



## **Status, Challenges and Advances in Global Aquaculture**

**Dr. George W. Chamberlain** *Global Aquaculture Alliance and Hendrix Genetics*

Global seafood demand is strong, but aquaculture growth is slowing. Disease is the major challenge, but others include environmental and social issues, limited supply of fishmeal and oil, financing, and market acceptance. Aquaculture must become more efficient and productive to deal with tightening resources associated with population increase. Promising technological advances are occurring in diagnostics, breeding, nutrition, water reuse, area management, and open ocean systems. Some sectors, such as seafood processing, need further improvement in automation and mechanization to reduce reliance on manual labor. Environmental, social, and food safety issues continue to limit consumer acceptance. Certification programs have arisen as a means of providing consumer assurances, but gaps and overlaps still exist. The ominous issue of global warming looms and threatens our planet. Can aquaculture find a breakthrough solution such as large-scale seaweed production to sequester carbon and provide an alternative feed ingredient? The challenges and opportunities for aquaculture have never been greater.



**A possible connection between oxidative stress and production related diseases in Atlantic salmon  
(Salmo salar L)**

**Prof. Kristin Hamre** *The Institute of Marine Research* **Dr. Eystein Oveland** *The Institute of Marine Research* **Dr. Giulia Micallef** *Gildeskål Forskningsstasjon AS* **Dr. Sofie Remø**  
*The Institute of Marine Research* **Dr. Sylvian Merel** *The Institute of Marine Research* **Dr. Marie Hillestad** *Biomar*  
**Mr. Rune Waagbø** *The Institute of Marine Research* **Mr. Robin Ørnsrud** *The Institute of Marine Research*

Poor pigmentation, fillet melanin spots and cataracts are well known challenges in Atlantic salmon farming. The poor pigmentation occurs during spring, thus called “the spring drop”. The increasing day length and temperature give a strong growth stimulus to the fish. Due to increased consumption of vitamin C in salmon during the first spring in seawater (Hamre et al 2016), we hypothesized that this period is characterized by oxidative stress in the fish and that the spring drop could be caused by oxidation of astaxanthin. The origin of melanin spots is not known, but oxidative stress caused by deficiency of vitamin E and selenium causes muscular damage. The invasion of melanocytes and the development into red and black spots may be a response to such damage. Melanin spots appear in approximately 20% of slaughtered salmon in Norway. Cataracts are also known to be induced by oxidative stress. In this study, we have sampled fish from a sea-cage experiment every month from April until August and then every second month until December. We registered colour, fillet melanin spots and cataracts. We analysed the levels of TBARS, glutathione and dietary antioxidants in the liver, fillet and lens of the fish and we plan to analyse oxidation products of astaxanthin in the fillet. Feed analyses were also performed to verify that feed composition was stable. Initial results show that cataracts were absent in the beginning of the year, increased in abundance until July and then decreased again. The fillet colour was stable from April until July and increased thereafter. Numerous small melanin spots were present initially. During the experimental period, the number of spots decreased and the severity of each spot increased. Chemical analyses are in progress and the results will be reported at the meeting.



**DIETARY PREBIOTICS AND PHYTOGENICS IN LOW FISH MEAL AND FISH OIL BASED DIETS FOR EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*): EFFECTS ON STRESS RESISTANCE**

**Dr. daniel montero** *University of Las Palmas de Gran Canaria* **Mr. Antonio Serradell (1)** **Dr. Alex Makol Delacon**  
*Biotechnik GmbH* **Dr. Victoria Valdenegro**  
*Biomar A/S. BioMar AS* **Prof. Marisol Izquierdo (1)** **Dr. Silvia Torrecillas (1)**

To replace marine raw materials by meals and oils of terrestrial origin is determinant to achieve a sustainable development of the fish feed production sector. However, its supplementation can have implications of fish growth performance, stress and disease resistance. Indeed, the physiological response to a stressful factor has a highly complex relationship with the immune system, and can have a severe impact on fish disease resistance, health and welfare (Montero and Izquierdo, 2010). Particularly in marine fish species, reductions in dietary n-3 LC-PUFA induce a chronic elevation of plasma cortisol basal levels and alter post confinement cortisol pattern of response, considering its reduction per se as a stressor-like process (Montero and Izquierdo, 2010). Similarly, high dietary levels of vegetable meals increase fish susceptibility to stressful situations (Mahmoud et al., 2015).

In this sense, the use of functional ingredients, such as prebiotics and phytogenics, in low fish meal and oils diets may be an effective tool to help fish to fight against stressful situations and to safeguard fish welfare.

In the present study, one control and three experimental diets containing galactooligosaccharides (GOS) and a mixture of essential oils (PHYTO) or their combination (GOSPHYTO) were fed to European sea bass for 9 weeks. Afterwards, fish were challenged by confinement or by confinement combined with an experimental infection *Vibrio anguillarum* for 7 days. Local and systemic immunocompetence as well as primary and secondary stress parameters were evaluated along the stress-infection panel. Head kidney gene expression of steroidogenesis-related genes was also evaluated. GOS, PHYTO and their combination reduced gut *V. anguillarum* translocation rates after 7 days of challenge test. Gene expression, immune and stress parameters results obtained were correlated to these findings.



**Effect of a specific composition of short- and medium- chain fatty acid 1-Monoglycerides on growth performances and gut microbiota of gilthead sea bream (*Sparus aurata*).**

**Ms. Emi Gliozheni** *University of Insubria, Department of Biotechnology and Life Sciences (DBSV)* **Dr. Simona Rimoldi (1)** **Ms. Chiara Ascione (1)** **Dr. Elisabetta Gini (1)** **Prof. Genciana Terova (1)**

Fish gut microbiota is involved in the anaerobic fermentation of otherwise indigestible dietary components. The bacterial metabolism of these substrates yields bioactive metabolites, such as the short-chain fatty acids (SCFAs). Being organic acids, SCFAs decrease pH in the gut and are therefore believed to prevent the overgrowth of pH-sensitive pathogenic bacteria. Because SCFAs are also antimicrobials, they may affect commensal microbiota. Accordingly, the present study aimed to evaluate the potential beneficial effects of SCFA monoglycerides used as a feed additive on fish growth performances, and intestinal microbiota composition. For this purpose, a specific combination of short and medium chain 1-monoglycerides (SILOhealth108) was tested in juvenile gilthead sea bream (*Sparus aurata*) fed a plant-based diet.

Fish were fed for 3 months with two different feed formulations. The control fish group received a plant-based diet without any form of butyrate supplementation, whereas the other group received the control feed supplemented with 0.5% of SILOhealth108. The Illumina MiSeq platform for high-throughput amplicon sequencing of 16S rRNA gene, and QIIME pipeline were used to analyse and characterize the whole microbiome associated to both, feeds and *S.aurata* intestine. We identified 259 OTUs at 97% identity in sea bream faecal samples; 105 OTUs constituted the core gut microbiota, i.e. those OTUs found in at least 80% of the samples per dietary group and shared irrespective of the diet. The dominant phyla in both experimental groups were represented by Firmicutes, Proteobacteria and Actinobacteria. In summary, our findings clearly indicated that SILOhealth108 positively modulated the fish intestinal microbiota. The specific composition of 1-monoglycerides of short and medium chain fatty acid contained in SILOhealth108 could have thus a great potential as feed additive in aquaculture.



## Autochthonous intestine bacteria used as microbial feed additives confer some protection to Senegalese sole with the infectious agent *Photobacterium damsela* sp. piscicida

**Dr. Sónia Batista** CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental **Prof. Ariadna Sitjà-Bobadilla** Instituto de Acuicultura Torre de la Sal **Prof. Belén Fouz** Universidad de Valencia **Prof. Maria dos Anjos Pires**

CECAV – Centro de Ciência Animal e Veterinária, UTAD **Prof. Viswanath Kiron** FBA - Faculty of Biosciences and Aquaculture, Nord University **Prof. Ana Gomes** Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina **Prof. Jorge Fernandes** FBA - Faculty of Biosciences and Aquaculture, Nord University **Prof. Luisa Valente** CIIMAR/ICBAS – Universidade de Porto **Prof. Rodrigo Ozório** CIIMAR/ICBAS – Universidade de Porto

Probiotics may be useful prophylactic agents to combat diseases, improve growth and stimulate the immune response in fish, minimizing the emergence of drug-resistant microorganism and accumulation antibiotic residues. The benefits of using autochthonous bacteria as a dietary probiotic in Senegalese sole (*Solea senegalensis*) were evaluated after a 36-day growth trial (22 fish/tank; 22.65±0.15g) followed by a 17-day LD10-LD20 bacterial challenge with *Photobacterium damsela* sp. piscicida (Phdp).

Two bacterial strains, *Enterococcus raffinosus* (PB1) and *Pseudomonas protegens* (PB2) were isolated from the intestine of healthy juvenile sole and tested in vitro for antagonistic effects against pathogens; their viability during processing and storage of feed was also assessed. A control diet (57% crude protein, 9% crude lipid) was formulated without probiotic and compared with two experimental diets supplemented with either 1.6×10<sup>10</sup> (PB1) or 1.3×10<sup>10</sup> CFU kg feed<sup>-1</sup> (PB2).

At the end of the growth trial, all fish had similar body weight and displayed comparable humoral immune responses. However, the bacterial challenge resulted in a decrease of total peroxidase activity in infected fish, evidencing their susceptibility to the Phdp infection. Intestinal microbiota was similar between fish fed probiotic diets, and differed from those fed the CTRL, evidencing the PB1 and PB2 effectiveness in stimulating the establishment of similar bacterial communities. Intestinal microbiota in challenged fish was extremely similar among all dietary treatments, probably due to the successful colonization and subsequent dominance of Phdp.

The histological observation of fish intestine showed that fish fed PB1 diet had a significantly thicker muscular layer and higher number of goblet cells in the epithelium, compared to other diets, associated with increased mucus production and a thicker protective mucus layer.

The Phdp was effective in causing an infection, even at a LD10-LD20 dose, resulting in a trend for fish fed PB1 diet to have a smaller cumulative mortality (6.7%) than other groups (PB2 13.3%, CTRL 10.0%).

In conclusion, increased intestinal protective barrier and higher mucus production capacity in Senegalese sole fed PB1 may have enhanced protection against Photobacteriosis challenge.

Work supported by Project INNOVMAR (NORTE-01-0145-FEDER-000035) within the line "INSEAFood, Innovation and valorization of seafood products", funded by NORTE2020 through the ERDF.



## **Recovery effect of dietary $\beta$ -glucan on the hypersaline stress induced immunity damage and gut microbiota in Nile tilapia**

**Prof. Erchao Li** *Department of Aquaculture, College of Marine Sciences, Hainan University* **Ms. Yantong Suo** *East China Normal University* **Dr. Chang Xu** *East China Normal University* **Dr. Xiaodan Wang** *East China Normal University* **Prof. Liqiao Cehn** *East China Normal University*

The effects of dietary  $\beta$ -glucan supplementation (0g, 2g or 4g/kg diet) for 8 weeks on the immunity of Nile tilapia from the aspect of hematology, histology, transcriptomic and immune related gene expressions, were systemically studied, and the gut microbiota change was also explored to partially explained the prebiotic effect of  $\beta$ -glucan on the immunity enchantment in Nile tilapia under hypersaline condition (salinity 16 versus freshwater). Eight weeks exposure of Nile tilapia to water with salinity 16 did not affected fish growth and survival, but induced splenomegaly, reduced coagulation function, enhanced phagocytic activity and down-regulated the complement pathway in the spleen. These adverse effects were alleviated by dietary supplementation of  $\beta$ -glucan higher than 2g/kg diet in tilapia under hypersaline stress, and no differences were found when compared these parameters with the control tilapia grown in freshwater fed diet without  $\beta$ -glucan. Besides, tilapia at hypersaline stress fed diet without  $\beta$ -glucan had damaged mid gut, including felt away, shortened gut villus, fewer goblet cells between epithelial cells, and loosely arranged intestinal epithelium, while, tilapia fed  $\beta$ -glucan higher than 2g/kg diet had similar healthy gut structure as the control fish in freshwater, and no any adverse effects were found as tilapia grown under hypersaline condition fed the diet without  $\beta$ -glucan. The alleviation effects can be explained partially from the aspect of  $\beta$ -glucan as a prebiotic, because the gut microbiota analysis showed that dietary  $\beta$ -glucan supplementation increase the hypersaline stress deduced gut beneficial bacteria, and meanwhile decreased the hypersaline induced opportunistic pathogenic bacteria in tilapia. All the results suggest that long-term salinity will cause many potential immunity problems in fish, however the damage caused from salinity stress in tilapia can be alleviated through nutritional modulation, and dietary  $\beta$ -glucan is a good candidate additive.



**Bidirectional mechanism of astaxanthin in growth performance, immune capacity, gut morphology and intestinal microbiota mediation of golden pompano (*Trachinotus ovatus*)**

**Dr. Jin Niu** Sun Yat-sen University **Dr. Shi-Wei Xie** Sun Yat-sen University(1) **Prof. Li-Xia Tian** (1) **Prof. Yong-Jian Liu** (1)

**Introduction:** As the nature's strongest pigment antioxidant, astaxanthin has beneficial effect on colour, growth and immunity of vertebrates, but the underlying mechanism is unknown, moreover, excessive astaxanthin produce what effect is also not reported.

**Objective:** We used golden pompano to test the hypothesis that astaxanthin influences growth and immunity by modulating gut morphology, intestinal microbiota community and reactive oxygen species (ROS) scavenging.

**Methods:** Fish were fed six diets containing six levels of astaxanthin (0%, 0.005%, 0.01%, 0.05%, 0.1% and 1%) in triplicate for 8 weeks. Growth performance, pigmentation, carotenoids contents and retention efficiency, gene expression associated with colour, enzyme expression and ROS production, gut morphology and gut microbiota community, and apparent morphology of fish eyeball involved in the topic were measured.

**Results:** Compared with fish no astaxanthin supplementation diet (0%), those fed suitable astaxanthin supplementation diets (0.005%-0.1%) had higher growth performance; however, growth performance and survival of fish were suddenly deteriorated in the excessive astaxanthin-supplemented group (1%). Similar, lysozyme (LYZ) activity in the excessive dietary astaxanthin-supplemented (1%) group was suddenly decreased and was significantly lower than other groups. ROS production was also sharply decreased under nonphysiological levels in the highest astaxanthin-supplemented group (1%). More profoundly, the indistinct boundary of conjunctiva and sclera with the middle pupil was discovered by accident in the highest astaxanthin-supplemented group (1%). The gut villus length and width and microvillus length in the suitable astaxanthin supplementation (0.005%-0.1%) groups were significantly higher than those in the highest astaxanthin-supplemented group (1%). The suitable astaxanthin supplementation (0.005%-0.1%) groups increased *Bacillus*, *Pseudomonas*, *Oceanobacillus*, *Lactococcus*, *Halomonas*, *Lactobacillus* and *Psychrobacter* and decreased *Vibrio* and *Bacterium*, while excessive astaxanthin supplementation (1%) group showed a sharp decline of *Pseudomonas* and *Lactobacillus* and sharp increase of *Vibrio*.

**Conclusions:** This is the first report targeting the negative effect of excessive astaxanthin supplementation by altering gut microbiota community and gut morphology in detrimental way, and decreasing the lysozyme activity and scavenging ROS under nonphysiological level and subsequently affecting eye morphology more seriously. These results offer new insight into nutraceuticals supplements for people and further investigating the modes of action of astaxanthin in the treatment of ophthalmological disease.