



USE OF MICRODIETS ENRICHED WITH PROBIOTICS (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) AS GROWTH AND SURVIVAL PROMOTERS FOR PIKE SILVERSIDE LARVAE (*Chirostoma estor*)

Ms. Edith Castillo *Instituto de Investigaciones Agropecuarias y Forestales UMSNH* **Mr. Eduardo Martínez-Angeles** *Instituto de Investigaciones Agropecuarias y Forestales UMSNH* **Dr. Gisela Ríos-Durán** *Instituto de Investigaciones Agropecuarias y Forestales UMSNH* **Dr. Pamela Navarrete-Ramírez** *Instituto de Investigaciones Agropecuarias y Forestales UMSNH*

CONACYT-Instituto de Investigaciones Agropecuarias y Forestales UMSNH **Dr. Luciana Raggi** *CONACYT-Instituto de Investigaciones Agropecuarias y Forestales UMSNH* **Prof. Carlos Martínez-Palacios** *Instituto de Investigaciones Agropecuarias y Forestales UMSNH*

Larviculture of many fish species is susceptible to a variety of lethal diseases caused by different types of pathogenic microorganisms. One alternative to replace the use of antibiotics to control diseases outbreak are probiotics. In recent years, bacteria like *Lactobacillus* sp., have been reported as potential probiotics candidates in fish farming because they are able to increase growth, promote survival and enhance the immune response. An experiment was carried out to evaluate the effect of different proportions of *Lactobacillus acidophilus* (LA) and *Lactobacillus plantarum* (LP) on growth and survival of pike silverside larvae. Seven microdiets with different probiotic inclusion levels were evaluated: Two were added with 0.1 and 0.5% LA, two with 0.1 and 0.5% LP, two with a combination of both probiotic strains (0.1% LA/0.1% LP and 0.5% LA/0.5% LP) and one microdiet without probiotics. A control treatment was evaluated using live rotifers as only feeding along the experiment. Newly-hatched larvae were fed only with rotifers as first feed until 7 day post hatching (dph) and weaned with the different experimental diets at 10 dph. Larvae were fed with the microdiets until 30 dph. The dietary inclusion of both probiotics significantly improve growth and survival of *C. estor* larvae. Body weight was higher in larvae fed diets with both LA inclusion levels ($p < 0.05$), than that obtained for larvae fed microdiets supplemented with LP, those fed diets without probiotic supplementation and those fed rotifers; however, survival was significantly higher ($p < 0.05$) with the dietary inclusion of 0.1% LP, than that obtained with all other treatments. Growth in fish fed diets with a combination of 0.1% LA/0.1% LP was not significantly different from growth obtained in fish fed LA diets ($p > 0.05$). Survival and growth were higher ($p < 0.05$) in larvae fed with diets supplemented with a combination of both probiotic strains at high levels (0.5% LA/0.5% LP) than those obtained for larvae fed all the other diets. In conclusion, the dietary inclusion of both probiotics, alone or in combination, improve growth and survival of *C. estor* larvae. A supplementation of 0.5% LA/0.5% LP in microdiets increase growth and survival of pike silverside.



Effect of yeast (*Xanthophyllomyces dendrorhous*) and plant (Saint John's wort, lemon balm, and rosemary) extract-based functional diets on antioxidant and immune status of Atlantic salmon (*Salmo salar*) subjected to crowding stress.

Dr. Eva Vallejos-Vidal *Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08913 Bellaterra, Spain* **Dr. Sebastián Reyes-Cerpa** *Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor, Chile* **Dr. Diego Pérez-Stuardo** *Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor, Chile* **Dr. Monica Imarai** *Laboratorio de Inmunología, Centro de Biotecnología Acuícola, Universidad de Santiago de Chile* **Dr. Ana María Sandino** *Laboratorio de Virología, Centro de Biotecnología Acuícola, Universidad de Santiago de Chile* **Dr. Felipe E. Reyes-López** *Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, 08913 Bellaterra, Spain*

Atlantic salmon (*Salmo salar*) production has increased strongly in recent decades thanks to the expansion in the northern Europe and in North and South America with Norway and Chile as the main world producers. In this context, salmon farming may face stress due to the intensive culture conditions with negative impacts on fish welfare and overall performance. In this aspect, the functional feeds have reported that improve not only the basic nutritional requirements but also the health status and fish growth. However, to date no studies have been carried out to evaluate the effect of functional diets in salmon subjected to crowding stress. Thus, the aim of this study was to evaluate the effect of yeast (*Xanthophyllomyces dendrorhous*; diet A) and the combination of plant extracts (common Saint John's wort, lemon balm, and rosemary; diet B) on the antioxidant and immune status of Atlantic salmon grown under normal cultured conditions and then subjected to crowding stress. Fish were fed with functional diets during 30 days (12 Kg/m³) and then subjected to crowding stress (20 Kg/m³) for 10 days. The lipid peroxidation in gut showed that both diets induced a marked decrease on oxidative damage when fish were subjected to crowding stress. The protein carbonylation in muscle registered at day 30 a marked decrease in both functional diets that was more marked on the stress condition. The expression of immune markers (IFN γ , CD4, IL-10, TGF- β , IgMmb, IgMsec T-Bet and GATA-3) on spleen indicated the upregulation of those associated to humoral-like response (CD4, IL-10, GATA-3) when fish were subjected to crowding stress. These results were confirmed with the expression of secreted IgM. Altogether, these functional diets improved the antioxidant status and increased the expression of genes related to Th2-like response suggesting a protective role on fish subjected to crowding stress.



SHORT-TERM EFFECTS OF FUNCTIONAL ADDITIVES IN GUT HEALTH, OXIDATIVE STATUS AND INNATE IMMUNITY OF GILTHEAD SEABREAM JUVENILES

Mr. Bruno Reis *Centro interdisciplinar investigação Marinha (CIIMAR); ICBAS Universidade Porto; Sparos Lda; Sorgal S.A.* **Mr. Lourenço Ramos-Pinto** *Centro interdisciplinar investigação Marinha (CIIMAR); ICBAS Universidade Porto; Sparos Lda* **Mr. Jorge Dias** *Sparos Lda* **Mr. Elisabete Matos**

Sorgal S.A **Mr. Juan Mancera** *Faculty of Marine and Environmental Sciences, Campus de Excelencia Internacional del Mar (CEI-MAR), University of Cádiz* **Mr. Benjamín Costas** *Centro interdisciplinar investigação Marinha (CIIMAR)*

Balanced diets are paramount for fish growth and welfare, since fish are often subjected to stressful conditions in aquaculture. To enhance fish disease resistance and general health, feeds maybe supplemented with health promoting additives reducing the need to use chemotherapeutics. Several compounds with proven antioxidant and/or immunostimulating effect on fish can be found in readily available commercial additives like beta-glucans, inulin, FOS, polyphenols amongst others. The present work aims to evaluate the effects of a short-term dietary supplementation with different commercial additives on immune related genes expression, gut health biomarkers and plasma innate immune parameters in gilthead seabream juveniles.

Five isonitrogenous (45% protein) and isolipidic (18% fat) diets were formulated. CTRL (10% FM diet) and the CTRL diet supplemented with 4 different additives: BG (0.1% beta-glucans), ART (0.6% artichoke extract), SH (3% marine protein hydrolysate) and PRE (0.1% prebiotic). Diets were assigned to triplicate groups of 20 *Sparus aurata* (IBW: 88.7 ± 2.3g). After 12 and 26 days of feeding 4 fish per tank were euthanized. Blood was collected for haematological procedures according to Machado et al., (2015). Innate immune parameters were analysed on blood plasma to evaluate fish immune status.

Total white blood cells and haemoglobin concentration remained unchanged among fish fed the different dietary treatments across 26 days of feeding. However, over time both mean corpuscular haemoglobin (MCH) and protease activity decreased, while total red blood cells (RBC) and anti-protease activity increased. Fish fed PRE diet showed lower total RBC compared to CTRL fed fish at 26 days. Also, fish fed this same diet showed higher anti-protease activity than ART fed fish at 12 days, while exhibiting lower bactericidal activity at both time points.

Overall results showed an effect over time on haematological and innate immune related parameters in consequence of fish growth and development. Nonetheless, different dietary treatments affected fish haematology with PRE diet leading to lower RBC count after 26 days. Yet, it was not possible to ascertain a clear effect on innate humoral parameters. Histological and molecular biology samples were collected and are currently being analysed to obtain an overall picture of fish innate immune



Effects of dietary immunostimulants on the immune condition in gilthead sea bream (*Sparus aurata*)

Mr. Firmino Joana *IRTA, Centre de Sant Carles de la Ràpita, Unitat de Cultius Experimentals* **Dr. María Ángeles Esteban** *Universidad de Murcia* **Ms. Gloria Asencio** *Centro de Investigaciones Biológicas del Noroeste (CIBNOR)* **Dr. Felipe Reyes** *Universitat de Autònoma de Barcelona (UAB)* **Dr. Lluís Tort** *Universitat de Autònoma de Barcelona (UAB)* **Dr. Enric Gisbert** (1)

The concept of maintaining the health of fish through the best possible nutrition is well accepted in modern aquaculture. Scientific evidence clearly indicates that dietary nutrients as well as additives have the ability to stimulate the immune system and to protect the fish from pathogenic diseases. A common strategy for maintaining fish health and to improve performance, immunostimulants have been used as dietary additives to improve weight gain, feed efficiency, and/or disease resistance in cultured fish. Under this context, an immunostimulant containing *Echinacea purpurea*, mannan oligosaccharides, β -glucans and vitamin C (INMUNOTEC[®], TECNOLOGÍA & VITAMINAS, S.L., Spain) was evaluated in seabream during 75 days. The inclusion of the additive was tested at two doses (0.15 and 0.3%) and compared to a control diet devoid of the feed additive. Each diet was tested with four replicates (basal diet: 48% crude protein, 17% crude fat, energy: 21.7 MJ/kg feed). Preliminary results showed that the group supplemented with Immunotec at 0.15% were 5% heavier in body weight (82.0 ± 1.3 g) when compared to the control group (78.1 ± 1.0 g) ($P < 0.05$), while the BW in fish the diet containing the feed additive at 0.3% was similar to the control group. In addition, the effect of the feed additive was evaluated in terms of oxidative stress markers (SOD, CAT, GPX, GST, TAC, TBARS), several non-specific serological immune parameters (lysozyme, complement, bacteriolytic activity), as well as the transcriptomic profiling (microarray 4x44K, Agilent) of selected target tissues (head kidney and intestine). Results from different analytical approaches will be integrated and discussed in order to evaluate the performance and immune competence of fish fed this functional feed with the purpose of addressing new nutritional strategies to improve aquaculture productivity and solutions against different stressors that concern the sector.



Effect of β -glucans extracted from yeast cell-wall mutants on the survival and expression of immune-related genes in gnotobiotic *Artemia franciscana* upon *Vibrio harveyi* infection

Mr. Biao Han *Lab of Aquaculture & Artemia Reference Center, Ghent University* **Mr. Kartik Baruah** *Swedish University of Agricultural Sciences* **Mr. Peter Bossier** *Lab of Aquaculture & Artemia Reference Center, Ghent University(1)*

Diseases caused by infectious microorganisms are known to be one of the major constraint in the aquaculture industry for past many years. β -Glucans, which are glucose polymers that are major structural components of the cell wall of yeast, fungi or bacteria, have been proven by various studies as a potent, valuable, and promising immunostimulant for improving immune status and controlling diseases in aquaculture. However, most studies focused on the doses or the administration way of β -glucans when conducted in vivo experiments, ignoring the different β -glucans which vary in origin, particle size, chemical structure or linkage patterns would affect physical and possibly physiological properties. In this study, we found that the structural variation in the β -glucans extracted from different yeast mutants, which can influence their physiological and immune functions. Hence the study was conducted under the gnotobiotic conditions, the axenic hatching *Artemia* was used as the model animal, avoiding experimental noise of changing microbial communities. The diameter of glucan particles was determined after 1min's sonication. Subsequently, in the interest of checking whether particle size of β -glucan could affect the bioactivity, glucan was homogenized using a microfluidizer to make the particle size almost the same between treatments. The results showed β -glucans extracted from mutant yeast Gas1 increased the survival and related immune genes expression significantly in *Artemia* when challenged with pathogen *Vibrio harveyi*. Although more studied in other species are needed, it is tempting to suggest that the structure of dietary of β -glucans need to be consider when using this compound to prime the host immune system and increase resistance against invading pathogens.



DIETARY PREBIOTICS AND PHYTOGENICS IN LOW FISH MEAL AND FISH OIL BASED DIETS FOR EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*): AN EFFECTIVE TOOL TO BOOST MUCOSAL TISSUES HEALTH AND DISEASE RESISTANCE?

Dr. Silvia Torrecillas *Grupo de Investigación en Acuicultura (GIA), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria* **Mr. Antonio Serradell (1)** **Dr. Alejandro Makol** *Delacon Biotechnik GmbH* **Prof. Genciana Terova** *Department of Biotechnology and Life Sciences, University of Insubria* **Dr. Victoria Valdenegro** *Biomar A/S*. **Dr. Daniel Montero (1)** **Ms. Elisabetta Gini** *Department of Biotechnology and Life Sciences, University of Insubria Varese Italy* **Dr. Tatiana Kalinowski** *Grupo de Investigación en Acuicultura (GIA), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria (ULPGC)* **Prof. Marisol Izquierdo** *Grupo de Investigación en Acuicultura (GIA), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria (ULPGC)*

The successful replacement of fishmeal (FM) and fish oil (FO) by land-based meals and oils in feeds for marine fish species is a determining factor to achieve a sustainable aquaculture sector development. However, their use has been associated with variable side-effects on fish growth performance and health. For European sea bass (*Dicentrarchus labrax*) gut in particular, marine raw materials dietary replacement causes gut lamina propria and submucosa engrossment, increases mucus production, up-regulates several inflammation related genes, alters microbiota populations and increase gut bacterial translocation rates [Torrecillas et al., 2017]. Therefore, it is necessary to develop functional diets as part of a management strategy targeting mucosal health and disease incidence when low FM/FO formulations are used. In this sense, prebiotics and phytogenics may be potential candidates to safeguard fish mucosal tissues health and increase disease resistance [Reverter et al., 2014; Thanigaivel et al., 2016; Guerreiro et al., 2017]. 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 before challenging the fish via gut inoculation with *Vibrio anguillarum* in combination with a stress panel by confinement. At the end of the feeding trial, growth performance, systemic immunocompetence, gut morphology and associated lymphoid tissue (GALT) status were evaluated by immunological, morphological and immunohistochemical methods. Gut morphological pattern and anti-immunopositivity patterns for several immune related molecules (anti-iNOS, anti-TNF, anti-PCNA) were studied along the challenge test. Despite individual GOS and PHYTO supplementation reduced SGR ($P < 0.05$), fish fed diets with a combination of both presented similar growth to fish fed control diet ($P > 0.05$). Furthermore, GOS, PHYTO and their combination reduced gut *V. anguillarum* translocation rates and fish mortality after 7 days of challenge test. Gut morphological, immunohistochemical findings, as well as gene expression of several gut junctional complexes-related genes were related to these protective effects.

References

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Exopolysaccharides from *Lactobacillus rhamnosus* GG ameliorate hepatic steatosis in HFD-fed zebrafish in collaboration with gut microbiota

Mr. Zhen Zhang *Feed Research Institute, Chinese Academy of Agricultural Sciences* **Mr. Chao Ran (1)** **Ms. Chengyao Lu (1)** **Ms. Qianwen Ding (1)** **Mr. Hongling Zhang (1)** **Mr. Zhigang Zhou (1)**

Probiotics have the ability to improve NAFLD, obesity and other metabolic syndrome, and study on the mechanisms of the beneficial effects of probiotics has been mainly focusing on gut microbiota modulation and probiotics-derived metabolites, such as SCFA. However, the roles of structural compounds of probiotic in the beneficial effects are less investigated. Here, we show that isolated exopolysaccharides (EPS) from *Lactobacillus rhamnosus* GG, which was widely known as a probiotic strain, had the anti-hepatic steatosis effect in high-fat diet-fed zebrafish through reduction of lipogenesis and enhancement of energy expenditure. LGG EPS supplementation alters the gut microbiota in HFD-fed zebrafish. We further show that LGG EPS inhibited lipogenesis in HFD-fed germ-free zebrafish larvae through TLR4ba, but had no effect on energy expenditure. LGG EPS induced gut microbiota ameliorated hepatic steatosis in HFD-fed GF zebrafish by inhibiting lipogenesis and increasing energy expenditure, and the effect of EPS induced microbiota involved an induction of Fiaf expression in zebrafish. The involvement of TLR4ba and Fiaf in the anti-hepatic steatosis effect of LGG EPS were further confirmed in conventional HFD-fed zebrafish. Our results indicate that oral LGG EPS has the potential for therapeutic development to intervene NAFLD.



USE OF POLYPHENOLS FROM AGRICULTURAL BY-PRODUCTS FOR FUNCTIONAL FEED APPLICATIONS

Prof. Marina Paolucci *Department of Sciences and Technologies* **Dr. Maria Grazia Volpe** *Institute of Food Sciences (ISA-CNR)* **Prof. Omid Safari** *Department of Fishery, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad* **Mr. Marina Paolucci** *Faculty of Fisheries, Department of Aquaculture, University of Çukurova*

Introduction

Agricultural by-products are a rich source of biomolecules, such as polyphenols, with antimicrobial, immunostimulant, antitumoral and antioxidants properties. The use of polyphenols as dietary supplements in animal feed may improve health and limit antibiotics.

Objectives

Evaluation of possible immunostimulant activity *in vitro* on leukocytes isolated from blood and GALT of the rainbow trout *Oncorhynchus mykiss* and *in vivo* on the narrow clawed *Astacus leptodactylus* in a 24 week-feeding trial.

Methodology

Polyphenols from chestnut shells (PECS) and olive mill waste water (OMWW) were extracted by organic solvents and characterized by HPLC/DAD, MALDI-TOF/MS and ATR-FTIR. Polyphenols were tested in *in vitro* assays with rainbow trout (*Oncorhynchus mykiss*) blood and intestine leukocytes. Cellular uptake was determined by HPLC. Superoxide anion was determined as the reduction of NBT and phagocytosis was quantified by flow cytometry. The crayfish *Astacus leptodactylus* was fed on OMWW diets for 24 weeks. Oxidative stress enzyme activity was determined with spectrophotometric enzymatic assays. Microbiota was evaluated by growing assays on selective media. Histological analyses were carried out according to the conventional procedures. Fatty acid analysis was carried out with GC. Metabolites were analyzed by ¹H-NMR and ATR-FTIR.

Results

Our results indicated that PECS caused the increase of superoxide anion production and phagocytosis activity in both blood and GALT leukocytes. Polyphenols were also able to modulate immune-related gene expression of the pro-inflammatory cytokine TNF- α and anti-inflammatory cytokine IL-10.

Crayfish fed diets enriched with polyphenols from OMWW, showed significant improvement of growth performances (specific growth rate; feed conversion ratio; Protein, Lipid and Carbohydrate efficiency ratio), activity of oxidative stress enzymes glutathione reductase and catalase in the hepatopancreas, as well as of the immunological parameters (total haemocytes, phenoloxidase activity and superoxide anion production). Moreover, in crayfish fed on polyphenol-enriched diet, total intestinal microbiota decreased. A significant increase in the general metabolism was registered in the hepatopancreas.

Conclusions

The emerging picture indicates that polyphenols had beneficial effects in both *in vitro* and *in vivo* experiments and hence could be considered a novel strategy of development of the feed industry sector.



Diets administrated with hot water extracts of *Morinda citrifolia* (noni) leaves for a short term feeding trial enhance immunocompetence of the giant freshwater prawn, *Macrobrachium rosenbergii*

Ms. Zhong-Wen *Chang* *National Pingtung University of Science and Technology* **Prof. Chin-Chyuan Chang**
National Pingtung University of Science and Technology

The giant freshwater prawn *Macrobrachium rosenbergii*, a commercially important cultured species in Taiwan, had been severely impacted by epidemics and caused serious economic losses. The antibiotic medications or chemicals for disease control had been conducted in aquaculture; however, the excessive use and misuse caused the antibiotic residuals in food and the spread of antibiotic-resistant pathogens in the aquatic environment. In recent, shrimp researchers and farmers tried to find environmentally friendly approaches to disease management such as immunostimulant. *Morinda citrifolia* (noni), the most significant source of traditional medicines among Pacific Island societies, has a pantropical distribution. Some reports indicated that plant extracts were able to be as sustainable and effective substitutes for veterinary drugs and vaccines in aquaculture, and therefore, in the present study, hot water extracts of *M. citrifolia* leaves administrated into diet was evaluated to be as functional feed for disease control and prevention in prawn cultivation. Invertebrates lack an adaptive immune system and rely on their effective cellular and humoral innate immune responses to combat invading microbes, and the indicators including total hemocyte count, phenoloxidase activity, respiratory burst, and phagocytic activity are common applied to assess the immunocompetence of prawn. Prawn fed with hot-water extracts of leaves of noni at 0, 0.6, 3 and 6 g/kg diet for 0, 1, 3, 5 and 7 days were sampled for immunocompetence assessment. The results showed that prawn fed with hot water extracts of noni leaves containing diets at 0.6 g/kg diet within 7 days of feeding trial were able to significantly increase total hemocyte count and phagocytic activity, and furthermore, no significant decrease in the performance of immunocompetence was detected in all treatments compared to the control. It is therefore concluded that hot water extracts of noni leaves administrated into diets for *M. rosenbergii* at 0.6 g/kg diet within 7 days of feeding trial can enhance the immunological responses especially in phagocytic activity, which might be as a function feed to prevent and control bacterial infection in prawn cultivation.



Use of Phytobiotic additives in the prevention of infection by *Sparicotyle chrisophrii*

Dr. Alicia Estevez *IRTA Centre of San Carlos de la Rápita* **Mr. Alvaro Rodriguez** *LIPTOSA* **Mr. Antonio Martinez**
LIPTOSA **Ms. Olga Bellot**
IRTA(1) **Ms. Noelia Gras** *IRTA(1)* **Ms. Marta Sastre (1)**

Three different additives (Liptocitro 193, 194 and 195 MMM, Liptosa, Spain) were used in the formulation of a diet for gilthead seabream. The diets were used to feed 150 g gilthead seabream for 60 days. The experiment was carried out in triplicate tanks in IRTA using recirculation units. After 60 days of feeding part of the fish were used in a cohabitation trial with fish infected with *S. chrisophrii* in a ratio 3 non parasitized fish (receptors) : 1 infected fish (donors), with a total N=27 fish per tank. The cohabitation trial was carried out for 30 days. At the end, the fish were sampled from each tank separating receptors and donors. The fish were weighted, measured, individually stored in labeled bags and frozen at -20°C. In addition a blood sample of 7 fish per tank was extracted in pre-heparinized syringes. The number of parasites in each of the gills was determined in each individual fish, with 3 categories: adults, juveniles and eggs according to morphological characteristics. The fish from the control group not fed the phytobiotics showed the lowest hematocrit with clear signs of anemia (Fig 1). Liptocitro 195 MMM fed fish had a significantly lower number of adults, mostly immature than the rest of the treatments whereas control and Liptocitro 193 fish presented a significantly higher number of adults and juveniles (Fig 2). The use of a diet enriched with Liptocitro 195 MMM preventatively allows a parasite much lower than would occur without the addition of the product.



Aloe Vera product and by-product for aquaculture feeds: preliminary study on mugilids (*Liza aurata*)

Ms. Raquel Quirós Pozo *Aquaculture Research Group (GIA), ECOAQUA institute at the Marine Scientific Technological Park of the University of Las Palmas de Gran Canaria* **Mr. Lidia Robaina (1)** **Mr. Sara Ramirez Bolaños (1)** **Mr. Anais Ventura (1)**

Aloe Vera's production is an important economic activity in many temperate places. Its benefits are directly related to the treatment and prevention of many health disorders, mainly in humans (Sahu et al., 2013). These benefits have raised the interest for aquaculture, but as far as we know there is no work done testing the Aloe Vera by-product, which is generated in an important quantity by the aloe industry, representing waste management problems. One of the objectives of the global political agenda is to move the primary sector into a more circular way of production, and this is the context of the project MAC/1.1.a/207 "R+D+I towards aquaponic development in the up islands and the circular economy" in which the present study is involved.

The aim of the present work was to evaluate the best processing methods for the Aloe Vera product (AP) and by-product (AB-P), to obtain adequately dried small size meals to later test different levels of both, AP and AB-P, in diets for mugilids (*Liza aurata*).

For this purpose AP and AB-P locally produced were biochemically analyzed, polyphenol content (González-Montelongo et al., 2009) included, before and after being processed (Table 1 & 2). Five diets (40/15) were formulated based on the polyphenol concentrations. A control diet (C) was tested against 3 dietary levels (2%, 4%, 6%) of the AB-P (B-P2; B-P4; B-P6); a diet with 2% of the AP (AP2) was also used as a positive control according to reported results in other fish species (Gabriel et al., 2015). The trial was performed at the Aquaculture Research Facilities, belonging to the ECOAQUA Institute of the ULPGC. Sixteen fishes per tank (8.93 ± 1.88 g; 10.08 ± 0.95 cm), were randomly distributed in 200 litre tanks in triplicates. Fish were manually fed twice a day, six days a week, for 91 days.

After 91 days there were no rejections for the inclusion levels of AP and AB-P, with similar FCR for the 4 tested diets respect to the control one, and no differences in fish weight and length were observed (Fig 1 & 2). Trial analyses continue on going to deeper knowledge of the effect of the tested ingredients in fish.



The dietary inclusion of curcumin powder from *C. longa* and conjugated linolenic acid reduced the oxidative stress caused by aflatoxin-induced toxicity in juvenile white shrimp, *L. vannamei*

Ms. Lucia Marlen Guerrero-Guerrero *Universidad Autonoma de Nuevo Leon, Facultad de Medicina Veterinaria y Zootecnia, Laboratorio de Producción acuícola* **Dr. Oscar Daniel García-Pérez** *Universidad Autonoma de Nuevo Leon, Facultad de Medicina Veterinaria y Zootecnia, Laboratorio de Producción acuícola* **Dr. Julio Cesar Cruz-Valdez** *Universidad Autonoma de Nuevo Leon, Facultad de Medicina Veterinaria y Zootecnia, Laboratorio de Producción acuícola* **Dr. Mireya Tapia-Salazar** *Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biológicas, Programa Maricultura* **Dr. Martha Guadalupe Nieto-López** *Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biológicas, Programa Maricultura* **Dr. Lucia Elizabeth Cruz-Suarez** *Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biológicas, Programa Maricultura*

Feeding diets containing aflatoxins to white shrimp juveniles *Litopenaeus vannamei* reduces feed intake, growth rate, survival and increases enzymatic and non-enzymatic antioxidant systems. The inclusion of some antioxidant compounds may be an alternative to revert aflatoxicosis in shrimp. The purpose of this study was to investigate the possible preventive role of curcumin and conjugated linolenic acid (CLA) in the performance and oxidative stress biomarkers in white shrimp aflatoxin-induced toxicity.

A trial was set up to evaluate the response of shrimp *L. vannamei* juveniles to dietary aflatoxin and to test the preventive effect of curcumin powder from *C. longa* and CLA. Triplicate groups (12 shrimp per tank) of shrimp (70 mg ABW) were fed diets containing 120 mg/kg of total aflatoxins alone (CD), or along with curcumin (0.15, 0.20 and 0.30 g/kg) or CLA (4, 5 and 6 g/kg) for a period of 6 weeks. Growth parameters were measured every 14 days. At the end of the experiment, hepatopancreas were taken to measure glutathione-S-transferase (GST) and alkaline phosphatase (ALP) activities. One-way ANOVA with Tukey's post-test was used to analyse the data ($P \leq 0.05$).

Feeding diets containing 120 mg/kg of total aflatoxins to shrimp *L. vannamei* reduces significantly feed intake (26.5%) and growth rate (26%). The dietary inclusion 0.2 g/kg curcumin to the CD produced a significantly higher growth rate than CD diet ($P < 0.05$), and it was statistically similar to the NCD (no contaminated diet). The inclusion of 0.2 g/kg curcumin and 4 g/kg CLA in CD diets resulted in similar feed intake than shrimp fed NCD. No significant effect on survival and feed conversion ratio was observed among test diets. The activity of GST (1.52 fold) and ALP (1.4 fold) was significantly higher in shrimp fed CD. Feeding shrimp with CD supplemented with curcumin (0.15 and 0.20 g/kg) and CLA (4 and 5 g/kg) reduced significantly the activities of GST (40-46%) and ALP (43-54%).

This study showed that curcumin from *C. longa* or CLA have a protective effect against AF-induced toxicity and oxidative stress.



The growth promoting effect of dietary nucleotides in fish is associated with an intestinal microbiota-mediated reduction in energy expenditure

Dr. Chao Ran Feed Research Institute, Chinese Academy of Agricultural Sciences

Introduction

Nucleotides (NTs) have been used as functional nutrients to improve growth and health of animals including fish. The mechanism involved in the growth promotion effect of nucleotides is still unclear.

Objective

We investigated the bioenergetic mechanism underlying the growth promotion effect of NTs in zebrafish and the associated roles played by the intestinal microbiota.

Methods

Larval zebrafish were fed control or 0.1% mixed-NTs supplemented diet for 2 weeks. Standard metabolic energy, the minimal rate of energy expenditure by animals at rest, was evaluated by oxygen consumption using a respirometer. The expression of fasting-induced adipose factor (Fiaf), inflammatory cytokines, and genes involved in fatty acid oxidation was tested by qRT-PCR. The intestinal microbiotas from the NTs fed fish or control fish were transferred to 3-d postfertilization (dpf) germ-free (GF) zebrafish in which oxygen consumption and expression of inflammatory cytokines and fiaf were evaluated.

Results

Compared with control, NTs supplementation at 0.1% increased the weight gain and energy gain of zebrafish by 10% and 25%, respectively ($P < 0.01$). The standard metabolic energy was 28% lower in NT fish compared with control ($P < 0.001$). NTs supplementation down-regulated the inflammatory tone in the head kidney of fish. Moreover, NTs fed fish had 51% lower intestinal expression of the fiaf gene compared with controls ($P < 0.05$), which accorded with decreased expression of key genes involved in fatty acid oxidation (*cpt1a* and *mcad*) in liver and muscle. GF zebrafish colonized with microbiota from fish fed NTs had 25% lower standard metabolism compared with those colonized by control microbiota ($P < 0.01$), while direct NTs feeding of GF zebrafish did not affect standard metabolism relative to GF controls that were not fed NTs. Furthermore, GF zebrafish colonized with NT microbiota exhibited down-regulated inflammatory tone and 33% lower fiaf expression compared with control microbiota colonized counterparts.

Conclusion

The growth promoting effect of dietary NTs involves two intestinal microbiota-mediated mechanisms that result in reduced standard metabolic energy: (i) lower inflammatory tone; and (ii) reduced fatty acid oxidation associated with increased microbial suppression of intestinal



DIETARY SUPPLEMENTATION OF A MIXTURE OF VEGETAL FLAVONOIDS AND TERPENOIDS (ENARECOX[®]) INCREASES EUROPEAN SEABASS (DICENTRARCHUS LABRAX) STRESS RESISTANCE.

Dr. Silvia Torrecillas *Grupo de Investigación en Acuicultura (GIA), IU-Ecoaqua* **Grupo de Investigación en Acuicultura (GIA), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, , 35214 Telde, Las Palmas, Canary Islands, Spain** **Mr. Manuel Martín** *European Natural Additives (ENA)* **Mr. Federico Astorga** *European Natural Additives (ENA)* **Dr. Lorena Román**
(1) Dr. Daniel Montero (1)

Physical stressors associated to intensive fish production may derive in a chronic stress situation involving suppressive effects on the fish immune system, disease resistance and energetic metabolism. Terpenoids and flavonoids are plant compounds with strong antioxidant, anti-inflammatory, anxiolytic and sedative properties, which at adequate doses may help fish to reduce chronic stress side effects by, for example, reducing circulating plasma glucocorticoid concentration. Thus, the objective of this study was to evaluate the effect of a dietary supplementation of a mixture of plant terpenoids and flavonoids (ENARECOX²[®]) at 100 ppm in European sea bass (*Dicentrarchus labrax*) stress resistance by a series of experimental and commercial trials.

For Experiment I, European sea bass juvenile's plasma cortisol acute and adaptive pattern of response to confinement was evaluated after feeding with ENARECOX[®] diets for 7 days in experimental tanks. For Experiment II, after feeding adult European sea bass in sea cages farm conditions with ENARECOX[®] for 7 days, scales chronic cortisol content was evaluated. For Experiment III, after feeding adult European sea bass in commercial conditions with ENARECOX[®] for 15 days, samples of plasma (1h after starting to harvest) and scales were evaluated for cortisol levels. In experiment I, fish fed ENARECOX²[®] diet presented lower ($P < 0.05$) basal cortisol levels (0h) and reduced ($P < 0.05$) cumulative mortality percentage compared to fish fed control diet. However, all fish evaluated presented the typical pattern of response to acute stress by confinement (2h) and recovered basal levels after 3 days of challenge. In Experiment II, scales of fish fed ENARECOX²[®] diet presented lower accumulation of cortisol than fish fed control diet. In experiment III, no differences in plasmatic cortisol ($P > 0.05$) levels were found, presenting all fish evaluated a similar pattern of response to stress by manipulation (commercial harvesting) in agreement with the results found in Experiment I. Besides, scales chronic cortisol levels of fish fed ENARECOX²[®] diet were reduced by a 25% compared to fish fed control diet in agreement with Experiment II findings.



Effects of *Ulva clathrata* inclusion in the fresh diet on reproductive performance, biochemical composition and fatty acid profile of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) broodstock

Ms. Cristina Corral-Rosales *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* **Dr. Elena Palacios** *Centro de Investigaciones Biológicas del Noroeste* **Dr. Denis Ricque-Marie** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* **Dr. Lucía Elizabeth Cruz-Suárez** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León*

The effect of supplementing a fresh maturation diet with dehydrated seaweed *Ulva clathrata* (20 g kg⁻¹ fresh squid) was evaluated in a commercial hatchery on the reproductive performance of a *Litopenaeus vannamei* broodstock, and on the females' biochemical parameters and fatty acid profile. The broodstock fed *Ulva* produced more eggs (+26%, $P < 0.05$) and nauplii per day (+33%, $P < 0.05$), the hatching rate being increased by 7% ($P < 0.001$). In females fed with *Ulva*, the gonads showed higher levels of protein ($P = 0.012$), lipids ($P = 0.044$) and triglycerides ($P = 0.035$); the hepatopancreas presented lower levels of triglycerides ($P = 0.037$). The consumption of *Ulva* increased the concentration of linolenic acid and the ratio 20:4(n-6)/20:5(n-3) in polar lipids of the ovaries, and the concentration of arachidonic acid in the neutral fraction (acylglycerides). In hepatopancreas of females fed with *Ulva*, the lipids in the neutral fraction showed increased concentrations of docosahexaenoic acid, of the sum of highly polyunsaturated fatty acids and in particular omega 3, and a decreased proportion of saturated fatty acids. The muscle fatty acid profile did not present significant differences in relation to *Ulva* supplementation. Therefore, *U. clathrata* appears to be a functional additive with a positive effect on the reproductive performance (fecundity and fertility) of shrimp. This effect could be associated to differences in the accumulation or metabolism of fatty acids in the gonad and hepatopancreas.



Changes in the fecal microbiota of the white shrimp (*Litopenaeus vannamei*) generated by presence or absence of *Ulva ohnoi* in diet

Mr. Elias Salce-Ruiz *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León* **Mr. Bruno Gomez-Gil Rodriguez-Sala** *Centro de Investigación en Alimentación y Desarrollo* **Ms. Martha Nieto-Lopez** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León(1)* **Mr. Denis Ricque-Marie** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León(1)* **Ms. Mireya Tapia-Salazar** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León(1)* **Mr. Lucia-Elizabeth Cruz-Suarez** *Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León*

Diet is considered one of the major modulators of the gut ecosystem. Seaweeds are one of the natural resources with more active compounds, which can be used to develop functional foods (prebiotics) that improve the health and production variables.

The aim of this study was to demonstrate that the switch from a diet with 4% of seaweed *Ulva ohnoi* to a control diet without *Ulva* modifies the fecal microbiota of *Litopenaeus vannamei*; and also, to demonstrate the impact of individual variation on the fecal microbiota fluctuation.

A metagenomic analysis amplifying the 16S rRNA V3 region of fecal samples from 4 shrimps confined individually and fed successively for four days with two treatments (diet with 4% of *U. ohnoi* and control diet) was carried out to track the change in fecal microbiota of each shrimp. We also carry out metagenomic analysis of feces from other four shrimp that were fed only with the control diet.

The taxonomic structure of the fecal microbiota demonstrated that richness and diversity of the fecal microbiota with the control diet was greater than with the diet with *U. ohnoi*. When comparing the sequences obtained from feces collected under the two different treatments in same shrimp (individual comparison), proportions of families and genera were significantly different ($p < 0.05$), the Pseudoalteromonadaceae appearing in greater proportion with *Ulva*, while the Vibrionaceae, Alteromonadaceae and Pasteurellaceae were dominating under the control diet. The genera *Vibrio* (most frequent pathogen bacteria in shrimp culture) and *Pseudoalteromonas* appeared in less proportion under the *Ulva* treatment. When comparing the samples in groups according to the treatment, only the Vibrionaceae family and *Vibrio* genus were significantly less abundant under the *Ulva* treatment. Finally, when comparing different groups of samples according to the treatment, there were no significant differences.

With the results of the present study it can be concluded that the switch from a diet with 4% of seaweed *Ulva ohnoi* to a control diet without *Ulva* significantly modifies the fecal microbiota. We also observed that it is necessary the individual monitoring with change of diet to observe significant changes in the microbiota.



Are *Ulva lactuca* and *Chondrus crispus* potential functional ingredients for gilthead seabream?

Dr. Inês Guerreiro CIMAR/CIIMAR – *Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto* **Mr. Rui Magalhães (1) Dr. Filipe Coutinho (1) Dr. Ana Couto**

(1) Prof. Aires Oliva-Teles CIIMAR and *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto*

Dr. Helena Peres (1)

In the last year's seaweeds begun to be used in aquaculture both as a potential alternative protein source and as a functional ingredient. While, improvements in fish performance were already observed in several fish species fed seaweeds [1-3], worse performance was reported for instance in European sea bass (*Dicentrarchus labrax*) fed 10% *Gracilaria cornea* or in Nile tilapia, (*Oreochromis niloticus*) fed 10% *Gracilaria vermiculophylla* [2, 3], reinforcing the need of performing more studies.

Therefore, the aim of the present study was to assess the effect of incorporating 5% of *Ulva Lactuca*, *Chondrus crispus*, or a mix of both algae on gilthead seabream (*Sparus aurata*) juveniles' growth, metabolism, and oxidative status.

Seabream (15g) were fed isoproteic (46% protein) and isolipidic (18% lipids) diets with fish meal and plant feedstuffs (providing 27% and 73% of protein, respectively) as main protein sources (control diet). Three other diets were formulated similar to the control but including 5% of *Ulva lactuca*, 5% of *Chondrus crispus*, or a mixture of 2.5% of each seaweed (diets *Ulva*, *Chondrus*, and *Mix*, respectively), in expense of wheat. Each diet was fed to triplicate groups of fish twice daily until visual satiation, 6 days a week, during 8 weeks. At the end of the trial, fish were bulk-weighed after 1 day of feed deprivation and sampled for body composition. Three fish per tank were also sampled 4 hours after the morning meal for collection of blood for plasmatic metabolites, and livers for intermediary and stress oxidative enzymes.

Fish fed *Chondrus* and *Mix* diets presented lower final body weight, body lipid composition, and plasmatic glucose. In fish fed *Mix* diets pyruvate kinase and 3-hydroxyacyl-CoA dehydrogenase activities were reduced comparatively to the control. Fish fed *Ulva* presented also decreased plasmatic glucose level. Antioxidant enzymes activities were not affected by dietary seaweeds incorporation, but liver peroxidation level was higher in fish fed seaweeds. Overall, no beneficial effects of including the tested algae in the diets was observed, while oxidative status was negatively affected.



Evaluation of dietary inclusion of ALL-G-RICH[®], a microalgae rich in docosahexaenoic acid, for Nile tilapia: performance, body composition, and fatty acid retention

Dr. Vitor Augusto Giatti Fernandes *Aquaculture Department, Federal University of Santa Catarina* **Mr. Fernando Dutra Brignol** *(1)* **Dr. Bruna Mattioni** *Aquaculture Department, Federal University of Santa Catarina* **Ms. Renata Oselame Nobrega**

(1) Prof. Débora Machado Fracalossi (1)

This study evaluated the inclusion of ALL-G-RICH[®], a source of docosahexaenoic acid (22:6 n-3, DHA) in the diet of Nile-tilapia and its effect on growth, feed conversion, body accumulation and retention of fatty-acids. A feeding trial with juvenile tilapia (mean initial weight 8.35 g) tested different inclusions of ALL-G-RICH[®]: 0.0, 0.5, 1.0, 2.0 and 4.0 %. A control diet was also included, containing cod liver oil (CLO), whose DHA level was comparable to the diet 1% ALL-G-RICH[®]. The inclusion of ALL-G-RICH[®] did not affect any growth variable, but influenced body fatty-acid profile and retention, promoting the accumulation of DHA. There was a significantly higher accumulation and retention of DHA in CLO-fed fish compared to those fed similar DHA content in the form of ALL-G-RICH[®]. Selected nutrient digestibilities were also performed with ALL-G-RICH[®], using yttrium oxide as an inert marker. The apparent coefficient digestibility was high for DHA (96.10%). However, it was low for palmitic acid (70.81%), another fatty-acid found in considerable amount in ALL-G-RICH[®]. Our findings show that the inclusion of up to 4% ALL-G-RICH[®] in the diet did not affect the performance of Nile tilapia and was very efficient to further accumulation of body DHA.



Effects of dietary raffinose on growth, non-specific immunity, intestinal morphology and microbiome of juvenile hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂)

Prof. Lin Luo *Beijing Fisheries Research Institute* **Dr. Guanling Xu** *Beijing Fisheries Research Institute* **Ms. Wei Xing**
Beijing Fisheries Research Institute **Mr. Tieliang Li**
Beijing Fisheries Research Institute **Prof. Zhihong Ma** *Beijing Fisheries Research Institute* **Ms. Na Jiang** *Beijing Fisheries Research Institute* **Prof. Lin Luo** *Beijing Fisheries Research Institute*

This study was performed to determine the efficacy of raffinose on the growth, non-specific immunity, intestinal morphology and microbiota of juvenile hybrid sturgeon, (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂). Hybrid sturgeons were divided into 2 groups and each group was fed with diets supplemented with or without raffinose for 56 days. Hybrid sturgeon fed diet supplemented with raffinose had significantly higher final body weight (FBW), specific growth rate (SGR), and weight gain ratio (WGR) than fish fed the control diet ($P < 0.05$). Raffinose in diet had no negative effect on feed intake (FI) and feed conversion ratio (FCR) ($P > 0.05$). Compared with the control diet, the myeloperoxidase (MPO) and respiratory burst (NBT) activities were significantly higher in sturgeon fed the raffinose supplemented diet ($P < 0.05$). The increasing of intestinal villi area and mucosal folds were observed in intestinal tract of sturgeon when they fed the raffinose supplemented diet. Meanwhile, the residual bait of intestinal tract was relatively lower in sturgeon with raffinose treatment. High-throughput sequencing revealed that majority of reads derived from the sturgeon digesta were constituted by members of Proteobacteria, Firmicutes, Fusobacteria and Actinobacteria. Shannon's diversity index existed significant difference among dietary treatments indicating that the overall microbial community was modified to a large extent by dietary raffinose. In conclusion, supplementation of the diet with raffinose is capable of improving hybrid sturgeon growth performances and intestinal morphology, modifying the intestinal microbial composition.



Iron Amino Acid Complex (Availa[®]Fe) and Zn-L-Selenomethionine (Availa[®]Se) in diets

Enhance Cooked White Shrimp (*Litopenaeus vannamei*) Color

Dr. Orapint Jintasataporn *Department of Aquaculture, Faculty of Fisheries, Kasetsart University* **Dr. Terry Ward**
Zinpro Corporation, USA **Ms. Sawinee Chalemlap (1)** **Dr. Srinoy Chumkam**
Faculty of Agricultural Technology, Valaya Alongkorn Rajabhat University,

The color of shrimp is one of the first criteria that consumers consider in making their buying decision. Coloration depends on content of carotenoid pigment in shrimp and has significant impact on market value. To enhance the red color in shrimp, carotenoids like astaxanthin or other pigments are applied in shrimp feed. Due to the high cost of astaxanthin and sometimes shortages in the market, some forms of supplemental mineral may be used to promote the expression of cooked shrimp color, especially red color. This study was conducted to investigate mineral supplementation in shrimp diets that may affect red color in white shrimps *Penaeus vannamei*. This study was a completely randomized design (CRD) with 5 treatments and 4 replications per treatment. Iron amino acid complex and Zn-L-selenomethionine (Zinpro Performance Minerals[®], USA) were applied in shrimp diets which contained 75 mg/kg astaxanthin (Behn Meyer, Indonesia) and 115.5 mg/kg xanthophyll in form of lutein (Kemin, Singapore). The control diet had 0 mg/kg pigment and 0 mg/kg mineral amino complex (T1NC). The other treatment diets contained 75 mg/kg astaxanthin and 115.5 mg/kg xanthophyll. The astaxanthin diet without mineral amino acid complex supplementation was designated as T2As. The astaxanthin diets with 200 mg/kg iron as iron amino acid complex (T3AsFe200), astaxanthin diet with 0.3 mg/kg selenium as Zn-L-selenomethionine (T4AsSe300) and combination of 200 mg/kg iron amino acid complex and 0.3 mg/kg selenium as Zn-L-selenomethionine (T5AsFeSe). The experimental period was 6 weeks. Shrimp fed T4AsSe had better feed utilization and survival rate ($P < 0.05$). Immunity was highest ($P < 0.05$) in shrimp fed T5AsFeSe in terms of number of haemocyte count and PO activity, while total protein was not different ($P > 0.05$). Moreover, cooked shrimp color in shrimp fed T5AsFeSe diet had the highest redness (a^* value, $P < 0.05$) and enhanced the cooked shrimp color from SalmonFan number 23.3 to 30.9 in 6 weeks. Shrimp fed the diet containing astaxanthin and xanthophyll in combination with Availa-Fe and Availa-Se improved the cooked shrimp color to consumer acceptance score within two to four weeks.



Dietary methionine supplementation, but not tryptophan or arginine, improves disease resistance in the European seabass

Dr. Benjamin Costas *CIIMAR* Ms. Marina Machado *CIIMAR* Dr. Rita Azeredo *CIIMAR* Mr. Luis Conceição *SPAROS* Dr. Jorge Dias *SPAROS* Dr. Nuno Santos *Instituto de Investigação e Inovação em Saúde (i3S)*

Arginine, methionine and tryptophan are examples of indispensable amino acids with recognized roles in the immune system and their dietary supplementation proved to increase mammalian host immunity. Therefore, the present study aimed to evaluate the health promoting effects of dietary arginine, methionine and tryptophan supplementation on the European seabass (*Dicentrarchus labrax*). Three independent studies were designed to determine the modulatory effects of arginine, methionine and tryptophan dietary supplementation in the European seabass disease resistance. For each trial, three diets were manufactured: a control diet (CTRL) was formulated to meet the indispensable amino acids profile established for seabass. Based on this formulation, two other diets were supplemented with: i) arginine at two different levels (0.5 % and 1 % of feed, ARG1 and ARG2, respectively); ii) methionine at two different levels (0.5 % and 1 % of feed, MET1 and MET2, respectively); and iii) tryptophan (0.35 % and 0.39 % of feed, TRP1 and TRP2, respectively). Fish were fed these diets for 4 weeks until visual satiation under controlled conditions. At the end of the feeding trial, fish from all dietary treatments were intraperitoneally injected with *Photobacterium damsela* piscicida and cumulative mortality was followed during 21 days.

In response to the Phdp insult, fish fed MET2 increased disease resistance compared to fish fed CTRL and MET1. In contrast, arginine and tryptophan supplementation led to an increased disease susceptibility compared to fish fed CTRL diets. These results suggest that both dietary arginine and tryptophan supplementation may compromise the European seabass disease resistance and the level of both arginine and tryptophan should be carefully considered in diets for European seabass.

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Effect of tryptophan supplementation and handling stressors in meagre (*Argyrosomus regius*) on gut immune gene expression

Ms. Gloria Asencio *CIBNOR, Laboratorio de Fisiología Comparada y Genómica* **Dr. Karl B. Andree** *IRTA, Unitat de Cultius Experimentals* **Ms. Inmaculada Giráldez** *Faculty of Experimental Sciences. University of Huelva* **Ms. Joana Firmino**

IRTA, Unitat de Cultius Experimentals **Dr. Marcelino Herrera** *IFAPA, Centro Agua del Pino* **Dr. Enric Gisbert** *IRTA, Unitat de Cultius Experimentals*

Meagre (*Argyrosomus regius*) is one of the most important new aquaculture species in Southern Europe and several studies are focusing on the optimization of its culture under intensive conditions. Nevertheless, stressors like handling during transport or culture maintenance may affect the immune system impairing some immune responses or provoking cellular damage. One strategy that has been used to avert this type of negative stress response is the supplementation of amino acids to improve resistance to stress. In this experiment, fish were fed for a 7 days period with 1% of tryptophan in a commercial diet and a control diet without tryptophan, a one-way experimental design was conducted, comparing the gene expression levels in fish under air exposure and confinement/netting stress in the different times of sampling (1 hour and 6 hours after stress). For air exposure fish were netted and kept out of the water for 3 minutes, and then taken back to the tanks. Confinement/netting stress was achieved by decreasing the water level (20 cm) and fish being chased with a net (without exposing them to the air) for 3 min repeating every 10 min for one hour. The foregut of individual fish was sampled at 1 and 6 hours post-stress ($n = 7$ per condition), to study gene expression of immune effectors (C3 complement, cyclooxygenase, defensin, glyceraldehyde-3-phosphate dehydrogenase, hepcidin, inteferon type 1, interleukin 1B, interleukin 10, lysozyme, MX protein, piscidin and tumor necrosis factor 1a, normalized with elongation factor 1a and hypoxanthine-guanine phosphoribosyltransferase). Results showed that tryptophan supplementation in feed modulated gut immune gene expression in fish submitted to confinement/netting stress.



Improved feed intake and growth in Snakehead fish (*Channa maculate*♀×*C. argus*♂) fed low fishmeal diets through dietary supplementation with an umami Functional Palatability Enhancer

Dr. Yirong Le *Lucta (Guangzhou) Flavours Co. Ltd.* **Dr. Yuping Sun** *Institute of Animal Science* **Mr. Jesús Matas**
Lucta (Guangzhou) Flavours Co. Ltd. **Dr. Sofia Morais**

Lucta S.A., Innovation Division **Mr. Mingzheng Jiang** *Lucta (Guangzhou) Flavours Co. Ltd.* **Mr. Tiejun Huang** *Lucta (Guangzhou) Flavours Co. Ltd.*

A feeding trial was conducted to test the application of a Functional Palatability Enhancer (FPE) containing umami compounds in improving the feed palatability and performance of Snakehead fish fed low-fishmeal diets. Seven isonitrogenous (43% crude protein) and isolipidic (7% total lipid) test diets were formulated, where diet 1 was a high-fishmeal diet (positive control - PC) containing 25% fishmeal, and diets 2 to 7 were prepared by replacing 10% fishmeal contents with soybean protein concentrate, and supplementing with 0 (negative control - NC), 0.05, 0.08, 0.11, 0.14 or 0.2% of the umami FPE. Triplicate groups of fish (average initial weight 93.5 ± 0.01 g) were randomly stocked in twenty-one 300-l tanks at a stocking density of 30 fish per tank. The fish were fed to satiation by hand twice daily, 7 days per week, for a period of 56 days. Results showed that weight gain (%) and specific growth rate (SGR) of fish were significantly reduced in fish fed the low-fishmeal diet (NC), and increased in a dose-responsive manner with the supplementation of the FPE, being significantly higher than the NC and non-significantly different from the PC at the 0.2% inclusion level. The improved growth was mostly attributable to an enhanced feed intake, which was significantly reduced in the NC but recovered to levels identical to the high fishmeal PC when the FPE was added to the diet at 0.14 and 0.2%. Feed conversion ratio (FCR) was not significantly different between treatments but results showed a trend for a reduced feeding efficiency (increased FCR) when dietary fishmeal level was reduced, while the supplementation with 0.2% FPE reduced the FCR to the same level as the PC. Survival rate was high and not influenced by the dietary treatments. Therefore, based on the overall performance of fish in this study, it can be concluded that Functional Palatability Enhancers containing umami compounds can act as attractants in low-fishmeal diets, and supplementation with this type of products can be an effective strategy for reducing the inclusion levels of marine-based ingredients in fish diets, while maintaining normal feeding behavior and growth in juvenile snakehead fish.



Supplementation of squid flavour and umami palatability enhancers to fishmeal-reduced diets increased feed palatability and growth performance of White shrimp (*Penaeus vannamei*)

Dr. Guangxu Liu *College of Animal Science* **Dr. Haifeng Wang** *College of Animal Science* **Dr. Jiahuang Rong** *College of Animal Science* **Dr. Sofia Morais**
Lucta S.A., Innovation Division **Dr. Maolong He** *Lucta (Guangzhou) Flavours Co. Ltd.* **Mr.**

Feed additives including flavours and umami compounds could be beneficial for improving palatability and functionality of fish diets, especially when dietary fishmeal is replaced by plant-based protein sources. Aimed to apply the proposed new strategy in shrimp production, the present study was designed to test the effect of supplementation of a squid flavour and umami palatability enhancers to fishmeal-reduced diets on feed intake and growth performance of shrimp. White shrimp with average initial body weight of 7.3g were randomly distributed in 21 tanks (40 shrimps/tank) with continuous flowing brackish water. They were provided with one of the following 7 diets (3 tanks/group) for 4 weeks: standard diet containing 30% fishmeal; two fishmeal-reduced diets containing 18% or 6% fishmeal; fishmeal-reduced diets with 0.1% squid flavour plus 0.3% umami; fishmeal-reduced diets with 0.1% squid flavours plus 0.6% umami. A feeding behavior trial was also carried out comparing a control low (6%) fishmeal diet with the same diet with 0.1% squid flavour plus 0.6% umami supplementation. Statistical analyses including one-way and two-way ANOVA were used for testing treatment effects on growth performance among all groups, and between the main factors of dietary fishmeal level, palatability enhancers' supplementation and their interaction. The study found that supplementation of 0.1% squid flavour plus 0.6% umami products reduced ($P<0.01$) shrimp waiting time for approaching and start eating feed. Fishmeal-reduced diets resulted in lower ($P<0.05$) growth rates compared to the standard 30% fishmeal diet, whereas the supplementation with the two palatability enhancers diminished such differences. The analysis on the main factors also found that the supplementation with either 0.3% or 0.6% umami plus 0.1% squid flavour increased ($P<0.05$) shrimp growth performance and net meat percentage. Comparison between treatments with reduced-fishmeal found that the 6% fishmeal diets caused lower ($P<0.05$) growth rates than the 18% fishmeal treatments. There was no significant difference on body protein and amino acid compositions among the groups. In conclusion, the study suggests that diet supplementation with squid flavour and umami products can be an effective strategy to counteract the reduction in feed palatability and growth performance of white shrimp fed fishmeal-reduced diets.



Taste matters? Molecular characterization of taste receptor family TAS1R in seabream (*Sparus aurata*) towards future functional studies

Dr. Anna Rita Angotzi *Department of Fish Physiology and Biotechnology, Institute of Aquaculture of Torre la Sal, Spanish National Research Council (IATS-CSIC)* **Ms. Sara Puchol** *Department of Fish Physiology and Biotechnology, Institute of Aquaculture of Torre la Sal, Spanish National Research Council (IATS-CSIC)* **Mr. José Miguel Cerdá-Reverter** *Department of Fish Physiology and Biotechnology, Institute of Aquaculture of Torre la Sal, Spanish National Research Council (IATS-CSIC)* **Dr. Sofia Morais**
Lucta S.A., Innovation Division

Over the past decade, sustainable aquaculture production promoting increasing dietary replacement of fisheries by plant ingredients has steadily grown, often reducing the palatability of fish diets and animal performance. However, very little information exists on taste-related mechanisms possibly underlying these effects.

Vertebrate taste perception is largely controlled by two families of G-protein-coupled receptors, taste receptor 1 (TAS1R) and taste receptor 2 (TAS2R), involved in recognition of sweet/umami and bitter tastes, respectively. Tastant selectivity through the TAS1R family is determined by the nature of combinatorial arrangement of TAS1R1, TAS1R2 and TAS1R3 heterodimeric subunits. The mammalian TAS1R1/TAS1R3 receptor responds to umami compounds such as amino acids, whereas TAS1R2/TAS1R3 is activated by sweet substances. Nevertheless, in some teleost fish, TAS1R2 duplicated genes revealed species-specific functional plasticity with a prominent response to amino acid ligands.

In order to investigate TAS1R functional properties and their potential distinct evolutionary trajectories in fish, we cloned the full-length cDNA of five TAS1R genes in *Sparus aurata* (sa), including the specific heterodimer subunit of the umami taste (saTAS1R1), three novel sweet taste duplicate genes (saTAS1R2x, saTAS1R2y, saTAS1R2z) and the saTAS1R3 gene common to both umami and sweet taste heterodimers.

Gene expression analyses indicate that, although to a different extent, saTAS1R1, saTAS1R2z, saTAS1R2y and saTAS1R3 genes are expressed in oral, gut and brain tissues. Interestingly, the absence of saTAS1R2x mRNA expression in all tissues investigated and the strikingly high levels of saTAS1R2y gene expression in oral and gut tissues indicate that saTAS1R2 paralogs may be under differential evolutionary pressure and may show functional specialization.

Furthermore, we identified and cloned two G-protein alpha subunit genes (saG(i) α 1 and saG(i) α 2) putatively involved in the first steps of intracellular taste signaling in fish. Heterologous transfection studies on multiple saTAS1R heterodimers coupled to saG(i) α 1 or saG(i) α 2 have just begun to be performed and will contribute to advance important knowledge on fish taste physiology.

This study provides an essential groundwork to further elucidate the evolution and functional roles of the TAS1R gene repertoire in seabream, to ultimately develop products enhancing the attractability of fish diets and possibly also regulating digestive/absorptive functions and metabolism through gut sensing mechanisms.



Tilapia protein hydrolysate improves growth performance, incorporation of tissue protein and antioxidant status in South American catfish

Dr. Juliano Uczay *Universidade Federal de Santa Maria* **Dr. Rafael Lazzari (1)** **Dr. Marcos Luiz Pessatti**
Universidade do Vale do Itajaí **Dr. Thiago El Hadi Perez Fabregat**
Universidade do Estado de Santa Catarina

Fish protein hydrolysate is an ingredient with great potential for use in aquaculture feeds. It may have different biological properties and act as a growth promoter, as the result of the formation of biologically active peptides during the enzymatic hydrolysis. The objective of this study was to evaluate different inclusion levels of tilapia waste protein hydrolysate in diets for South American catfish juveniles (*Rhamdia quelen*), in order to evaluate its effects on growth, blood parameters, biochemical parameters and biomarkers of oxidative stress. South American catfish juvenile (6.42 ± 0.10 g) were divided into 20 experimental aquariums (250 L) connected in a water recirculation system equipped with mechanical and biological filter. Four levels of inclusion (0, 5, 10, 15 and 20%) of SPH were evaluated in substitution to the fish meal. Fish were fed twice a day to apparent satiety with isoproteic (37.0%) and isoenergetic (3500.00 kcal.kg⁻¹). The hydrolysate was produced by enzymatic hydrolysis of carcasses of Nile tilapia (*Oreochromis niloticus*) exposed to swine pepsin enzyme (1:250, Enzyme: Fish) at 36°C for four hours. The experimental design was completely randomized with five treatments and four replications. An inclusion of 20% of tilapia waste protein hydrolysate in the diets increased the growth of South American catfish juveniles. At levels above 5%, there was an increase in protein incorporation in the tissues and increase in the efficiency of oxidative metabolism. Tilapia waste protein hydrolysate can be used as a growth and health promoter for South American catfish juveniles.



Effects of dietary tributyrin supplementations on growth performance, feed utilization and digestive enzyme activities of juvenile hybrid grouper (male *Epinephelus lanceolatus* × female *E. fuscoguttatus*)

Mr. Jin Zibo *Hainan University* **Dr. Wu Xiaoyi** *Hainan University* **Dr. Gao Yujie** (1) **Ms. Li Xiaojun**
(1) **Ms. Dong Yu** (1) **Mr. Zhou Zhiyu** (1) **Mr. Yao Wei** *Hainan University*

A 9-week feeding trial was conducted to evaluate the effects of dietary tributyrin supplementations on growth performance, feed utilization and digestive enzyme activities of juvenile hybrid grouper (male *Epinephelus lanceolatus* × female *E. fuscoguttatus*). Five isoenergetic (340 kcal per 100 g dry matter), isoproteic (53.5% of dry matter) and isolipidic (8.1% of dry matter) experimental diets were formulated to contain graded supplementations of tributyrin (0%, 0.05%, 0.1%, 0.2% and 0.4% of dry matter), and these experimental diets were alleviated as T0, T0.05, T0.1, T0.2 and T0.4, respectively. Each experimental diet was fed to triplicate groups of 11 hybrid grouper juveniles (average initial weight of 4.90 g / fish) which were stocked into small floating cages (L 120cm × W 70cm × H 50cm).

Values of weight gain percentage (WG%) of fish fed T0.05 and T0.1 were higher ($P < 0.05$) than those of fish fed T0, T0.2 and T0.4. Daily feed intake (DFI) of fish displayed a similar variation trend as WG%. Feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish showed no significant ($P > 0.05$) differences among all experimental treatments. The pH values of intestinal contents were positively correlated with the concentrations of dietary tributyrin. Specific activities of trypsin, α -Amylase and lipase in intestine of fish fed T0.05 and T0.1 were higher than those of fish fed T0, T0.2 and T0.4. No obvious correlations were observed in pH values of gastric contents or specific activities of gastric α -Amylase and lipase between fish fed different dietary tributyrin contents. Whole-body compositions were not affected by different experimental treatments. Taken all results into account, it appears that 0.05% of tributyrin supplementation to the diets was suitable for good growth of hybrid grouper juveniles.



Evaluation of the immune-stimulatory effects of dietary housefly larvae meal on juvenile Rainbow trout

Mr. Nathaniel *Sibinga* Cornell University Dr. H  l  ne Marquis (1)

Insect larvae are a promising new feed source for aquaculture. In addition to their nutritional value, insect larvae and insect-derived ingredients appear to have immune-stimulatory properties in fish and other livestock. We and others have shown that the nutritional value of insect larvae varies depending on the substrate used to rear them. We speculate that their immune-stimulatory properties might also be influenced by rearing conditions. In this study, we aimed to assess the immune-stimulatory effect of larva meal (LM) produced from housefly (*Musca domestica*) larvae reared on dairy cow manure. Two experimental diets - 5% and 30% inclusion of LM - were fed to juvenile rainbow trout (*Oncorhynchus mykiss*) for two or eight weeks. Growth and health were monitored over the course of the experiment. At the end of the study, blood and tissue samples were collected. A 20% mortality rate was observed in the 5% LM inclusion diet, as opposed to less than 5% in the control diet and 30% LM inclusion groups. This difference in mortality rate was statistically significant but not readily explainable by technical factors or necropsy of dead and moribund fish. Serum lysozyme activity was significantly increased after two weeks in both experimental diets but stabilized to control levels after eight weeks of feeding. Tissue samples are being processed for RNAseq to assess the expression levels of immune-associated genes. Hopefully, these results will shed light on the potential of LM as an immunostimulant. Future studies will include experiments assessing the resistance of fish fed LM diet to infectious diseases.



NANOTECHNOLOGY: AN EMERGING TOOL FOR NUTRACEUTICALS AND DRUG DELIVERY IN AQUAFEED

Prof. SUBODH GUPTA *Principal Scientist* **Mr. S. Ferosekhan** *Grupo de Investigación en Acuicultura, EcoAqua Institute, University of Las Palmas de Gran Canaria* **Ms. P.M. Nuzaiha** *Fish Nutrition, Biochemistry & Physiology Division, ICAR-Central Institute of Fisheries Education*

Since aquatic animals live worst environmental conditions, they are highly susceptible to diseases. Aquaculture production needs the intensive system of management practices, where antibiotics, drugs and chemicals are frequently used to prevent fish diseases caused by environmental stress and other factors. However, these have been found to be effective only for a short duration besides enhancing the risk of bioaccumulation in environment. Hence, use of nutraceuticals in aquafeed are considered to be safe and effective against various pathogens. These problems can be rectified by incorporating appropriate nutraceuticals in aquafeeds. Aquaculture nutritionists are trying their best to explore new nutraceuticals for aquaculture practices. Nanotechnology may play a vital role in delivery of nutraceuticals and other biomolecules for aquatic animals. Recently we have successfully developed chitosan based nano-sized delivery for yeast RNA as nutraceuticals (Ferosekhan et al., 2014) and trypsin as exogenous enzyme supplement (Kumari et al., 2013). These formulated nanoparticles have shown better performance in compare to their bared forms. These finding are encourage us to work in direction of further improvement to make efficient and environmental friendly nano-sized delivery for other nutraceuticals and pharmacological important molecules. Because nutraceuticals in aquafeed have certain limitations due to its thermolabile nature, pH specificity, etc. To overcome these problems, an efficient delivery system is essential to maximize the efficiency of nutraceuticals in aquafeed.