infected fish, however proteases populations differed in metalloproteases and serine proteases when comparing infected and noninfected fish.
Keywords: Greater amberjack, Mucus, Skin, Proteomic, Ectoparasites

Corresponding author.
E-mail address: alvaro.montero@ulpgc.es (A. Fernández-Montero).
* These authors have contributed equally to this work.

O-115.

Comparative transcriptome analysis of pilchard orthomyxovirus (POMV) and infectious salmon anaemia virus (ISAV)

F. Samsing [1], J. Hoad [2], P. Mohr [2], M. Dearnley [2], J.W. Wynne [1,#].

[1] CSIRO Agriculture and Food, Aquaculture program, Hobart, Tasmania, Australia
[2] CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia

Abstract
Pilchard orthomyxovirus (POMV) is an emerging virus of concern to the Tasmanian
Atlantic salmon industry. Originally isolated from pilchards in South Australia in 1998, this virus has now caused several high mortality events in Tasmanian farmed Atlantic salmon. Despite its classification as an orthomyxovirus, POMV isphylogenetically divergent from ISAV. While previous research has produced a formal case definition for clinical POMV, the molecular events that underpin viral infection have not been characterized. To this end we have undertaken a comparative transcriptome analysis of the response of Atlantic salmon kidney cells (ASK) to both POMV and ISAV. Despite their genetic divergence, both orthomyxoviruses induced significant, and in some cases similar, innate antiviral responses. Early up-regulation of the host pathogen recognition receptors, RIG-I and TLR3, was observed in response to both viruses and triggered downstream interferon responses. Analysis of transcription factor binding sites in the up-regulated gene sets revealed that the host response to both viruses was largely driven by interferon regulatory factor 1 and 2. Unique host responses were also observed for each virus which are likely a consequence of virus divergence. The potential to exploit these early host response genes as subclinical biomarkers specific to POMV will be discussed.
Keywords: Orthomyxovirus, transcriptome, interferon, biomarker, host-pathogen interaction

Corresponding author.
E-mail address: james.wynne@csiro.au (J.W. Wynne).

O-116.

Characterization of flounder (*Paralichthys olivaceus*) CD4+ T lymphocyte subsets in response to Th-type antigens

Hongfei Tian [1], Jing Xing [1,2,#], Xiaoqian Tang [1], Xiuzhen Sheng [1], Wenbin Zhan [1,2].

[1] Laboratory of Pathology and Immunology of Aquatic Animals, KLMME, Ocean University of China, Qingdao, 266003, PR China
[2] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, No. 1 Wenhai Road, Aoshanwei Town, Qingdao, China

Abstract
The CD4+ T lymphocytes play crucial roles in the adaptive immune system. Naive CD4+ Th cells differentiate into a variety of effector T lymphocytes subsets, such as Th1, Th2, Th17 and regulatory (Treg) cells. These CD4+ T cells widely involved in immune regulation, immune pathogenesis and host defence through subsequent secretion of effector and regulatory cytokines. Two CD4 homologues have been reported in flounder (*Paralichthys olivaceus*), one is like mammalian CD4 molecules, containing four extracellular Ig-like domains, named as CD4-1, and the other is a CD4-like molecule, containing two or three Ig-like domains, termed CD4-2. In this study, identification of CD4-1+ and CD4-2+ T lymphocytes subsets and the immune response to Th-type antigens in flounder were investigated. The epitopes peptides of CD4-1 and CD4-2 molecule were screened with high hydrophilicity, accessibility, flexibility, antigenicity and specificity. Two peptides were synthesized and immunized to the mouse, and then the monoclonal antibodies (mAbs) against flounder CD4-1 and CD4-2 were produced, respectively. The mAbs had high specificity in identifying flounder CD4-1+ and CD4-2+ T lymphocytes subsets. And then, three Th-type antigens, poly (I:C), PMA and β-glucan, were injected to flounder, respectively, the percentages of CD4-1+ and CD4-2+ T lymphocytes and the transcription factors and cytokines in sorted CD4+ cells subsets were detected. The results showed, CD4-1+ and CD4-2+ cells in peripheral blood, spleen and head kidney were all increased after stimulation. Notably, CD4-2+ cells were give stronger response to poly (I:C), which indicated that CD4-2+ cells may play a main role in the Th1-related immune responses. While the proliferation of CD4-1+ cells were showed no difference to three antigens. The Th cells transcription factors and related cytokines in sorted CD4+ cells were sharply up-regulated. These results demonstrate that the CD4+ cells in flounder have potentials to differentiate into different Th cells similar to mammalian.
Keywords: CD4+ T lymphocytes; monoclonal antibody; antigens; immune response

Corresponding author.
E-mail address: xingjing@ouc.edu.cn (J. Xing).

O-117.

Functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on immune response, stress and disease resistance

A. Serradell [1,#,], S. Torrecillas [1,*], A. Makol [2,*], F. Acosta [1,*], V. Valdenegro [3,*], D. Montero [1,*].*

[1] Grupo de Investigación en Acuicultura. Instituto Ecoaqua.Universidad de Las Palmas de Gran Canaria, Gran Canaria, Spain
[2] Delacon Biotechnik GmbH, Weissenwolffstrasse 14, 4221 Steyregg, Austria
[3] Biomar A/S. BioMar AS, POB 1282 Sluppen, N-7462, Trondheim, Norway

Abstract
The use of terrestrial raw materials to replace fish meals and oils in fish diets may affect fish growth performance and health. In the last years functional additives have been profiled as good candidates to reduce the effects on health and disease resistance derived from this replacement, via reinforcement of the fish immune system. On the present study four isoenergetic and isonitrogenous diets with 10% FM and 6% FO levels supplemented with 5000 ppm galactomannanoligosaccharides (GMOS), 200 ppm of a mixture of essential oils (PHYTO) and a combination of both products, 5000 ppm galactomannanoligosaccharides plus 200 ppm of a mixture of essential oils (GMOSPHYTO). Fish were fed the experimental diets in triplicate for 9 weeks and then fish were subjected to a stress confinment (S treatment) challenge combined or not with an experimental intestinal infection with *Vibrio anguillarum* (SI treatment). Along the challenge test, selected stress and immunological parameters were evaluated at 2h, 24h and 7 days post S or SI treatment. As stress indicators, plasmatic cortisol and glucose levels as well as gene expression of *cyp11β-hydroxylase, hypoxia.inducible factor, steroidogenic acute regulatory protein, heat shock protein 70 and heat shock protein 90* (CYP11β, HIF, StAR, HSP70 and HSP90) were measured. As immune response markers, serum and skin mucus lysozime levels, bactericidal and peroxidase activities as well as gen expression of *Caspase -3* (Casp 3) and *interleukin 1β* (IL-1β) were measured. Besides, fish survival rate to *V. anguillarum* was monitored at the end of the challenge test. Fish fed GMOS and PHYTO diets increased fish relative

percent survival in relation to fish fed control diet. PHYTO diet reinforced fish capacity of stress response via protection of head kidney leucocytes from stress-related apoptotic processes. Additionally, dietary supplementation with GMOS and PHYTO compounds increased fish serum lysozime and peroxidase activities.

Keywords: Functional feeds, prebiotics, phytogenics, stress response, immune response

Corresponding author.
E-mail address: antonio.serradell101@alu.ulpgc.es (A. Serradell).
* These authors have contributed equally to this work.

O-118.

Dietary and phytogenics in low fish meal and fish oil diets for *Dicentrarchus labrax*: An effective tool to gut health and disease resistance

S. Torrecillas [1,#], A. Serradell [1], A. Makol [2], G. Terova [3], E. Gini [3], V. Valdenegro [4], M.S. Izquierdo [1], F. Acosta [1], D. Montero [1].

[1] Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214, Telde, Las Palmas, Canary Islands, Spain
[2] Delacon Biotechnik GmbH, Weissenwolffstrasse 14, 4221, Steyregg, Austria
[3] Department of Biotechnology and Life Sciences, University of Insubria, Via J.H. Dunant, 3, 21100 Varese, Italy
[4] Biomar A/S. BioMar AS, POB 1282 Sluppen, N-7462, Trondheim, Norway

Abstract
Fish intestinal mucosal surface supposes a potential route of entrance for pathogenic bacteria. An inflammatory gut reaction can be induced by a variety of factors, such as infection, stress or changes in feed composition. Particularly, for European sea bass (*Dicentrarchus labrax*) feeding low fishmeal (FM) and fish oil (FO) diets results in a gut inflammation-like status. The use of functional additives such as prebiotics and phytogenics simultaneously with a low FM/FO based diet may help to buffer these associated gut health negative-side effects. Four low FM/FO (10%/6%) diets for European sea bass containing galactomannan oligosaccharides (GMOS), a mixture of garlic oil and labiatae oils (PHYTO) were fed for 63 days before exposition to an intestinal *Vibrio anguilarum* infection in combination with a crowding stress. In order to evaluate functional diets efficacy in terms of gut mucosal health maintenance, structural, cellular and immune intestinal status was evaluated by optical and electron microscopy and gene expression analyses. A semi-automated software was adapted to determine variations on goblet cells area and mucosal mucus coverage along the challenge test. Functional diets fed did not affect growth performance, however PHYTO and GMOS dietary inclusion reduced European sea bass susceptibility to *V. anguillarum* after 7 days of challenge test. Rectum (post-ileorectal valve) presented longer ($p=0.001$) folds than posterior gut (pre-ileorectal valve), whereas posterior gut presented thicker submucosa ($p=0.001$) and higher mucus coverage as a result of an increased cell density compared to rectum. Functional diets did not affect mucosal folds length or the grade of granulocytes and lymphocytes infiltration in both intestinal segments. However, fish fed GMOS ($F=14.53$; $p=0.001$) and PHYTO ($F=5.52$; $p=0.019$) presented less posterior gut fold area covered by goblet cells. PHYTO ($F=3.95$; $p=0.049$) reduced posterior gut goblet cell size and increased rodlet cells density ($F=3.604$; $p=0.068$). Dietary GMOS reduced submucosa thickness ($F=51.31$; $p=0.001$) and increased rodlet cells density ($F=3.604$; $p=0.068$) in rectum. Structural TEM analyses revealed a normal intestinal morphological pattern. GMOS increased rectum microvilli length. PHYTO increased ($p\leq0.10$) *Ocln*, *N-Cad* and *Cad-17* posterior gut gene expression. After bacterial intestinal inoculation posterior gut of fish fed PHYTO responded in a more controlled and belated way in terms of goblet cell size and mucus coverage in comparison to other treatments. Rectum pattern of response was similar for all dietary treatments

Keywords: European sea bass, functional additives, gut health, mucus production, disease resistance

Corresponding author.
E-mail address: silvia.torrecillas@giaqua.org (S. Torrecillas).

O-119.

Health status of Senegalese sole (*Solea senegalensis*) post-larvae fed diets with microalgae inclusion

D. Peixoto [1,2,#], W. Pinto [5], M. Machado [1,2,3,4], B. Reis [1,2,5,6], J. Silva [7], J. Navalho [8], J. Dias [5], L.E.C. Conceição [5], B. Costas [1,2].

[1] CIIMAR - Centro Interdisciplinar de Investigacão Marinha e Ambiental, Matosinhos, Portugal
[2] ICBAS - Instituto de Ciências Biomedicas Abel Salazar, Universidade do Porto, Porto, Portugal
[3] i3S -Instituto de Investigacão e Inovacão em Saude, Universidade do Porto, Porto, Portugal
[4] IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
[5] SPAROS Lda, Area Empresarial de Marim, Lote C, Olhão, Portugal
[6] Sorgal S.A., EN 109- Lugar da Pardala, 3880-728, São João de Ovar, Portugal
[7] CMP, Unidade de Produção de Microalgas, Fábrica Cibra Pataias, 2445-287, Pataias, Portugal
[8] Necton S.A., Belamandil s/n, 8700-152, Olhão, Portugal

Abstract
Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species targeted for aquaculture diversification in Southern-European countries and, as most farmed fish, are potentially subjected to stress and pathogens due to environmental factors (Reis et al., 2017; Pinto et al., 2018). Previous studies reported that algae can be used as antioxidant additive, high-quality dietary protein or source of bioactive compounds, thus promoting optimal growth and health in farmed fish (Becker, 2007; Teimouri et al., 2013). For these reasons, this study intended to evaluate the effects of dietary microalgae inclusion in both health status and growth performance of Senegalese sole post-larvae. Individuals with 41 days after hatching (DAH) were randomly distributed among 12 tanks with an initial density of 3000 post-larvae/m2 and four experimental diets were randomly distributed by triplicate groups of tanks. Three experimental diets (CHLO-*Chlorella* sp., fermented; PHAEO-*Phaeodactylum* sp. and NANNO-*Nannochloropsis* sp.) were formulated to include 3% of each algal biomass to a basal diet, which served as CONTROL. The experimental diets were supplied through automatic feeders set up to supply 8 meals in a 24 h period. At 50 DAH, 20 post-larvae/tank were collected for analysis of immune and oxidative
status. Also, at 61 DAH the total length, dry weight and survival were assessed. Homogenates had to be performed for the analyses of immune (i.e. lysozyme and protease activities) and oxidative stress (i.e. catalase activity) related parameters. Survival, relative growth rate and total length of individuals, at 61 DAH were not altered by the dietary treatments. However, post-larvae fed NANNO and CHLO dietary treatments increased dry weight at 61 DAH compared to those fed the CONTROL diet. Neither immune or oxidative stress status were altered by dietary treatments. According to these results, *Nannochloropsis* sp. and *Chlorella* sp. are potential candidates for inclusion in microdiets for Senegalese sole. Further analyses are being carried out to confirm the bioactive potential of these biomasses and optimal dietary inclusion levels.

Keywords: Early feeding, Nannochloropsis, Chlorella, immune status, catalase.

Corresponding author.
E-mail address: dpeixoto@ciimar.up.pt (D. Peixoto).