	up
unknown	1.45E-03	2.07	up
unknown	4.77E-04	2.06	up
unknown	8.17E-03	2.01	up
unknown	5.32E-03	2.00	up
unknown	2.52E-03	1.99	up
unknown	5.99E-03	1.99	up
unknown	5.30E-04	1.99	up
similar to RIKEN cDNA 4933411K20 [Rattus norvegicus]	6.63E-03	1.98	up
unknown	2.21E-03	1.98	up
unknown	7.54E-03	1.98	up
unknown	7.00E-03	1.97	up
unknown	6.38E-03	1.96	up
unknown	3.60E-04	1.96	up
unknown	1.96E-04	1.96	up
unknown	7.73E-05	1.95	up
Mon2 protein [Mus musculus]	1.25E-04	1.95	up
ablinteractor 1, isoform CRA_b [Mus musculus]	6.50E-03	1.95	up
unknown	5.95E-03	1.95	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
PCAF [Danio rerio]	2.15E-03	1.94	up
unknown	1.42E-03	1.94	up
unknown	8.76E-03	1.94	up
unknown	9.00E-03	1.94	up
unknown	3.94E-03	1.93	up
unknown	9.93E-04	1.93	up
unknown	7.29E-03	1.93	up
Paralichthys olivaceus insulinlike growth factor I mRNA, complete cds	4.93E-03	1.92	up
unknown	9.77E-03	1.92	up
low density lipoprotein receptor [Danio rerio]	5.21E-03	1.92	up
unknown	3.20E-03	1.92	up
unknown	4.28E-03	1.91	up
unknown	3.24E-03	1.91	up
unknown	2.04E-03	1.91	up
unknown	3.92E-03	1.91	up
unknown	3.09E-03	1.89	up
unknown	4.42E-03	1.89	up
unknown	1.39E-05	1.88	up
isoenzyme 2 [Danio rerio]	3.68E-03	1.88	up
unknown	5.41E-03	1.87	up
tp53induced glycolysis and apoptosis regulator a [Danio rerio]	1.41E-03	1.85	up
unknown	1.88E-03	1.84	up
unknown	3.72E-03	1.83	up
unknown	9.20E-03	1.83	up
unknown	1.13E-03	1.83	up
unknown	7.20E-03	1.83	up
ribosomal protein S6 kinase alpha3 [synthetic construct]	7.06E-03	1.82	up
unknown	5.29E-03	1.82	up
unknown	5.56E-04	1.82	up
ectonucleotide pyrophosphatase/phosphodiesterase 3 [Mus musculus]	2.39E-03	1.82	up
unknown	3.73E-04	1.82	up
unknown	3.14E-03	1.82	up
unknown	6.96E-03	1.82	up
unknown	7.67E-03	1.81	up
unknown	5.84E-03	1.80	up
vacuolartype H+ transporting ATPase B1 subunit [Anguilla anguilla]	3.55E-03	1.80	up
unknown	6.89E-03	1.80	up
unknown	2.99E-03	1.80	up
unknown	7.50E-03	1.80	up
unknown	3.10E-03	1.79	up
Transmembrane protein 184A [Salmo salar]	1.68E-04	1.79	up
unknown	6.60E-03	1.79	up
unknown	1.88E-03	1.79	up
unknown	4.87E-03	1.78	up
unknown	1.01E-03	1.78	up
unknown	1.42E-03	1.77	up
unknown	5.85E-03	1.77	up
Rho GTPase activating protein 29 [Danio rerio]	9.15E-03	1.76	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
tumor necrosis factor receptor1 [Paralichthys olivaceus]	3.53E-04	1.76	up
unknown	9.17E-03	1.76	up
unknown	7.76E-03	1.76	up
sodium bicarbonate cotransporter 1 [Zoarces viviparus]	2.68E-03	1.75	up
unknown	4.42E-03	1.75	up
unknown	1.06E-03	1.75	up
unknown	6.89E-03	1.74	up
unknown	4.40E-03	1.74	up
unknown	6.19E-03	1.74	up
class I helical cytokine receptor number 29 [Tetraodon nigroviridis]	3.39E-03	1.74	up
beta polypeptide [Danio rerio]	3.17E-03	1.74	up
unknown	1.19E-03	1.73	up
unknown	4.92E-03	1.72	up
unknown	4.40E-03	1.72	up
unknown	5.27E-03	1.72	up
unknown	1.21E-03	1.72	up
unknown	3.98E-03	1.71	up
unknown	3.23E-03	1.71	up
unknown	8.68E-03	1.71	up
unknown	5.84E-03	1.71	up
unknown	6.44E-03	1.71	up
unknown	9.72E-03	1.70	up
unknown	2.13E-03	1.70	up
unknown	2.33E-03	1.69	up
unknown	3.96E-03	1.69	up
unknown	5.44E-03	1.69	up
unknown	2.74E-03	1.69	up
GDPLfucose synthetase [Salmo salar]	7.43E-03	1.69	up
unknown	7.74E-04	1.68	up
unknown	2.97E-03	1.68	up
unknown	1.56E-03	1.68	up
unknown	9.69E-04	1.68	up
unknown	3.67E-03	1.68	up
transposase [Rana pipiens]	9.47E-03	1.67	up
unknown	8.12E-03	1.67	up
unknown	8.76E-03	1.67	up
hypothetical protein [Danio rerio]	3.03E-04	1.67	up
unknown	3.95E-03	1.67	up
Deubiquitinating enzyme 47	4.12E-04	1.67	up
unknown	4.09E-03	1.67	up
unknown	7.53E-03	1.66	up
unknown	6.34E-03	1.66	up
Pirin [Salmo salar]	2.97E-03	1.66	up
unknown	4.56E-03	1.66	up
unknown	1.78E-03	1.66	up
unknown	5.13E-03	1.66	up
unknown	3.34E-03	1.65	up
unknown	1.47E-03	1.65	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.48E-04	1.65	up
Transmembrane BAX inhibitor motifcontaining protein 1 [Salmo salar]	8.35E-03	1.65	up
unknown	5.96E-03	1.65	up
unknown	3.00E-03	1.65	up
CCAAT/enhancerbinding protein beta 2 [Epinephelus coioides]	1.58E-03	1.64	up
Lithognathus mormyrus clone lithmor170 mRNA sequence	1.35E-03	1.64	up
unknown	5.26E-03	1.64	up
unknown	2.64E-03	1.64	up
unknown	9.49E-03	1.64	up
unknown	4.29E-03	1.64	up
unknown	5.77E-03	1.64	up
unknown	3.71E-03	1.64	up
Lithognathus mormyrus clone lmos7p10f12 mRNA sequence	4.95E-03	1.63	up
unknown	8.12E-03	1.63	up
unknown	8.43E-03	1.63	up
unknown	8.54E-03	1.63	up
Ddx5 protein [Danio rerio]	9.89E-03	1.63	up
unknown	5.09E-03	1.63	up
Mphase phosphoprotein 1 [Rattus norvegicus]	3.23E-03	1.63	up
glutathione peroxidase [Thunnus maccoyii]	5.97E-03	1.62	up
unknown	3.67E-03	1.62	up
unknown	9.45E-03	1.62	up
unknown	4.02E-03	1.61	up
serum and glucocorticoidregulated kinase [Fundulus heteroclitus]	5.33E-03	1.61	up
unknown	3.36E-03	1.59	up
unknown	1.12E-03	1.59	up
unknown	5.31E-03	1.59	up
unknown	6.12E-03	1.59	up
unknown	8.56E-04	1.59	up
pappalysin 1 (PAPPA) [Danio rerio]	9.04E-03	1.59	up
unknown	3.37E-03	1.59	up
unknown	5.92E-03	1.59	up
unknown	1.13E-03	1.58	up
rCG33097 [Rattus norvegicus]	7.17E-03	1.58	up
Chemokine (CXC motif) receptor 7b [Danio rerio]	1.60E-03	1.58	up
unknown	5.80E-03	1.57	up
unknown	6.38E-03	1.57	up
unknown	5.90E-03	1.57	up
unknown	9.79E-04	1.57	up
unknown	1.70E-03	1.56	up
unknown	7.27E-03	1.56	up
unknown	9.87E-03	1.56	up
unknown	2.52E-03	1.56	up
unknown	7.96E-03	1.56	up
unknown	6.52E-03	1.55	up
unknown	1.26E-04	1.55	up
unknown	8.23E-03	1.55	up
unknown	1.93E-03	1.54	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	3.40E-03	1.54	up
unknown	1.58E-03	1.54	up
unknown	1.52E-03	1.54	up
Chain A, Solution Structure Of The Uas Domain Of Human Ubx Domain Containing Protein 7	6.75E-03	1.53	up
unknown	6.44E-03	1.53	up
novel protein similar to vertebrate fibronectin type III domain containing 1 (FNDC1) [Danio rerio]	4.37E-03	1.53	up
unknown	2.11E-03	1.53	up
unknown	5.61E-03	1.53	up
unknown	3.98E-03	1.52	up
Glutathione reductase, mitochondrial precursor [Salmo salar]	6.37E-03	1.52	up
DNA segment, Chr 8, Brigham & Women's Genetics 1414 expressed [Mus musculus]	3.47E-03	1.52	up
unknown	4.49E-03	1.51	up
unknown	9.62E-03	1.51	up
unknown	4.21E-03	1.50	up
alpha 2,3sialyltransferase ST3Gal V [Takifugu rubripes]	2.11E-03	1.50	up
Cell cycle control protein 50B [Rana catesbeiana]	4.45E-04	1.50	up
urocanase domain containing 1 [Mus musculus]	5.57E-03	1.50	up
unknown	6.31E-03	1.49	up
unknown	6.02E-03	1.49	up
unknown	9.54E-03	1.49	up
Splicing factor 3B subunit 3 [Salmo salar]	5.15E-04	1.48	up
Multidrug and toxin extrusion protein 1 [Salmo salar]	1.17E-03	1.48	up
unknown	5.19E-03	1.48	up
Lithognathus mormyrus clone lmos2p09h05 mRNA sequence	4.83E-03	1.48	up
unknown	3.03E-03	1.48	up
unknown	7.47E-03	1.48	up
unknown	6.67E-03	1.48	up
rCG24089, isoform CRA_c [Rattus norvegicus]	9.52E-04	1.48	up
unknown	5.28E-03	1.48	up
unknown	9.03E-03	1.47	up
solute carrier family 31 (copper transporters),member 1 [Sparus aurata]	4.19E-03	1.47	up
Translocationassociated membrane protein 2 [Salmo salar]	1.01E-04	1.47	up
unknown	7.83E-03	1.46	up
lysophosphatidic acid receptor 2 [Xenopus (Silurana) tropicalis]	7.73E-03	1.46	up
unknown	6.98E-03	1.46	up
Peroxisomal 3,2transenoylCoA isomerase [Salmo salar]	3.47E-03	1.46	up
unknown	9.40E-03	1.46	up
Hypoxia upregulated protein 1 precursor [Salmo salar]	6.61E-03	1.45	up
TFIIAgamma	6.26E-03	1.45	up
unknown	3.09E-03	1.45	up
unknown	5.77E-03	1.45	up
unknown	1.78E-03	1.45	up
pob [Salmo salar]	6.11E-03	1.44	up
pumiliolike protein 1 [Xenopus laevis]	2.09E-03	1.44	up
unknown	7.15E-03	1.44	up
unknown	1.55E-03	1.44	up
unknown	4.67E-03	1.44	up
chromatin modifying protein 4C [Danio rerio]	3.82E-03	1.43	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	1.21E-03	1.43	up
Stromal cellderived factor 2like protein 1 [Salmo salar]	9.92E-03	1.43	up
Vacuolar proteinsortingassociated protein 36 [Salmo salar]	1.24E-03	1.43	up
deltex homolog 3 [Xenopus (Silurana) tropicalis]	5.09E-03	1.43	up
Arntl2 protein [Danio rerio]	9.45E-03	1.43	up
unknown	5.39E-03	1.42	up
unknown	1.36E-03	1.42	up
unknown	4.84E-03	1.42	up
unknown	7.42E-03	1.42	up
Srp72 protein [Danio rerio]	3.02E-03	1.42	up
unknown	7.30E-03	1.42	up
unknown	4.99E-03	1.42	up
3phosphoinositidedependent protein kinase 1 [Salmo salar]	5.45E-03	1.42	up
unknown	2.54E-03	1.42	up
unknown	3.19E-03	1.41	up
Fucose1phosphate guanylyltransferase [Salmo salar]	3.29E-03	1.41	up
unknown	3.92E-03	1.41	up
Oryzias latipes eya1 mRNA for eyes absent 1, partial cds	2.93E-03	1.41	up
unknown	3.21E-03	1.41	up
unknown	4.45E-03	1.40	up
unknown	9.41E-03	1.40	up
Nicotinic acid receptor 1 [Salmo salar]	4.25E-03	1.40	up
unknown	5.94E-03	1.40	up
1110020G09Rik protein [Mus musculus]	6.60E-04	1.40	up
unknown	7.57E-03	1.40	up
unknown	1.10E-03	1.40	up
unknown	6.99E-03	1.40	up
Transposable element Tc1 transposase [Rana catesbeiana]	5.39E-03	1.39	up
Suppressor of actin mutations 1like protein B	3.37E-03	1.39	up
unknown	2.85E-03	1.39	up
unknown	2.91E-03	1.39	up
unknown	1.19E-03	1.39	up
cohesin subunit XSA2 [Xenopus laevis]	1.11E-03	1.39	up
Takifugu rubripes TPM41 mRNA for tropomyosin41, complete cds, spliced variant:exon 1b, 3, 4, 5, 6b, 7, 8 and 9d	3.83E-04	1.39	up
protein kinase C substrate 80KH [Danio rerio]	2.50E-03	1.38	up
unknown	2.03E-03	1.38	up
unknown	5.02E-03	1.38	up
unknown	4.22E-03	1.38	up
Endoplasmic reticulumGolgi intermediate compartment protein 2 [Salmo salar]	7.48E-03	1.37	up
unknown	6.60E-03	1.37	up
unknown	2.54E-03	1.37	up
Sec23like protein B [Danio rerio]	3.28E-03	1.37	up
Eptatretus stoutii nonfunctional variable lymphocyte receptor B (VLRB) gene, complete sequence	4.41E-03	1.37	up
unknown	8.08E-03	1.36	up
unknown	4.90E-03	1.36	up
Sorting nexin14 [Salmo salar]	2.38E-03	1.36	up
unknown	9.37E-04	1.36	up
Rana lessonae mRNA for Pl10related protein (pl10 gene)	5.68E-03	1.36	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
Protein transport protein Sec61 subunit gamma	8.01E-03	1.36	up
unknown	1.38E-03	1.36	up
unknown	2.05E-03	1.35	up
unknown	9.08E-03	1.35	up
Mus musculus chromosome 8, clone RP23161H3, complete sequence	2.50E-03	1.35	up
reverse transcriptaselike protein [Salmo salar]	1.78E-03	1.35	up
unknown	8.87E-03	1.35	up
unknown	4.55E-03	1.35	up
unknown	7.89E-03	1.35	up
unknown	7.94E-03	1.35	up
unknown	6.97E-03	1.34	up
unknown	1.15E-03	1.34	up
unknown	4.82E-03	1.34	up
heat shock protein 10 [Monopterus albus]	8.57E-03	1.34	up
proline rich Gla (Gcarboxyglutamic acid) 4 (transmembrane) [Homo sapiens]	4.97E-03	1.34	up
39S ribosomal protein L14, mitochondrial precursor [Salmo salar]	3.90E-03	1.34	up
unknown	6.78E-03	1.34	up
unknown	8.07E-03	1.34	up
unknown	9.06E-03	1.34	up
mannosePdolichol utilization defect 1b [Danio rerio]	4.23E-04	1.33	up
unknown	3.86E-03	1.33	up
unknown	7.25E-03	1.33	up
unknown	8.37E-03	1.33	up
RIKEN cDNA 2700097O09 gene [Mus musculus]	6.59E-03	1.32	up
Sparus aurata growth hormone gene, complete cds	8.27E-03	1.32	up
caspase9 [Dicentrarchus labrax]	1.41E-03	1.32	up
unknown	6.23E-03	1.32	up
UPF0420 protein C16orf58 homolog	6.71E-03	1.31	up
Mitochondrial inner membrane protease subunit 1 [Salmo salar]	2.45E-03	1.31	up
Probable palmitoyltransferase ZDHHC4 [Salmo salar]	1.02E-03	1.31	up
protein inhibitor of activated STAT, 3, isoform CRA_a [Homo sapiens]	9.22E-03	1.31	up
unknown	8.30E-03	1.31	up
unknown	7.05E-03	1.30	up
Hydroxysteroid (17beta) dehydrogenase 7 [Xenopus tropicalis]	3.42E-03	1.30	up
Angelman syndrome 2 [Danio rerio]	4.07E-03	1.30	up
Vacuolarsorting protein SNF8 [Salmo salar]	6.31E-03	1.30	up
unknown	4.94E-04	1.30	up
Sparus aurata growth hormone receptor type II (GHRII) gene, promoter region and exon 1	8.88E-03	1.30	up
unknown	9.99E-03	1.29	up
unknown	3.56E-03	1.29	up
unknown	1.86E-03	1.29	up
15 kDa selenoprotein precursor [Oncorhynchus mykiss]	4.25E-03	1.29	up
unknown	7.72E-03	1.29	up
unknown	7.19E-04	1.29	up
RASIP1 protein [Homo sapiens]	2.09E-03	1.28	up
unknown	6.33E-04	1.28	up
unknown	7.31E-04	1.28	up
unknown	7.13E-03	1.28	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.89E-03	1.28	up
Transposable element Tcb1 transposase [Salmo salar]	3.30E-03	1.27	up
unknown	2.13E-03	1.27	up
unknown	9.21E-03	1.26	up
2	4.86E-03	1.26	up
histone lysine demethylase [Danio rerio]	7.80E-03	1.26	up
unknown	3.71E-03	1.26	up
unknown	9.32E-03	1.26	up
Nuclear receptor subfamily 3 group A member 1	7.97E-03	1.25	up
unknown	7.50E-03	1.25	up
Cyclindependent kinase 2interacting protein [Salmo salar]	7.94E-03	1.25	up
magnesium transporter 1 [Homo sapiens]	9.63E-03	1.24	up
unknown	6.37E-03	1.24	up
Leydig cell tumor 10 kDa protein [Oncorhynchus mykiss]	6.22E-03	1.24	up
SNAP23 [Lateolabrax japonicus]	9.97E-03	1.24	up
PREDICTED: similar to lysosomal alphaNacetyl glucosaminidase [Danio rerio]	4.23E-03	1.23	up
BCL2like 12 [Bos taurus]	2.81E-03	1.23	up
CDK5 regulatory subunitassociated protein 1like 1 [Salmo salar]	5.76E-03	1.23	up
Ncadherin precursor zebra fish	8.32E-03	1.23	up
unknown	5.02E-03	1.23	up
unknown	6.91E-04	1.23	up
novel protein similar to vertebrate ER degradation enhancer, mannosidase alphalike 3 (EDEM3) [Danio rerio]	4.66E-03	1.22	up
unknown	5.68E-03	1.22	up
4 [Salmo salar]	6.24E-03	1.22	up
PostGPI attachment to proteins factor 2 [Salmo salar]	3.84E-03	1.22	up
unknown	4.41E-03	1.22	up
protein prenyltransferase alpha subunit repeat containing 1, isoform CRA_d [Rattus norvegicus]	4.08E-04	1.22	up
Antizyme inhibitor 1 [Salmo salar]	5.36E-03	1.21	up
unknown	3.22E-04	1.21	up
oxidativestress responsive 1a [Danio rerio]	7.25E-03	1.21	up
PREDICTED: similar to novel G proteincoupled receptor protein [Danio rerio]	6.56E-03	1.21	up
Transmembrane protein 49 [Salmo salar]	9.64E-03	1.20	up
wibg homolog [Salmo salar]	2.80E-03	1.20	up
unknown	3.14E-03	1.20	up
unknown	5.22E-03	1.20	up
Nicotinamide mononucleotide adenylyltransferase 1 [Salmo salar]	4.90E-03	1.19	up
beta 1 [Danio rerio]	2.45E-04	1.19	up
unknown	2.84E-03	1.19	up
PHD fingerlike domaincontaining protein 5A [Rana catesbeiana]	4.20E-03	1.19	up
unknown	3.49E-03	1.19	up
unknown	5.39E-03	1.17	up
leucine aminopeptidase 3 [Danio rerio]	9.48E-03	1.17	up
YQ007 protein [Salmo salar]	7.24E-03	1.16	up
Interferonrelated developmental regulator 1 [Salmo salar]	7.55E-04	1.16	up
unknown	4.30E-03	1.15	up
unknown	1.67E-03	1.15	up
unknown	7.84E-03	1.14	up
Dolichol phosphatemannose biosynthesis regulatory protein [Oncorhynchus mykiss]	8.27E-03	1.13	up

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
TM2 domaincontaining protein 1 [Salmo salar]	7.09E-03	1.13	up
E3 ubiquitinprotein ligase MARCH3 [Salmo salar]	8.12E-03	1.12	up
unknown	7.76E-03	1.12	up
cytoplasmic [Salmo salar]	9.65E-03	1.11	up
4activating kinase	3.42E-03	1.11	up
Homo sapiens striatin, calmodulin binding protein 3 (STRN3), transcript variant 1, mRNA	3.84E-03	1.11	up
unknown	4.19E-03	1.08	up
Chain A, Crystal Structure Of HoloCrbp From Zebrafish Chain A, Crystal Structure Of ApoCrbp From Zebrafish	7.91E-03	15.50	down
unknown	2.47E-04	9.30	down
secretory calciumbinding phosphoprotein 5 [Takifugu rubripes]	2.71E-05	6.76	down
alcohol dehydrogenase Class VI [Oryzias latipes]	1.48E-03	6.19	down
unknown	6.50E-03	5.93	down
agouti related protein 2 [Takifugu rubripes]	2.56E-05	5.63	down
Retinoidbinding protein 7 [Salmo salar]	3.14E-03	5.55	down
unknown	5.66E-03	4.62	down
Adipophilin [Salmo salar]	3.49E-04	4.47	down
unknown	7.93E-03	4.26	down
unknown	5.33E-03	4.00	down
unknown	9.32E-03	3.53	down
ubiquitin [Dictyostelium discoideum AX4]	2.14E-03	3.45	down
unknown	9.60E-03	3.02	down
Ntd5 protein [Danio rerio]	3.11E-03	2.83	down
unknown	5.87E-03	2.83	down
unknown	3.45E-03	2.74	down
unknown	2.00E-03	2.51	down
unknown	8.29E-03	2.48	down
unknown	4.28E-03	2.46	down
myocilin [Danio rerio]	3.41E-03	2.27	down
unknown	7.85E-03	2.25	down
unknown	6.05E-03	2.25	down
suppressor of cytokine signaling 1 [Danio rerio]	2.06E-03	2.19	down
unknown	1.96E-03	2.13	down
Lithognathus mormyrus clone lmos2p03c10 mRNA sequence	1.67E-03	2.11	down
unknown	4.71E-04	2.07	down
unknown	4.25E-04	2.06	down
granzymelike III [Ictalurus punctatus]	8.46E-03	2.04	down
unknown	2.55E-03	2.01	down
Lipocalin precursor [Salmo salar]	2.87E-03	2.00	down
FBP32 precursor [Morone saxatilis]	5.48E-03	2.00	down
unknown	2.32E-03	1.99	down
VHSVinduced protein [Epinephelus coioides]	6.28E-03	1.94	down
unknown	1.09E-03	1.93	down
unknown	4.17E-03	1.89	down
zinc finger protein 706, isoform CRA_c [Mus musculus]	6.07E-03	1.87	down
unknown	4.78E-03	1.87	down
myelin associated glycoprotein isoform a precursor variant [Homo sapiens]	9.36E-03	1.85	down
unknown	5.98E-03	1.84	down
Lithognathus mormyrus clone lmos8p01h10 mRNA sequence	2.90E-03	1.77	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	5.46E-03	1.76	down
GH17343 [Drosophila grimshawi]	3.81E-03	1.76	down
unknown	9.68E-03	1.74	down
unknown	9.10E-03	1.73	down
COMM domain containing 10, isoform CRA_c [Rattus norvegicus]	7.02E-03	1.73	down
unknown	3.26E-03	1.72	down
unknown	8.57E-03	1.72	down
FAM60A [Salmo salar]	5.67E-03	1.72	down
unknown	1.30E-03	1.71	down
CAPN1 protein [Danio rerio]	8.39E-03	1.70	down
unknown	3.57E-03	1.70	down
unknown	6.53E-04	1.69	down
unknown	9.08E-03	1.69	down
unknown	9.98E-04	1.69	down
unknown	9.01E-03	1.68	down
unknown	7.59E-03	1.67	down
SH3 protein expressed in lymphocytes [Salmo salar]	3.09E-03	1.66	down
viral Atype inclusion protein [Trichomonas vaginalis G3]	4.46E-03	1.66	down
unknown	5.20E-03	1.65	down
unknown	2.36E-04	1.64	down
vascular endothelial growth factor 189 [Ochotona curzoniae]	1.30E-03	1.64	down
unknown	2.76E-03	1.64	down
unknown	6.53E-04	1.64	down
unknown	1.70E-03	1.63	down
unknown	2.42E-03	1.60	down
CD8 alpha [Sparus aurata]	9.36E-03	1.60	down
unknown	8.45E-03	1.60	down
GATS [Salmo salar]	8.56E-03	1.59	down
Dicentrarchus labrax aquaporin 3 (AQP3) mRNA, complete cds	1.26E-03	1.59	down
unknown	1.66E-03	1.58	down
unknown	8.89E-03	1.58	down
unknown	9.97E-03	1.58	down
15hydroxyprostaglandin dehydrogenase [Salmo salar]	6.51E-04	1.58	down
1433 protein gamma2 [Salmo salar]	1.76E-03	1.57	down
adrenomedullin1 [Takifugu rubripes]	8.81E-04	1.57	down
exosomal core protein CSL4 [Bos taurus]	6.45E-04	1.57	down
Ebox binding protein 2 [Ictalurus punctatus]	1.45E-03	1.56	down
unknown	2.47E-03	1.56	down
unknown	5.53E-03	1.54	down
unknown	9.23E-03	1.53	down
unknown	1.71E-03	1.53	down
unknown	2.66E-03	1.53	down
unknown	2.29E-03	1.53	down
unknown	3.62E-03	1.52	down
Salmo salar clone ssalrgf001325, novel cds	7.19E-03	1.52	down
unknown	3.16E-03	1.51	down
unknown	5.72E-03	1.51	down
Aa2141 [Rattus norvegicus]	9.61E-03	1.51	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	4.78E-03	1.51	down
Salmo salar clone ssalrgf502253 Transmembrane protein 16H putative	4.98E-03	1.51	down
Astatotilapia burtoni early growth response 1 mRNA, complete cds	3.65E-03	1.50	down
lysosomalassociated membrane protein 3 [Bos taurus]	4.65E-03	1.50	down
Lipocalin precursor [Esox lucius]	5.46E-03	1.49	down
unknown	5.02E-03	1.49	down
unknown	3.21E-04	1.49	down
unknown	4.26E-03	1.48	down
unknown	7.47E-03	1.48	down
unknown	5.06E-03	1.47	down
unknown	3.65E-03	1.47	down
unknown	2.66E-03	1.47	down
unknown	1.45E-03	1.47	down
catalase [Oplegnathus fasciatus]	8.98E-03	1.46	down
unknown	1.73E-03	1.46	down
Tcell surface glycoprotein CD3 zeta chain precursor [Oncorhynchus myl	1.47E-03	1.46	down
unknown	3.03E-03	1.45	down
unknown	9.69E-03	1.45	down
unknown	9.47E-04	1.45	down
unknown	1.62E-03	1.45	down
unknown	9.82E-04	1.45	down
Samd9l protein [Xenopus tropicalis]	7.36E-03	1.44	down
unknown	3.82E-03	1.44	down
unknown	5.99E-04	1.43	down
unknown	3.76E-03	1.43	down
unknown	2.66E-03	1.43	down
Charged multivesicular body protein 5 [Oncorhynchus mykiss]	8.97E-03	1.43	down
Tcell receptor beta chain [Sparus aurata]	8.04E-04	1.43	down
RIKEN cDNA B230219D22 [Mus musculus]	7.01E-03	1.43	down
unknown	9.45E-03	1.42	down
unknown	5.31E-03	1.42	down
CDC42 effector protein (Rho GTPase binding) 3 [Bos taurus]	4.79E-03	1.42	down
unknown	5.49E-03	1.42	down
unknown	1.23E-03	1.42	down
Transcription factor PU.1 [Salmo salar]	2.88E-03	1.41	down
unknown	4.79E-03	1.41	down
unknown	7.24E-04	1.41	down
unknown	2.48E-03	1.41	down
unknown	1.60E-03	1.40	down
b [Danio rerio]	7.13E-03	1.40	down
unknown	9.94E-03	1.40	down
Lithognathus mormyrus clone lithmor94 mRNA sequence	5.70E-04	1.40	down
unknown	6.56E-03	1.39	down
dsRNAdependent protein kinase [Paralichthys olivaceus]	3.92E-03	1.39	down
unknown	8.85E-04	1.39	down
unknown	8.94E-04	1.39	down
unknown	7.24E-03	1.39	down
unknown	8.72E-03	1.39	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	7.59E-03	1.38	down
unknown	7.94E-03	1.38	down
unknown	9.03E-03	1.38	down
Endonuclease domaincontaining 1 protein precursor [Salmo salar]	6.85E-06	1.38	down
PREDICTED: im:6912447 [Danio rerio]	8.77E-03	1.37	down
Dicentrarchus labrax caspase3 (CASP3) gene, complete cds	6.46E-03	1.37	down
unknown	8.68E-03	1.37	down
unknown	7.88E-03	1.37	down
unknown	5.77E-03	1.36	down
unknown	2.24E-03	1.36	down
unknown	5.79E-03	1.36	down
unknown	2.68E-03	1.36	down
unknown	5.49E-03	1.36	down
unknown	9.97E-03	1.36	down
unknown	6.88E-04	1.36	down
unknown	8.76E-03	1.35	down
Cell division protein kinase 2 [Salmo salar]	2.42E-03	1.35	down
unknown	6.91E-03	1.35	down
unknown	5.42E-03	1.34	down
unknown	1.73E-03	1.34	down
TIP41like protein [Salmo salar]	2.93E-03	1.34	down
Prefoldin subunit 3 [Salmo salar]	3.95E-03	1.34	down
Transcription factor Adf1 [Esox lucius]	7.67E-03	1.34	down
Thyroid transcription factor 1associated protein 26 homolog [Salmo sal	9.60E-03	1.33	down
unknown	8.94E-04	1.33	down
unknown	3.34E-03	1.33	down
unknown	8.53E-03	1.33	down
unknown	1.54E-03	1.33	down
unknown	5.77E-03	1.33	down
CD18 protein [Oncorhynchus mykiss]	6.45E-03	1.33	down
unknown	8.37E-03	1.33	down
Fugu rubripes gammaaminobutyric acid receptor beta subunit gene, par	3.92E-03	1.33	down
unknown	5.09E-03	1.32	down
member 1 [Danio rerio]	5.12E-03	1.32	down
BEN domain containing 3 [Mus musculus]	2.98E-03	1.32	down
unknown	3.36E-03	1.32	down
unknown	3.59E-03	1.31	down
PHD finger protein 2 [Mus musculus]	1.27E-03	1.31	down
unknown	1.24E-04	1.31	down
receptorlike tyrosine kinase [Rattus norvegicus]	6.72E-03	1.31	down
unknown	4.03E-03	1.31	down
Myosin9 [Salmo salar]	6.32E-04	1.30	down
unknown	6.63E-03	1.30	down
unknown	2.17E-03	1.30	down
unknown	5.49E-03	1.30	down
Anaphasepromoting complex subunit 13 [Salmo salar]	3.11E-03	1.30	down
unknown	4.99E-03	1.30	down
Response gene to complement 32 protein [Salmo salar]	5.03E-03	1.29	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
ATAD2 protein [Homo sapiens]	1.09E-03	1.29	down
mFLJ00348 protein [Mus musculus]	7.84E-03	1.29	down
unknown	2.40E-03	1.29	down
unknown	5.54E-03	1.29	down
guanine nucleotide binding protein [Takifugu rubripes]	3.22E-03	1.29	down
unknown	9.73E-04	1.29	down
unknown	1.06E-04	1.29	down
telomeric Rap1 [Xenopus laevis]	8.50E-03	1.29	down
unknown	1.22E-03	1.28	down
unknown	8.20E-03	1.28	down
unknown	1.34E-03	1.28	down
rCG62161, isoform CRA_a [Rattus norvegicus]	5.78E-03	1.28	down
Paralichthys olivaceus mRNA for perforin, complete cds	9.22E-04	1.28	down
radixin isoform 1 [Danio rerio]	7.17E-03	1.28	down
unknown	5.34E-03	1.28	down
unknown	6.54E-03	1.28	down
unknown	9.09E-03	1.27	down
unknown	3.68E-03	1.27	down
gammalike [Danio rerio]	7.18E-03	1.27	down
unknown	1.48E-03	1.27	down
unknown	5.31E-04	1.27	down
kelch domain containing 4 [Danio rerio]	7.07E-03	1.27	down
unknown	5.29E-03	1.27	down
unknown	6.01E-03	1.26	down
member 1b [Danio rerio]	3.63E-03	1.26	down
ZNF554 protein [Homo sapiens]	5.68E-03	1.26	down
unknown	7.17E-03	1.26	down
unknown	9.08E-03	1.26	down
unknown	5.02E-04	1.26	down
DNAbinding protein [Takifugu rubripes]	4.40E-03	1.25	down
unknown	2.53E-03	1.25	down
Interactor protein for cytohesin exchange factors 1 [Salmo salar]	1.56E-03	1.25	down
unknown	9.53E-05	1.25	down
IK cytokine [Danio rerio]	5.55E-03	1.25	down
CCHC domain containing 8 [Danio rerio]	6.67E-03	1.24	down
unknown	5.98E-03	1.24	down
unknown	2.08E-03	1.24	down
cohesin subunit Rad21 [Oryzias latipes]	4.05E-03	1.24	down
Marosatherina ladigesi large subunit ribosomal RNA gene, partial seque	8.41E-04	1.24	down
unknown	9.09E-03	1.24	down
unknown	1.76E-03	1.24	down
unknown	2.96E-03	1.23	down
unknown	2.91E-05	1.23	down
unknown	2.19E-03	1.23	down
unknown	1.61E-03	1.23	down
septin 2 [Danio rerio]	5.22E-03	1.23	down
17 beta hydroxysteroid dehydrogenase 4 [Salmo trutta fario]	2.00E-03	1.23	down
unknown	5.81E-03	1.23	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
unknown	4.32E-03	1.22	down
unknown	6.79E-03	1.22	down
unknown	4.18E-03	1.22	down
unknown	5.92E-03	1.22	down
Chain A, Solution Structure Of Three ZfC2h2 Domains From Mouse Prot	8.50E-03	1.22	down
unknown	6.77E-03	1.21	down
YY1 associated factor 2 [Danio rerio]	2.13E-03	1.21	down
unknown	8.47E-03	1.21	down
unknown	1.32E-03	1.21	down
unknown	8.49E-03	1.21	down
endoalpha [Mus musculus]	6.69E-03	1.21	down
unknown	7.09E-03	1.20	down
Danio rerio ankyrin repeat and FYVE domain containing 1 (ankfy1), mR	2.91E-03	1.20	down
copine III [Danio rerio]	1.84E-03	1.20	down
Cbx5 [Astatotilapia burtoni]	5.03E-03	1.20	down
sorbin and SH3 domain containing 3 [Danio rerio]	5.71E-03	1.19	down
unknown	9.38E-03	1.19	down
unknown	8.47E-03	1.19	down
unknown	6.86E-03	1.18	down
3 complex subunit 4 [Salmo salar]	1.32E-03	1.18	down
unknown	8.78E-03	1.18	down
Mediator of RNA polymerase II transcription subunit 22 [Esox lucius]	5.74E-04	1.18	down
unknown	8.41E-03	1.18	down
potassium channel tetramerisation domain containing 6 [Danio rerio]	7.48E-03	1.18	down
unknown	8.22E-03	1.18	down
unknown	3.12E-03	1.18	down
unknown	6.70E-03	1.17	down
unknown	5.97E-03	1.17	down
peroxisomal membrane protein 2, isoform CRA_b [Rattus norvegicus]	2.23E-03	1.17	down
unknown	3.20E-03	1.17	down
SMC3 protein [Takifugu rubripes]	1.68E-03	1.17	down
unknown	3.67E-03	1.17	down
unknown	5.36E-03	1.16	down
unknown	9.72E-03	1.16	down
unknown	5.35E-03	1.16	down
Abhydrolase domaincontaining protein 14A [Salmo salar]	1.68E-03	1.15	down
unknown	4.47E-03	1.15	down
unknown	4.12E-04	1.15	down
unknown	4.99E-03	1.15	down
UNQ655/PRO1286 precursor [Salmo salar]	7.45E-03	1.15	down
Transmembrane protein 120A [Salmo salar]	3.39E-03	1.14	down
unknown	8.32E-03	1.14	down
unknown	5.51E-03	1.14	down
unknown	9.41E-03	1.13	down
unknown	3.20E-03	1.13	down
Rho GTPase activating protein 27 [Rattus norvegicus]	1.91E-03	1.12	down
unknown	7.97E-03	1.12	down
unknown	1.81E-03	1.11	down

TABLE S8: (*...continued*)

Diet B day 14 vs Diet B day 28			
Description	**p-value**	**FCA**	**Regulation**
Endothelial differentiationrelated factor 1 homolog [Salmo salar]	8.75E-03	1.10	down
unknown	1.84E-04	1.10	down
unknown	5.94E-03	1.10	down
unknown	7.49E-03	1.10	down
unknown	4.06E-03	1.10	down
unknown	2.03E-03	1.10	down
unknown	9.56E-03	1.09	down
Takifugu rubripes HoxDa gene cluster, complete sequence	5.37E-03	1.09	down
transcription factor XlDP1 [Xenopus laevis, embryos, Peptide, 409 aa]	8.53E-03	1.09	down
unknown	3.19E-03	1.09	down
Xiap protein [Danio rerio]	3.62E-03	1.09	down
unknown	8.43E-03	1.09	down
Salmo salar clone ssalrgf505003 Active breakpoint cluster regionrelatec	3.23E-03	1.07	down