



## **IX WORKSHOP**

**15<sup>th</sup> SEPTEMBER 2022**

**Salão Nobre, ICBAS-UP**

**Rua de Jorge Viterbo Ferreira 228**

**Also transmitted online via ZOOM**

**Porto, Portugal**



## **WORKSHOP of the Animal Science Doctoral Programme 2022**

The daily life adjustments caused by COVID-19 resulted in a wide range of experiences and emotions. The transition into a new normal was a major challenge for many PhD students that struggled to find solutions to continue carrying on their research project and settle into their new routines. In such times adapting to change, enthusiasm for upskilling and emotional intelligence were key soft skills that gained relevance. In the Animal Science Doctoral Programme, we were very committed to provide training courses in such areas and help students succeed with their career plans. During this last year, effective communication was also a major target and outcomes will hopefully be delivered during this IX workshop.

Students put together a challenging programme where their newest data will be shared among the academia, industry and general society. Under a climate challenge scenario, we will be honoured by the presence of two key note speakers, David R. Yanez-Ruiz from CSIC in Spain, and Margareth Øverland from the Norwegian University of Life Sciences, that will address major challenges facing the livestock production and the global raw material crisis. A final round table chaired by the journalist Manuel Molinos will gather industrial partners (Ingrid Van Dorpe from Premix, Tiago Aires from Sorgal and Carolina Castro from Flatlantic) to share their vision on this problematic. Ana Sofia Santos from a Collaborative laboratory (FeedInov) and a PhD student (Raquel Rodrigues) will also have the opportunity to participate in this debate.

The mission of this School of Medicine and Biomedical Sciences (ICBAS) is reflected in its innovative educational, scientific and cultural project. The series of seminars “From Science to business: Success Strategies for Winning at Life” put together former well succeeded students from our University and student enrolled in the programme that shared their own experience and vision considering new future scenarios and career prospects! During this workshop, you all will also have the opportunity to meet many industrial partners and highly recognised researchers in a friendly environment. So, I wish you all a vary participative and enthusiastic workshop where besides science you can all establish positive interactions, develop a personal bond, share a bit of your personal life and form a friendship!

Luisa M.P. Valente

Director of the Animal Science Doctoral Programme, ICBAS-UP, 15<sup>th</sup> September, 2022

## **ANIMAL SCIENCE DOCTORAL PROGRAMME VIII WORKSHOP**

**15<sup>th</sup> September 2022**

### **Organization**

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

**António José Mira da Fonseca**, *ICBAS-UP & REQUIMTE-LAQV*

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

**Daniela Resende**, *CIIMAR, ICBAS-UP, CBQF-UCP, GREENUPORTO & SENSE TEST, Lda*

**Inês Ferreira**, *CIIMAR, ICBAS-UP, IBMC & i3S*

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

**Diogo Peixoto**, *ICBAS-UP, CIIMAR & INMAR-CEIMAR*

**Joana Fernandes**, *REQUIMTE-LAQV, ICBAS-UP & SOJA DE PORTUGAL*

**João Estêvão**, *CIIMAR & ICBAS-UP*

### **Conference Chairpersons**

**Margarida Maia**, *ICBAS-UP & REQUIMTE-LAQV*

**Sofia Costa Lima**, *FFUP & REQUIMTE-LAQV*

**Alexandra Correia**, *ICBAS-UP & i3S*

**Rita Azeredo**, *CIIMAR*

**Marta Monteiro**, *CIIMAR*

**Andreia Silva**, *ICBAS-UP & CIIMAR*

**Manuel Molinos**, *JORNAL DE NOTÍCIAS*

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

**Ingrid Van Dorpe**, *PREMIX*

**Tiago Aires**, *SORGAL*

**Raquel Rodrigues**, *LAQV-REQUIMTE, ICBAS-UP, CAVC-CRL & AGROS-UCRL*

**Carolina Castro**, *FLANTLANTIC*

**Ana Sofia Santos**, *COLAB FEEDINOV*

### **Steering Committee**

**Leonor Nunes**, *CIIMAR*

**Luis Mira Vieira**, *ICBAS-UP*

**Ingrid Van Dorpe**, *PREMIX*

**Ana Sofia Santos**, *COLAB FEEDINOV*

## INVITED SPEAKERS

### **Professor Doctor David R. Yanez-Ruiz**



David R. Yanez-Ruiz has a veterinary degree in Animal Production from the University of Cordoba (Spain, 1997). He has a PhD in Animal Nutrition comparing the effect on rumen fermentation of using olive by-products in the diet of sheep and goats (Estacion Experimental del Zaidin, CSIC, 2003). He worked during 4 years with Prof. Jamie Newbold at Aberystwyth University (UK, 2003-2007) on the use of molecular techniques to gain insight in the microbial processes associated to fatty acids bio-hydrogenation and methane emissions. He is established as senior scientists at EEz-CSIC (Grnada, Spain) since 2007 and leads a team on the use of nutritional interventions applied in early life of ruminants to enhance animal productivity during the adult life.

### **Professor Doctor Margareth Øverland**



Margareth Øverland has a BSc and MSc in animal nutrition from Montana State University in Montana USA and a PhD in animal nutrition from the Norwegian University of Life Sciences (NMBU). Today, she is a professor in aquaculture nutrition at NMBU and she is the Center Director of a Center for Research-based Innovation (CRI), Foods of Norway. Margareths' research focus is to develop high-quality novel feed ingredients based on renewable natural resources such as tree biomass, marine macroalgae, and animal and fish co-products by use of advanced biotechnology and biorefinery technology. The research also aims to improve feed efficiency by innovative feed-processing technology and by use of advanced genomic analysis to support genetic adaptation of fish and other farm animals to the novel feed ingredients.

## AGENDA

### 09:00 • WORKSHOP OPENING Henrique Cyrne Carvalho (*ICBAS Director*)

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

Luísa Valente, *CIIMAR & ICBAS-UP*  
António Mira da Fonseca, *ICBAS-UP & REQUIMTE - LAQV*  
Ana Rita Cabrita, *ICBAS-UP & REQUIMTE - LAQV*  
Benjamin Costas, *CIIMAR & ICBAS-UP*  
Leonor Nunes, *CIIMAR*

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

Luísa Valente, *CIIMAR & ICBAS-UP*  
Luís Mira Vieira, *REQUIMTE-LAQV, ICBAS-UP & SOJA DE PORTUGAL*  
Ricardo Pereira, *CIIMAR, CBQF-UCP & ICBAS-UP*

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

Luísa Valente (*Animal Science Doctoral Programme Director*)

#### SESSION I

**Chairpersons:** Margarida Maia (*ICBAS-UP & REQUIMTE-LAQV*) & Sofia Costa Lima (*FFUP & REQUIMTE-LAQV*)

**09:30 • Plenary lecture:** The rumen microbiome and its relevance in livestock husbandry to mitigate climate change, David R. Yanez-Ruiz (*Estación Experimental del Zaidín, EEZ; Consejo Superior de Investigaciones Científicas, CSIC*)

**10:15 • Oral presentation:** Emission of greenhouse gases and ammonia from naturally ventilated dairy buildings, Raquel Rodrigues (*LAQV-REQUIMTE, ICBAS-UP, CAVC, CRL & AGROS, UCRL*)

**10:30 • Oral presentation:** Whole conventional, whole pasture-based, and fat-free cow's milk: effect in mRNA expression of human gastric epithelium, Susana Pinho (*LAQV-REQUIMTE, ICBAS-UP & SOJA DE PORTUGAL*)

**10:45 • Oral presentation:** Effect of dietary algae blend supplementation to intensive reared lambs and of gender on meat quality and intramuscular fatty acid composition, Cátia Mota (*LAQV-REQUIMTE, ICBAS-UP, ALGAPLUS & ALLMICROALGAE*)

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

**Chairpersons:** Alexandra Correia (*i3S*) & Rita Azeredo (*CIIMAR*)

**11:45 • Oral presentation:** Assessment of *in vivo* immune and health status parameters in Holstein-Friesian calves fed milk replacer supplemented with *Chlorella vulgaris*, Ana Pedro (*ICBAS-UP, i3S, LAQV-REQUIMTE, CAVC & ADM Portugal*)

**12:00 • Oral presentation:** Empowering evaluation of intestinal health status merging traditional and cutting-edge technologies, Mariana Ferreira (*ICBAS-UP & CIIMAR*)

**12:15 • Oral presentation:** Early mucosal immune responses in European seabass (*Dicentrarchus labrax*) following *Tenacibaculum maritimum* infection, *Inês Ferreira (CIIMAR, ICBAS-UP, IBMC & i3S)*

**12:30 • Oral presentation:** Dietary modulation strategies in gilthead seabream (*Sparus aurata*) following intestinal inflammation, *Carla Teixeira (CIIMAR, ICBAS-UP & SPAROS)*

### **12:45 • LUNCH & POSTER SESSION**

### **SESSION II**

**Chairpersons:** *Marta Monteiro (CIIMAR) & Andreia Silva (ICBAS-UP & CIIMAR)*

**14:15 • Plenary lecture:** How can we solve the global raw material crisis?, *Margareth Øverland (Norwegian University of Life Sciences, NMBU)*

**15:00 • Oral presentation:** Impact of partial and total fishmeal replacement by defatted *Tenebrio molitor* larvae meal on short and mid-term homeostatic regulation of food intake in European sea bass (*Dicentrarchus labrax*), *Ana Basto (CIIMAR, ICBAS-UP & PHYStoFISH - U. Vigo)*

**15:15 • Oral presentation:** Evaluation of sardine cooking wastewaters as feed intake modulators for European seabass diets, *Daniela Resende (CIIMAR, ICBAS-UP, CBQF-UCP, GreenUPorto & Sense Test)*

**15:30 • Oral presentation:** Towards optimal calibration of Nile tilapia growth models, *Andreia Raposo (ICBAS-UP & SPAROS)*

**15:45 • Oral presentation:** Accumulation of microplastics in tissues of farmed European seabass and risks for the consumers, *Ricardo Matias (ICBAS-UP & CIIMAR)*

### **16:00 • ROUND TABLE “A PRODUÇÃO ANIMA NUM CENÁRIO DE ALTERAÇÕES CLIMÁTICAS”**

**Chairpersons:** *Manuel Molinos (Jornal de Notícias)*

#### **Participants:**

*Ingrid Van Dorpe (Premix)*

*Tiago Aires (Sorgal)*

*Raquel Rodrigues (LAQV-REQUIMTE, ICBAS-UP, CAVC, CRL, AGROS, UCRL)*

*Carolina Castro (Flatlantic)*

*Ana Sofia Santos (CoLab FeedInov)*

### **17:00 • BEST PARTICIPANT, POSTER AND ORAL PRESENTATION AWARDS**

#### **Jury:**

*Maria Leonor Nunes (CIIMAR)*

*Ingrid Van Dorpe (Premix)*

*Luís Mira Vieira (ICBAS-UP)*

*Ana Sofia Santos (CoLab FeedInov)*

### **17:00 • WORKSHOP CLOSING**

# **PLENARY SESSIONS**

**The rumen microbiome and its relevance in livestock husbandry to mitigate climate change**

*David R. Yanez-Ruiz*

Estación Experimental del Zaidín (EEZ), Consejo Superior de Investigaciones Científicas (CSIC)

Abstract

Keywords



## How can we solve the global raw material crisis?

Margareth Øverland<sup>1</sup>\*, S.Rocha<sup>1</sup>, J.O. Agboola<sup>1</sup>, B. Morales-Lange<sup>1</sup>, L.T. Mydland<sup>1</sup>

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### Introduction

The aquaculture industry is the fastest-growing food sector in the world and is playing an increasing role in meeting the global protein demand. Continued growth depends on high-quality feed resources. Climate changes, competition for land, water and energy, and fully exploited capture fisheries, emphasize the need for sustainable feed ingredients from under-utilized renewable natural resources.

### Discussion

While marine ingredients such as fishmeal are limited, increased use of some plant proteins in aquafeeds is questionable from a sustainability standpoint. Reducing competition with human food resources will be key for sustainability, and microbial ingredients (MI) like yeast or filamentous fungi can play an important role. MI are high-quality protein sources that can meet the high protein demand of fish and they contain bioactive components with beneficial health effects. MI have rapid growth rates, do not require agricultural land, use little fresh water, and can be produced from under-utilized biomass such as forestry and seaweeds [1-4].

Insects, another promising sustainable feed resource, can up-circulate low-value waste streams into high-value feed ingredients that otherwise can represent an environmental concern. Black soldier fly (*Hermetia illucens*) (BSF) is a promising insect species with high nutritional value, efficiency in bioconversion and high potential for upscaling. BSF has high content of fat and protein and contains the bioactive compounds chitin, lauric acid and antimicrobial peptides with potential beneficial health effects [5,6]. However, regulatory constraints on rearing substrates limit large-scale insect production. Insect production in Europe is still too low to compete with conventional feed sources on volumes and costs. Access to newer methods for processing and risk assessment would lead to a broader range of substrates and facilitate upscaling of insects.

### Conclusion

As biotechnology advances and competition for natural resources increase, a broader range of substrates from various waste streams will be used for alternative protein sources. With technology advances, production of MI will shift from dependence on photosynthesis as substrate toward cheaper input factors (e.g., organic acids, CH<sub>4</sub>, H<sub>2</sub> and CO<sub>2</sub> gas from industrial waste). Before we can replace conventional protein sources, we need new policies and alternative sources need to be up-scaled, which takes time. To solve the raw material crises, we also need to maximize the use of existing raw material such as plant, animal, and marine by-products to meet the growing demand for aquafeeds.

**Keywords:** aquafeeds, protein-rich ingredients, microbial ingredients, insects, circular proteins

**Acknowledgements:** This study was funded by the Foods of Norway – a Centre for Research-based Innovation (RCN 237841/030) and the Trained Immunity and Nutritional Programming for Resilient Salmon (RCN 294821).

### References:

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# **ORAL COMMUNICATIONS**

## **Emission of greenhouse gases and ammonia from naturally ventilated dairy buildings**

Ana Raquel F. Rodrigues<sup>1,2,3,†,\*</sup>, M.E. Silva<sup>4</sup>, V.F. Silva<sup>5</sup>, A. Gomes<sup>2</sup>, L. Ferreira<sup>3</sup>, M.R.G. Maia<sup>1</sup>, A.R.J. Cabrita<sup>1</sup>, H. Trindade<sup>6</sup>, A.J.M. Fonseca<sup>1</sup>, J.L. Pereira<sup>6,7</sup>

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Strategies to increase sustainability and efficiency of milk production encompass the mitigation of greenhouse gases (GHG) and ammonia (NH<sub>3</sub>) emissions from dairy cattle houses [1]. Factors as animal housing characteristics, diet and climate parameters, among others, have been reported to affect GHG and NH<sub>3</sub> emissions [2], resulting in differences among countries. Monitorization of these emissions is pivotal to improve the emission factors used in national inventories and thus to adopt specific abatement techniques [3] to face the challenges of the dairy sector. To date, few studies have determined NH<sub>3</sub> emissions from dairy buildings in Portugal and none have yet assessed GHG emissions. This work addressed this knowledge gap by monitoring GHG and NH<sub>3</sub> emissions in naturally ventilated dairy buildings of three farms with distinct feeding systems for at least 7 days in each season (spring, summer, autumn and winter), for two years (2017 and 2018). In each building, air samples from 5 indoor locations were drawn by a multipoint sampler (INNOVA 1409) to a photoacoustic infrared multigas monitor (INNOVA 1412). Indoor temperature and relative humidity, milk average production, days in milk and dietary ingredient composition were also recorded. In each period, diets were sampled and chemically analyzed. GHG concentrations (mg/m<sup>3</sup>) varied among buildings during 2017 from 1.63 to 30.9 for methane and from 0.73 to 1.03 for nitrous oxide and from 2.52 to 37.9 for methane and from 0.50 to 0.84 for nitrous oxide in 2018. NH<sub>3</sub> concentrations (mg/m<sup>3</sup>) varied from 0.34 to 1.80 in 2017 and from 0.25 to 2.49 in 2018. This work contributed to the accomplishment of emission factors for Portuguese dairy cows houses needed to be considered in the definition of a national action plan.

**Keywords:** Ammonia, Greenhouse gases, Mitigation, Sustainability

**Aknowledgements:** Funding of FCT, AGROS and CAVC to ARFR (PDE/BDE/114434/2016), FCT to VFS (SFRH/BD/139630/2018), MRGM (DL57-Norma Transitória), REQUIMTE (UIDB/50006/2020), CITAB (UIDB/04033/2020) and LIAAD-INESCTEC (LA/P/0063/2020) is acknowledged.

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## Whole conventional, whole pasture-based, and fat-free cow's milk: effect in mRNA expression of human gastric epithelium

Susana C.M. Pinho<sup>1,2\*</sup>, Miguel A. Faria<sup>1</sup>, Rui Alves<sup>3</sup>, Ana R.J. Cabrita<sup>2</sup>, António J.M. Fonseca<sup>2</sup>, Isabel M.P.L.V.O. Ferreira<sup>1</sup>

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Dairy foods are a foundational group in many food-based dietary guidelines due to their high-quality protein and calcium contributions to the human diet. Dairy guidance is, however, often accompanied by messages to avoid dairy foods rich in saturated fat, which are linked to negative health effects [1]. Bovine milk contains 70% of their total fatty acids as saturated fatty acids, but pasture feeding has been demonstrated to increase the content of healthy omega-3 polyunsaturated fatty acids and conjugated linoleic acid [2]. Milk fat digestion starts in the stomach and short-chain fatty acids are uptaken through the membrane of gastric epithelial cells. Responding to nutrients availability is an important homeostatic mechanism of cells [3], but literature lacks information concerning the impact of milk on gastric cells function.

The present work aimed to compare the effect of homogenized UHT whole conventional (i.e., housed cows offered conserved forages and concentrate feeds), whole pasture-based, and fat-free milk on messenger ribonucleic acid (mRNA) expression of membrane fatty acids receptors GPR41, GPR84, antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, transcription factor NFκB p65, and pro-inflammatory cytokines IL1β, IL6, IL8, TNFα in the gastric epithelium. A combined model of semi-dynamic *in vitro* digestion (INFOGEST protocol) with cell lines (NCI-N87 monolayer) and long-term testing were used. In addition, subsequent inflammatory stimulation with 30 ng/mL IFN-γ that enhances intracellular production of reactive oxygen species was applied to enable a better understanding of the responses induced by *in vitro* digested milk in the NCI-N87 monolayer.

Milk samples had no effect on mRNA expression of membrane fatty acids receptors GPR41 and GPR84, neither superoxide dismutase nor glutathione peroxidase ( $P > 0.05$ ), but up-regulated mRNA expression of catalase after both treatments ( $P < 0.05$ ). The whole milk digested samples induced higher mRNA expression of NFκB p65 and IL-1β than the fat free milk ( $P < 0.05$ ); and no differences were found between the whole conventional and whole pasture-based milk ( $P > 0.05$ ). After IFN-γ-stimulation of NCI-N87 monolayer, no differences were observed in the expression of NFκB p65 and IL1β ( $P > 0.05$ ). Concerning the mRNA expression of IL6, IL8 and TNFα, no differences were observed after both treatments ( $P > 0.05$ ). In conclusion, at gastric epithelial level, whole conventional and whole pasture-based milks up-regulate genes involved in redox homeostasis and inflammation, while fat-free milk only up-regulates genes involved in redox homeostasis. However, this study showed that whole milk does not enhance subsequent inflammatory processes.

**Keywords:** Dairy management, human simulated gastric digestion, milk composition, NCI-N87 monolayer, quantitative PCR

**Acknowledgements:** Susana C. M. Pinho thanks Fundação para a Ciência e a Tecnologia, SANFEED Doctoral Programme, and SORGAL (Sociedade de Óleos e Rações S.A., S. João Ovar, Portugal) the Ph.D. grant PD/BDE/135539/2018 and COVID/BDE/152525/2022. Miguel A. Faria acknowledges FCT/MEC - Fundação para a Ciência e Tecnologia, Ministério da Educação e Ciência - the researcher contract. This work was financed by FEDER - Fundo Europeu de Desenvolvimento Regional funds through the

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## Effect of dietary algae blend supplementation to intensive reared lambs and of gender on meat quality and intramuscular fatty acid composition

Cátia S. C. Mota<sup>1\*</sup>, Ângelo S. José<sup>2</sup>, Vasco A. P. Cadavez<sup>2</sup>, R. Domínguez<sup>3</sup>, José M. Lorenzo<sup>3</sup>, Ursula Gonzales-Barron<sup>2</sup>, Ana R. J. Cabrita<sup>1</sup>, Helena Abreu<sup>4</sup>, Joana L. Silva<sup>5</sup>, António J. M. Fonseca<sup>1</sup>, Margarida R. G. Maia<sup>1</sup>

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Meat from lambs raised in extensive systems are enriched in n-3 polyunsaturated fatty acids (PUFA), whereas those raised in intensive systems have higher saturated fat [1], associated with increased incidence of metabolic diseases in humans [2]. Dietary supplementation of oilseeds and algae have been proposed to promote the unsaturation and n-3 PUFA content of meat [3]. Thus, this study aimed to evaluate the supplementation of a commercial algae blend composed of macro- and microalgae (Algaessence™) on meat quality and fatty acids (FA) profile of lambs reared in intensive production system. Gender effects were also assessed. In this trial, three groups of 10 Bordaleira-de-Entre-Douro-e-Minho lambs, males and females, were assigned to one of three feeding systems: i) traditional extensive production (pasture by day and indoors by night); ii) intensive production fed a concentrate diet, and iii) intensive production fed a concentrate diet with 5% algae blend. All animals had *ad libitum* access to water and meadow hay. After 60 days, lambs were slaughtered in a commercial abattoir, the carcass was chilled at 4 °C for 24-h and *Longissimus lumborum* muscle collected for analysis. Meat proximate composition was similar among feeding groups and genders, no interaction between feeding and gender being observed ( $P > 0.05$ ). Loin FA profile was greatly affected by feeding and sparsely by gender, with only three minor FA being affected by feeding and gender interaction. Meat from extensive reared lambs had the highest PUFA ( $P < 0.001$ ), in particular n-3 PUFA that was 3-fold higher than in intensive meat ( $P < 0.001$ ). Algae blend lowered the n-6/n-3 ratio of intensive reared meat but the proportion of health promoting 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA) was similar. Female lambs meat presented higher proportions of the bioactive FA 20:4n-6 (ARA), EPA, DPA and DHA than male lambs. Lipid nutritional quality indices were affected by feeding system but not by gender or their interaction. Extensive reared meat presented the lowest atherogenicity index (AI,  $P = 0.015$ ) and thrombogenicity index ( $P < 0.001$ ) and the highest hypocholesterolemic to hypercholesterolemic ratio (h/H,  $P = 0.005$ ), peroxidation index ( $P < 0.001$ ) and desirable fatty acids ( $P = 0.001$ ) compared to intensive meat. Algae blend supplementation reduced AI and increased h/H but did not statistically differ from extensive and intensive meats. Overall results revealed a marked effect of production system on intramuscular FA profile, while algae blend had a modest effect on its modulation.

**Keywords:** Algae blend, fatty acids, gender, lambs, meat quality

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## Assessment of *in vivo* immune and health status parameters in Holstein-Friesian calves fed milk replacer supplemented with *Chlorella vulgaris*

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Morbidity in newborn calves, although difficult to precise and extremely variable among regions, has devastating effects on animal welfare and on long-term productivity and profitability. In addition to good management practices, strategies that may enhance calves' immunity are crucial to reduce morbidity rates. In this context, dietary supplementation of microalgae has gained increasing attention as these ubiquitous photosynthetic microorganisms have been reported to exert functional activities in cellular models, including anticancer, antimicrobial, anti-inflammatory, and immunomodulatory [1]. This work aimed at evaluating the dietary supplementation of *Chlorella vulgaris* on performance, health and immune parameters of newborn calves fed milk replacer.

Fourteen male Holstein-Friesian calves, 10 days old, were used in the trial. Prior to the beginning of the trial, animals were weighted and clinical evaluation was performed. After acclimation, calves were randomly allocated to control (milk replacer) and experimental (milk replacer with 1% *C. vulgaris*) groups and fed 7 L of milk replacer (140 g/L) daily in two equal meals for 42 days. Starter feed, meadow hay and freshwater were provided *ad libitum*. Milk intake and refusals, faecal scores and health parameters were recorded. At the end of the trial, calves were weighted, faeces collected for pH, short-chain fatty acids and microbiome profiling and blood collected for hemogram and immune parameters determination. Serum cytokine were evaluated and peripheral blood mononuclear cells (PBMC) isolated for assessment of proliferation and cytokine production in response to mitogens. Peripheral blood monocytes were challenged with several toll-like receptor (TLR) and C-type Lectin-like receptor (CLR) agonists to evaluate cytokine expression, and monocyte-derived macrophages (MDM) were used for phagocytosis assays. *In vivo* supplementation of *C. vulgaris* had no negative impact on milk replacer palatability, being well accepted. Moreover, it had no effect on average feed intake, average daily gain, faecal scores and health parameters, both groups having good performances and low morbidity. Serum cytokines and innate immune cells' response (cytokine mRNA and protein expression, phagocytosis) were similar between control and experimental groups. Also, no differences were found between groups regarding MDM phagocytosis capacity or PBMC proliferation. Data analysis of faecal pH, short-chain fatty acids and microbiome is ongoing.

Supplementation of calves' milk replacer with 1% *C. vulgaris* did not result in significant alterations in the evaluated performance, health and immune parameters. Higher inclusion levels may be necessary to

evidence putative immunomodulatory effects of *C. vulgaris* supplementation on immune and growth parameters in newborn calves.

Keywords: Bovine, microalgae, dietary supplementation, cytokines

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## Empowering evaluation of intestinal health status merging traditional and cutting-edge technologies

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**Introduction:** The intestine health status of aquatic animals is becoming one of the most studied topics in aquaculture, as feeds largely impact intestinal physiology. Integrated approaches using histomorphological and molecular biomarkers are emerging, however, not only morphologic traits vary along the intestinal tract but also analysis rely on traditional and laborious methods. An in-depth characterization of European seabass intestine was hence carried out using a holistic approach: fast-track image analysis combined with traditional histology methods and gene expression analysis.

**Materials/Methods:** Anterior, medium, posterior, and rectal sections of intestine, from European sea bass fed a commercial-based diet with or without a 2% supplementation of a blend of micro- and macroalgae, were fixed in formaldehyde for histological evaluation or deep-frozen for expression of immune, nutrient transport, and oxidative stress genes by RT-PCR. Classical staining and immunohistochemistry methods were applied to evaluate the gut histomorphology.

**Results/Discussion:** The experimental diet had no impact on fish growth performance or feed utilization parameters. The impact of feeds on fish intestinal health often relies on semi-quantitative/score-based analyses that require highly-trained specialists. We validated a reliable and reproducible quantitative approach to evaluate several gut health parameters. A dedicated image software was successfully used for automatic counting of goblet cells (GC), absorption area estimation, and evaluation of cellular proliferation. Based on a principal component analysis (PCA), the anterior intestine was associated with parameters related to absorption (absorption area, villus length, neutral GC, *fabp2* and *malt*), oxidative stress (*sod* and *cat*), lamina propria leukocytes and cellular proliferation (PCNA<sup>+</sup> cells); while rectum samples were related to immune genes, muscularis thickness, microvillus height, acid GC and expression of aquaporin and alkaline phosphatase. Posterior intestine and rectum samples were positioned in the same PCA quadrants, but medium samples stood in a separated quadrant. Interestingly, the medium intestine was the section where the algae-supplemented diet had a higher impact resulting in decreased villus length, wider lamina propria and submucosa, lower number of acid GC, higher number of leukocytes, higher number of PCNA<sup>+</sup> cells, and increased expression of immune (*il-8*, *dic*, *pisc1*) and oxidative stress (*gpx*) genes. Most studies evaluating fish feed utilization and immune status rely on anterior or posterior/rectal sections, respectively. However, in this study, the subtle differences between diets could only be perceived in the medium intestine. This might be associated with a tradeoff strategy between intestinal digestion and assimilation processes where the functional diet favored a slower excretion rate and hence higher contact with digesta.

**Keywords:** Functional Feeds; Gut morphology; Image analysis; Intestinal absorption area  
Histomorphology; Intestinal quantitative analysis.

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## Early mucosal immune responses in European seabass (*Dicentrarchus labrax*) following *Tenacibaculum maritimum* infection

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In the last years, the aquaculture industry has been witnessing a worldwide emergence of tenacibaculosis, a poorly understood pathology that affects commercially important fish [1] Despite several putative *T. maritimum*'s virulence factors have been proposed, its virulence mechanisms and interaction with the host remain unknown. The present study aimed to evaluate the short-term innate immune response of European seabass (*Dicentrarchus labrax*) after bath-challenge with *T. maritimum*.

A time-course trial was performed, in which groups of seabass were bath-challenged with *T. maritimum* (challenged fish) or mock-challenged. Undisturbed fish were used as controls (time 0). At 0 h (control) and at 6, 24, 48 and 72 h post-challenge, 12 fish from each treatment were randomly selected, euthanized and blood and mucosal tissues were collected. Skin, gills and posterior gut were used for analysing the expression of immune-related genes by RT-qPCR. Blood samples were used for assessing haematological parameters and for collecting plasma to evaluate innate humoral parameters. To determine the severity of the challenge, cumulative mortality was followed in a parallel trial, using the same bacterial inoculum/challenge protocol used for the time-course trial.

Challenge with *T. maritimum* induced 30% mortality, whereas no mortality occurred in mock-challenged fish. The molecular analysis of the skin revealed an increased expression of *il-1 $\beta$*  and *mmp9* in challenged fish, when compared to control (time 0) and mock-challenged fish at 6 and 24 h post-challenge, and also an increased expression of *il-8* when compared to mock-challenged fish at all time points. All these genes participate in the inflammatory response, recruitment of monocytes and neutrophils and activation of phagocytosis in macrophages [2]. The gills and posterior-gut samples are currently under analysis, to disclose the local response triggered by *T. maritimum* in these tissues. Analysis of the systemic response showed that circulating neutrophil and monocyte numbers increased in challenged fish at 48 and 72 h post-challenge comparing with control and mock-challenged fish. In contrast, erythrocytes decreased at 6, 24 and 48 h when compared with control and mock-challenged fish. Bactericidal and lysozyme activity also decreased in challenged fish at 6 and 24 h post-challenge. These results indicate that upon bath infection, *T. maritimum* induces a local innate immune response in the skin, likely triggered by the *T. maritimum*'s capacity to adhere, colonize and damage the skin, which ultimately leads to a systemic response, as suggested by data from haematological and humoral parameters.

**Keywords:** Tenacibaculosis, Aquaculture, Mucosal Immunity, Innate Immunity, Gene expression

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## **Dietary modulation strategies in gilthead seabream (*Sparus aurata*) following intestinal inflammation**

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A common practice to achieve economic sustainability and maximum production in aquaculture involves the use of functional feeds.  $\beta$ -glucans and curcumin are compounds with immunomodulatory abilities known to increase growth performance, stimulate immunity, improve general health, and enhance disease resistance in fish. The present study aimed to evaluate the effects of dietary  $\beta$ -glucans and curcumin on gilthead seabream juveniles' health status before and after an intestinal inflammatory stimulus. Three experimental diets were formulated: a control diet (CTRL, a practical commercial-type diet),  $\beta$ -glucan diet (BG, control diet supplemented with 1 % microalgae  $\beta$ -glucans extract), and curcumin diet (CUR, control diet supplemented with 0.2 % of curcumin). After a 30-day feeding trial, fish were sampled and subjected to a dietary administration of 1 % dextran sodium sulphate (DSS) to induce intestinal inflammation. Four dietary treatments were considered. While a group of fish continued to be fed on the control diet (CTRL), the remaining groups were exposed to DSS: CTRL-D (CTRL + DSS), BG-D (BG + DSS), and CUR-D (CUR + DSS) for 6 days. Growth, plasma and gut humoral immune parameters, liver and gut oxidative stress biomarkers, and intestinal gene expression were evaluated. No significant differences were found in growth, after the feeding trial, however, seabream fed BG decreased anti-protease activity and nitric oxide concentration in plasma, while their counterpart's fed CUR increased the mRNA levels of *tnfa*, *csf1r* and *hep* genes. Following the intestinal inflammatory stimulus, haematocrit was enhanced in the groups BG-D and CUR-D, while red blood cells numbers increased in the CTRL-D. Superoxide dismutase activity decreased in the intestine of all DSS groups, while lipid peroxidation in the gut increased in the CTRL-D and BG-D groups. Additionally, mRNA expression levels of *csfr1* and *sod* genes decreased in the CTRL-D and BG-D groups, respectively. Despite the intestinal inflammatory condition induced by the DSS, BG and CUR were able to partially ameliorate its intestinal and systemic effects.

**Keywords:** Dextran sodium sulphate; Gilthead seabream (*Sparus aurata*), DSS intestinal inflammation;  $\beta$ -glucans; curcumin.

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## **Impact of partial and total fishmeal replacement by defatted *Tenebrio molitor* larvae meal on short- and mid-term homeostatic regulation of food intake in European sea bass (*Dicentrarchus labrax*)**

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Understanding the underlying mechanisms responsible for central regulation of feeding behavior in fish, namely stimulation (orexigenic) or inhibition (anorexigenic) of appetite is crucial for proper formulation of aquafeeds and consequently ensure the best growth performance of fish [1]. Over the last decade, the study of the impact of insect meal-based diets on growth performance and voluntary feed intake of several fish species have emerged, but little is known about their influence on anorexigenic potential. According to our previous study [2], up to 80% fishmeal (FM) replacement by defatted *Tenebrio molitor* larvae meal (*d*TM) (36% *d*TM inclusion) does not affect central homeostatic regulation of food intake of European sea bass (*Dicentrarchus labrax*). However, the study was carried out after 10 weeks of feeding and possible changes in mechanisms involved in the short- and mid-term regulation of food intake might have been overlooked. This is the first study evaluating short- and mid-term responses of European sea bass fed diets not only with partial, but also total FM replacement by *d*TM. A FM-based diet was used as a control (CTRL) and two other diets were formulated to replace 50% and 100% of FM (20% and 40% *d*TM inclusion, respectively). The short-term response was evaluated immediately after exposing fish to a single meal with the experimental diets; the mid-term response was evaluated after 7 days of feeding those experimental diets. In both short- and mid-term trials food intake was registered; hepatic and plasmatic metabolites and the expression of hypothalamic and telencephalic neuropeptides involved in food intake were assessed 2 and 24 hours post-feeding. No differences occurred in food intake, neither in the short- nor mid-term. In the short-term, plasmatic glucose levels increased in fish fed TM100, regardless post-feeding sampling time. In the mid-term, fish fed TM100 had the highest levels of triglycerides in liver and the lowest levels of  $\alpha$ -amino-acids in plasma, irrespectively of post-feeding sampling time. At central level, dietary treatment did not alter the expression of neuropeptide Y (*npv*), agouti-related protein-2 (*grp2*), pro-opio melanocortin a (*pomca*) or cocaine- and amphetamine-related transcript-2 (*cartpt2*) in hypothalamus and telencephalon. The obtained results suggest that partial and total FM replacement by *d*TM do not seem to influence short- and mid-term homeostatic regulation of food intake in sea bass.

**Keywords:** AgRP/NPY; appetite; aquafeeds; insect meal; POMC/CART.

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## Evaluation of sardine cooking wastewaters as feed intake modulators for European seabass diets

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There has been a tendency to replace marine ingredients in aquafeeds, namely fishmeal, with more sustainable vegetable protein sources. However, this replacement often reduces diet palatability, with a subsequent decrease in feed intake and growth and/or alteration of flesh quality. The goal of this work was to evaluate the effectiveness of aromatic mixtures extracted from sardine cooking wastewaters, a by-product of the canning industry, as feed intake stimulants in highly vegetable diets for European seabass (*Dicentrarchus labrax*).

Sardine cooking wastewaters were either used directly (CW) or after processing by vacuum condensation (VC) or liquid/liquid extraction with soybean oil (LLE). The chemical profile of the extracts differed, but the most abundant compound identified in all extracts was the 1-penten-3-ol, hence selected as marker and included at 2 µg/g in plant protein-based diets. Thus, four isolipidic and isoproteic diets (one for each aroma sample and a non-supplemented control) were produced. Diets were assigned to triplicate groups of fish (initial weight 95.7 ± 13.5 g) that were hand-fed twice daily until apparent satiation in a recirculating saltwater system at 21 °C. After 18 weeks, fish growth performance and nutrient utilisation were evaluated. Flesh colour and textural properties were assessed instrumentally and by sensory analysis using a consumer panel.

Fish fed LLE displayed a significantly higher voluntary feed intake than those fed diet CW, although neither differed from the control. LLE diet also resulted in increased feed conversion ratio, but final weight, daily growth rate, whole-body composition, and nutrient gain remained similar among diets. No differences were also found in fish skin or muscle colour. Despite a lower hardness in fillets of fish fed LLE when compared to those fed the control, no significant differences could be perceived by the sensory panel; global liking of samples was similar among treatments, being all generally well accepted. Additionally, the taste and odour of all samples was similar with a “characteristic fish” and “soft” odour/taste. Overall, results suggest that the aromas from sardine cooking wastewaters can modulate feed intake, but further optimization of either the processing and/or incorporation levels seems required to potentiate their effectiveness on fish growth. The physiological mechanisms underlying the modulation of appetite also merits further evaluation.

Keywords: Circular economy; feed intake; palatability; sustainability.

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## **Towards optimal calibration of Nile tilapia growth models**

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Traditionally, empirical mathematical models (e.g. RGR, TGC, FCR) have been used in aquaculture to predict growth due to their simplicity and ease of parameter determination. However, such models often ignore important aspects, such as feeding information (e.g. feed quantities and properties), and/or lack clear physical/biological support, being inadequate as general context-independent prediction models. On the other hand, bioenergetic models or nutritional models take into consideration feed quantities and, at least some of its properties, which allow more precise growth predictions, and may also provide other important information concerning feed utilisation (e.g., environmental impact due to waste and excretion losses).

In this work, different combinations of models and calibration methods were developed and compared, using objective criteria based on cross-validation, to determine the best combination for Nile tilapia.

Nile tilapia growth data were collected from 27 publications, covering sizes from 0.51 g to 457 g. Models such as, bioenergetic and energy-protein flux models were developed and tested with different calibration methods. Several “model + calibration method” combinations were evaluated through different cross-validation methods (leave-one-out cross-validation, repeated k-fold cross-validation with k=10, k=5 and k=2), to objectively evaluate their predictive capacity. This evaluation was performed considering both qualitative (diagnostic plots) and quantitative aspects (calibration and cross-validation RMSE, MAPE and CRM).

Considering the results of cross-validation, nutritional models, like the energy-protein flux (EP model), appear to provide better predictions than the simpler bioenergetic models; the EP models showed cross-validation errors (e.g. MAPE) on the order of  $\approx 8\%$  and  $\approx 14\%$ , whereas the bioenergetic model had  $\approx 13\%$  and  $\approx 14\%$ , for the body weight and body composition predictions, respectively (in a 5-fold cross-validation). Regarding the calibration methods, an important finding was that models have better growth predictions when calibrated assuming the standard body weight exponents of 0.8 and 0.7 for maintenance costs (energy and protein, respectively), than when the estimated exponents are used.

The fact that EP models have a better predictive ability than bioenergetic models shows that protein intake is an important component to consider when estimating growth in Nile tilapia.

Overall, in this study, the use of evaluation methods that consider cross-validation error metrics enabled the clarification of important scientific (e.g., best model to predict growth) and technical (e.g., best calibration method) questions, providing a meaningful contribution to a more widespread adoption of highly-predictive nutrient-based fish growth models.

**Keywords:** Mathematical models, Growth, Tilapia, Regression analysis, Prediction tools

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## Accumulation of microplastics in tissues of farmed European seabass and risks for the consumers

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Seafood contamination by microplastics (MPs, plastic particles inferior to 5mm) is a growing concern in global food safety that has permeated the aquaculture sector, currently producing over 50% of the fish available in markets. MP ingestion and translocation across tissues have been reported in several commercial fish species, potentially leading to high consumer exposure to MPs. Within production systems, recirculating aquaculture systems (RAS) provide constant and controlled conditions in an almost closed circuit where fish are particularly exposed to plastic components (e.g., tanks, pipes, filters, nets) which constitute potential sources of MPs. Likewise, the surrounding atmosphere, water and aquafeed, also constitute potential MP sources. These particles can then be uptaken and bioaccumulated in fish tissues.

A total of 55 specimens (fish weight: 122.0±0.7g) of European seabass (*Dicentrarchus labrax*) reared in RAS for 8 months were sampled, for MP extraction, quantification and characterization. Tank water and aquafeed were also collected. From each fish, MPs were extracted from several tissues: gastrointestinal tract, gills, liver and dorsal muscle. Recovered suspect-plastic particles were quantified and characterized according to size, shape and colour, under a stereomicroscope. Then, 50% were chemically identified by micro-Fourier Transform Infrared Spectroscopy (μFTIR) and the spectra were compared with reference libraries. The remaining particles were compared with previously μFTIR-analysed particles.

A total of 431 particles were extracted from fish tissues with the gastrointestinal tract (n=149) and muscle (n=124) presenting the highest particle count, followed by gills (n=82) and liver (n=76). Despite the high MP count, muscle presented the lowest MP concentration (0.4±0.3 MP items/g), similar to the one reported in the dorsal muscle of wild specimens from the NE Atlantic Ocean [1]. All examined fish had particles in their tissues, ranging from 3 to 15 in total. Considering EFSA's recommendations on fish consumption for adults of 300 g/week [2], the human exposure scenario is estimated at 5616 MP items/year from RAS-farmed seabass fillet consumption. Fibres and fragments accounted for 70% of the particles found, ranging from 30 to 5038 μm. Blue and black colourations were the most common. Among tissues, natural/synthetic cellulose, polyethylene terephthalate fibres, and polyvinyl chloride fragments were the most common. Similar particles were recovered from water and aquafeed. Although MP contamination levels presently reported correspond well to those found in wild specimens' dorsal muscle samples, the prevalent polymer types differed. The practical implications of these findings warrant further studies.

**Keywords:** Microplastics, European seabass, Recirculation aquaculture systems (RAS), μFTIR, 'One Health' concept

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# **POSTERS**



## **Does *Salicornia ramosissima* improves immune response of European seabass?**

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One of the main challenges of modern aquaculture is to find new ingredients and additives able to improve aquafeeds sustainability, without compromising fish health and performance. Halophytes are rich in antioxidant compounds as polyphenols and hydroxycinnamic acids and many others that could be beneficial to fish. Despite scarce, studies approaching the outcome of these plants as feed additives, on immune responses and fish susceptibility to disease have been recently performed. Therefore, in the present work, the effects of *Salicornia ramosissima* fractions in metabolic and immune pathways of interest on European seabass (*Dicentrarchus labrax*), upon stimulation with bacteria. Viability assays performed with head kidney leukocytes showed that *S. ramosissima* fractions do not seem to be harmful to cells within the tested concentrations, instead, an improved cell viability was perceived. Results on ATP and nitric oxide production assays will be presented. Also, gene expression data will put into evidence the of key metabolic- and immune-related pathways modulated.

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## ***Chlorella vulgaris* extracts as modulators of gilthead seabream juveniles (*Sparus Aurata*) health status and inflammatory response**

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This study aimed to evaluate the effects of short-term supplementation with 2% *Chlorella vulgaris* (*C. vulgaris*) biomass and two 0.1 % *C. vulgaris* extracts, in gilthead seabream (*Sparus aurata*) health status (experiment 1) and inflammatory response (experiment 2). The trial comprised 4 isoproteic (50 % crude protein) and isolipidic (17 % crude fat) diets. A fishmeal-based (FM), practical diet was used as control (CTR) whereas 3 experimental diets based on CTR were further supplemented with a 2 % inclusion of *C. vulgaris* biomass (Diet D1); 0.1 % inclusion of *C. vulgaris* peptide-enriched extract (Diet D2), and finally 0.1 % inclusion of *C. vulgaris* insoluble fraction (Diet D3). Diets were randomly assigned to quadruplicate groups of 97 fish/tank (IBW:  $33.4 \pm 4.1$  g), fed to satiation three times a day in a recirculation seawater system. In experiment 1, seabream juveniles were fed for 2 weeks and sampled for tissues at 1 week and at the end of the feeding period. Afterwards, randomly selected fish from each group, were subjected to an inflammatory insult (experiment 2) by intraperitoneal injection of inactivated gram-negative bacteria, following 24 and 48 h fish were sampled for tissues. Blood was withdrawn for haematological procedures, whereas plasma and gut tissue were sampled for immune and oxidative stress parameters. Anterior gut was also collected for gene expression measurements. After 1 and 2 weeks of feeding, fish fed D2 showed higher circulating neutrophils than seabream fed CTR. In contrast, dietary treatments induced mild effects on the innate immune and antioxidant functions of gilthead seabream juveniles fed for 2 weeks. In the inflammatory response following the inflammatory insult, mild effects could be attributed to *C. vulgaris* supplementation either in biomass form or extract. However, the *C. vulgaris* soluble peptide-enriched extract seems to confer a protective, anti-stress effect in the gut at the molecular level, which should be further explored in future studies.

## **New ingredients with functional properties from *Hermetia illucens* and *Tenebrio molitor* through the enzymatic hydrolysis with corolase**

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Edible insects have been studied as a new sustainable source of protein and recent works demonstrated their potential to originate bioactive peptides with potential application in food and feed industries. This study aimed to investigate the optimal enzymatic hydrolysis conditions of *Hermetia illucens* and *Tenebrio molitor* with corolase enzyme (porcine pancreas) to obtain bioactive hydrolysates. A combination of different times of hydrolysis (30, 240 and 450 min) and enzyme concentration (0.5%; 1.5% and 2.5%) were performed and the degree of hydrolysis, protein concentration (BCA) and antioxidant activity (ABTS) of the obtained hydrolysates were evaluated. The practical results were statistically analyzed through two Box–Behnken and the optimal conditions of hydrolysis to get the best outcome in the three variables (DH, antioxidant activity and protein concentration) were set with 2.5% of corolase during 385 min for *Hermetia illucens* and 2.1% of corolase during 360 min for *Tenebrio molitor*. Using these hydrolysis conditions, a DH of 65%, a protein concentration of 11,3 mg/ml and an antioxidant activity of 19654 µmol/L were obtained for *Hermetia illucens* and a DH of 67%, a protein concentration of 13,1 mg/ml and an antioxidant activity of 23483,7 µmol/L for *Tenebrio molitor*. Due to the antioxidant activity observed for both hydrolysates obtained under optimal conditions, they may have application as bioactive compounds to be used in functional foods for human and animal nutrition.

Keywords: Antioxidant activity; Corolase; Enzymatic hydrolysis; Insect protein; Protein hydrolysate.

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## Central immune and neuroendocrine gene expression patterns of European seabass (*Dicentrarchus labrax*) undergoing chronic inflammation and fed tryptophan supplemented diets

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Amino acids (AA), such as tryptophan, play several functions on key metabolic pathways important to immune and neuroendocrine responses. The dietary supply of AA could be applied in specific situations when requirements are higher, such as stressful conditions and inflammation. This study aimed to contribute to this endeavour by assessing the immune-endocrine interactions of European seabass (*Dicentrarchus labrax*) fed tryptophan supplemented diets undergoing chronic inflammation.

European seabass juveniles ( $33.0 \pm 3.78\text{g}$ ) were distributed in 12 tanks. At the onset of the trial, half of the fish were intraperitoneally injected with 100  $\mu\text{L}$  of Freund's Incomplete Adjuvant solution to induce a peritoneal inflammation, and the other half with Hanks' Balanced Salt Solution (sham group). Two dietary treatments were evaluated in triplicate groups: a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP). Fish were fed these diets for 4 weeks, twice a day (2% body weight). Fish was sampled at 1, 2, 3 and 4 weeks. Plasma was used for plasma cortisol evaluation while immune- and neuroendocrine-related gene expression was analysed in both the hypothalamus and pituitary gland.

Plasma cortisol levels were not modulated by diet nor by stimulation. Still, cortisol concentration gradually increased over time until 3 weeks, decreasing back to basal levels at 4 weeks. A similar time-dependent increase was observed in hypothalamic *htr1a $\beta$* , *il6* and *mcsfr1* mRNA expression levels of FIA-injected fish fed TRP. Regarding pituitary gland *il1 $\beta$* , mRNA levels significantly decreased over time, from 1 to 4 weeks post-injection, irrespectively of dietary treatment or injection nature.

A general inflammatory response was observed by regulatory mechanisms that were triggered upon neuroendocrine stimulation in brain and in pituitary gland by TRP supplementation with the up-regulation of the pro-inflammatory cytokine *il1 $\beta$*  produced at the inflammation site, contributing to induce the expression of others such as *il6* as neuroendocrine-immune response. However, the increase serotonergic activity by the up-regulation of *htr1a $\beta$*  in fish fed TRP did not result in a decrease in plasma cortisol levels, but the opposite. The absence of a stronger neuroendocrine response and TRP-mediated effects might be explained to the fact that there was no particular stressful condition, besides the intra-peritoneal injection and the development of an inflammatory response.

**Keywords:** Stress response, HPI-axis, functional feeds, immune response, serotonergic activity

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## Marine sources in dog feeding: nutritive value and palatability of squid meal and shrimp hydrolysate

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The quality and sustainability of pet food ought to be improved due to the increased population of companion animals and the growing demand for an optimal nutrition by owners. The study of alternative and functional protein feed sources will contribute for the pet food sustainability, providing optimal nutritional value and promoting animal health. The present study evaluated the palatability and nutritive value of alternative protein sources of marine origin, squid meal and shrimp hydrolysate, namely proximate composition, amino acid profile, antioxidant activity, palatability, and *in vivo* digestibility in adult Beagles. Chemical composition and antioxidant activity substantiated both squid meal and shrimp hydrolysate as valuable feed sources of protein with functional properties. Both sources showed high levels of crude protein (810.0 g kg<sup>-1</sup> dry matter basis, DM, in squid meal; 657.5 g kg<sup>-1</sup> DM in shrimp hydrolysate), with arginine, lysine, and leucine being the essential amino acids presented at higher concentrations, ranging 45.7-57.9 g kg<sup>-1</sup> DM in squid meal and 30.3-38.5 g kg<sup>-1</sup> DM in shrimp hydrolysate. Based on the minimum recommended levels for adult dogs by FEDIAF (maintenance energy requirement of 110 kcal/kg<sup>0.75</sup>), amino acid scores were above 100, except for methionine+cystine in both sources, and threonine in shrimp hydrolysate. Shrimp hydrolysate presented higher antioxidant activity than squid meal in all methods analysed (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS<sup>•+</sup>) scavenging activity, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>) scavenging activity, ferric reducing antioxidant power (FRAP), and Folin-Ciocalteu reducing capacity). The palatability tests on adult Beagles showed preference for a commercial diet over 15% dietary inclusion of either source, with no differences for first approach and taste. Dietary inclusion of 5, 10, and 15% of either marine sources had no effect on *in vivo* diet digestibility and metabolizable energy content, with these feeds presenting high DM (79.5-80.3%) and crude protein (87.0%) digestibility. Consistency of faeces was not affected by dietary inclusion of 5, 10, and 15% of marine sources and ranged between 3.1-3.3 for diets with squid meal, and 3.2-3.4 for diets with shrimp hydrolysate, corresponding to soft, shaped, and moist stools leaving spots on the floor (3.0) to approximately firm, shaped, and dry stools (3.5). While further research is needed, the results suggest the potential of squid meal and shrimp hydrolysate as novel feed sources in dog nutrition and the need for strategies to increase palatability.

**Keywords:** Marine sources, Pet food, Sustainability, Nutritive value, Palatability

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## **Tolerance signatures to *Perkinsus olseni* parasite infection in *Ruditapes decussatus* clams**

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### **Introduction**

The grooved carpet shell (*Ruditapes decussatus*) is a bivalve mollusc species with a distribution from NE-Atlantic coast and Mediterranean Sea. This species is highly desirable and sold at high prices being Portugal the top-seller<sup>1</sup>. A decline of the populations happened since the 90's due to biotic and abiotic factors, such as parasite infection, degradation of the environment and competition with alien species. Infection by *Perkinsus olseni* parasite is the main biotic factor that causes decrease of populations. It was first diagnosed in the 1980s, possibly after introduction of the invasive species *Ruditapes philippinarum* from Asia for intensive production in Europe<sup>2,3</sup>. It has been observed that *Perkinsus* causes changes in humoral and cellular responses and a delay in gonad maturation and reproductive status in infected individuals causing recruitment problems<sup>4</sup>.

Several studies focused on host-parasite early interaction and markers of resistance<sup>5-7</sup>, while none of them have addressed the presence of tolerance/susceptibility signatures in different populations. Thus, this study seeks to identify markers of tolerance/susceptibility in populations of *R. decussatus* affected by *P. olseni* by looking at the single nucleotide polymorphism (SNPs) profile of tolerant individuals in comparison with susceptible ones.

### **Materials and methods**

Clams from five populations with high prevalence of the parasite across Europe namely, Pontevedra (Spain), Algarve (Portugal), Naples (Italy), Venice (Italy), and Izmir (Turkey) were sampled. Also, a *Perkinsus*-free population (Noia, Spain) was used as control.

After *Perkinsus spp.* diagnosis by RFTM, 30 adult individuals (>40 mm) were chosen from each population for DNA extraction from foot tissue. Chosen individuals included all the infection stages according to the mackin scale for *Perkinsus* infection quantification<sup>8</sup>, except for Noia (only non-infected individuals) and for Venice that included medium to highly infected individuals. DNA quality was assessed by gel electrophoresis and quantified on a DeNovix instrument. Extracted DNA samples were pooled by population, the respective library prepared and pool quality assessed according to Vera et al.<sup>9</sup>. Illumina sequencing of the pooled samples was performed at FISABIO facilities (Valencia, Spain). SNP identification and analysis will be elaborated according to Vera et al.<sup>9</sup>.

### **Expected outcomes**

This work aims to obtain three major outcomes. The first one will be the knowledge of the genetic signature of each population which will allow us to know in depth how the populations of *R. decussatus* are connected

among them. Also, it will be possible to obtain a set of characteristic SNPs for population determination that could be useful for determination of the origin of clams for alimentary industry. Finally, we hope to obtain a set of SNPs that indicates a genetic signature for resistance to *P. olsenii* that could allow to perform in the future a breeding program based on these results.

**Keywords:** *Ruditapes decussatus*; *Perkinsus olsenii*; host-parasite interaction; genomics; innate immunity; SNPs

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## Beyond methionine: new tools to improve fish robustness and disease resistance

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Infectious diseases and feed management are among the major challenges of the aquaculture sector. To overcome these constraints, the enrichment of feeds with additives that improve fish health, such as certain amino acids, is becoming common in modern farming [1]. Methionine is among the amino acids with recognised roles in the immune system, notoriously improving fish immune response to infection and disease resistance [2-4]. However, methionine role on mucosal immune machinery and in response to pathogens still needs further investigation. Furthermore, despite the recognised link between nutrition and immune system [5], few studies have combined immunisation strategies with nutrient supplementation. In this scenario, methionine appears to be promising [2]. Therefore, the present work plan aims to provide a better understanding of the underpinning mucosal immune mechanisms of fish fed methionine supplemented diets and to evaluate methionine potential for improving immunization efficiency of commercial vaccines. Two fish models, European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*), were selected as important species for European aquaculture and will be fed diets containing graded levels of methionine (i.e., 1 and 2 % of feed) for short-term feeding periods. Both the systemic and mucosal immune responses will be assayed, as well as disease resistance. After selected feeding times, fish will be bath challenged with bacteria and the inflammatory response and mortality will be evaluated. Based on the outcomes of a first battery of trials, a methionine supplementation level and feeding period will be selected, and experiments will be conducted to study the synergistic effects of dietary methionine supplementation and vaccination against bacterial or viral pathogens.

**Keywords:** Functional feeds; amino acids; mucosal immunity; vaccination

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## **Pineapple byproducts in aquafeeds: impact on the radical-scavenging potential of diets and stress-response of farmed European seabass**

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The fruit industry generates millions of tons in surplus peels rich in natural antioxidants, i.e. polyphenols, vitamins and carotenoids. Including small amounts of these byproducts in aquafeeds might help reduce fish oxidative stress and delaying feed/flesh oxidation during storage, while meeting consumer preferences for natural products. In a previous study, we have addressed natural antioxidants in aquafeeds and, more importantly, how manufacturing temperature impacts the preservation of these compounds. Surprisingly, results revealed that conventional extrusion at high temperatures (110°C) followed by quick drying at 60°C better retain the antioxidant properties of the resulting mixtures compared to lower processing temperatures (25°C and 35°C, respectively). Moreover, inclusion of 2% pineapple peel flour in mixtures for European seabass (*Dicentrarchus labrax*) improved antioxidant potential of resulting extruded diets. Based on these findings, a subsequent study was designed for evaluating how dietary inclusion of pineapple flour impacts fish growth performance, stress response, and immunological and antioxidant defences. A commercial-based diet for European seabass without antioxidant supplementation was used as negative control (CTRL) and compared to a diet containing 100 mg/kg of Vitamin E (VITE). Two experimental diets were formulated by adding 2% of pineapple peel or stem flour to the VITE mixture, generating diets PP and PS, respectively. All diets were processed using high temperatures (110°C extrusion; 60°C drying), and remained isoproteic and isoenergetic. Flours and diets were analysed for proximate composition, free/bound polyphenols, carotenoid profile, TBARS, DPPH, ABTS and ORAC over storage. Diets were stored in vacuum for 3 months, at 4 and 24°C, simulating optimal storage and practical farm conditions, respectively. Afterwards, diets' antioxidant content and potential was assessed. In parallel, an experimental trial was carried out with European sea bass juveniles (initial weight 13.5±0.8g). Each diet was assigned to quadruplicate groups of 17 homogeneous fish distributed in 50L tanks. Fish were fed to apparent satiety with automatic feeders until tripling their initial body weight. After this trial, all fish were weighed and measured, and 20 fish p/treatment sampled for proximate composition. Additionally, 16 fish per treatment were immediately sampled or exposed to a stress challenge, i.e. confinement stress (5 min; 100 kg/m<sup>3</sup>) followed by air exposure (1 min), replicating capture in an aquaculture scenario. Immunological status, blood stress biomarkers, liver antioxidant enzymes, muscle/liver TBARS, and muscle DPPH, ABTS and ORAC will be evaluated in both non-stressed and stressed fish to ascertain the potential of pineapple byproducts as natural sources of antioxidants in aquafeeds.

**Keywords:** natural antioxidants; functional feeds; circular economy; oxidative stress

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