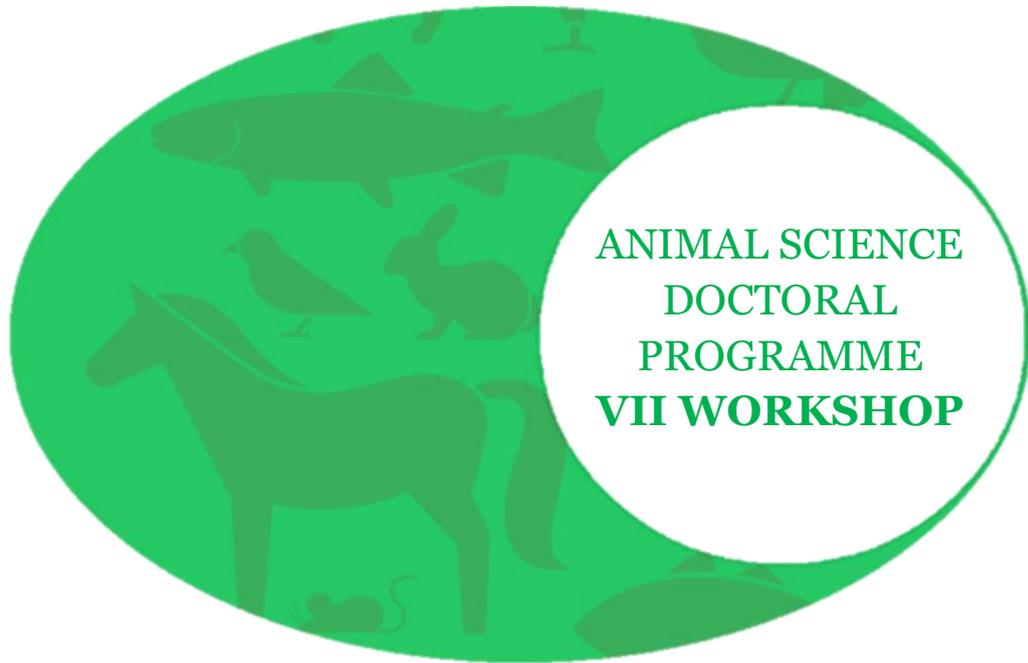


**U. PORTO**



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR  
UNIVERSIDADE DO PORTO



**18<sup>th</sup> SEPTEMBER 2020**

**Salão Nobre, ICBAS-UP**

**Rua de Jorge Viterbo Ferreira No 228**

**Transmitted online via ZOOM for attendees**

**Porto, Portugal**

## The Animal Science Doctoral Programme



The Doctoral Programme in Animal Science of the Faculty of Biomedical Sciences Abel Salazar (ICBAS) of the University of Porto aims to promote advanced training in the field of Animal Science through research. This programme was designed to achieve levels of excellence, with the supervision of researchers associated with research institutions, including several associated laboratories of recognized merit at national and international levels in the different specialities of Animal Science.

ICBAS is recognized for its multidisciplinary characteristics within the Life Sciences, which is reflected in its innovative educational, scientific and cultural project, that presides over the formation of the institution. Being the only Portuguese School which offers courses in Medicine and Veterinary Medicine in parallel with Biochemistry, Bioengineering and Aquatic Sciences, ICBAS is particularly well placed to effectively contribute to the new challenges of Animal Science: the sustainable production of safe and healthy animal food products, using innovative production techniques able to respect animal welfare, increase efficiency and protect the environment. Moreover, a branch of this programme is in an industrial setting, establishing a highly innovative programme (SANFEED - Sustainable Animal Nutrition and Feeding) funded by the Portuguese Foundation for Science and Technology (FCT). SANFEED research themes are generated by its consortium that comprises top-level academic and business partners, bringing business into the research and innovation chain, and promoting the transfer of new ideas from science into technology.

Every year, all students of the Doctoral Programme in Animal Science organise a Workshop to disseminate their newest data among the academia, industry and general society. This year, in the 18<sup>th</sup> September, an exciting high quality programme also includes two plenary lectures with international experts and a round table focused on University-Industry Technology Transfer.

For more information about this programme please access our webpage and follow us on social media at <https://animalscience-phd.pt>.

Luisa M.P. Valente

Director of the Animal Science Doctoral Programme, ICBAS-UP, 18<sup>th</sup> September, 2020

# **ANIMAL SCIENCE DOCTORAL PROGRAMME**

## **- VII WORKSHOP**

**18<sup>th</sup> September 2020**

### **Organization**

**Luísa Maria Pinheiro Valente, CIIMAR & ICBAS-UP**

**Luis Mira Vieira, ICBAS-UP**

**Ana Basto, CIIMAR & ICBAS-UP**

**Cátia Mota, CIIMAR & ICBAS-UP**

**Joana Fernandes, ICBAS-UP**

**Luís Baião, ICBAS-UP, CIIMAR & SENSE TEST**

**Ricardo Pereira, CIIMAR & ICBAS-UP**

**Vítor Silva, ICBAS-UP & SORGAL**

### **Conference Chairpersons**

**Elisabete Matos, B2E CoLAB**

**Joana Silva, Allmicroalgae**

**Ana M. Pereira, ICBAS-UP**

**Margarida Maia, ICBAS-UP**

**Lourenço Ramos-Pinto, CIIMAR**

**Marina Machado, CIIMAR**

### **Conference Round-table Guest Participants**

**Joana Resende, U. Porto, UPTEC & UPin**

**Joana Silva, Allmicroalgae**

**Sara Magalhães, Soja de Portugal S.A.**

**Ana Catarina Guedes, Inclita Seaweed Solutions**

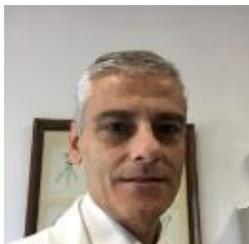
### **Steering Committee**

**Maria Teresa Dinis, CCMAR-UALG**

**Leonor Nunes, CIIMAR**

**Luis Mira Vieira, ICBAS-UP**

## INVITED SPEAKERS



### **Univ. Prof. Dr. Giacomo Biagi**

Professor Dr. is Associate Professor at the Department of Veterinary Medical Sciences, Faculty of Veterinary Medicine - University of Bologna – Italy, head of the Service of Animal Production and Food Safety (SPASA), and member of the FEDIAF Scientific Advisory Board. Since November 2017 he is President of the Italian Society of Nutrition and Animal Nutrition - SIANA. He acquired his doctorate at the University of Bologna, Italy. He was also auxiliary officer program at the veterinary military school "Scuola del Corpo Veterinario Militare" in Pinerolo, Torino (Italy). His Ph.D. Degree as obtained at the Department of Veterinary Physiology and Animal Production, University of Bologna, Italy. Since 2001, he has been responsible for numerous contracts with Italian and foreign companies, in the form of consultancy and research contracts. He was also a lecturer in a myriad of different and distinguished universities worldwide. At present, lecturer at the University of Bologna of four different courses related to animal nutrition.



### **Univ. Prof. Dr. Dušan Palić**

Professor Dr. Dušan Palić comes from a long line of veterinarians and educators, being third generation professor of veterinary medicine. He teaches at the Faculty of Veterinary Medicine at the Ludwig-Maximilians University of Munich, where he is Chair of Fish Diseases and Fisheries Biology. He received D.V.M. and MVSc degrees from Faculty of Veterinary Medicine, Belgrade, Serbia, and Ph.D. from Iowa State University College of Veterinary Medicine, specializing in innate immunology and specifically neutrophil function, with broader focus in aquatic animal veterinary medicine, biosecurity and education. Prof. Palić is a Certified Aquatic Veterinarian (by World Aquatic Veterinary Medical Association). He is currently Vice-President of the Center for Excellence in Aquatic Veterinary Medicine, Biosecurity and Education, and served as Executive Director of the International Aquatic Veterinary Biosecurity Consortium, Vice President of the European College for Aquatic Animal Health, as well as President (2012) and Distinguished Fellow of World Aquatic Veterinary Medical Association.

## AGENDA

### 9:00 • WORKSHOP OPENING

Henrique Cyrne Carvalho (ICBAS Director)

#### Scientific Committee Doctoral of the Programme in Animal Science:

Luísa Valente (ICBAS-UP / CIIMAR)

António Mira da Fonseca (ICBAS-UP / REQUIMTE)

Ana Rita Cabrita (ICBAS-UP / REQUIMTE)

Benjamin Costas (CIIMAR / ICBAS-UP)

Leonor Nunes (CIIMAR)

#### Accompaniment Committee Doctoral of the Programme in Animal Science:

Luísa Valente (ICBAS-UP / CIIMAR)

Luís Mira Vieira (ICBAS-UP)

Luís Baião (ICBAS-UP / CIIMAR / SENSETEST)

Vitor Silva (ICBAS-UP / CIIMAR / SORGAL)

### 09:15 • THE ANIMAL SCIENCE DOCTORAL PROGRAMME IN ICBAS - UNIVERSITY OF PORTO, ASSOCIATE RESEARCH CENTERS AND INDUSTRIAL PARTNERS – INTRODUCTION

## SESSION I

**Chairs:** Elisabete Matos (B2E CoLAB) & Ana M. Pereira (ICBAS-UP)

09:50 • **Plenary lecture:** Nutritional management of chronic enteropathies in dogs, Giacomo Biagi (School of Agriculture and Veterinary Medicine, University of Bologna)

10:20 • **Oral presentation:** Targeted metabolomics characterization of biological samples of dairy cow, Liliana Cordeiro (REQUIMTE/LAQV | AGROS UCRL | ALLTECHADITIVOS)

10:35 • **Oral presentation:** Exploring the benefits of natural and synthetic antioxidant in diets for European sea bass *Dicentrarchus labrax*, Ricardo J. Pereira (CIIMAR | ICBAS-UP)

10:50 • **Pitch presentation:** Microbial community monitoring in a recirculating aquaculture system, Diana Almeida (ICBAS-UP | CIIMAR | SEA8)

### 11:00 • COFFEE BREAK & POSTER SESSION

**Chairs:** Margarida Maia (ICBAS-UP) & Lourenço Ramos-Pinto (CIIMAR)

11:45 • **Oral presentation:** Beyond cortisol – glucocorticoid ratios in an endangered mammal, Alexandre Azevedo (Leibniz-Institute for Zoo- and Wildlife Research | ICBAS-UP)

12:00 • **Oral presentation:** Dietary oxytetracycline treatment can modulate gut health condition in the Nile tilapia (*Oreochromis niloticus*), Carla Teixeira (CIIMAR | ICBAS-UP | SPAROS)

12:15 • **Oral presentation:** Nile Tilapia body composition models and calibration methods, Andreia I. G. Raposo (ICBAS-UP | SPAROS)

12:30 • **Pitch presentation:** Impact of *Tenebrio molitor* larvae meal on muscle growth of European seabass (*Dicentrarchus labrax*), Ana Basto (CIIMAR | ICBAS-UP)

## SESSION II

**Chairs:** Joana Silva (Allmicroalgae) & Marina Machado (CIIMAR)

14:00 • **Plenary lecture:** Healthy fish for healthy people: role of aquaculture in achieving global goals, Dusan Palic (Faculty of Veterinary Medicine, Ludwig-Maximilians University of Munich)

14:30 • **Oral presentation:** Improving immune function through animal nutrition: could customized diets be the future? Ana R. V. Pedro (I3S | ICBAS-UP | REQUIMTE/LAQV)

14:45 • **Oral presentation:** Effect of natural extracts as modulators of growth performance and oxidative status in fish, Maria Xavier (CCMAR | CIIMAR | ICBAS-UP | SPAROS)

15:00 • **Oral presentation:** Inclusion of blood hydrolysates in European seabass (*D. labrax*) diets: impact on nutrient retention and fish resistance to *T. maritimum* infection, Daniela Resende (ICBASUP | CIIMAR)

15:15 • **Oral presentation:** Algal blend: Effects on feed intake, growth performance and whole-body composition of European seabass (*Dicentrarchus labrax*) juveniles, Cátia S. C. Mota (REQUIMTE/LAQV | ICBAS-UP)

15:30 • **Pitch presentation:** Feeds with a micro- and macroalgae blend to increase fish robustness, Mariana Ferreira (CIIMAR | ICBASUP)

### 15:40 • ROUND TABLE “University – Industry Technology Transfer”

**Chair:** Elisabete Matos (Coordinator of B2E CoLAB)

#### **Participants:**

**Joana Resende** (Pro-Rector of U. Porto for Strategic Planning | Director of UPTEC & Coordinator of UPin)

**Joana Silva** (Plant Manager of Allmicroalgae)

**Sara Magalhães** (Sales & Technical Manager of Soja de Portugal S.A.)

**Ana Catarina Guedes** (CTO & Co-founder of ISS – Inclita Seaweed Solutions)

### 16:50 • BEST POSTER, ORAL AND PITCH PRESENTATION AWARDS

**Jury:** Maria Teresa Dinis (CCMAR-UALG), Leonor Nunes (CIIMAR) & Luis Mira Vieira (ICBAS-UP)

### 17:00 • WORKSHOP CLOSING

# **PLENARY SESSIONS**

## **Dietary treatment of canine chronic enteropathies**

*Giacomo Biagi*

<sup>1</sup>Department of Veterinary Medical Sciences, University of Bologna, Italy

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### Abstract

Canine chronic enteropathies (CE) represent a set of inflammatory diseases affecting the intestine that are difficult to manage for both the veterinarian and the owners. Recently, evidence has been gained that dysbiosis of the intestinal microbiota may play an important role as a pathophysiological factor causing chronic inflammation of the intestine. At present, CE are classified on the basis of the therapeutic response: food responsive enteropathy (FRE), antibiotic responsive enteropathy (ARE) and steroid-responsive enteropathy (SRE) (1). In dogs with CE, the improvement of symptoms following the change of diet (usually consisting in the transition to an exclusion diet) is observed in about 70% of cases (2).

Major clinical signs in the presence of CE include diarrhea, increased frequency of defecation, tenesmus, mucous faeces and hematochezia. However, the clinical symptoms can be variable and vomiting, dysorexia and weight loss may also appear. A clinical finding that must be carefully evaluated is the presence of itching and other skin symptoms associated with gastrointestinal clinical signs, as this concomitance must lead to the hypothesis of an allergic FRE. The diagnosis of FRE is based on the response to a dietary trial. Although numerous laboratory tests have been proposed, to date, the gold standard for the diagnosis of FRE is the resolution of symptoms with the transition to a new diet and the possible recurrence of symptoms with the reintroduction of the old diet. An exclusion diet ideally consists of a ration (commercial or home-made) based on ingredients that the animal has never received and which excludes the ingredients contained in the previous diet. Alternatively, it may consist of a commercial diet containing only hydrolyzed proteins. Usually, in presence of FRE, symptom resolution occurs quickly if a proper new diet has been chosen.

With regard to their nutritional properties, diets fed to dogs suffering from CE should be rich in protein and moderate in fat (3). However, the diet may be added with specific sources of fat, such as fish oil (for its antiinflammatory properties) and coconut oil (rich in medium-chain tryglycerides that are easily digested and absorbed). Dietary fat content should be strongly limited if a protein-losing enteropathy has been diagnosed (in this case, fat should not exceed 5% of dry matter, in order to reduce the intestinal flow of lymph). Although fiber is not an essential nutrient in dogs, its addition in moderate quantities to the diet can have positive effects. In fact, fermentable fiber (beet pulp, fiber from legumes and some cereals, such as barley and oats) and prebiotic molecules (fructo-oligosaccharides and others) can stimulate the multiplication of beneficial bacteria which produce volatile fatty acids, among which butyrate is particularly interesting as it has a strong trophic effect on the epithelium of the ileum and large intestine. The use of insoluble fiber (such as wheat bran) can also play a positive role on the clinical picture of the animal, regulating intestinal peristalsis, absorbing toxins and retaining water.

Dietary modulation of the intestinal microbiota is an innovative aspect of CE therapy as it has been seen that the composition of the microbiota plays an important role in the pathogenesis of

CE (3). For this reason, the use of probiotic strains of bacteria and yeasts may become part of the therapy, especially when the use of the exclusion diet does not lead to the resolution of the clinical picture. The use of tyndallized probiotics (probiotics that have been heat-inactivated) has recently attracted attention, as they are capable of maintaining the immunomodulatory properties of vital bacteria. While viable probiotics are not easily added to pet food and are classified as additives (as such requiring specific authorizations), tyndallized strains are simple ingredients and do not require to be protected from all the factors that affect bacterial viability.

In conclusion, canine CE is a relatively frequent pathology that, in most cases, can be managed with a nutritional approach, with no need of drugs. In particular, the use of antibiotics for the treatment of canine CE should be strongly discouraged due to their consolidated dysbiotic effect and the risk of antibiotic resistance.

Keywords: Dogs, Chronic enteropathy, Food-responsive enteropathy, Exclusion diet, Intestinal microbiota

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## **Healthy Fish for Healthy People: Aquatic Veterinarians Help Global Goals**

*Dušan Palić*<sup>1\*</sup>

<sup>1</sup> Chair for Fish Diseases and Fishereis Biology, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Kaulbachstr. 37, 80539 Munich, Germany

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### Abstract

As world aquaculture is steadily growing and overtaking terrestrial protein production, it is becoming clear that sustainable and safe use of ocean resources must be achieved in order to support global goals to provide safe and affordable food for increased human population. What recent history taught us, is that such global expansion of aquaculture is prone to catastrophic crashes due to emerging and re-emerging diseases. Overuse of antibiotics with and without veterinary supervision poses a serious problem to human health as multi-drug resistance is increasing. All of the above comes together in one health approach, in which major pillar is identified to be disease prevention, control and possible eradication through biosecurity approaches that are in accordance with International standards and National legislations, and with utilization of veterinary workforce. As plenary speaker, Dr Palić will provide insights on how aquatic veterinary biosecurity could contribute to sustainable and safe increase in marine resources use for aquaculture.

Intensive aquaculture systems create a highly stressful environment for fish. Crowding, handling, and manipulation suppress the immune response, and the suppression may be further augmented due to exposure to poor environmental conditions and pollutants. Fish kept under immunosuppressive conditions become highly susceptible to disease. Traditional use of synthetic chemicals and antibiotics to prevent or treat fish diseases has achieved partial success. The emergence of antibiotic-resistant microbes and rising concerns about the effects of chemotherapeutics on the surrounding ecosystems present today's industry with challenges to find alternative approaches to prevent diseases. The use of vaccines in aquatic animal disease prevention and control has advanced in recent years, but with limited success. An alternative practical approach to increase disease resistance and vaccination success with reduced use of chemotherapeutics is necessary for continuous growth of the aquaculture industry globally. These alternative approaches and new technologies, such as use of nanomaterials, modification of existing materials, as well as introduction of novel “-biotics” and immunomodulators, can be used to complement and potentially improve chemotherapeutics treatments, vaccination success, disease resistance, and well-being of fish in intensive aquaculture operations, but also in ornamental fish trade, retail and home aquariums. Therefore this presentation also reviews major advances in feed additives in aquaculture.

Keywords: Global Aquaculture, Sustainability Goals, Veterinary Medicine, Functional Feeds, Additives

## Acknowledgements

The author would like to thank the organizers for the invitation to give the plenary lecture. Special recognition goes to all current and past Chair members and collaborators who contributed to the information and results presented here.

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# **ORAL COMMUNICATIONS**

## Targeted metabolomic characterization of biological samples of dairy cows

Liliana Cordeiro<sup>1,3,4\*</sup>, Hugo M. Oliveira<sup>2</sup>, Ana Rita J. B. Cabrita<sup>1</sup>, Margarida R. G. Maia<sup>1</sup>, Luís Ferreira<sup>3</sup>, Francisco Castanheira<sup>4</sup>, José A. Rodrigues<sup>5</sup> António J. M. Fonseca<sup>1</sup>, Inês M. Valente<sup>1,5</sup>

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### Abstract

Metabolic and digestive disorders have a high health and economic impact on the dairy production sector [1]. The high energetic demand of dairy cows during lactation are commonly met by increasing the diet energy density namely by increasing the proportion of rumen fermentable starch that might increase the probability of developing health complications (e.g., ruminal acidosis) [2]. The diagnosis of most of the digestive pathologies is invasive and nonspecific [3, 4]. An alternative strategy focused on the study of potential biomarkers present in biological samples has been proposed over the last years [3, 5] This strategy can improve and complement the current diagnostic methods, maximizing the chances of a highly selective and early-stage diagnosis.

Therefore, this work aimed to characterize the metabolomic composition of different biological samples (rumen fluid, feces and urine) collected from three rumen fistulated multiparous Holstein dairy cows fed diets with different forage: concentrate ratios. The extraction procedure was based on a protocol previously developed [6]. Briefly, acidified samples were extracted by a miniaturized liquid-liquid extraction protocol with diethyl ether and ammonium sulfate. After agitation and centrifugation, the upper phase was collected and analysed by gas-chromatography with flame ionization detection (GC-FID).

In ruminal fluid, the major fraction of the identified compounds was the volatile fatty acids (VFA), *p*-cresol, 1-phenylethanol, and dimethyl sulphide. The compounds determined in feces had a profile similar to the ruminal fluid, yet other compounds such as 2-propanol, 1-phenylethanol, dimethyl disulphide, *tert*-butyl methyl sulphide, and 3-ethylphenol were also identified. The profile of the urine samples was less complex since the most abundant compounds consist of VFA (at lower levels than those determined in the other two samples), *trans*-2-nonenal, 2,6-dimethoxyphenol, and eugenol.

In all biological samples, the majority of the analysed metabolites were related to the digestion and metabolism of carbohydrates and proteins, especially VFA, aldehydes, and alcohols. The

relationship of the compounds detected in the urine and faeces samples with the fermentative processes in the rumen suggests the applicability of those compounds as biomarkers for feed conversion efficiency studies, which will certainly impact the early-stage diagnosis of health disorders caused by unbalanced diets.

Keywords: Biomarkers, Compounds, Dairy cows, Digestive disorders, GC-FID.

### Acknowledgements

This work was financed by National Funds through FCT/MCTES – Portuguese Foundation for Science and Technology within the scope of the project UIDB/50006/2020. LEC also thanks FCT, SANFEED Doctoral Programme, AGROS UCRL and Alltech for her PhD grant PD/BDE/136294/2018. IMV and MRGM acknowledge the funding program (DL 57/2016 – Norma transitória) supported by Fundação para a Ciência e a Tecnologia I.P.

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## Exploring the benefits of natural and synthetic antioxidants in diets for European sea bass *Dicentrarchus labrax*

Ricardo J. Pereira<sup>\*1,2</sup>, Luís F. Baião<sup>1,2,3</sup>, Sónia Batista<sup>2</sup>, Sergio Fernández-Boo<sup>1</sup>, Tiago Sá<sup>1</sup>, Benjamín Costas<sup>1,2</sup>, Rui C. Lima<sup>3</sup>, Luís M. Cunha<sup>4</sup>, Mónica Costa<sup>5</sup>, Miguel Pereira<sup>6</sup>, Débora Campos<sup>6</sup>, Manuela Pintado<sup>6</sup>, Luísa M.P. Valente<sup>1,2</sup>

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<sup>4</sup> GreenUPorto & LAQV/REQUIMTE, DGAOT, Faculty of Sciences of University of Porto, Vila do Conde, Portugal

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<sup>6</sup> CBQF, Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Rua de Diogo Botelho, 1327 4169-005 Porto, Portugal

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### Abstract

The feed industry increasingly relies on the positive effects of antioxidants for feed conservation, fish health and fillet quality traits. Synthetic Vitamin E is one of the most widely used antioxidants in aquafeeds, but recent consumer trends mixed with concerns regarding potential health hazards of synthetic antioxidant sources caused a shift towards natural sources. Annual plant production has passed 600 million tonnes, generating large amounts of byproducts that are important sources of natural antioxidants, e.g. carotenoids. In this study, a commercial-based diet supplemented with a regular dose of synthetic vitamin E (100 mg.kg<sup>-1</sup>) was used as control (CTRL), and compared with a diet containing a higher dose of this vitamin (500 mg.kg<sup>-1</sup>; VITE). Three natural antioxidant sources (tomato, carrot and coriander) were added to the control diet at 2%, originating three experimental diets: TO, CA and CO, respectively. Carotenoid content and antioxidant potential of selected byproducts and diets were analysed. All five isoproteic and isolipidic diets were fed *ad libitum* to *Dicentrarchus labrax* (IBW = 114g) during 85 days. Growth performance, body composition, liver antioxidant enzyme activity, lipid oxidation, immunomodulatory effects, flesh quality and consumer acceptance were evaluated after the trial. Lipid peroxidation, muscle and skin colour, and flesh texture were also evaluated in fillets stored on ice for 8 days. Tomato, carrot and coriander were rich sources of lycopene,  $\alpha$ -Carotene and lutein, respectively. Results showed that the DPPH radical scavenging potential of CA and CO diets more than tripled that displayed by the CTRL. CO diet also had the highest ABTS value.

All diets were well accepted by fish (FBW = 237-248 g). Growth, nutrient utilization and whole body composition did not differ significantly among treatments. Likewise, the immune status and plasma bactericidal activity of European seabass was unaffected by the diets. Vitamin E deposition in muscle reflected dietary levels, being significantly higher in fish fed VITE compared to remaining treatments. Liver antioxidant enzyme activity and total antioxidant potential revealed no differences among treatments. Moreover, no differences were observed in terms of ABTS radical scavenging potential or lipid oxidation (TBARS) of fillets after 8 days of storage. Despite observed fillet colour alterations due to byproduct inclusion, consumer acceptance was

unaffected. Overall, results suggest that neither a high dose of Vitamin E (500 mg.kg<sup>-1</sup>) nor a 2% inclusion of natural carotenoid sources resulted in antioxidant benefits in cultured sea bass compared to diets supplemented with 100 mg.kg<sup>-1</sup> of Vitamin E.

Keywords: natural antioxidants, Vitamin E, circular economy, functional feeds

#### Acknowledgements

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## Microbial community monitoring in a recirculating aquaculture system

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Recirculating aquaculture systems (RAS) provide opportunities to reduce water usage and to improve waste management and nutrient recycling, making intensive fish production compatible with environmental sustainability. Managing disease outbreaks poses specific challenges in RAS, in which a healthy microbial community contributes to water purification and water quality. How the different factors interact and influence the fish and the various purification reactors, is still poorly understood. The main objective of this PhD project is to monitor and model the water quality and microbial communities of a seawater based RAS, with special emphasis on the biofilter, in order to control water quality and bacterial infections throughout the production cycle. The work is being developed in a sole hatchery where a comprehensive characterization of the microbial communities from all RAS compartments, that includes microbiome of the biofilters, water, biofilms and physicochemical parameters was conducted. Total DNA was isolated from the different matrices and the V4-V5 region of the 16S rRNA gene was sequenced using Illumina MiSeq® platform and the output analysed in the DADA2 pipeline, with the SILVA database. This study showed a highly dynamic prokaryotic community in the RAS, that develops different taxonomic profiles in separate compartments, even when they have the same water supply. The prokaryotic community shifts were found to be modelled by water parameters such as salinity, temperature, pH and nutrient load. Currently we are analysing the microbiome data sets to extract scientific knowledge on microbial interactions within this RAS unit to apply it in laboratory experiments to model the biofilter microbial communities for their effectiveness in the control of water quality and the mobility of pathogenic bacteria. At a later stage, the modelling process will be optimized at laboratory trials using fish, water and biofilm inoculum from the hatchery, and finally implemented in the real system in order to validate its efficiency.

**Keywords:** Recirculating aquaculture systems, hatchery, microbial community

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## **Beyond cortisol – glucocorticoid ratios in an endangered mammal**

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### Abstract

Cortisol is the main effector of the neuro-endocrine stress response in mammals and has thus been measured in several matrices in order to quantify “stress”. Hair cortisol measurement has emerged as a non-invasive tool to quantify “stress” in wild species. However, several questions remain concerning cortisol incorporation into hair including local mechanisms at play. Radiometabolism studies have demonstrated that cortisol is partly incorporated into hair as cortisone and other glucocorticoids [1,2]. In some human studies, cortisol-cortisone and cortisol-DHEA ratios showed to be more predictive of allostatic load and stress-related pathology than single hormone measurements [3,4]. These findings raise questions whether local cortisol-to-cortisone interconversion by 11 $\beta$ -hydroxysteroid-dehydrogenase (11 $\beta$ -HSD) isoenzymes, possibly influenced by dihydroepiandrosterone (DHEA), is involved in the regulation of hair cortisol concentrations.

In this study we quantified cortisol, cortisone and DHEA in Iberian lynx hair (n=12) using UHPLC-MS/MS. Next, we assessed the variation in steroid hormones and their ratios between sexes and captive-bred and wild-caught animals of the conservation breeding programme. Comparisons were performed using Mann-Whitney U-tests.

DHEA was the most abundant steroid in Iberian lynx hair, followed by cortisone and finally cortisol. When comparing between sexes, females presented significantly higher cortisol and cortisol-cortisone ratios than males. When comparing origins, there was a trend toward higher cortisol in wild-caught animals. However, when using cortisol-cortisone and cortisol-DHEA ratios, this difference between wild-caught and captive-bred animals became highly significant. Our results add to the evidence of radiometabolism studies and are consistent with the hypothesis of an integrated local mechanism of cortisone and cortisol interconversion through 11 $\beta$ -HSD enzymes regulated by DHEA. To this date validation studies using repeated ACTH challenges and known stressors have focused solely on cortisol. A more comprehensive approach comparing the simultaneous variations in hair cortisol, cortisone and DHEA (and their ratios) in response to repeated ACTH challenges and known stressors will be necessary to clarify these questions.

Keywords: cortisol, cortisone, DHEA, 11 $\beta$ -HSD, glucocorticoid ratios

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## **Dietary oxytetracycline treatment can modulate gut health condition in the Nile tilapia (*Oreochromis niloticus*)**

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### Abstract

Bacterial richness and diversity in the gut microbiota are extremely important for normal metabolic and immunological functions. Antibiotics, psychological and physical stress, and certain dietary components can contribute to intestinal dysbiosis, which is an imbalance between commensal and pathogenic bacteria, that can contribute to inflammatory diseases. This study was carried out to evaluate the immunomodulatory effects of oxytetracycline (OTC) dietary treatment on gut gene expression in Nile tilapia (*Oreochromis niloticus*) juveniles. A group of 315 tilapia (ABW: 118 ± 0.8 g) was randomly distributed among 9 tanks (35 fish each tank), in a freshwater recirculating system (23 °C, 10 h light /14 h dark) at UTAD (University of Trás-os-Montes e Alto Douro) facilities. All animals were acclimatized to the experimental conditions and fed with a control diet (CTRL) that fulfilled the known nutritional requirements for Nile tilapia, during 15 days. Thereafter, animals from 6 tanks were fed with a medicated feed (OTC) at a therapeutic dose (55 mg Kg<sup>-1</sup> body weight day<sup>-1</sup>), whereas fish from the other 3 tanks were fed with the control diet (CTRL). During OTC treatment, water from those tanks was not shared and freshwater was continuously added daily to allow water renewal (50 % d<sup>-1</sup>). After 10 days, fish from OTC treatment shifted to the control diet (OTC-CTRL) or an extreme diet (OTC-ED). The feeding trial lasted for 55 days. Nile tilapia juveniles were fed twice a day, until apparent satiety. Several samplings (n = 9 per tank) were performed: i) at 15 days to determine gut health condition before OTC treatment; ii) at 25, 26 and 33 days to evaluate the effects of the medicated feed; iii) at 40 and 55 days to evaluate recovery after OTC treatment. Anterior and posterior portions of the intestinal tissue were collected to evaluate the expression of immune-related genes, as described by Machado *et al.* (2020)<sup>1</sup>. Among the 3 treatments, fish from OTC-ED group showed an up-regulation in the expression levels of genes related to innate immune, inflammatory and anti-oxidant responses in the posterior intestine, at 26 days (CAT), at 33 days (TNF $\alpha$ , IL1 $\beta$ , CAT, PNCA, IL10), at 40 days (IL1 $\beta$ , CAT), and at 55 days (G6PD, CASP3). Further analyses will be performed to evaluate the effects of oral administration of OTC in the gene expression of the anterior intestine.

Keywords: Oxytetracycline, Immunity, Intestinal inflammation, Gene expression, *Oreochromis niloticus*

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## Nile Tilapia body composition models and calibration methods

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### Abstract:

Proximal body composition analysis is an important tool to evaluate fish condition and understand their nutritional requirements. Usually, this type of analysis is performed using standard methods [1], but it requires animal sacrifices, involves toxic chemicals and can be expensive and time-consuming for fish farmers. Prediction tools can be used to overcome some of these problems, by leveraging collected data in mathematical models. The most common models use linear regression analysis to predict the body composition of animals, assuming either isometric or allometric relationships between body weight and body components (i.e. moisture, crude protein, crude lipid, ash) [2]. In the specific case of Nile tilapia (*Oreochromis niloticus*), there are several published models and it is interesting to note that some authors use different models for different components (Van Trung et al. 2011), being the justification for these choices, and the consequences in terms of predictive capability, unclear.

The objective of this work was to compare and evaluate different types of models and calibration methods, to obtain a Nile tilapia body composition model with a demonstrable high predictive capacity.

For this purpose, Nile tilapia whole-body composition and corresponding whole-body weight data were collected from 141 publications (926 measurements, in total), covering fish sizes from 0.01 g to 2400 g. Regression analyses were performed for each body component and both isometric and allometric models were tested with different calibration methods. The “model + calibration method” combinations were evaluated with qualitative (diagnostic plots) and quantitative methods (RMSE, MAE and MAPE). Then, a repeated K-fold cross-validation was performed for all combinations, in order to objectively evaluate their predictive capacity. After determining the optimal “model + calibration method” combination for tilapia, optimal models were calibrated using all data.

The results showed that the prediction capability is greatly affected not only by the type of model, but also by the calibration method used. In particular, models calibrated under the assumption of multiplicative noise seem to have a better prediction capability than those where noise was assumed to be additive, displaying more reasonable and stable predictions for body weights below 100 g. This suggests that performing the regression in “log space” is not only convenient, but advisable, even for isometric models.

Concluding, this study shows the importance of testing body composition models and their assumptions, in order to ensure high predictive capacity.

Keywords: Mathematical models, Body composition, Tilapia, Regression analysis, Prediction tools

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## Impact of *Tenebrio molitor* larvae meal on muscle growth of European seabass (*Dicentrarchus labrax*)

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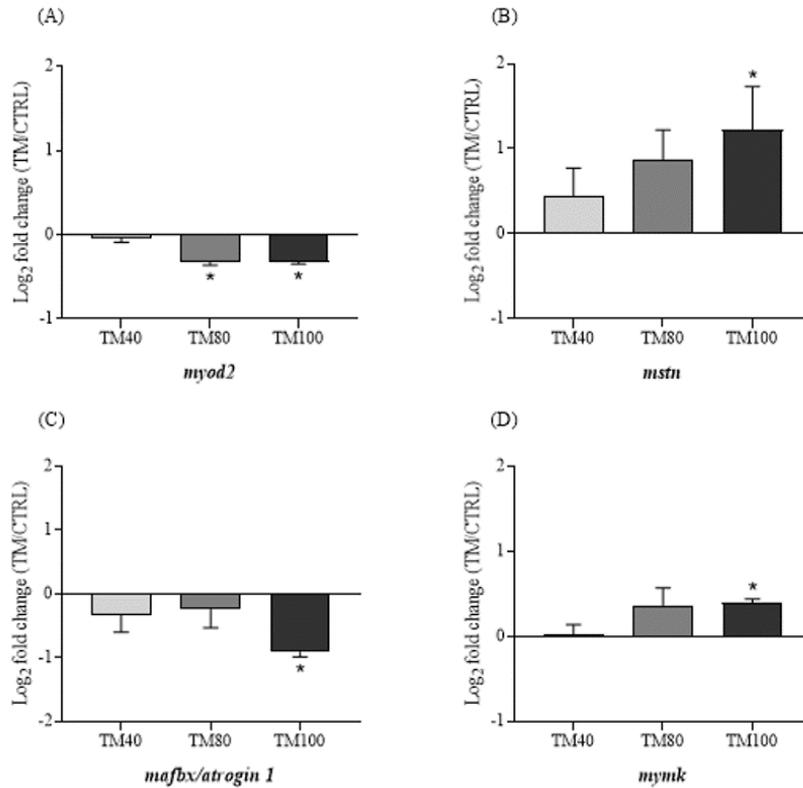
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In 2017, the use of insects as protein source for aquafeeds was authorized by European Union. *Tenebrio molitor* larvae meal (TM) is a valuable protein source (up to 70% DM) with well-balanced amino acid profile, and a well-established industrial production. The success of aquaculture not only depends on fish growth, but also on the ability to produce high quality products for consumers. Muscle tissue represents the edible part of fish, being flesh firmness one of the most appreciated characteristics. Fillet texture is largely dependent on muscle cellularity which also determines fish growth potential. Thus, this study aimed to evaluate the impact of defatted TM (TMd), to substitute fishmeal (FM) in diets for European seabass. Four isonitrogenous diets were formulated to replace increasing levels of FM by TMd: 0, 40, 80 and 100% (CTRL, TM40, TM80 and TM100, respectively). Each diet was assigned to triplicate groups of 25 fish (55±5g) fed 3 times daily, in a recirculating saltwater system (35‰, 22±1°C). After 10 weeks of feeding, fish were individually weighed and measured. Muscle from 3 fish/tank was collected for evaluation of gene expression, cellularity, colour and texture profile. Overall, fish tripled their weight. Feed intake was lowest in fish fed TM100, but these fish had the best FCR resulting in similar final body weight and final condition factor between treatments. Fish fed TM80 and TM100 had lower expression of myogenic factor 2 (*myod2*) than those fed CTRL, while myostatin (*mstn*) expression only was lower in fish fed TM100 when compared to CTRL (Figure 1). A compensatory expression of muscle atrophy F-box/atrogenin 1 (*mafbox/atrogenin1*) and myomaker, myoblast fusion factor (*mymk*) was observed, being the expression of these genes higher in fish fed TM100 than CTRL (Figure 1). Muscle cellularity, texture and colour parameters were similar between fish fed different treatments. These results indicate that the observed gene expression modulation wasn't enough to produce a significant alteration in muscle phenotype, suggesting a high potential of TMd as protein source for aquafeeds.



**Figure 1.** Fold-changes (TM/CTRL) of differentially expressed genes in muscle tissue: *myod2* (A), *mstn* (B), *mafbx/atrogen 1* (C), *mynk* (D). The asterisks indicate statistically significant differences (\*,  $P < 0.05$ ; t-test) between European seabass fed TM and CTRL. Values  $> 1$  indicate up-regulated genes in TM fish; values  $< 1$  indicate down-regulated genes in TM fish.

**Keywords:** Insect protein; muscle growth; European seabass

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## Improving immune function through animal nutrition: could customized diets be the future?

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### Abstract

Dietary supplements, such as yeast cell wall-based products, have long been used to improve immune function, growth and performance and to reduce negative effects associated with stress and disease. Such an approach may be particularly useful in calf rearing, where infectious diseases (such as neonatal diarrhoea and bovine respiratory syndromes) are major health concerns and the main cause of calf mortality [1,2]. Several studies support the benefits of yeast-based supplementation in animal nutrition by modulating immune function [3]. Some of these compounds have been shown to reprogram immune cells through “trained immunity”, a mechanism by which innate immune cells show enhanced activity after a first exposure to antigens [4]. In ruminant nutrition however, most of the studies regarding supplementation as a means to enhance immune function are observational, lacking mechanistic data and very often presenting confounding results. Our aim was to understand the mechanisms by which bovine cells respond to yeast-derived  $\beta$ -glucans *in vitro* and to explore what could explain the differences observed among individuals, namely by the study of the bovine  $\beta$ -glucan receptor: Dectin-1. Peripheral blood monocytes of Holstein-Friesian cows were *in vitro* stimulated with soluble  $\beta$ -glucans, particulate  $\beta$ -glucans and zymosan. Cytokine and Dectin-1 mRNA expression were evaluated by qRT-PCR. Data was analysed using the mixed model of the GraphPad Prism 8.2.1 software including the fixed effect of treatment, the random effect of the animal, and the random residual error. Cytokine expression was positively correlated with Dectin-1 expression, suggesting an immunomodulatory effect of yeast-derived products through Dectin-1-signalling in bovine monocytes. Cytokine profile differed among  $\beta$ -glucans, which could be explained by the activation and downstream cell signalling through other receptors (rather than Dectin-1 alone). These results suggest that the magnitude of the cell’s response to yeast-derived products may be different among animals according to its Dectin-1 expression. Our results also suggest that the effects observed, namely the cytokine profile, may depend on the  $\beta$ -glucan used to stimulate the cells.

Keywords: Calves, yeast-derived beta-glucans, bovine dectin-1

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## Effect of natural plant extracts as modulators of growth performance and oxidative status in fish

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Plant based extracts such as green tea, curcumin and grape seeds are known for their high amount and diversity of polyphenols. These bioactive compounds have high antioxidant capacity making them good additives to include in fish diets, potentially improving the antioxidative status of fish, therefore, enhancing growth and stress resistance of farmed fish.

The objective of our study was to assess the effect of natural antioxidants in growth performance, muscle morphometry, oxidative status and thermal stress resistance of Senegalese sole postlarvae. A commercial diet (CTRL) and three experimental diets supplemented with curcumin (CC), green tea (GT) and grape seeds (GS) extracts were fed to sole postlarvae for 25 days. Growth performance, muscle cellularity, oxidative status and the expression of muscle growth and antioxidant defences related genes were assessed. The remaining fish were then submitted to a thermal stress after which oxidative status of the fish was evaluated.

The diets CC and GS significantly improved growth performance of fish compared to the CTRL diet. Sole fed CC diet had larger muscle cross sectional area, a higher number of muscle fibres and an increased proportion of large-sized fibres (>25 µm) compared to the CTRL. In addition, the inclusion of curcumin resulted in a significant up-regulation of the *myogenic differentiation 2* (*myod2*) and the *myomaker* (*mymk*) transcripts. The CC sole showed a tendency for lower lipid and protein oxidative damage, however, not statistically different from the CTRL group. In opposition, juveniles fed GT and GS diets presented higher lipid peroxidation and protein carbonilation, suggesting a potential pro-oxidant effect of these dietary supplements. However, after thermal stress, fish from GT and GS diets were able to revert such pro-oxidant effect and evidenced improved oxidative status.

The results demonstrate that the dietary supplementation with curcumin and grape seed extract promoted Senegalese sole growth. In the case of curcumin, enhanced growth was associated with both hyperplasia and hypertrophy of muscle fibres, through an up regulation of myogenic factors. However, the use of green tea and grape seed extracts in fish diets will require further evaluation to fully explore the potential of these natural antioxidants and identify the most adequate inclusion level for Senegalese sole diets.

Keywords: Supplemented diets; Plant extracts; Muscle growth; Oxidative status

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## **Inclusion of blood hydrolysates in European seabass (*D. labrax*) diets: impact on nutrient retention and fish resistance of to *T. maritimum* infection**

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### Abstract

Bioactive peptides are small amino acid chains with interesting properties (beyond their nutritional value), including antioxidant, mineral-binding, immunomodulatory or antimicrobial activities [1]. In this study, we hypothesised that including blood hydrolysates (BH) in aquafeeds could promote fish robustness, increase the economic value of animal blood and minimize waste, contributing to a circular economy. Additionally, BH with antimicrobial properties may address the *T. maritimum* infections that occur in aquaculture farms, with high impact on fish health, well-being and aquaculture production.

Three fractions of swine BH obtained by autohydrolysis (AH) or enzymatically were selected. AH was oven dried. The BH obtained enzymatically were further submitted to a micro- (MF) and nanofiltration (NF) and freeze-dried. Dried hydrolysates were then included in five isolipidic and isoproteic diets for European seabass: a fishmeal (FM) based diet (positive control, PC), a commercial-based diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 juveniles (initial weight 12.3±1.4 g), fed three times daily until apparent satiation in a recirculating saltwater system. Growth, nutrient utilisation and whole-body composition were evaluated after 12 weeks. At the end of the trial, ten fish per tank were infected with *Tenacibaculum maritimum* ( $3.5 \times 10^5$  cfu/L), in a two-hour water bath, and mortality was assessed for 8 days.

Fish fed PC had the highest final weight, followed by NC and NF. The NC had a significantly higher weight than the MF and AH groups. Specific growth rate (SGR) and feed conversion ratio (FCR) of fish fed NF showed no significant differences from those fed PC and NC. Fish fed AH also displayed similar SGR to those fed NF and NC, but differed significantly from the MF. Despite no significant differences among treatments regarding final body composition, lipid and energy retention and gain were lowest in fish fed MF, which also had the lowest condition factor. Protein retention was unaffected by dietary treatments. Regarding the infectious challenge, NF had the lowest mortality rate that differed significantly from the NC and MF groups. Mortality for fish fed AH and PC did not differ from NF. MF showed the highest mortality. Overall, results indicate that dietary supplementation with NF clearly improves the resistance of European seabass to *T. maritimum* without affecting growth rate and can hence become a valuable ingredient for this species.

Keywords: Blood hydrolysates; fish robustness; circular economy; bioactive peptides; *Tenacibaculum maritimum*

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## Algal blend: Effects on feed intake, growth performance and whole-body composition of European seabass (*Dicentrarchus labrax*) juveniles

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### Abstract

Microalgae and macroalgae are valuable sources of nutrients and bioactive compounds of interest for aquaculture [1-3]. Individual microalgae or macroalgae species have been shown to exert positive effects on feed efficiency, growth performance and carcass quality but in a dose-dependent and species-specific manner; best results being reported up to 10% inclusion level [4-7]. However, the combined effects of microalgae and macroalgae species are still largely unknown. This study aimed to evaluate the effects of a commercial blend targeted for the feed market (Algaessence™; ALGAplus/Allmicroalgae, Portugal), composed of two microalgae (*Chlorella* sp. and *Nannochloropsis* sp.) and two macroalgae (*Ulva* sp. and *Gracillaria* sp.) species, on feed intake, growth performance and whole-body composition of European seabass (*Dicentrarchus labrax*) juveniles. Four iso-proteic (52.8% dry matter, DM), iso-lipidic (15.3% DM) and iso-energetic (21.7 kJ GE/g DM) diets were formulated with increasing levels of algae blend (0, 2, 4, and 6%). Each diet was fed to triplicate groups of 42 fish (11.3 ± 2.7 g) for 12 weeks. Data were analysed using the General Linear Model procedure of SPSS software (IBM SPSS statistics V26, USA). The statistical model included the fixed effect of diet and the random residual error. Multiple comparison of means was performed with Tukey's test. Effects were considered significant when  $P \leq 0.05$  and a trend when  $0.05 < P \leq 0.10$ . After 12 weeks, fish final body weight and length increased with algae blend inclusion, the highest value being observed with 6% ( $P < 0.001$ ). Compared to the control (0% algae blend), fish fed with Algaessence had higher voluntary feed intake, specific growth rate, and daily growth, viscerosomatic and hepatosomatic indexes ( $P < 0.001$ ). The lowest feed conversion ratio was observed with 2% and 4% algae blend ( $P = 0.024$ ); protein efficiency ratio being the highest with 4% ( $P < 0.001$ ). Whole-body composition was affected by algae blend supplementation, which increased energy and lipid contents and reduced moisture content ( $P < 0.001$ ). Algae blend increased the daily gain of DM, protein, lipid and energy, compared to control ( $P \leq 0.002$ ) and the retention:consumption ratio of DM, lipid and energy ( $P < 0.001$ ). Conversely, protein retention:consumption ratio and whole-body content were unaffected ( $P \geq 0.098$ ). Overall results suggest that the dietary supplementation

of a micro and macroalgae blend improved feed utilisation, growth performance, daily gain and retention:consumption ratio of nutrients on European seabass juveniles. However, at 6% inclusion, algae blend promoted the highest lipid whole body content and daily gain, suggesting that lower levels should be used to prevent excessive fat accumulation in adults.

Keywords: Algae blend, European seabass, Feed utilisation, Growth, Whole-body composition

### Acknowledgements

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## Feeds with a micro- and macroalgae blend to increase fish robustness

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European seabass (*Dicentrarchus labrax*) is an important species of the Mediterranean region, but intensification of its farming often increases disease outbreaks. Due to the growing antimicrobial resistance, a prudent use of antibiotics in animals is advised. Algae are valuable sources of bioactive compounds with antibacterial activity, being promising products to improve fish health. This work aims to unravel the antimicrobial potential of commercially produced algae and their impact on *D. labrax* immune status and health using a double approach. Firstly, the *in vitro* bactericidal activity [1] of two microalgae (*Nannochloropsis oceanica* and *Chlorella vulgaris*), two macroalgae (*Gracilaria gracilis* and *Ulva rigida*), and a blend of these algae was tested against main pathogenic bacteria for farmed fish (*Vibrio anguillarum*, *Photobacterium damsela* subsp. *piscicida*, *Tenacibaculum maritimum*, *V. harveyi*, *V. parahaemolyticus*, *Aeromonas hydrophyla*, *Yersinia ruckeri* and *Edwardsiella tarda*). Four isoproteic and isolipidic diets were then formulated with increasing amounts of the algae blend (0, 2, 4 and 6%) for an *in vivo* study. After feeding diets to triplicate groups of European seabass for 12-weeks, fish were subjected to a bacterial bath challenge (*T. maritimum*) and reallocated into 2 new systems: one to register mortalities over 8 days, triplicates of 10 fish; and other to collect samples after 48h, duplicates of 10 fish. For the 48h-challenge, a non-infected group with similar handling was also included. Blood was collected from both groups to analyse haematological profile: total white (WBC) and red (RBC) blood cells counts, haematocrit (Ht), haemoglobin (Hb) and mean corpuscular volume (MCV), haemoglobin (MCH) and haemoglobin concentration (MCHC) [1]. One-way or two-way ANOVA followed by Tukey's test was performed and cumulative mortality was analysed using Chi-square test. Results showed that algae displayed bactericidal activity against most groups of pathogenic bacteria, but were unable to kill *V. anguillarum*, *A. hydrophyla* and had limited effects against *Y. ruckeri*. The highest activity (68%) was observed with *N. oceanica* against *E. tarda*, followed by a 40-45% activity with all algae and blend against *T. maritimum*. After the 48h-challenge, Ht (regardless of infection treatment) and WBC (non-infected group) were increased in fish fed 6% diet compared to those fed CTRL (0%). RBC and MCV were higher and lower, respectively, in infected group compared to non-infected. The cumulative mortality was lowest in fish fed 4% diet (33%)<6% (50%)<Ctrl (53%)<2% (63%). Further studies are required to confirm the potential of algae to improve fish health and disease resistance.

**Keywords:** European seabass, algae, fish robustness, bacterial disease

### Acknowledgements

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# **POSTERS**

**Dietary supplementation with *Gracilaria* sp. by-products modulates stress response, antioxidant and immune systems in gilthead seabream (*Sparus aurata*) exposed to crowding**

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Abstract

The use of seaweeds (SW) by-products appears as a promissory source of bioactive compounds for application in aquaculture. This study evaluated the effects of dietary supplementation with by-products obtained from the *Gracilaria* sp. on the stress response, antioxidant and immune systems in seabream (*Sparus aurata*) exposed to an acute crowding event. Seabream were fed to apparent satiety for 59 days a diet containing a commercial antioxidant (CTR), and this diet without antioxidant addition (NO-AOX) or supplemented with 0.5% ethanolic extract (0.5% EE), 5% ethanolic waste (5% EEW), 2.5% or 5% of agar waste (2.5% AW and 5% AW, respectively). After this period, fish were exposed to 1 h of crowding (100 Kg m<sup>-3</sup>) and sampled immediately after (0 h) and at 24 h post-stress. The effect of crowding on several stress markers were assessed by t-tests, by comparing crowded and no-crowded groups of fish fed the CTR diet at 0 h or at 24 h, as well as between times within each condition. Differences in physiological responses of fish exposed to crowding between experimental diets were analysed by a one-way ANOVA, and a Tukey test was used to identify pairwise differences. Differences over time for each experimental diet were analysed by a t-test. Data were checked for normality and homogeneity of variances (Shapiro-Wilk test and Brown-Forsyth test, respectively). Significant differences were considered when  $P < 0.05$ .

Fish fed AW and EEW diets displayed lower plasma cortisol levels than those fed the CTR diet, suggesting an improved stress response to crowding on these groups. No differences were detected on hepatic lipid peroxidation (LPO) levels between dietary groups after crowding. However, the seabream fed 0.5% EE diet displayed lower hepatic glutathione reductase (GR) activity than fish fed the NO-AOX diet. Additionally, glutathione peroxidase (GPx) activity was decreased in the liver of fish fed 2.5% and 5% AW diets compared to the other dietary groups. Seabream fed the 2.5% AW diet displayed higher plasma peroxidase activity than fish fed NO-AOX or 5% EEW diets, suggesting a modulatory effect of dietary AW at 2.5% in the humoral immune response in fish exposed to crowding. Results suggest that dietary supplementation with AW, particularly when supplemented at 2.5%, could be used as a dietary tool to mitigate oxidative stress when improving immune response in seabream exposed to a stressor.

Keywords: Seabream, by-products; *Gracilaria* sp., stress response, antioxidant compounds, immune stimulant compounds.

## SYSTEMIC IMMUNE RESPONSES OF GILTHEAD SEABREAM *Sparus aurata* JUVENILES FED MICROALGAE-DERIVED BETA-GLUCANS

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### Abstract

The present work aimed to evaluate the effects of both short- and long-term dietary supplementation with microalgae (*Phaedactylum tricorutum*) extracted  $\beta$ -glucans on immune related genes expression, oxidative stress biomarkers and plasma immune parameters in gilthead seabream (*Sparus aurata*) juveniles.

The trial comprised four isonitrogenous (63% crude protein) and isolipidic (17% crude fat) diets. A practical diet was used as CTRL, whereas 3 others based on CTRL were further supplemented with a constant dose of  $\beta$ -glucans, derived from *Saccharomyces cerevisiae* (diet YEAST) and different extracts of *P. tricorutum* (diets PH21 and PH37). Diets were randomly assigned to quadruplicate groups of 200 gilthead seabream (initial body weight:  $4.1 \pm 0.1$ g) that were fed to satiation three times a day for 8 weeks in a pulse feeding regimen. Fish were first fed the different experimental diets intercalated with the CTRL diet every 2 weeks. After 2 and 8 weeks of feeding, 3 fish/tank were sampled for blood and tissues collection.

All groups showed equal feed conversion ratio (FCR) and relative growth rate (RGR) values (1.2 and 3.8 %/day, respectively) and attained similar final body weight (FBW) (CTRL:  $41.4 \pm 1.6$  g; C+:  $42.5 \pm 0.9$  g; PH21:  $42.1 \pm 1.1$  g and PH37:  $41.9 \pm 1.9$  g). Regarding immune parameters, no changes were observed in plasma bactericidal and anti-protease activities and IgM levels among different dietary groups. However, the relative proportion of circulating lymphocytes increased in seabream fed the diet PH21 compared to fish fed the CTRL diet after 2 weeks. In contrast, seabream fed the 3 supplemented diets decreased the relative proportion of peripheral lymphocytes compared to those fed CTRL at the end of the feeding period. Liver lipid peroxidation, catalase and superoxide dismutase activities showed no differences among dietary treatments.

$\beta$ -glucan supplementation did not affect fish growth performance. The absence of clear effects on plasma immune parameters and oxidative stress biomarkers may suggest that dietary treatments did not activate a humoral immune response. However, dietary treatments appeared to modulate peripheral lymphocyte numbers with opposite effects. Results pointed to an immunostimulatory effect of diet Ph21 after 2 weeks of feeding, but a long term feeding (i.e. 8 weeks) with  $\beta$ -glucan supplementation seems to negatively affect the adaptive arm of the immune system with a drop in circulating lymphocytes. Still, and having in mind the nature of  $\beta$ -glucan stimulus, it is reasonable to expect a more evident local immune modulation in the gut. Further analysis on gut samples are currently underway.

Keywords: Microalgae  $\beta$ -glucan; Pulse feeding; immunostimulatory

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## **Impact of presentation on liking, acceptance and sensory profiling of sea urchin gonads from the North Atlantic coast of Portugal**

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### Abstract

Sea urchin gonads are receiving increasing attention for their exclusive sensorial attributes. However, the sensory profile of this highly demanded gourmet product is not well described yet, and it may potentially help enhancing gonads marketability and acceptability. The main goal of this study was to build a sensory profile of sea urchin (*Paracentrotus lividus*) gonads, to evaluate differences between sex, harvest location, as well as the impact of presentation on consumers acceptance. Sixty untrained panellists (regular consumers of seafood) were asked to evaluate eight samples of raw sea urchin gonads, divided by sex, harvest location and presentation (in a clear translucent glass bowl or inside a sea urchin test). The panellists evaluated overall liking and acceptance (Food Action Scale), followed by a Check-All-That-Apply (CATA) ballot with a list of 38 sensory attributes divided into four dimensions: appearance (9), odour (8), texture (7) and taste (14), launched in Compusense. From a three-way ANOVA, no significant effect of sex, harvest location and presentation were identified for both liking and acceptance, although a significant interaction between presentation and sex was identified: presentation in test favoured female gonads and undervalued male gonads. Sensory profiling clearly separates gonads according to sex, with females being more appealing than males that presented a milky white fluid. Gonads were also separated according to the presentation method: the white background of the bowl highlighted the orange colour of females and masked the male white fluid, while the gourmet presentation was correlated with a fresh, tropical and pleasant odour. Moreover, the gourmet presentation favoured the females in relation to the males, mainly because the male gonad colour and white exsudate were emphasized by the background of the sea urchin test. This study concluded that sea urchin with an orange gonad and a sweet, fresh and tropical flavour is preferable, which will allow future nutritional research efforts to be focused in the enhancement of these gonad attributes set.

Keywords: CATA; *P.lividus*; sea urchin; seafood; sensory characterization; aquaculture

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## **Microbiome in poultry: influence of nutritional factors**

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### Abstract

Poultry has a major relevance for human feeding, being one of the most important protein sources. Over the past century poultry has transitioned from predominantly breeding layers to breeding a mixture of both layers and broilers, based on the consumers demand evolution. Optimization of zootechnical performance through diet adjustment is one pivotal focus point for the industry. Chicken's digestive system is adapted to extract energy and nutrients from difficult to digest food sources, which requires an interplay between the chicken and the microbiota present within the gastrointestinal tract (GIT). While supplementing the chicken's diet with prebiotics and probiotics, it is expected that the zootechnical performance is improved through positive selection of the GIT microbiota. Also, genetic potential is tough not yet achieved. Protein and Aminoacid levels' fine tuning may help understand how far optimal growth is. Recent advances in culture-independent techniques allowed the identification of new taxa within the microbiota. The chicken GIT harbors a very diverse microbiota that aids in the breakdown and digestion of food and comprises over 900 species of bacteria, which contain both human and animal pathogens. Unlike mammals, caecum has an important role in birds' digestion, being a fermentation powerhouse due to the presence of a dense microbial community. The main objective of this work is to obtain information regarding the chicken GIT microbiome and selectively increase the chicken growth process performance. Histological samples will be scanned to evaluate possible morphological changes regarding the different feedings. Villi height and quantity was assessed as well as the different cell types present. Combined with the different microbiome analysis results, genetic diversity of the gut microbiome, obtained via Next Generation Sequencing (NGS), will give the possibility to infer which supplements will have a positive effect on chickens' growing and nutrient absorption as well as improved wellbeing and reducing the stress and cost of production.

Keywords: Poultry; Microbiome; Livestock; Feeding efficiency; Nutrition

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## **Novel functional ingredients in pet food - a multifactorial study of alternative diets for dogs**

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### Abstract

Pets, especially cats and dogs, are kept as companion animals in most of households in developed countries [1,2], and, as a result of the continuous growth of the global population, their number is increasing worldwide. Consequently, awareness of sustainability of pet industry has increased, and efforts to make environmentally, economically and socially sustainable pet food are needed. Indeed, this sector faces several challenges such as the competition for ingredients with livestock or human food system [3], the anthropomorphism of dogs and cats, resulting in higher requirements concerning quality of ingredients [4-6], and the negative impact on the environment. Among macronutrients, protein is the most expensive in economic and ecological terms, thus being arguably the nutrient requiring the most attention as it pertains to sustainability. In typical pet foods, the most currently protein sources are chicken, beef, pork, lamb, turkey, salmon, menhaden, meat by-products and poultry by-products, among the animal sources (generally with a high carbon footprint), and soybean meal and corn gluten meal, among the plant sources [7]. Thus, the study and development of novel and alternative ingredients and functional foods will contribute for the sustainability of the pet food sector, providing optimal nutritional value and promoting animal health. Alternative protein ingredients with a lower impact on the environment may include underexploited by-products (e.g. shellfish and molluscs by-products), microalgae, macroalgae, and lower order animals [3-10]. Additionally, the development of nanoparticles for oral delivery of bioactive compounds has been proposed as an emerging solution to improve bioavailability of nutritive elements and the development of functional ingredients. Indeed, it is possible to develop biocompatible [9] and biodegradable[10] nanoparticles that respond to external or internal stimuli, such as pH or oxidative stress, and, consequently, control or target the release of the bioactive ingredients [11].

This project aims to investigate sustainable and alternative ingredients to include in dog food and develop nanoparticles to improve bioavailability of nutrients of interest. The key research question is: Does the dietary inclusion of alternative feed ingredients has a positive impact on dog performance and health? The work plan comprises three main experimental tasks: 1) Screening of alternative ingredients; 2) *In vivo* evaluation of dietary inclusion of selected feed ingredients on dog performance and health; and 3) Development of nanoparticles for oral delivery of bioactive compounds. This project will contribute to a more sustainable pet food industry by addressing a major constraint in the sector through the evaluation of alternative ingredients under a circular economy perspective.

Keywords: Pet food, Sustainability, Alternative ingredients, Nanoparticles, Bioavailability

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**Tenacibaculum maritimum pathogenesis: crosstalk between host and pathogen and beyond**Ferreira I.A.<sup>1,2,3\*</sup>, Guardiola F.A.<sup>1,4</sup>, Vale A<sup>3</sup>, Costas B.<sup>1,2</sup>

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Abstract

One of the most devastating bacterial diseases of wild and farmed marine fish is tenacibaculosis, which causative agent is *Tenacibaculum maritimum* [1]. This pathogen has a wide range of host species and a worldwide distribution, inducing ulcerative lesions mainly on host skin and fins [2]. Due to the high mortality and economic losses associated with this bacterium it is relevant to gather more knowledge for more effective methods to avoid the spread of tenacibaculosis. This pathogen has the ability of producing extracellular polymers and other extracellular products (ECPs) containing exotoxins, which allow it to persist in aquatic environments and provoke cellular necrosis in internal organs with highly toxic effects [3] as well as potent cytotoxicity [4]. Although it is known that ECPs are important to a successful invasion of the host, the identity of the ECPs' components remain unknown. Therefore, it is imperative to develop studies to isolate and characterize *T. maritimum* ECPs' to better comprehend its virulence mechanisms. To address this issue, we are developing a work plan that includes several tasks. To detect putative protein toxins secreted by *T. maritimum*, concentrated culture supernatants from a virulent strain will be resolved by native-PAGE and protein fractions tested *in vivo* for the capacity to induce harmful/toxic effects in European seabass and Senegalese sole. Afterwards, ECPs from virulent and non-virulent strains will be compared by SDS-PAGE and proteins exclusively present or enriched in the ECPs from virulent strains will be identified LC-MS/MS. To evaluate *T. maritimum* evading mechanisms, immune-related parameters of European seabass and Senegalese sole will be assessed with virulent and non-virulent strains, through *ex vivo* method and live pieces of skin tissue (maintained according to the superfusion technique described by Ganga *et al.* (2006) [5]) with evaluation of gene expression profile, cellular response parameters and apoptosis. *In vivo* time-course challenges of European seabass and Senegalese sole with *T. maritimum* non-pathogenic and pathogenic strains will be performed. To identify genes/gene pathways possibly related with virulence, the *in vivo* induced antigen technology (IVIAT) will be used [6]. Briefly, the serum collected from infected fish will be utilized to identify *T. maritimum* components that are immunogenic and expressed *in vivo*. By identifying important virulence factors of the pathogen and defining the host immune response against infection, this work will

increase the knowledge on *T. maritimum* pathogenesis and will contribute to the development of future strategies to prevent or treat tenacibaculosis.

Keywords: Host defence, Virulence factors, Immunology, Aquaculture

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