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Cooperativa Agrícola de Vila do Conde

**Vila do Conde, Portugal**

**ANIMAL SCIENCE DOCTORAL PROGRAMME:  
BOOK OF ABSTRACTS OF THE X WORKSHOP  
13<sup>th</sup> October 2023, Cooperativa Agrícola de Vila do Conde  
Vila do Conde  
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**Jury for the best participant, poster and oral presentation awards:**

Maria Coelho (*B2E CoLab*)  
Johan Schrama (*Wageningen University*)

## **EDITORIAL**

### **Bridging Academia and Animal science: the X Animal Science Workshop**

In a world where academia often stays within university walls, there are moments when innovation beckons us to venture beyond. It is in this spirit of breaking new ground and fostering interdisciplinary collaboration that we proudly present the X Edition of the Animal Science Workshop.

This year, we embark on a journey that transcends academia and merges with the practical world of agriculture. We have joined hands with a non-academic partner of our doctoral program, the Cooperativa Agrícola of Vila do Conde, in organizing this groundbreaking event.

Our journey begins with a focus on animal nutrition. Professor Johan Schrama, our distinguished guest, will discuss the importance of using net energy values in feed formulation, a topic of immense importance in the ever-evolving field of animal nutrition. We will transition to the presentation of the most recent research findings by our talented students. Many of these projects have been conducted in collaboration with industrial partners, creating a dynamic exchange of ideas and knowledge. Next, Professor Carlos Soares, a distinguished member from the University of Porto's Faculty of Engineering, will offer a personal perspective on the application of Artificial Intelligence in both the realms of science and industry. The final segment of our workshop promises to be a thought-provoking and enlightening experience. We are honored to have António Bob dos Santos from the Foundation for Science and Technology (FCT) to discuss the future of funding for doctoral students, particularly in non-academic environments, offering insights that will shape the high educational landscape. Additionally, we have assembled a remarkable panel of experts, including CEOs and professionals from various companies and associations, to engage in a lively debate. This panel will explore the critical relevance of innovation in the agrofood sector and tackle the emerging challenges it faces. Their expertise and diverse perspectives promise to enrich our understanding of the industry's evolving landscape.

The X Edition of the Animal Science Workshop is designed to be an enriching and informative experience, bridging academia and industry to shape the future of our field. We look forward to your participation and engagement in this dynamic event.

Luísa M.P. Valente

Director of the Animal Science Doctoral Programme, ICBAS-UP

## INVITED SPEAKERS

### Professor Johan Schrama



Johan Schrama studied animal science and got his PhD at Wageningen University on nutrition and thermoregulation of young calves. Before joining the Aquaculture and Fisheries group at Wageningen University (WU) in 2001, he was assistant professor at the Animal Husbandry group working on energy metabolism in warm-blooded (farm) animals. From 2001 onward he moved to aquaculture focusing on nutrition of fish. One of the focus areas in his research is the impact of carbohydrates on various aspects of nutrition, like feed intake, digestion, faecal waste production and energy metabolism in fish. Next to feed-fish interaction, the feed-fish-system interaction have a central place in his research, like the topic how and what to feed fish for “clean” water.

### Professor Carlos Soares



Carlos Soares is an Associate Professor at the Faculty of Engineering of U. Porto, where he served in several management roles, including Subdirector of the Department of Informatics Engineering and Director of the Ph.D. Programme in Informatics Engineering. Carlos is also an External Advisor for Intelligent Systems at Fraunhofer Portugal AICOS, a researcher at LIACC and a collaborator at LIAAD-INESC TEC. He is also a lecturer at the Porto Business School. The focus of his research is on metalearning/autoML but he has a general interest in Data Science. He has participated in 20+ national and international R&ID as well as consulting projects, published/edited several books and 150+ papers in journals and conferences and supervised 10+/50+ Ph.D./M.Sc. thesis. In 2009, he was awarded the Scientific Merit and Excellence Award of the Portuguese AI Association.

## AGENDA

### 09:00 • WORKSHOP OPENING :

António Rodrigues Balazeiro (CAVC)

### SESSION I – IN ENGLISH

**Chairs:** Jorge Dias (Sparos) & Rita Cabrita (ICBAS / REQUIMTE-LAQV)

**09:15 • Plenary lecture 1:** The importance of using net energy values in feed formulation – **Johan Schrama** (WUR, Netherlands)

**10:00 • Oral presentation 1** Improving fish growth and composition models - tools to optimize fish nutrition and rearing practices – **Andreia Raposo** (ICBAS/SPAROS)

**10:15 • Oral presentation 2** Unveiling shrimp hydrolysate as a novel protein source for pet food – **Joana Fernandes** (REQUIMTE-LAQV/ICBAS/SORGAL)

**10:30 • Oral presentation 3** Dietary algae blend modulates the *in vitro* rumen fermentation depending on the inclusion level and the inocula basal diet – **Cátia Mota** (REQUIMTE-LAQV/ICBAS/ALGAplus/ALLMICROALGAE)

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

**Chairs:** Marta Monteiro (ICBAS / CIIMAR) & Margarida Maia (ICBAS / REQUIMTE-LAQV)

**11:30 • Oral presentation 4** Impact of insect hydrolysates supplementation on diet palatability, digestibility and faecal characteristics of adult dogs – **Daniela Leal** (REQUIMTE-LAQV/ICBAS)

**11:45 • Oral presentation 5** *Tenacibaculum maritimum* can boost an inflammatory response in European seabass (*Dicentrarchus labrax*) upon peritoneal injection but cannot trigger tenacibaculosis disease – **Inês Ferreira** (ICBAS/CIIMAR/ i3S)

**12:00 • Oral presentation 6** Effects of pineapple by-products in aquafeeds: influence on the stress response of European seabass – **Ricardo Pereira** (CIIMAR/CBQF/ICBAS)

**12:15 • Oral presentation 7** Dietary supplementation of tryptophan alleviates gene expression changes induced by stress and inflammation in the hypothalamic-pituitary-interrenal (HPI) axis of a teleost – **Diogo Peixoto** (CIIMAR/ICBAS/UCA)

**12:30 • Oral presentation 8** From seashore to seafood: a study into the effects of *Aster tripolium* inclusion on *Penaeus vannamei* health – **Ana Garcia** (CIIMAR/ICBAS)

**12:45 • Oral presentation 9** Tolerance signatures of selection of *Ruditapes decussatus* against *Perkinsus olseni* infection – **João Estêvão** (CIIMAR/ICBAS)

### 13:00 • LUNCH & POSTER SESSION

**14:30 • Oral presentation 10** Boosting fish robustness: algae feeds as a promising strategy for enhancing intestinal health and performance – **Mariana Ferreira** (CIIMAR/ICBAS/NORD)

**14:45 • Oral presentation 11** How do consumers perceive farmed fish and how to improve that perception? A literature review – **Daniela Resende** (ICBAS/CIIMAR/SenseTest/GreenUPorto)

## SESSION II – IN PORTUGUESE (EM PORTUGUÊS)

**15:00 • Palestra Plenária 2:** A inteligência Artificial no Meio Científico e nas Empresas – **Carlos Soares** (FEUP)

**15:45 • O programa doutoral em Ciência Animal do ICBAS - Universidade do Porto – um exemplo de uma relação bem-sucedida entre instituições de I&D e parceiros industriais** – Luísa Valente (Animal Science Doctoral Programme Director)

## 16:00 • MESA REDONDA: CIÊNCIA ANIMAL - AS LIGAÇÕES ENTRE ACADEMIA E INDÚSTRIA

**Moderadora:** Luísa Valente (ICBAS /CIIMAR)

**Participantes:** António Bob dos Santos (FCT Board Member); António Isidoro (CEO, Soja de Portugal); Ingrid Van Dorpe (CEO, Premix); Jaime Piçarra (IACA), Jorge Dias (CEO, Sparos), Ana Gomes (CAVC)

## 17:45 • BEST PARTICIPANT, POSTER AND ORAL PRESENTATION AWARDS

## 18:00 • SESSÃO DE ENCERRAMENTO

Henrique Cyrne Carvalho (ICBAS Director)

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## POSTERES

- Immunostimulatory capacity of grapeseed extracts as feed additives in European seabass (*Dicentrarchus labrax*) juveniles – **André Cunha** (CIIMAR/ICBAS/ GreenCoLab)
- Innate immune response and disease resistance of European seabass (*Dicentrarchus labrax*) juveniles after *Tetraselmis* sp. extracts dietary supplementation – **Paulo Santos** (CIIMAR/ICBAS/ GreenCoLab)
- Microplastics and metals in European seabass from different aquaculture systems: dietary exposure and toxicological risks for humans - **Ricardo Matias** (ICBAS/CIIMAR)
- Effects of dietary methionine supplementation on European seabass mucosal immune response and disease resistance – **Inês Carvalho** (CIIMAR/ICBAS)
- Innovative hydrolysates as functional nutritional supplements to promote seabass robustness and flesh quality addressing the European zero-waste strategy – **Luciano Santos** (CIIMAR/ICBAS/CBQF/ETSA)

# **PLENARY SESSIONS**

## **The importance of energy evaluation for making balanced aqua-feeds: passed present and future.**

*Johan W. Schrama*<sup>1\*</sup>

<sup>1</sup> Aquaculture and Fisheries Group, Wageningen University, The Netherlands

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### Abstract

Energy content is an important factor for making balanced diets, thus also for balanced aqua-feeds. Energy is required to sustain vital life process and to fuel growth. Between the step of energy intake (gross energy) via the feed to energy gain in the fish, various steps of energy losses occur. For the formulation of balanced diets regarding energy it is important to have knowledge about these various losses. Evaluation of energy of diets/ingredients in fish is currently mainly based on digestible energy (DE). DE evaluation only takes difference in digestibility into account, thus estimating the energy losses via faeces. In a DE evaluation, the dietary DE content is calculated from the relationship between DE and retained energy (RE). In this way, the amount of energy needed for maintenance and the amount of energy required for growth are predicted. DE evaluation systems, assume that the relationship between DE and RE are not influenced by dietary macronutrient composition. However, various studies in different fish species have shown that this assumption is not valid. In fact, the utilization efficiency of DE for growth ( $k_{gDE}$ , the slope of the relationship between DE and RE) is influenced by the macronutrient composition of the diet. Currently and in the future, the ingredient composition of fish feed formula is and will further diversify. Related to this diversification, also the macronutrient composition can increase in variability. Consequently, there is an increasing need to evaluate fish feeds/ingredients by an alternative way than the DE approach. Therefore, recently net energy (NE) evaluation has been considered for fish. In NE evaluation the potential for energy gain (RE) is predicted from the digested protein (dCP), digested fat (dFat) and digested carbohydrate (dCarb) content of diets. In an NE approach, RE is related to dCP, dFat and dCarb by multiple regression. This results in estimates of energy utilization of digested protein, fat and carbohydrate separately ( $k_{NE;dCP}$ ,  $k_{NE;dFat}$  and  $k_{NE;dCarb}$ , respectively). Comparison of NE equations is made between different fish species. Furthermore, the evaluation of DE versus NE for practical feed formulation is addressed.

Keywords: Energy evaluation, Digestible nutrients, Bioenergetics, Net energy, Digestible energy.

### References

1. Kaushik, S.J., Panserat, S., Schrama, J.W., 2022. Fish Nutrition, Fourth Edition - Chapter 2 – Bioenergetics. <https://doi.org/10.1016/B978-0-12-819587-1.00011-2>



## **A Personal Perspective on the use of Artificial Intelligence in Science and Industry**

*Carlos Soares<sup>1</sup>*

<sup>1</sup>Lab. Artificial Intelligence and Computer Science/Faculdade de Engenharia, Universidade do Porto

External Advisor for Intelligent Systems, Fraunhofer AICOS Portugal

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### Abstract

In this talk, I will provide a very personal perspective on AI. I will start with a brief introduction of what AI. Then, I will discuss the use of AI for science and for industrial applications, with a focus on some of the opportunities and challenges. A particular emphasis will be given to the responsible use of AI systems.

Keywords Artificial Intelligence, Machine Learning

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# **ORAL COMMUNICATIONS**

## **From seashore to seafood: A study into the effects of *Aster tripolium* inclusion on *Penaeus vannamei* health**

Ana Garcia<sup>1,2\*</sup>, S. Fernández-Boo<sup>2</sup>, A. Stensballe<sup>3</sup>, K. Viswanath<sup>4</sup>, M. Thomsen<sup>3</sup>, S. Kohnen<sup>5</sup>, R. Rocha<sup>6,7</sup>, B. Costas<sup>1,2</sup>

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### Abstract

Some plant-derived oils have been studied for inclusion in shrimp diets both as an alternative to reduce the dependence on fish oil and as a source of health promoting compounds. The aim is to promote sustainability in the aquaculture industry while still providing essential nutrients and also bioactive compounds for shrimp to grow and thrive. *Aster tripolium* is a halophyte containing several bioactive compounds that have potential to benefit shrimp health. The primary objective of this study was to evaluate the effect of *A. tripolium* lipid extract on the immune system and intestinal microbiota of whiteleg shrimp (*Penaeus vannamei*).

The trial was conducted in a recirculating system composed of 40L tanks, with 22 shrimp per tank and 6 tanks per treatment. Experimental diets were prepared including 0.1% and 1% of *A. tripolium* lipid extracts in soybean oil to the control diet. After one month of feeding trial, three shrimps per tank were sampled, and the remaining animals were subjected to a bacterial challenge with *Vibrio parahaemolyticus*. At 24 and 48 hours after challenge, three shrimps per tank were sampled. In all sampling points, haemolymph was collected for immune parameters assessment, and gut was collected for microbiome evaluation and proteomic expression.

Regarding growth parameters, no differences were founded among treatments. Protein content and innate immune parameters in haemolymph showed no significant differences between shrimps fed with *A. tripolium* lipid extracts and those fed a control diet. These findings suggest that the inclusion of *A. tripolium* does not have harmful effects on shrimp immune status. Proteomics data revealed a higher abundance of 195 and 29 proteins in the 0.1% and 1% *A. tripolium* supplemented diet, respectively, when compared to control (AVG Log<sub>2</sub> Ratio > 2, p < 0.05). Still, other 79 and 114 proteins were less abundant in the 0.1% and 1% supplemented diet, respectively, compared to the control (AVG Log<sub>2</sub> Ratio < -2, p < 0.05). Additional data on proteomics and also microbiome analysis are currently being analyzed and may provide further information to complement this study.

Keywords: halophytes, aquafeeds, whiteleg shrimp

### Acknowledgements

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## **Improving fish growth and composition models - tools to optimize fish nutrition and rearing practices.**

Andreia I. G. Raposo<sup>\*1,2</sup>, F. Soares<sup>2</sup>, L. E. C. Conceição<sup>2</sup>, L.M.P. Valente<sup>1,3</sup>, T. S. Silva<sup>2</sup>

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### Abstract:

The main focus of this work was to explore different mathematical models to predict the growth and body composition of Nile tilapia (*Oreochromis niloticus*), to optimize feed formulations and rearing practices for this species. The work address models used commonly in the industry and compare them with more complex ones that attempt to overcome the simple models' limitations. This work also establishes a structured and objective way to approach design and model comparison by taking objective measures of generalization capacity (e.g., cross-validation error) into account. This work focused not only on Nile tilapia, but also on other economically relevant species (e.g., gilthead seabream, European seabass, rainbow trout, Atlantic salmon, sole and turbot), in order to assess their similarity.

In this study, we tested static body compositions and found that the body composition components of Nile tilapia follows a quasi-isometric allometric pattern. Strong correlations exist between fish body weight and relative water and lipid content, while the relationships for relative protein and ash content are sometimes indistinguishable from isometry. Therefore, utilizing isometric models or ensemble averaging models combining both allometric and isometric predictions may be suitable for estimating relative protein and ash contents in Nile tilapia and other studied species.

Dynamic body composition models – which consider intrinsic (e.g., body weight) and extrinsic factors (e.g., environmental conditions and nutrition) over time – were also used in combination with the Energy-protein growth models (EP). The results showed that updating water and ash retention rates in EP models using protein retention improves understanding of body composition changes during fish growth. Additionally, using information on protein intake alongside energy intake enhances fish growth estimations. The observed linear relationship between protein and fat gain in Nile tilapia may not apply to high protein and/or fat intakes, suggesting the suitability of linear-plateau or other saturating models, especially for carnivorous species or those with high protein requirements. Furthermore, the results on the evaluation of the patterns and parameters of growth models for different species challenge the concept of universal metabolic allometry found in some literature.

This study showcased advancements in mathematical models to accurately predict fish body composition and growth. It provided valuable reference models for Nile tilapia precision farming, which have been thoroughly validated with independent data. Furthermore, the research highlighted the significance of assessing species similarity to understand their metabolic differences, and assess how this information can be relevant for modelling the metabolism of other economically-relevant species.

Keywords: Mathematical models, Body composition, Precision farming, Regression analysis, Dynamic models, Growth

### Acknowledgements

A. Raposo acknowledges financial support by Grant PD/BDE/150525/2019 (SANFEED Doctoral program, with support by FCT and SPAROS Lda, Portugal). This work was also funded by project E!12516 - FEEDNETICS 4.0\_40813, supported by EUROSTARS-2 programme, Portugal and European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020.

## **Dietary algae blend modulates the *in vitro* rumen fermentation depending on the inclusion level and the inocula basal diet**

Cátia S. C. Mota<sup>1\*</sup>, Ana R. J. Cabrita<sup>1</sup>, Helena Abreu<sup>2</sup>, Joana L. Silva<sup>3</sup>, António J. M. Fonseca<sup>1</sup>, Margarida R. G. Maia<sup>1</sup>

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### Abstract:

Urgent actions are needed to improve ruminant production efficiency and environmental impact to meet the UN 2030 Agenda goals (1). Dietary inclusion of alternative and more sustainable feeds, as locally produced algae, may effectively contribute to achieve these goals. Micro- and macroalgae have been reported to modulate the rumen fermentation and methanogenesis (2, 3), but the mixture of micro- and macroalgae species has yet to be assessed. Thus, this work aimed to evaluate the effects of ruminal inocula from dairy cows fed forage-based diets, differing only on basal forage proportion [i.e., maize silage (MS) and haylage (HL); 100MS, 70MS+30HL, 30MS+70HL, and 100HL], on the rumen fermentation parameters of a basal substrate (MS:HL, 50:50) supplemented with increasing levels (0, 5, 10, and 15%, dry matter basis) of a commercial microalgae (*Chlorella vulgaris* and *Nannochloropsis oceanica*) and macroalgae (*Ulva* sp. and *Gracilaria gracilis*) blend, after 24-h *in vitro* incubations. Briefly, basal substrate was supplemented with algae blend and inoculated with individual rumen inocula, in duplicate, under anaerobic conditions. After 24-h incubation at 39 °C, total gas was measured and methane production, pH, volatile fatty acids (VFA), and ammonia-N were analysed (4, 5). Two incubations were run in independent days. Data were analysed using the GLM model of the SAS software, with the fixed effects of cow, rumen inocula, algae blend, incubation run, interaction between rumen inocula and algae blend, and the random residual error. Fermentation pH was higher with inocula from cows fed HL-based diets ( $P<0.001$ ). Gas and methane yield were lower with inocula from cows fed HL-based diets ( $P<0.001$ ) and with 10 and 15% algae blend supplementation ( $P<0.001$ ). Ammonia-N content was only affected by rumen inocula, following the opposite trend of pH ( $P<0.001$ ). Total VFA production was affected by inocula and blend interaction, being the lowest with 15% algae incubated with 100HL ( $P<0.001$ ). Molar proportion of acetate was the highest for 15% algae blend incubated with 100MS inoculum ( $P=0.009$ ), no differences observed within HL-based inocula. Similarly, propionate proportion was only affected with 100MS inoculum, the lowest value being found with 15% blend ( $P=0.006$ ). Butyrate proportion was affected by inocula ( $P<0.001$ ) but not by algae blend ( $P=0.119$ ). Overall, results highlight the impact of rumen inocula when assessing the fermentability of a novel feed and unveil the potential of algae blend as a natural modulator of the rumen fermentation and methanogenesis.

### Keywords:

Algae blend; alternative feed; fermentation pattern; *in vitro*; rumen inocula.

### Acknowledgements:

This work was supported through the project UIDB/50006/2020 | UIDP/50006/2020 (REQUIMTE), funded by Fundação para a Ciência e Tecnologia (FCT)/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds, and the project R&W Clean - New solutions for environmental and biological parameters sensing to support the demedicalisation of the agricultural sector (POCI-01-0247-FEDER-070109). CSCM acknowledges FCT, SANFEED Doctoral Programme, ALGAplus and Allmicroalgae for funding her PhD grant (PD/BDE/150585/2020). MRGM acknowledges FCT for funding

through program DL 57/2016 – Norma transitória (SFRH/BPD/70176/2010). Authors acknowledge the valuable technical expertise of Sílvia Azevedo, Daniela Flores and Marta Azevedo, from ICBAS-UP.

#### References

1. United Nations. (2015). Transforming our World: The 2030 Agenda for Sustainable Development. NY, USA: United Nations.
2. Meehan D.J., Cabrita A.R.J., Silva J.L., Fonseca A.J.M. & Maia M.R.G. (2021). Effects of *Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp. supplementation levels on *in vitro* rumen fermentation. *Algal Research*, 56, 102284. doi: 10.1016/j.algal.2021.102284
3. Maia M.R.G., Fonseca A.J.M., Oliveira H.M., Mendonça C. & Cabrita A.R.J. (2016). The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. *Scientific Reports*, 6, 32321. doi: 10.1038/srep32321
4. Maia M.R.G., Fonseca A.J.M., Cortez P.P. & Cabrita A.R.J. (2019). *In vitro* evaluation of macroalgae as unconventional ingredients in ruminant animal feeds. *Algal Research*, 40, 101481. doi: 10.1016/j.algal.2019.101481
5. Chaney A.L. & Marbach E.P. (1962). Modified reagents for determination of urea and ammonia. *Clinical chemistry*, 8, 2, 130.

## Impact of insect hydrolysates supplementation on diet palatability, digestibility and faecal characteristics of adult dogs

Daniela Leal<sup>1</sup>\*, D. Pereira<sup>1</sup>, S. Borges<sup>2</sup>, M.R.G. Maia<sup>1</sup>, C. Barros<sup>1</sup>, A. Almeida<sup>3</sup>, M. Pintado<sup>2</sup>, A.J.M. Fonseca<sup>1</sup>, A.R.J. Cabrita<sup>1</sup>

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### Abstract

In recent years, edible insects have garnered attention as a sustainable alternative protein source for animal feed (Hawkey, 2021). Nevertheless, the use of insect hydrolysates as functional supplements in dog food requires further investigation. *In vitro* studies suggest potential bioactive properties, including antioxidant, anti-hypertensive and anti-inflammatory activities (Pan, 2022). This study aimed to assess the effects of dietary supplementation with hydrolysates from *Tenebrio molitor* and *Hermetia illucens* on palatability, diet digestibility and faecal characteristics of adult Beagle dogs. Hydrolysates derived from *T. molitor* and *H. illucens* were obtained through enzymatic hydrolysis with alcalase or corolase. Twelve Beagle dogs were used in four two-bowl tests to determine palatability by pairwise comparison of a commercial diet supplemented with 3% of shrimp hydrolysate (control diet) with the commercial diet supplemented with 3% of each insect hydrolysate (experimental diets). Diet first-approach and first taste results were submitted to the Chi-square test and the intake ratio to the Student's t-test, both at 5% probability level. The impact on food intake, faecal characteristics, diet digestibility and estimated metabolizable energy content of both the control and experimental diets was evaluated in a digestibility trial carried out according to a replicated Latin square 5 x 5 with 10 Beagle dogs. Each experimental period lasted for 10 days, with five days for diet adaptation and five days for total faeces collection. Data were analysed using the GLM procedure of SAS software (2021, release 3.1.0., SAS Institute, Cary, NC, USA) with the fixed effects of the square, dog within the square, period, diet and the residual error. When differences were significant ( $P < 0.05$ ), the least significant difference test was used to compare means. Insect hydrolysate supplementation did not affect the first diet approached or tasted, but dogs showed a preference for the diet supplemented with *T. molitor* obtained through corolase hydrolysis. The dietary supplementation with insect hydrolysates kept unaffected food and nutrient intake, diet digestibility and faecal characteristics, except for a higher concentration of faecal caproate in dogs that were fed the control diet. In summary, dogs readily accepted diets supplemented with 3% insect hydrolysates without negative effects on food intake, faecal characteristics and diet digestibility, suggesting potential of insect hydrolysates to be used as functional supplements in dog feeding.

**Keywords:** insects, hydrolysates, digestibility, palatability, dog food

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### References:

- Hawkey, K. J., Lopez-Viso, C., Brameld, J. M., Parr, T., & Salter, A. M. (2021). Insects: A Potential Source of Protein and Other Nutrients for Feed and Food. *Annual review of animal biosciences*, 9, 333–354.
- Pan, J., Xu, H., Cheng, Y., Mintah, B. K., Dabbour, M., Yang, F., Chen, W., Zhang, Z., Dai, C., He, R., & Ma, H. (2022). Recent insight on edible insect protein: extraction, functional properties, allergenicity, bioactivity, and applications. *foods*, 11(19), 2931.

## **How do consumers perceive farmed fish and how to improve that perception? A literature review**

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### Abstract

Despite the continuous growth of the aquaculture sector, wild fish is still a reference for consumers. 72% of the EU's apparent per capita consumption of fishery products is still provided by wild-caught products<sup>1</sup>, and only 7% of European consumers (and 6% of Portuguese consumers) claim to prefer farmed products<sup>2</sup>. Thus, a literature review was performed to investigate the reasons for this biased perception of aquaculture fish.

The SCOPUS and Web of Science databases were searched, using the query “aquaculture AND fish AND consumer”. Following the Prisma approach<sup>3</sup>, a total of 1067 records were retrieved from SCOPUS and 970 from Web of Science. 694 duplicates were identified with the Endnote (v20.5; Clarivate, United Kingdom) software and excluded. Reports without an available abstract or full text, not in English, obviously irrelevant, reviews, or those that did not focus on finfish were excluded, thus resulting in 140 analysed studies.

Overall, the reviewed papers highlight a consumer preference for wild-caught fish. There is a strong belief that farmed fish contain harmful chemicals, such as antibiotics or preservatives and often this product is seen as unsafe. Farmed fish is also seen as “unnatural”. Wild fish is perceived as having better taste and quality, and being fresher. This idea is further strengthened by the perceived lower price of farmed fish, since consumers see price as a quality cue. The idea that wild fish is healthier and more nutritious was also frequently reported. Fisheries are seen as eco-friendlier, and consumers believe wild fish to have better welfare. Another aspect that stood out during this review was the low objective and subjective knowledge of consumers regarding aquaculture. In fact, despite the claimed preference for wild fish, sensory studies often reveal no differences between comparable wild and farmed products. The preference for wild products was generally associated with older age, more frequent fish consumption and the male gender. Consumers also tend to value domestic products. Finally, a growing interest in organic and sustainable fish products has been observed.

Therefore, we propose four main points for the improvement of aquaculture perception. First, aquacultures must minimize the use of harmful chemicals and consumers must be assured of that. Second, there should be a focus on sustainable and eco-friendly farming processes, which should be clearly labelled as such. Third, each region should promote their domestic aquaculture products. Finally, clear and concise information regarding aquaculture should be disseminated to increase consumers' knowledge.

Keywords: Attitudes; beliefs, consumer perception; farmed fish; wild fish.

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References

1. European Market Observatory for Fisheries and Aquaculture Products (2022). The EU fish market. doi:10.2771/716731
2. European Commission (2021). Special Eurobarometer 515 – EU Consumer habits regarding fishery and aquaculture products. doi:10.2771/87688
3. Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L. & PRISMA-P Group. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews*, 4, 1. doi:10.1186/2046-4053-4-1

## **Dietary supplementation of tryptophan alleviates gene expression changes induced by stress and inflammation in the hypothalamic-pituitary-interrenal (HPI) axis of a teleost fish**

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### Abstract

Amino acids, such as tryptophan, play several functions on key metabolic pathways important to immune and neuroendocrine responses. This study aimed to assess the links between tryptophan nutrition and the network that regulates the bi-directional pathways between neuroendocrine and immune systems in European seabass.

European seabass juveniles ( $12.02 \pm 2.77$  g) were distributed in two independent recirculating seawater systems with a density of 5 kg/m<sup>3</sup> (control) or a stressful density (10 kg/m<sup>3</sup>). Fish were fed a control diet (CTRL) and a CTRL-based diet supplemented with tryptophan (0.3 % DM basis; TRP) in quadruplicate for 15 days. Fish were sampled at the end of the feeding period and at 4, 24 and 72 hours post intraperitoneal infection with *Photobacterium damsela* *piscicida*. The hypothalamus, pituitary gland and head-kidney were sampled for gene expression analysis.

Hypothalamic *gr1* expression was significantly induced by injection in stressed fish fed CTRL, but no such response was observed in stressed fish fed TRP, which expression levels at 4 hours post-injection were lower than in CTRL counterparts. Moreover, as opposed to CTRL, TRP-fed groups did not show an increase in hypothalamic *il6* expression levels after injection. In the pituitary, the expression of *htr2a* was higher in stressed fish fed CTRL compared to those fed TRP. Moreover, *tph1a* was not induced by injection in stressed, TRP-fed fish, and was lower in these fish at 24 hours post-injection than in their CTRL counterparts.

Taking into account genes that are related with neuroendocrine and serotonergic pathways, in non-stressful conditions, TRP-fed fish displayed a molecular profile more similar to that of CTRL-fed, stressed fish (neuroendocrine activation and immunosuppression); in contrast, TRP in stressed fish promoted a scenario resembling that of non-stressed fish (lower cortisol and serotonin reactivity). Results unveil modulatory effects of tryptophan dietary intervention in molecular patterns that might have sustained changes in cortisol levels (parallel approach), pointing out a serotonergic activity (changes) as a key regulatory mechanism.

### Acknowledgements

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## ***Tenacibaculum maritimum* can boost an inflammatory response in European seabass (*Dicentrarchus labrax*) upon peritoneal injection but cannot trigger tenacibaculosis disease**

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### Abstract

Despite being a bacterial pathogen with devastating consequences, *Tenacibaculum maritimum*'s transmission, infection route and pathogenesis are not fully disclosed (Mabrok *et al.*, 2023).

The present study aimed to evaluate the short-term innate immune response of European seabass (*Dicentrarchus labrax*) after intraperitoneal-challenge with *T. maritimum*'s extracellular products (ECPs), whole cells without and with ECPs, as well as their ability to induce tenacibaculosis.

A time-course trial was performed in which groups of seabass (35.61±6.5 g) were intraperitoneally-challenged with 5.5x10<sup>5</sup> CFU mL<sup>-1</sup> *T. maritimum* with and without ECPs, ECPs alone or marine broth (sham control). A group of fish were also bath challenged with the same inoculum. Undisturbed fish were randomly selected just before infection as controls (time 0). Twelve fish per treatment were randomly selected, euthanized, and sampled at 0, 3, 6, 24 and 48 h post-challenge. Blood, liver and head-kidney samples were collected for assessing immune parameters, oxidative stress and gene expression, respectively. To determine the severity of both bath and intraperitoneal-challenges, cumulative mortality was followed for two weeks.

Tenacibaculosis symptoms, such as skin/fin abrasions and mortality were only observed in the bath-challenged fish, in which 100% mortality was recorded. It was observed an increase of immune cells in the peritoneal cavity for the fish challenged with bacteria plus ECPs, when compared with the other treatments. Blood circulating leukocytes, lymphocytes and thrombocytes had a significant decrease immediately after the challenge, mainly seen in fish challenged with bacteria plus ECPs. At 48 h post-challenge, bactericidal activity increased for the treatments with bacteria (with and without ECPs). The same tendency was seen for some of the oxidative stress parameters.

The results of mortality trial and the observed immune responses suggests that the infection route is a determinant factor regarding *T. maritimum*-induced pathogenesis. The intraperitoneal challenge may result in a fast recruitment of immune cells which can undermine the invasion of bacteria. The head-kidney samples are currently under analysis to attempt to disclose the systemic response triggered by *T. maritimum*.

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

### Acknowledgments

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References

1. Mabrok, M., Algammal, A.M., Sivaramasamy, E., Hetta, H.F., Atwah, B., Alghamdi, S., Fawzy, A., Avendaño-Herrera, R., Rodkhum, C. (2023). Tenacibaculosis caused by *Tenacibaculum maritimum*: Updated knowledge of this marine bacterial fish pathogen. *Frontiers in Cellular and Infection Microbiology*. 6, 12:1068000. doi: 10.3389/fcimb.2022.1068000.

## Unveiling shrimp hydrolysate as a novel protein source for pet food

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### Abstract

The pet population is increasing worldwide<sup>1,2</sup>, bringing concerns on the environmental impact in food production and consumption<sup>3</sup>. Marine ingredients, particularly lower order animals and underexploited by-products, have lower environmental impact than terrestrial ingredients<sup>4</sup>, being potentially a more sustainable food source for companion animals. The aim of the present study was to evaluate the effect of the dietary inclusion of a shrimp hydrolysate on diet palatability and digestibility, faecal characteristics, coat quality and concentration of oral volatile sulphur compounds of adult Beagle dogs. Two extruded complete diets for adult Beagle dogs were formulated without (control diet) and with 5% w/w inclusion of shrimp hydrolysate (experimental diet). A two-bowl test with twelve dogs was performed to determine palatability by pairwise comparison of the control and the experimental diets. Animals were then distributed into six blocks of two animals each, according to sex and body weight, and one animal of each block was randomly allocated to one of the two diets for a 12-week assay with data collected at 0, 4, 8 and 12 weeks. First diet approached and tasted and consumption ratio did not differ between diets. The interaction between time and diet was not significant for all the measured parameters. Dry matter and nutrient intake were unaffected by diet, but the inclusion of shrimp hydrolysate increased organic matter digestibility ( $P = 0.046$ ) and tended to increase the dry matter ( $P = 0.069$ ) and fibre digestibility ( $P = 0.085$ ) while lowering faecal production ( $P = 0.069$ ). Faecal score, dry matter content, pH and ammonia-N content were similar between diets. Inclusion of shrimp hydrolysate tended to decrease ( $P = 0.054$ ) the concentration of oral volatile sulfur one hour after the morning meal, with no effects on concentrations measured before and five hours after the morning meal. Coat gloss ( $P = 0.002$ ) and coat scale ( $P = 0.005$ ) were respectively decreased and increased with the inclusion of shrimp hydrolysate, without differences on the general coat quality. Overall, results support the potential of shrimp hydrolysate in pet food, with benefits on diet digestibility and promoting the sustainability of the sector. Further research is needed to fully evaluate the bioactive properties of shrimp hydrolysate for an optimum nutrition.

### Keywords

Shrimp hydrolysate, Dog, Novel ingredient, Nutritive value, Palatability

### Acknowledgements

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### References

1. FEDIAF. Annual Report 2022. Bruxelles, Belgium; 2022.
2. AVMA. AVMA Pet Ownership and Demographics Sourcebook. American Veterinary Medical Association; 2022.
3. Cucurachi S, Scherer L, Guinée J, Tukker A. Life Cycle Assessment of Food Systems. *One Earth*. 2019;1(3):292-7.
4. Newton RW, Maiolo S, Malcorps W, Little DC. Life Cycle Inventories of marine ingredients. *Aquaculture*. 2023;565:739096.

## **Tolerance signatures of selection of *Ruditapes decussatus* against *Perkinsus olseni* infection**

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### Abstract

*Ruditapes decussatus* is a commercially important bivalve species that suffered a decline caused by massive mortalities associated with synergetic factors (*Perkinsus olseni* infection, environment, etc.)<sup>1</sup>. Control and prevention of *P. olseni* infections in *R. decussatus* has relied on a combination of management strategies, including monitoring and surveillance of infected populations, proper handling and disposal of infected individuals, and minimizing stressors that may weaken the immune system of clams<sup>2</sup>. Genetic basis of resilience to parasite infection can aid to develop marker assisted selection (MAS) programs to obtain resilient strains to parasite infection. The aim of this study was to uncover the genetic structure of *R. decussatus* across the NE Atlantic (NE-A) and Mediterranean (Med) areas and to identify genomic regions related to infection susceptibility and tolerance to *P. olseni*.

Six parasite-affected beds (~30 clams / bed) distributed across the NE-A (Noia, Pontevedra, Algarve) and the Med (Sardinia, Venice, Turkey) areas were sampled, including one parasite-free bed. 2b-RADseq genotyping was performed taking the *R. decussatus* assembled draft genome as reference. The genotyping data was used to ascertain genetic diversity and population structure considering the geographical hierarchy. Outlier loci were identified across the species distribution range and considering the different degree of perkinsosis infection.

A total of 23,968 consistent SNPs were identified after filtering using the reference genome. Genetic diversity was significantly lower in the NE-A than in the Med beds (He: 0.137 vs 0.179). Genetic structure analyses differentiated two main clusters (NE-A and the Med beds), being Venice (Italy) a particular population in-between both main clusters, suggesting restocking with seed from both origins. Fifty-six outliers of divergent selection across different levels of infection were identified, suggesting their potential for marker assisted selection programs.

Two main regions were identified to be considered in restoration programs and a set of outlier markers could potentially help in the production of strains with improved resistance to perkinsosis.

### Keywords

*Ruditapes decussatus*; SNP genotyping; Population genomics; *Perkinsus olseni*; Disease tolerance

### Acknowledgements

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References

4. Choi, K. S., & Park, K. I., (2010). Review on the protozoan parasite *Perkinsus olseni* (Lester and Davis 1981) infection in asian waters. Coastal environmental and ecosystem issues of the East China Sea, 269-281.
5. Ramilo, A., Pintado, J., Villalba, A., & Abollo, E. (2016). *Perkinsus olseni* and *P. chesapeaki* detected in a survey of perkinsosis of various clam species in Galicia (NW Spain) using PCR–DGGE as a screening tool. Journal of Invertebrate Pathology, 133, 50-58. <https://doi.org/10.1016/j.jip.2015.11.012>

## **Boosting fish robustness: algae feeds as a promising strategy for enhancing intestinal health and performance**

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### Abstract

As the world population grows, aquaculture faces the challenge of meeting increasing fish demand. Intensified fish farming leads to disease outbreaks, resulting in high mortality rates and economic losses. With the rise of antimicrobial resistance, finding innovative approaches to improve fish robustness and welfare is crucial for aquaculture profitability. Exploring algae inclusion in aquafeeds offers a natural solution to enhance fish health, reduce antibiotic reliance, and meet the demand for healthier food.

In the initial phase of the thesis, we analyzed commercially available microalgae (*Nannochloropsis oceanica* and *Chlorella vulgaris*) and macroalgae (*Gracilaria gracilis* and *Ulva rigida*) species, alongside a blend of these algae. We aimed to determine their chemical and functional properties. Subsequently, we investigated the effects of these algae, individually or in combination, on the intestinal microbiota and structure of European seabass using 16S rRNA sequencing. To assess the algae's potential in enhancing fish robustness, we conducted a feeding trial utilizing algae-based feeds. Afterward, European seabass were exposed to a bacterial challenge with *Tenacibaculum maritimum*. We evaluated mortality rates and examined systemic and mucosal immune responses, analyzing the hematological profile, immune parameters in plasma, and gene expression patterns in the skin, intestine, and head kidney.

Both the individual microalgae, macroalgae, and their blend displayed antibacterial activities against several pathogenic bacteria, with particularly promising results observed against *T. maritimum*. The blend combination stood out as a well-balanced and nutrient-rich source of proteins, lipids, fatty acids, essential amino acids, and carbohydrates. Regarding the intestinal microbiota, individual algae inclusion decreased gut microbial diversity in European seabass. However, the inclusion of these algae in a blend mitigated these effects. Furthermore, algae inclusion exhibited a positive influence on the proliferation of bacteria capable of outcompeting fish pathogens, while concurrently decreasing the abundance of potentially harmful bacteria in the intestine. In terms of fish performance, including the algae blend in European seabass diets improved growth and elicited an appropriate mucosal immune response during *T. maritimum* infection. Notably, the blend stimulated a more pronounced immune response by facilitating the migration of monocytes and lymphocytes to mucosal tissues. This was accompanied by an upregulation of genes associated with cell migration, proliferation, and pro-inflammatory responses in both the skin and gut tissues.

In conclusion, the incorporation of a blend of micro- and macroalgae in aquafeeds holds promising potential for boosting fish robustness. It effectively modulates the intestinal bacterial communities in European seabass, resulting in improved host health, nutrition, and disease resistance. Including the algae blend in diets enhances fish growth, prevents pathogen adhesion and colonization on mucosal tissues, and reduces mortality rates when challenged with *T. maritimum*. These findings highlight the valuable benefits of including algae blends in fish diets, promoting overall fish performance and well-being.

Keywords: European seabass, algae, fish robustness, gut microbiota, bacterial disease

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## Effects of Pineapple By-products in Aquafeeds: Influence on the Stress Response of European Seabass

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### Abstract

Surplus by-products generated by the fruit industry are abundant in natural antioxidants, i.e. phenolic compounds. Inclusion of these by-products into aquafeeds may help mitigate oxidative stress in fish, while also meeting consumer demands for natural products. To assess the potential of pineapple by-products as natural sources of antioxidants for European seabass (*Dicentrarchus labrax*), a negative control diet (CTRL) based on a commercial formulation without antioxidants was utilized. This control diet was compared to a diet supplemented with 100 mg kg<sup>-1</sup> of Vitamin E (VITE). Additionally, two experimental diets were formulated by incorporating 2% of either pineapple peel flour or pineapple stem flour into the VITE mixture (P2 and S2, respectively). Diets were isolipidic, isoproteic and isoenergetic. An experimental trial was conducted using juvenile fish. Each diet was assigned to quadruplicate homogeneous groups (13.5 ± 0.8 g), reared in 50L tanks of a recirculating aquaculture system. Fish were fed *ad libitum* until tripling their initial body weight. At the end of the trial, 12 fish per treatment were sampled, while another 12 were subjected to a stress challenge involving air exposure (1 min) followed by confinement stress (5 min; 100 kg m<sup>-3</sup>), replicating common aquaculture practices. After recovering for one hour, fish were anaesthetized, weighed and measured to study growth performance. Moreover, fish were evaluated for liver antioxidant enzyme activity, plasma stress biomarkers, oxidation of muscle and liver, as well as muscle radical scavenging capacity of DPPH•, ABTS•+, and ORAC. Results demonstrated that the experimental diets were well accepted, as no differences were observed in terms of fish growth. Additionally, induction of stress was achieved, as lactate, glucose and cortisol were higher in the stressed group. However, none of the experimental diets showed benefits in terms of acute stress response. The tested diets modulated antioxidant enzyme response, as fish fed with VITE showed a lesser need for antioxidant enzyme production in order to maintain liver lipid oxidation to lower levels in both groups. Moreover, no significant differences were found in muscle compared to VITE. Although this reinforces the importance of incorporating vitamin E in aquafeeds, the impact of the diets with pineapple stem and peel inclusion was insufficient as to induce notable benefits in fish antioxidant defences. Further research is necessary to maximize antioxidant capacity, as well as experimenting with different inclusions percentages and diet manufacturing conditions, in order to assess the effects of these natural antioxidants on fish stress resistance.

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

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# **POSTERS**

## **Immunostimulatory capacity of grapeseed extracts as feed additives in European seabass (*Dicentrarchus labrax*) juveniles.**

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### Abstract

In aquaculture, feed engineering is one of the main ways to prevent disease outbreaks and improve health status, as well as optimising growth capacity (1). However, few commercial products with proven capacity to improve immunity are available. The aim of this work is to evaluate the capacity of a grapeseed extract supplemented diet to modulate European seabass (*Dicentrarchus labrax*) immune system.

A two-weeks feeding-trial was performed with European seabass juveniles (22.7±1.8 g). Fish were sorted and three diets were distributed into triplicates tanks (Control–commercial diet; positive control diet - supplemented with an immunostimulant; and a test diet supplemented with a grapeseed extract). Following 2-weeks of feeding, the remaining animals (90 per treatment) were i.p. inoculated with *Photobacterium damsela* piscicida strain MM1415 in a concentration of 2.62×10<sup>5</sup> CFU mL<sup>-1</sup> and randomly redistributed into triplicates for each diet. Mortalities were tracked for 14-days, to evaluate disease resistance. Fish were sampled (3 per tank) before and 6 hours after for the evaluation of the inflammatory response. At both sampling points fish were sampled for blood, plasma, mucus and tissue collection (liver, intestine and head-kidney) for immune parameters' assessment.

Preliminary data did not show significant changes regarding the hematological, oxidative stress and plasma humoral parameters of seabass fed dietary treatments for two weeks either before or following infection. While the positive control diet was not able to enhance disease resistance compared to the control, the diet supplemented with grape extract induced higher mortalities (72.2%) compared with control (54.4%). Nonetheless, more parameters are being analyzed to better understand the effects of dietary treatments.

Preliminary data suggest that dietary supplementation with a grapeseed extract does not modulate seabass immune status and seems to deteriorate seabass responses against *Photobacterium damsela* piscicida, at least under the conditions tested in the present study. More data is being gathered to confirm this hypothesis. In such a case, other supplementation levels, as well as, testing other feed additives will be considered in future studies.

**Keywords:** Functional Feeds, Immunomodulation, Survivability, *Dicentrarchus labrax*, Immunonutrition

### Acknowledgements

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### References

1 - Kiron V. Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology* (2012) 173:111–133

## **Innovative hydrolysates as functional nutritional supplements to promote seabass robustness and flesh quality addressing the European zero-waste strategy.**

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### Abstract

The challenge of feeding farmed fish with economically and environmentally sustainable products is a main concern of the aquaculture sector. Vegetable protein sources have been considered an alternative to fishmeal, however, plant ingredients often impair fish robustness and flesh quality. Every year, considerable amounts of animal by-products discarded by the agro-food sector could be valorised through hydrolysis, becoming promissory sources of bioactive peptides with antioxidant, antimicrobial and growth-promoting properties for aquafeeds. This thesis hypothesizes that the supplementation of vegetable-based diets with such hydrolysates could boost fish robustness and ultimately promote flesh quality through the modulation of physiological and metabolic mechanisms still unravelled. Cutting-edge approaches involving the combination of classic tools and omics will be used to evaluate fish growth, resistance to challenging environmental conditions and muscle quality, providing evidence for the potential of a novel renewable source of bioactive compounds for functional fish feeds.

Keywords: Innovative hydrolysates; Functional feed; Fish robustness; Flesh Quality; Circular Economy

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## **Innate immune response and disease resistance of European seabass (*Dicentrarchus labrax*) juveniles after *Tetraselmis* sp. extracts dietary supplementation**

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### Abstract :

Aquaculture high stocking densities and fish handling are stressful conditions that lead to unbalanced fish immune systems endangering fish farms worldwide. The occurrence of diseases in farmed fish is particularly relevant during early life stages and current prophylactic measures, such as the use of functional ingredients, aim to anticipate potential hazards and boost animal immunity.

Since macro- and microalgae are recognized to have anti-inflammatory, immunestimulant and antibacterial properties, recent approaches are testing several marine algae refined extracts (alone or in combination) as immune enhancers. Thus, the objective of this study was to evaluate the health-promoting effects of macro- and microalgae-derived compounds in diets for European seabass (*Dicentrarchus labrax*) juveniles.

The experimental trial was performed at CIIMAR facilities and was divided on a short-term feeding trial (14 days) followed by a bacterial challenge. After quarantine, 405 European seabass ( $22.7 \pm 1.8$  g) were randomly distributed in nine, 250L tanks (n=45 fish/tank) to which three experimental diets were assigned in triplicates. Animals were either fed a commercial diet (CTRL) or the CTRL diet supplemented with two different levels of a blend containing *Tetraselmis* sp. aqueous extract, sulphated polysaccharides and phycocyanin (DLow and DHigh). At the end of the feeding trial, a bacterial challenge was performed by intraperitoneal injection of 100 µl of a bacterial inoculum ( $2.62 \times 10^5$  CFU/mL of *Photobacterium damsela piscicida* strain MM415). Samplings were performed immediately before (immune status) and 6 hours after bacterial challenge (immune response). Fish were euthanized and samples were taken of mucus (humoral parameters), peritoneal exudates (leukocyte counts), blood (haematology and humoral parameters), liver (oxidative stress), intestine and spleen (molecular markers) (n=9 per experimental condition). The remaining fish were used for disease resistance assessment.

After evaluating the heamatological profile, plasma humoral parameters and disease resistance, no significant differences were found among different dietary treatments. Regarding total peritoneal leukocytes, it was possible to observe a significant increase in cell numbers after bacterial challenge in fish fed DHigh when compared with fish fed DLow.

Even though diets supplemented with microalgae-derived compounds did not enhance fish survival against the selected pathogen, there was a clear improvement of leukocyte recruitment to the inflammatory focus when the highest supplementation level was used. Samples of liver, intestine and head-kidney are currently being processed for analysis of antioxidant activity and immune-related gene expression.

Keywords: Immune modulation, disease resistance, blue biotechnology, marine valorization, European seabass.

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## **Microplastics and metals in European seabass from different aquaculture systems: dietary exposure and toxicological risks for humans**

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### Abstract

With the bioavailability of microplastics (MPs, plastic particles < 5 mm) in aquatic environments, the MP contamination on aquaculture species has become a growing concern in food safety worldwide [1]. Indeed, the proximity between aquaculture production systems and reservoir environments, as well as their use of plastic gear are likely to affect MP availability for uptake by fish [2]. MP occurrence has been reported in many wild-caught fish species and their farmed counterparts [3]. Therefore, our goal was to evaluate MP occurrence in European seabass (*Dicentrarchus labrax*) produced in three different aquaculture systems by analysing water, fish feed and seabass tissue samples, as well as evaluate trace and non-essential metal levels and assess human exposure and toxicological risk to these contaminants.

Approximately 50 European seabass specimens were obtained from 1) a cage farm, 2) a pond farm and 3) from a recirculating aquaculture system (RAS). MPs were quantified in the gastrointestinal tract (GIT) and muscle, and visually characterized according to their shape, colour, and size. The particles' composition was identified by Fourier Transform Infrared Spectroscopy (FTIR). Trace (Cr, Ni, Cu, Zn) and non-essential (Cd, Hg, Pb) metals were determined in seabass muscle through Atomic Absorption Spectrophotometry (AAS). Human exposure and toxicological risk to contaminants were estimated based on European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) data and using European Food and Safety Authority (EFSA) guidelines for metals.

In RAS water and feed, higher MP levels were detected than in the other systems. Fibres with blue and black colour, fibre-shaped, and composed of cellulose/rayon and polyester, were the most common MPs in the three systems. MP characteristics were generally similar among fish tissues. Cage-farmed seabass had the lowest MP occurrence, with 89% of the fish having at least one MP. RAS-farmed fish had the highest MP levels in muscle, while in GIT, the values were also higher in these fish but comparable to those observed in pond-farmed seabass. Cr, Ni, Zn, Cd, and Hg concentrations detected in muscle were below the maximum permissible concentrations established for this species. Cu and Pb were below detection limits. No significant differences were observed among systems, except for Zn with pond-farmed seabass displaying the highest values ( $5.5 \pm 0.7$  vs.  $3.6\text{-}4.6$   $\mu\text{g/g}$  wet weight). It was estimated a low toxicological risk related to farmed seabass fillet consumption. Moreover, monthly human exposure to MPs ranging from 1.8-9.3 per kg of consumer's body weight was estimated, depending on seabass consumption habits.

### Keywords

Microplastics, European seabass, Recirculation aquaculture system (RAS),  $\mu\text{FTIR}$ , 'One Health' concept

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References

- [1] Garrido Gamarro, E., & Constanzo, V. (2022) Microplastics in food commodities - A food safety review on human exposure through dietary sources. Food Safety and Quality Series FAO, Rome, Italy.
- [2] Chen, G., et al. 2021. Occurrence and ecological impact of microplastics in aquaculture ecosystems. Chemosphere 274, 129989.
- [3] Sequeira, I.F., et al. 2020. Worldwide contamination of fish with microplastics: A brief global overview. Marine Pollution Bulletin 160, 111681.

## Effects of dietary methionine supplementation on European seabass mucosal immune response and disease resistance

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### Abstract

Methionine is among the amino acids with recognized positive effects on immunity. Similar to the observed in other animals, dietary methionine supplementation has been shown to enhance the immune response and disease resistance in fish when faced with an inflammatory insult[1-3]. However, its role in mucosal immune machinery and response to pathogens requires further investigation. Therefore, this study aimed to investigate the effects of dietary methionine supplementation on mucosal immunity and disease resistance against *Tenacibaculum maritimum* in European seabass (*Dicentrarchus labrax*).

Triplicated tanks of juvenile European seabass were fed experimental diets containing increasing levels of methionine supplementation: 0, 1 or 2% DL-methionine (CTRL, MET1 and MET2, respectively), resulting in methionine concentrations of 8.6, 18.5 and 29.2 mg/g dry matter, respectively. After four weeks of feeding, samples of blood, skin mucus, gut and head kidney were collected and fish were bath-challenged with *T. maritimum*. The same tissues were collected 48 hours post-infection, coinciding with the peak of mortality. The haematological profile was evaluated and circulating leucocytes were counted and classified. Skin mucus samples were used to assess innate immune parameters, oxidative stress analysis was conducted on the gut and gene expression analysis on the head kidney. The obtained results were subjected to canonical discriminant analysis (DA).

A clear inflammatory response was observed in all experimental treatments following infection. Moreover, while before infection fish fed different dietary treatments clustered together, inflamed fish fed experimental diets were significantly separated from one another. At 48h post-infection, the MET1 was the furthest group, whereas the CTRL and MET2 groups were relatively close to each other. The discrimination of the MET1 group was driven by the positive load of monocyte counts, IgM and total peroxidase in the skin mucus, as well as interleukin 8 (*il8*) and interleukin-1 beta (*il1β*) expression in the head-kidney. On the other hand, the variables that contributed the most to the separation of the MET2 and CTRL groups were haemoglobin and mean corpuscular haemoglobin concentration (MCHC).

In conclusion, dietary methionine supplementation significantly influenced the immune response of the European seabass against *T. maritimum* infection. The great separation of MET1 from the other dietary groups suggests that there may exist an optimal or threshold level of methionine intake beyond which the immune response may experience diminishing returns[4]. Nevertheless, the MET1 diet seemed to positively impact the skin mucus immune-related mechanisms studied.

### Keywords

Functional feeds; amino acids; mucosal immunity

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#### References

1. Machado, M., R. Azeredo, P. Díaz-Rosales, A. Afonso, H. Peres, A. Oliva-Teles, & B. Costas. (2015). Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. *Fish & Shellfish Immunology*. 42 (2), 353-362. doi: 10.1016/j.fsi.2014.11.024.
2. Machado, M., R. Azeredo, F. Fontinha, S. Fernández-Boo, L.E.C. Conceição, J. Dias, & B. Costas. (2018). Dietary methionine improves the European seabass (*Dicentrarchus labrax*) immune status, inflammatory response, and disease resistance. *Frontiers in Immunology*. 9. doi: 10.3389/fimmu.2018.02672.
3. Machado, M., J. Moura, D. Peixoto, M. Castro-Cunha, L.E.C. Conceição, J. Dias, & B. Costas. (2021). Dietary methionine as a strategy to improve innate immunity in rainbow trout (*Oncorhynchus mykiss*) juveniles. *General and Comparative Endocrinology*. 302. doi: 10.1016/j.ygcen.2020.113690.
4. Shorter, K.R., M.R. Felder, & P.B. Vrana. (2015). Consequences of dietary methyl donor supplements: Is more always better? *Progress in Biophysics and Molecular Biology*. 118 (1), 14-20. doi: <https://doi.org/10.1016/j.pbiomolbio.2015.03.007>.