



VIII WORKSHOP

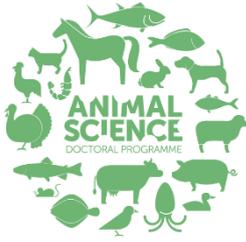
17th SEPTEMBER 2021

Salão Nobre, ICBAS-UP

Rua de Jorge Viterbo Ferreira No 228

Transmitted online via ZOOM for attendees

Porto, Portugal



The VIII WORKSHOP of the Animal Science Doctoral Programme

It was with great enthusiasm that our students organised the VIII WORKSHOP of the Animal Science Doctoral Programme to disseminate their newest data among the academia, industry and general society. This year, besides the exciting high quality programme, we have two plenary lectures, one dedicated to new protein sources for food and feed, and another that will help us defining a healthy diet. We will also have a round table focused on “One Health” where invited experts representing all food chain, including producers, distributors, researchers and academics will share their vision. Finally, the best oral presentation, best pitch and best poster will be awarded.

During the last year, we all lived challenging scenarios requiring from us skills that we have never imagined of. Doctorates will need for the post-Covid working world soft skills that we had already started offering during our training courses this past year (personal development and self-awareness, time management, management of anxiety and stress), and that will be maintained (e.g. communication module) during 2021/2022. Besides, all animal science doctoral student started having access to training courses in technical skills (entrepreneurship) that will continue. The university of Porto is also member of the European University Alliance for Global Health (EUGLOH) where regular activities are available to all students of this alliance. Finally, as I am very honoured to launch a new series of seminars this coming year “From Science to business: Success Strategies for Winning at Life”. We will start with former students of our University, with an Animal science background, that will join us in an informal get together to share their own experience and vision. This will be a great opportunity for students to come face to face with bright minds and start considering new future scenarios and career prospects! I dare you to join our invitees, and not miss this amazing opportunity to share a moment with people that once had a vision and make it happened!

For more information about the Animal Science Doctoral Programme follow our webpage and social media at: <https://animalscience-phd.pt/>

Luisa M.P. Valente

Director of the Animal Science Doctoral Programme, ICBAS-UP, 17th September, 2021

ANIMAL SCIENCE DOCTORAL PROGRAMME VIII WORKSHOP

17th September 2021

Organization

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

Luis Mira Vieira, *ICBAS-UP*

Ana Basto, *CIIMAR, Faculdade de Biologia - UVigo & ICBAS-UP*

Cátia Mota, *ICBAS-UP, REQUIMTE-LAQV, ALGAPLUS & ALLMICROALGAE*

Diogo Peixoto, *CIIMAR, Faculdade de Biologia - UVigo & ICBAS-UP*

Inês Ferreira, *CIIMAR & ICBAS-UP*

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

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

Conference Chairpersons

Alexandra Correia, *ICBAS-UP & i3S*

Cristina Velasco, *CIIMAR*

Inês Valente, *REQUIMTE – LAQV, ICBAS-UP & FCUP*

João Albuquerque, *i3S*

Rita Azeredo, *CIIMAR*

Sónia Gomes, *ICBAS-UP*

Conference Round-table Guest Participants

Lourenço Ramos Pinto, *CIIMAR*

Luís Pinho, *ICBAS-UP & SVAExpLeite, Lda.*

Maria Leonor Nunes, *CIIMAR*

Mayumi Thais Delgado, *SONAE MC*

Pedro Moreira, *FCNAUP*

Steering Committee

Maria Teresa Dinis, *CCMAR-UALG*

Leonor Nunes, *CIIMAR*

Luis Mira Vieira, *ICBAS-UP*

INVITED SPEAKERS



Professor Doctor Daniel Murta

Professor Doctor Daniel de Moura Murta completed his PhD in 2014 at the Faculty of Veterinary Medicine of the Technical University of Lisbon. In the same year, he joined as Assistant Professor at the Faculty of Veterinary Medicine at the Lusófona University of Humanities and Technologies (2014-2020), where he lectured Molecular and Cellular Biology and Physiology. He has published several technical/scientific works as a researcher at CIISA-FMV-ULisboa, being also responsible for coordinating MSc students. He has been a member of the Board of the Portuguese Society of Veterinary Sciences since 2013, having served in several position, including Editor of the Portuguese Journal of Veterinary Sciences. In 2014 he founded EntoGreen, a company dedicated to the production of Black Soldier Fly (*Hermetia illucens*) for animal feed. He is also the founder of Portugal Insect, the recently created association of insect production and processing for animal and human consumption. Currently, he is also the CEO of Ingredient Odyssey SA and Scientific Officer and Professor of Veterinary Medicine at Egas Moniz.



Professor Doctor Pedro Moreira

Pedro Alexandre Afonso de Sousa Moreira received his PhD in 2001, in Human Nutrition, with his work focusing on eating behavior and weight control, with a preference for the study of dietary intake and obesity-asthma, before and after birth, and the control of hydric and sodium-potassium balance (in collaboration with WHO) in the life cycle. He was Director of FCNAUP (2014-2018), President of the General Assembly of the Portuguese Association of Nutritionists (2010-2016), promoted the creation of the "Ordem of Nutritionists" and was one of the founding members of the Portuguese Society of Nutrition and Food Sciences. Currently, he is the Director of the Master's Course in Pediatric Nutrition, and President of the Pedagogical Council, as well as Full Professor of Human Nutrition at Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, Integrated Researcher at Instituto de Saúde Pública da Universidade do Porto (ISPUP), and collaborator at Centro de Atividade Física e Saúde e Lazer (CIAFEL). He is also a member of the Editorial Board of Nutrients.

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, *ICBAS-UP*
Joana Fernandes, *ICBAS-UP*
Ricardo Pereira, *CBQF-UCP, CIIMAR & 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: *Cristina Velasco (CIIMAR) & Inês Valente (REQUIMTE-LAQV / ICBAS-UP / FCUP)*

09:30 • Plenary lecture: Insects as new protein sources for food and feed, *Daniel Murta (ENTOGREEN, INGREDIENT ODYSSEY S.A.)*

10:15 • Oral presentation: Impact of defatted *Tenebrio molitor* on nutritional, organoleptic and sensory quality of market-sized European sea bass (*Dicentrarchus labrax*), *Ana Basto (ICBAS-UP / CIIMAR / UVIGO)*

10:30 • Oral presentation: Nutritive value and palatability of microalgae species in dog feeding, *Joana Fernandes (ICBAS-UP / REQUIMTE-LAQV / SOJA DE PORTUGAL)*

10:45 • Oral presentation: Algae blend in dog food: Effects on palatability, digestibility and faecal end-fermentation products, *Cátia Mota (ICBAS-UP / REQUIMTE-LAQV / ALGAPLUS / ALLMICROALGAE)*

11:00 • COFFEE BREAK & POSTER SESSION

Chairpersons: *Alexandra Correia (ICBAS-UP / i3S) & Rita Azeredo (CIIMAR)*

11:45 • Oral presentation: European seabass response to air exposure: impact of dietary inclusion of bioactive blood hydrolysates, *Daniela Resende (ICBAS-UP / CIIMAR / CBQF-UCP / SENSE TEST)*

12:00 • Oral presentation: Innate immune-related gene expression profiles in European seabass (*Dicentrarchus labrax*) bath challenged with *Tenacibaculum maritimum*, *Inês Ferreira (ICBAS-UP / CIIMAR / IBMC / i3S)*

12:15 • Oral presentation: Microalgae-induced cytokine and reactive oxygen species production by bovine monocyte-derived macrophages, *Ana Pedro (ICBAS-UP / i3S / REQUIMTE-LAQV / CAVC / ADM Portugal)*

12:30 • Pitch presentation: Search for biomarkers of resistance in *Ruditapes decussatus* to *Perkinsus olseni* parasite infection by shotgun proteomics, *João Estêvão (ICBAS-UP / CIIMAR)*

12:40 • LUNCH

SESSION II

Chairpersons: : *Sónia Gomes (ICBAS-UP) & João Albuquerque (i3S)*

14:00 • Plenary lecture: Defining a healthy diet: it's not just about the nutrients, *Pedro Moreira (FCNAUP)*

14:45 • Oral presentation: Optimizing extrusion and drying temperatures for maximizing the scavenging potential of aquafeeds with natural antioxidant inclusion from mango and pineapple peels, *Ricardo Pereira (ICBAS-UP / CIIMAR / CBQF-UCP)*

15:00 • Oral presentation: Conventional versus pasture-based cow's milk: simulated human gastric lipolysis and lipid oxidation, *Susana Pinho (ICBAS-UP / REQUIMTE-LAQV / SOJA DE PORTUGAL)*

15:15 • Oral presentation: Novel applications: hair glucocorticoids in a mammalian range expansion, *Alexandre Azevedo (ICBAS-UP / LEIBNIZ-INSTITUTE FOR ZOOAND WILDLIFE RESEARCH)*

15:30 • Oral presentation: Inclusion of macro- and microalgae in European seabass (*Dicentrarchus labrax*) diets: effects on intestinal microbiota, *Mariana Ferreira (ICBAS-UP / CIIMAR)*

15:45 • Pitch presentation: Immune and intestinal benefits of krill meal and krill oil in Pacific white shrimp (*Litopenaeus vannamei*) diets, *Carla Teixeira (ICBAS-UP / CIIMAR / SPAROS)*

15:55 • ROUND TABLE “One Health”

Chairpersons: *Manuel Vilanova (ICBAS-UP / i3S)*

Participants:

Mayumi Thais Delgado (SONAE MC)

Luís Pinho (ICBAS / SVAExpLeite, Lda.)

Maria Leonor Nunes (CIIMAR)

Pedro Moreira (FCNAUP)

Lourenço Ramos Pinto (CIIMAR)

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

Jury:

Maria Teresa Dinis (CCMAR-UALG)

Maria Leonor Nunes (CIIMAR)

Luís Mira Vieira (ICBAS-UP)

17:00 • WORKSHOP CLOSING

PLENARY SESSIONS

Insects as new protein sources for food and feed

Daniel Murta

EntoGreen - Ingredient Odyssey, SA. Rua Cidade de Santarém, Santarém, Portugal
CiiEM - Centro de investigação interdisciplinar Egas Moniz, Campus Universitário, Quinta da Granja Monte de Caparica, 2829 - 511 Caparica, Portugal.

Abstract

Together, world population growth, expected to reach approximately 10 billion in 2050, and food habits changes in developing countries, with an increase consumption of animal protein, have been pressing the increase of feed production, resulting in feed ingredient prices rise and agricultural production of feed material to be more and more intensive, what contribute to both agricultural land loss and desertification. Besides that, the present COVID-19 pandemic has shown how Europe is hostage of the international feed market, and as far as nutrition is concerned, protein is a huge problem to be solved. However, on the other hand, society continues to waste food products, contributing to a very inefficient agriculture vale chain in which more than 25% of food products can be lost.

Based on a one hundred percent circular economy-based approach, vegetable by-products can be converted into high valuable nutrient sources for both animals and plants. Insects can be the key for the transformation of this otherwise lost nutrients into new nutritional solutions not only for both humans and animals, but also for plants.

In a very short period of time, insect can convert a very large range low value by-products into high value insect protein and oil for animal and human nutrition and insect frass, an organic fertilizer, for plants.

With this process, now completely industrialized and at a full-scale level, it is possible to reduce the Europe dependency from the international feed and food markets, contributing to a local and more sustainable food production. On the other hand, this novel plant nutritional source can contribute to a wide range of soil solutions, from drought resistance and plant nutrition to even pest control and sprouting promotion.

However, this novel sector still faces several challenges, from legal to consumer acceptance and to industrialization and growth. Although the legal framework is changing and adapting to this new reality consumers still have to prepare for it, and insect producers have a lot to learn from other livestock and industrial sectors. Besides that, the use of insects as a tool to other applications is still in its infancy, as insects can be used from bioremediation in garbage disposal systems, to the production of new plastic solutions.

Keywords

Insects, feed, food, insect frass, organic fertilizer

Defining a healthy diet: It's not just about the nutrients

Pedro Moreira

Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

Abstract

In the context of unprecedented challenges related to climate change, conflicts, hunger, chronic diseases and their metabolic comorbidities, together with the current COVID-19 pandemic, it is urgent to improve the nutritional status of populations while exploring new definitions for the ever-changing concept of healthy eating.

When considering the nutritional and health effects of diet, it is important to recognise that foods contain a large number of nutrients and bioactive substances in a complex matrix, and their related health effects may differ from the outcomes obtained when studying these nutritional components alone. Within this framework, it is important to recover traditional and healthy eating patterns, and bring creative innovations to respond to important challenges around environmental sustainability, quality, functionality and health impact of foods, knowing also that the same meal, consumed by the same person, at different times of the day, may have different metabolic effects, depending on individual traits, such as the diet-gene interactions, gut microbiota, or lifestyle characteristics, such as sleep and physical activity.

In this context, improving access to safe and healthy and sustainable diets is essential, ensuring adequate nutrition during the first 8,000 days of life, and other critical periods of the life cycle, to ensure the health of populations and the prospects of future generations

Keywords:

Healthy eating; nutrients; nutritional status

ORAL COMMUNICATIONS

Impact of defatted *Tenebrio molitor* on nutritional, organoleptic and sensory quality of market-sized European sea bass (*Dicentrarchus labrax*)

A. Basto^{1,2*}, A. Marques¹, A. Silva^{1,2}, T. Sá^{1,2}, M.B.P.P. Oliveira³, V. Sousa¹, E. Matos⁴, L.M.P. Valente^{1,2}

¹ CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

² ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

³ REQUIMTE, Chemical Science Department of Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

⁴ SORGAL, Sociedade de Óleos e Rações, S.A., Estrada Nacional, 109, Lugar da Pardala, 3880-728 S. João de Ovar, Portugal

*Presenter author: anafbasto@gmail.com

Tenebrio molitor (TM) is one of the recently authorized species for use in aquafeeds. When defatted (*d*-) is particularly rich in highly digestible protein (up to 93% on a dry matter basis) and have a well-balanced amino acid profile able to meet European sea bass (*Dicentrarchus labrax*) requirements (Basto et al., 2020). In this study, we have evaluated the impact of replacing fish meal (FM) with *d*TM in European sea bass growth performance, nutrient utilization, skin and muscle quality traits. A FM-based diet with 47% of protein and 20% of fat was formulated and used as control (CTRL). Two other isoproteic and isolipidic diets were formulated to replace 50 and 100% of FM by *d*TM (TM50 and TM100, respectively). Each diet was assigned to quadruplicate homogeneous groups of 15 fish (69 ± 5 g) fed until apparent satiation for 16 weeks. Fish fed TM50 had a significantly lower voluntary feed intake (VFI) and feed conversion ratio (FCR) compared to the CTRL, resulting in similar final body weight and condition factor among treatments. The whole body lipids increased with dietary inclusion of *d*TM, mainly due to oleic acid (OA; 18:1n-9) gain and retention. Muscle total lipids levels remained similar among dietary treatments. The sum of saturated FA (SFA) increased in muscle of fish fed *d*TM, whereas monounsaturated FA (MUFA) didn't change. Despite the relative percentage (% total FA) of muscle EPA and DHA decreased in fish fed *d*TM, when expressed in wet weight muscle EPA + DHA final contents were similar among all fish (0.30 g 100 g⁻¹). Muscle cohesiveness and resilience were higher in fish fed TM50; this fish also had the largest-sized and lowest number of muscle fibers. Fish fed TM100 had a significantly lower number of smaller-sized muscle fibers (<25 µm) than those fed the CTRL. The hue angle (H°) was significantly lower in skin of fish fed TM100 compared to CTRL, but in muscle remained similar among all dietary treatments. The panelist could not detect any significant differences in fish global acceptance that remained high for all fish, but *d*TM samples were strongly associated with a “pleasant taste” and “juicy texture”. Altogether, these results evidence a great potential of *d*TM to fully replace FM in diets for European sea bass. However, the long term impact of feeding *d*TM diets still needs to be addressed.

Keywords

Docosahexaenoic acid, eicosapentaenoic acid, flesh colour, flesh texture, sensory profile.

Acknowledgements

Work supported by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF) and project ANIMAL4AQUA, funded by Portugal2020, financed by ERDF through the Operational Competitiveness Program (COMPETE) - POCI-01-0247-FEDER – 017610. AB was financially supported by FCT (SFRH/BD/138593/2018).

Nutritive value and palatability of microalgae species in dog feeding

J.G. Fernandes¹, M. Spínola¹, T. Aires², J.L.Silva³, A.J.M. Fonseca¹, S.A.C. Lima¹, M.R.G. Maia¹, A.R.J. Cabrita¹

¹LAQV, REQUIMTE, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal;

²SORGAL, Sociedade de Óleos e Rações S.A., Lugar da Pardala, 3880-728 S. João Ovar, Portugal;

³Allmicroalgae Natural Products S.A., Industrial Microalgae Production, Apartado 9, 2449-909 Pataias, Portugal;

⁴LAQV, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal;

*jmgmfernandes@gmail.com

Abstract

World population of companion animals is increasing along with the continuous growth of the human population. This increase is raising awareness about the quality and sustainability of pet food. Microalgae are highly efficient in converting inorganic or organic carbon sources into biomass rich in protein, lipids, and bioactive compounds. Studies that evaluated the use of microalgae in pet food are almost non-existent. Therefore, the present study evaluated the nutritive value and palatability of three microalgae species (*Nannochloropsis oceanica*, *Chlorella vulgaris*, and *Tetrademus obliquus*) offered to adult Beagles at different levels (0.5, 1.0 and 1.5% in substitution of the basal diet). Proximate composition, amino acids, fatty acids and minerals profiles, and antioxidant activity confirmed microalgae as valuable sources of macro and micronutrients with functional properties. Crude protein (CP) of microalgae varied from 32.2% (*N. oceanica*) to 57.4% (*C. vulgaris*). Arginine, lysine, and leucine were the essential amino acids present at higher concentrations, while cystine and histidine existed in lower concentrations. Glutamic acid was the non-essential amino acid occurring in the highest concentration. Regarding fatty acids profile, *T. obliquus* and *C. vulgaris* had higher content of polyunsaturated fatty acids (PUFA), particularly alpha-linolenic acid (ALA), and also linoleic acid in *C. vulgaris*, whilst eicosapentaenoic acid (EPA) was present at higher amounts in *N. oceanica*. Palmitoleic acid is the monounsaturated fatty acid in the highest concentration in *N. oceanica*. All microalgae had higher amounts of n-3 PUFA than n-6 PUFA, with *T. obliquus* presenting the lowest n-6/n-3 ratio. Concerning the mineral content, *N. oceanica* showed the highest concentration of essential macro minerals, particularly Na, while *T. obliquus* showed higher amounts of essential trace elements, particularly Fe. Moreover, extracts of microalgae showed high and similar 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS^{•+}) scavenging activity, whereas *C. vulgaris* had the highest 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) scavenging activity. *T. obliquus* presented the highest ferric reducing antioxidant power (FRAP). The inclusion of *N. oceanica* and *T. obliquus* up to 1.5% did not affect diet digestibility and metabolizable energy (ME) content whereas inclusion of *C. vulgaris* linearly increased diet organic matter and energy digestibility and ME content. Inclusion of 1.5% microalgae did not affect first approach and choice, but decreased intake ratio of diets with *N. oceanica* and *C. vulgaris*, no differences being observed for *T. obliquus*. Overall, results suggest that microalgae have high nutritive value for dogs, but strategies to increase palatability should be envisaged.

Keywords

Microalgae, Pet food, Sustainability, Nutritive value, Palatability

Acknowledgements

Financial support from Fundação para a Ciência e Tecnologia (FCT) through project UIDB/50006/2020|UIDP/50006/2020 and from Portugal 2020 program through project AlgaValor (grant agreement n° POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234) and individual funding from FCT and Soja de Portugal to JGF (PD/BDE/150527/2019)

and of FCT to SCL (CEECIND/01620/2017) and MRGM (DL 57/2016–Norma transitória) are greatly acknowledge.

References

1. FEDIAF. European Facts & Figures 2019 2019 [Available from: https://fediaf.org/images/FEDIAF_facts_and_figs_2019_cor-35-48.pdf.
2. Becker EW. Micro-algae as a source of protein. *Biotechnology Advances*. 2007;25(2):207-10.

Algae blend in dog food: Effects on palatability, digestibility and faecal end-fermentation products

Cátia S.C. Mota¹, Maria Spinola¹, Ana R.J. Cabrita¹, Helena Abreu², Joana L. Silva³, António J.M. Fonseca¹, Margarida R.G. Maia¹

¹REQUIMTE, LAQV, ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal;

²ALGAplus, PCI, Via do Conhecimento, 3830-352 Ílhavo, Portugal;

³ALLMICROALGAE - Natural Products, SA, R. 25 de Abril, 2445-413 Pataias;

*catiam04@gmail.com

Abstract

The increased concern on pet food sustainability and tutors desire to improve their animals' health has leveraged the interest on functional pet foods, in particular for dogs. In this context, algae (macro and microalgae) emerged as alternative ingredients for dog foods, due to their low carbon footprint and high nutritional and functional value, with reported antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, prebiotic, anticancer and antidiabetic properties [1-3]. Based on this reported effects, macroalgae (e.g., *Ascophyllum* sp.) or microalgae (e.g., *Chlorella* sp. and *Arthrospira* sp.) species are currently used in commercial complete dog foods. However, systematic studies on the effects of macroalgae or microalgae on dog's nutrition and health are scarce and none has yet evaluated the effects of combined macro and microalgae species in dog foods. Thus, this study aimed to evaluate, for the first time, the chemical composition, digestibility and faecal end-fermentation products of a commercial algae blend composed of two macroalgae (*Fucus* sp. and *Ulva* sp.) and one microalgae (*Chlorella* sp.) species (Algaessence food™; ALGAplus/Allmicroalgae, Portugal) offered at 0.5, 1.0 and 1.5% dry matter basis (in replacement of the commercial complete diet) to six adult Beagle dogs, for 10 days (5 days adaptation and 5 days of total faecal collection). Algae blend palatability was assessed using a two-plate test, comparing 0 and 1.5% inclusion, on two consecutive days. The proximate composition and amino acids, fatty acids and minerals content and profile suggest the high nutritional and functional value of the algae blend. Palatability was negatively affected by 1.5% algae blend inclusion as suggested by the lower consumption ratio ($P < 0.001$), albeit first choice and first approach were not affected ($P \geq 0.803$). Dry matter and organic matter digestibility of diets were improved by 1.0 and 1.5% algae blend inclusion ($P = 0.004$) while protein digestibility was the highest at 1.0% supplementation ($P = 0.023$). Fat and fibre digestibility were not affected ($P > 0.05$). Faecal total volatile fatty acids and acetate were promoted by 1.5% ($P \leq 0.008$) whereas propionate was promoted at 1.0 and 1.5% algae supplementation ($P = 0.002$). Faecal acetate to propionate ratio and pH were the highest at 0.5 and the lowest at 1.0% algae blend ($P \leq 0.027$). Ammonia was not affected by algae blend supplementation ($P > 0.05$). Overall, results suggest the algae blend to be an interesting ingredient for dog foods but strategies should be considered to overcome its low palatability.

Keywords

Algae blend, digestibility, dog, faecal fermentation products, palatability.

Acknowledgements:

The work was supported through the project UIDB/50006/2020 | UIDP/50006/2020 and AlgaValor project (POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234), funded by FCT/MCTES through national funds. 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).

References

- [1] M. de Jesus Raposo, A. de Morais, R. de Morais, Emergent sources of prebiotics: Seaweeds and microalgae, *Mar. Drugs* 14(2) (2016) 27.
- [2] R.R. Sonani, R.P. Rastogi, D. Madamwar, Chapter 5 - Natural Antioxidants From Algae: A Therapeutic Perspective, in: R.P. Rastogi, D. Madamwar, A. Pandey (Eds.), *Algal Green Chemistry*, Elsevier, Amsterdam, 2017, pp. 91-120.
- [3] G. Riccio, C. Lauritano, Microalgae with Immunomodulatory Activities, *Mar. Drugs* 18(1) (2020) 2.

European seabass response to air exposure: impact of dietary inclusion of bioactive blood hydrolysates

Daniela Resende^{1,2,3,6}, Ricardo Pereira^{1,2,3}, Cristina Velasco⁴, David Domínguez¹, Miguel Pereira³, Carlos Pereira⁴, Bianca Marques⁵, Cristina Rocha⁵, Rui Costa Lima⁶, Manuela Pintado³, Luísa M.P. Valente^{1,2}

¹CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal

²ICBAS, UP, Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal

³CBQF, Laboratório Associado, ESB-UCP, Rua Diogo de Botelho, 1327, 4169-005 Porto, Portugal

⁴Politécnico de Coimbra/ESAC, Bencanta, 3045-601 Coimbra, Portugal

⁵CEB, UM, Campus de Gualtar, 4710-057 Braga, Portugal

⁶Sense Test, Lda, Rua Zeferino Costa, 341, 4400-345 Vila Nova de Gaia, Portugal

*danielaresende@outlook.com

Abstract

In aquaculture farms, fish are subjected to stress situations, which induce oxidative stress through an imbalance between the generation of reactive oxygen species (ROS) and the scavenging activity of antioxidants. Diet supplementation with functional ingredients to modulate oxidative stress after air exposure was investigated, using bioactive peptides from swine blood hydrolysates (BH), within a circular economy context.

Three swine BH were obtained by autohydrolysis (AH) or enzymatically. The enzymatically produced BH were further submitted to a micro- (MF) or nanofiltration (NF). Five isolipidic and isoproteic diets for European seabass were developed: a fishmeal (FM) based diet (positive control, PC), a commercial-based diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 European seabass juveniles (initial weight 12.3 ± 1.4 g), and fed to apparent satiation in a recirculating saltwater system. After 12 weeks, 9 fish per treatment were either immediately sampled or air-exposed for 1 minute and let to recover for 6 hours prior to sampling, in which plasma and liver were collected.

Plasmatic cortisol and lactate were elevated for all diets after the air exposure, without differences among diets. Glucose levels were unaffected by diet or stress. Regarding liver oxidative stress markers, lipid peroxidation (LPO) tended to increase after air exposure. Considering the non-stressed fish, MF diet led to the lowest LPO, being significantly lower than the AH, and similar to the remaining diets. Protein oxidation decreased in stressed fish, but did not differ among diets. Liver catalase was significantly lower in NC, NF and AH groups compared to PC and increased after stress. Superoxide dismutase activity was lower for AH and PC diets than for the MF, and it was reduced in stressed fish.

The stress response triggered by air exposure involved increased cortisol levels, followed by an increment in plasma lactate. However, none of the BH improved plasma stress response. The stress challenge increased liver LPO through ROS accumulation under oxidative stress. Carbonyls decreased post-stress, likely due to a feedback interaction with the LPO radicals, which reduced protein oxidation. The NF, composed of smaller peptides than the other BH, may modulate European seabass antioxidant defences by lowering catalase levels without increasing LPO, suggesting it could provide exogenous antioxidants to counteract ROS-induced oxidative stress.

Keywords:

Blood hydrolysates; Bioactive peptides; European seabass; oxidative stress; lipid peroxidation.

Acknowledgements:

Work supported by Project MOBFOOD, POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT,

SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019). RP thanks FCT for his PhD grant (SFRH/BD/144631/2019).

Innate immune-related gene expression profiles in European seabass (*Dicentrarchus labrax*) bath challenged with *Tenacibaculum maritimum*

Ferreira I. A.^{1,2,3,4*}, Santos P.^{1,2,5}, Machado M.¹, Guardiola F. A.^{1,6}, do Vale A.^{3,4}, Costas B.^{1,2}

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto (Portugal).

²Abel Salazar Institute of Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal

³Fish Immunology and Vaccinology Group, IBMC-Instituto de Biologia Molecular e Celular, University of Porto, 4200-135 Porto, Portugal

⁴i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal

⁵MARE - Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, Peniche, Portugal

⁶Department of Cell Biology and Histology, Faculty of Biology, *Campus Regional de Excelencia Internacional "Campus Mare Nostrum"*, University of Murcia, 30100, Murcia, Spain

*Presenter author: ines.ferreira@ciimar.up.pt

Abstract

One of the most devastating bacterial diseases, associated with high mortality and economic losses, of wild and farmed marine fish is tenacibaculosis, caused by Gram-negative bacterium *Tenacibaculum maritimum*. The development of effective strategies to prevent and treat tenacibaculosis requires knowledge about the host-pathogen interactions occurring during infection. In this study, the expression of immune-related genes in European seabass (*Dicentrarchus labrax*) in response to infection with *Tenacibaculum maritimum* was evaluated. For this purpose, a time-course trial was performed, in which groups of seabass (31.9 ± 6.9 g) were bath-challenged for 2 h in aerated seawater with 5×10^5 CFU mL⁻¹ *T. maritimum* (challenged fish) or mock-challenged with marine broth medium (mock-challenged fish). Non-challenged fish randomly selected from the groups just before infection were used as controls (time 0). Following 4, 8, 24 and 48 h post-challenge, 8 fish from each group were randomly selected, euthanized and head-kidney (HK), distal gut and skin collected for total RNA extraction and subsequent cDNA synthesis. Gene expression was analysed by RT-qPCR and normalized with *40s* and *ef1b*. To determine the severity of the challenge, a lethality trial was performed in parallel, using the same bacterial inoculum/challenge protocol used for the time-course trial. Challenge with *T. maritimum* induced 40% mortality, whereas no mortality occurred after mock-challenge. An up-regulation of *il-1 β* gene expression was detected in the distal gut and HK at 8 h post-challenge, whereas *il-10* transcripts, were up-regulated at 8 h in the HK and at 24 h in skin and distal gut. The expression of *mmp9* gene also increased at 8 and 24 h post-challenge in distal gut and skin, respectively. Moreover, *cxc4* and *il-8* transcripts increased at 8 and 24 h in skin whilst in the HK the response was delayed for *il-8*, with an up-regulation at 48 h for the challenged fish. Preliminary data suggest that bath infection by *T. maritimum* is able to trigger a local immune response at mucosal tissues (i.e. gut and skin), but also a systemic response (HK). Further work will be required to disclosure the dynamics and timing established between the local and systemic immune responses.

Keywords

Tenacibaculosis, Gene expression, Immune response, Aquaculture, Mucosal Immunity

Acknowledgements

This work is partially supported by project BE4AQUAHEALTH (16-02-05-FMP-0013), funded by MAR2020 Operational Programme and the European Union through FEDER, and by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and

UIDP/04423/2020. I. Ferreira, A. do Vale and B. Costas benefited from grants by FCT (SFRH/BD/147750/2019, L57/2016/CP1355/CT0010 and IF/00197/2015, respectively).

Microalgae-induced cytokine and reactive oxygen species production by bovine monocyte-derived macrophages

A.R.V. Pedro^{1,2,3}, T. Lima,¹ C. Gonçalves⁴, A.J.M. Fonseca³, A.R.J. Cabrita³, I. Ramos⁵, E. Martins^{6,7,8}, M. Vilanova^{1,2}, M.R.G. Maia³, A. Correia^{1,2}

¹Grupo de Imunobiologia, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

²Laboratório de Imunologia, DIMFF, ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

³LAQV, REQUIMTE, ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal,

⁴Laboratório Ibérico Internacional de Nanotecnologia, Av. Mestre José Veiga, 4715-330 Braga, Portugal

⁵Cooperativa Agrícola de Vila do Conde, Vila do Conde, Portugal

⁶ADM Portugal, SA, Murte, Portugal

⁷EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal

⁸Escola Universitária Vasco da Gama, Coimbra, Portugal

*Presenter author: arpedro@i3s.up.pt

Abstract

Livestock farming sustainability greatly relies on adequate management and nutrition strategies to prevent the incidence of infectious disease. Although major changes have been achieved (LeBlanc et al., 2006), calf rearing is still a particular sensitive process. It is estimated that about 20% of the calves develop neonatal diarrhoea in their first weeks of life (Windeyer et al., 2014). Respiratory syndromes are also major causes of calf morbidity and mortality, although much more complex and of multifactorial origin (Bednarek et al., 2012). Immune system modulation through nutrition has long been used to reduce the incidence of disease and avoid the use of antibiotics. Among dietary ingredients, increasing interest is emerging on microalgae, since they contain compounds that stimulate the immune system, like beta-glucans, rhodopsins, phycobiliproteins, eicosapentaenoic acid, and docosahexaenoic acid (Wells et al., 2017; Bule et al., 2018, Villarruel-López et al., 2017). However, only few studies have evaluated the effects of microalgae species on innate immune system modulation.

Our aim was to evaluate the effect of digestion products of microalgae on bovine peripheral blood monocyte-derived macrophages production and expression of pro- and anti-inflammatory cytokines and on the production of reactive-oxygen species (ROS).

Digestion products of *Chlorella vulgaris*, *Nannochloropsis oceanica*, and *Tetraselmis* sp., obtained by an *in vitro* enzymatic method simulating monogastric digestion (Minekus et al., 2014) were used at two dilutions to stimulate *in vitro* bovine monocyte-derived macrophages (MDM). A 10- and 100-fold dilution were chosen to represent a milk replacer intake of 8 L supplemented with 2 and 0.2% microalgae, respectively. Cytokine gene expression (*IL6*, *IL8*, *IL10* and *IL12p40*) and production (TNF- α , IL-6, IL-8 and IL-10) were respectively evaluated by real-time polymerase chain-reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). ROS production assessment was performed using spectrophotometry, through an intracellular ROS assay kit (dichlorodihydrofluorescein diacetate).

Our results indicate that digestion products of *C. vulgaris*, *N. oceanica*, and *Tetraselmis* sp., when used at 10-fold dilution *in vitro* induced considerable cytotoxicity. However, when used at a higher dilution (100-fold), microalgae digestion products enhanced the production and expression of all cytokines evaluated and the production of ROS. These results indicate that microalgae supplementation exerts an immunomodulatory effect on bovine macrophages that could account for the activation of *in vivo* local immune effector mechanisms. The anti-inflammatory and antioxidant effects of microalgae products on bovine MDM are currently under evaluation.

Keywords

Calves; Cytokines; Microalgae; Monocyte-Derived Macrophages; Reactive-oxygen Species

Acknowledgements

ARVP acknowledges Fundação para a Ciência e a Tecnologia (FCT), CAVC CRL., and ADM Portugal SA. for funding through scholarship PD/BDE/135540/2018. MRGM acknowledges FCT for funding through program DL 57/2016 – Norma transitória (SFRH/BPD/70176/2010) and AC acknowledges FCT through Individual CEEC 2017 (CEECIND/01514/2017). The work was supported through the project UIDB/50006/2020 | UIDP/50006/2020, funded by FCT/MCTES through national funds. Microalgae were kindly provided by Allmicroalgae - Natural Products.

References

- Bednarek, D., Szymańska-Czerwińska M., Katarzyna, D. (2012) Bovine respiratory syndrome (BRD) etiopathogenesis, diagnosis and control. In A bird's-eye view of veterinary medicine. Perez-Marin, C.C. ed., InTech, U.S.A. 363-379. doi: 10.5772/33465
- Bule, M.H., Ahmed, I., Maqbool, F., Bilal, M., Iqbal, H.M.N. (2018). Microalgae as a source of high-value bioactive compounds. *Front. Biosci.* 10: 197-216. doi: 10.2741/s509
- LeBlanc, S. J., K. D. Lissemore, D. F. Kelton, T. F. Duffield and K. E. Leslie (2006). Major advances in disease prevention in dairy cattle. *J Dairy Sci* 89(4): 1267-1279. doi: 10.3168/jds.S0022-0302(06)72195-6
- Minekus, M., M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carriere, R. Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Menard, I. Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. Wickham, W. Weitschies and A. Brodkorb (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food Funct* 5(6): 1113-1124. doi: 10.1039/C3FO60702J
- Villarruel-López, A., Ascencio, F., Nuño, K. (2017). Microalgae, a potential natural functional food source – a Review. *Pol J Food Nutr Sci.* 67: 251–263. doi: 10.1515/pjfn-2017-0017
- Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A.G., Camire, M. E., Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol* 29:949-982. doi: 10.1007/s10811-016-0974-5
- Windeyer, M. C., Leslie, K. E., Godden, S. M., Hodgins, D. C., Lissemore, K. D., & LeBlanc, S. J. (2014). Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev Vet Med*, 113(2), 231-240. doi:10.1016/j.prevetmed.2013.10.019

Optimizing extrusion and drying temperatures for maximizing the scavenging potential of aquafeeds with natural antioxidant inclusion from mango and pineapple peels

Ricardo Pereira^{*1,2,3}, Cristina Velasco¹, Manuela Pintado², Luísa Valente¹

- ¹ CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões. Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.
 - ² Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal.
 - ³ ICBAS, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, R. de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.
- *ricardo.pereira@ciimar.up.pt

Abstract

The food industry generates high amounts of discarded fruit peels rich in antioxidants (e.g. polyphenols, carotenoids and vitamins)⁽¹⁾. These by-products could be a valuable source for ameliorating oxidative stress in fish and delaying feed/flesh oxidation during storage, while meeting consumer preferences for natural antioxidant sources⁽²⁾. However, there are knowledge gaps concerning the antioxidant characterization of fruit peel inclusion in aquafeeds, and few studies concerning the impact of manufacturing temperatures in feed antioxidant profile/potential, despite certain antioxidants being heat-sensitive. Thus, results from this study may shed light on how we can optimize this process in order to retain maximum antioxidant potential of aquafeeds with natural antioxidant inclusion.

For this study, dried mango and pineapple peels were turned into flour. 2% of each flour was added to an aquafeed mix. Both mixtures were subjected to different extrusion/drying temperatures, originating 8 isoproteic, isolipid and isoenergetic diets (4 for each fruit). Mango diets were: i) M_H60 (110 °C extrusion; 60 °C drying - conventional manufacturing temperatures), ii) M_H35 (110 °C; 35 °C), iii) M_C60 (25 °C; 60 °C) and iv) M_C35 (25°C; 35°C). Equal temperature conditions were applied to pineapple mix, generating P_H60, P_H35, P_C60 and P_C35, respectively. A commercial-based diet (110 °C; 60 °C) was used as control (CTRL). Peels, flours and diets were analysed for their polyphenol content (free and in antioxidant fibre), antioxidant potential (DPPH, ABTS⁺ and ORAC) and carotenoid quantification/identification.

Results showed that most antioxidant potential losses were originated by converting peel into flour. Moreover, free polyphenols showed >50% losses in mango flour, while carotenoids displayed >50% loss in both fruits. In diets, evidence of antioxidant degradation was found via absence of dietary β -carotene, despite being present in flours. Compared to CTRL, 2% pineapple flour inclusion had a positive impact on antioxidant potential. Specifically, AEQ60 displayed significantly higher DPPH, ABTS⁺, polyphenols and ORAC, the latter being a more realistic model for biological samples, as the oxygen radical forms naturally within cells.

Surprisingly, a 2-way ANOVA between diets revealed that conventional manufacturing temperatures are better for retaining antioxidant properties. Since diets must be dried until <8% moisture, short-term drying at high temperatures could be more effective than cooler, yet longer drying processes. Further research is required for minimizing antioxidant potential loss in processing peels into flour. Additionally, additional chemical and antioxidant characterization of diets, e.g starch, sugars and Vitamin C, is necessary before undergoing *in vivo* trials.

Acknowledgements:

This work is a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). The doctoral

scholarship of Ricardo Pereira was financed by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the grant SFRH/BD/144631/2019.

Keywords:

Circular economy; Aquaculture; Functional aquafeeds; Natural antioxidants; Antioxidant activity

References:

1. Aklakur, M., (2018). Natural antioxidants from sea: a potential industrial perspective in aquafeed formulation. *Reviews in Aquaculture*, 10(2), 385-399. doi: 10.1111/raq.12167
2. De Laurentiis, V., S. Corrado, and S. Sala (2018). Quantifying household waste of fresh fruit and vegetables in the EU. *Waste Management*, 77, 238-251. doi: 10.1016/j.wasman.2018.04.001

Conventional *versus* pasture-based cow's milk: simulated human gastric lipolysis and lipid oxidation

S. C. M. Pinho^{1,2}, M. A. Faria¹, S. Casal¹, M. Madalena C. Sobral¹, R. Alves³, A. R. J. Cabrita², A. J. M. Fonseca², I. M. P. L. V. O. Ferreira¹

¹LAQV/REQUIMTE, Department of Chemical Sciences, Laboratory of Food Science and Hydrology, Faculty of Pharmacy, University of Porto, Portugal

²LAQV/REQUIMTE, ICBAS, School of Medicine and Biomedical Sciences, University of Porto, Portugal

³SORGAL, Sociedade de Óleos e Rações S.A., Estrada Nacional 109 Lugar da Pardala, 3880-728 S. João Ovar, Portugal

*Presenter author: susanapinho7@hotmail.com

Abstract

Milk fatty acids (FA) have a dual origin: synthesis *de novo* in the mammary gland (\leq C14:0 and some C16:0; approximately 45% w/w) and the plasma lipids originating from feed (\geq C18:0 and also some C16:0; up to 60% w/w).¹ Importantly, the amount of C16:0 influences FA distribution within the milk triacylglycerol molecule,² possibly affecting the behaviour of milk fat in the human stomach.³ Differences on free FA (FFA) available for absorption can affect their metabolism rate and consequent physiological responses in humans. For instance, FA up to C12:0 are readily used as a source of energy, resulting in faster satiety; and long-chain FA are effective releasers of the cholecystokinin, an intestinal hormone recognized to suppress appetite.⁴

The present work aimed to study the gastric behaviour in humans (i.e. lipolysis and lipid oxidation) of commercial milks from cow subjected to different feeding practices. A UHT whole milk without information of management on the label (C milk) and a UHT whole milk pasture-based labelled (P milk) were compared over simulated gastric digestion taking into account gastric emptying, simulated by sampling 5 equal volumes of chyme each 16 min, based on the method described by Mulet-Cabero et al.⁵ Samples were analysed for FA relative release, absolute quantity of individual FFA, and formation of markers of oxidation in polyunsaturated FA (PUFA), namely conjugated dienes (abs233 nm) and trienes (abs268 nm). FFA were separated by solid-phase extraction and FA methyl-esters analysed by GC-FID. Concerning milk fat composition in C16:0, the C milk had higher proportion than the P milk ($P < 0.05$). Regarding gastric lipolysis, the P milk had a higher relative release of C8:0 and C18:2n-6, whereas the C milk had a higher relative release of C18:3n-3 and CLA $c9t11$ ($P < 0.05$). Alongside with the FA relative release, the individual FFA quantity over simulated human gastric digestion is also a valuable data. Specifically, the absolute quantity of free C18:3n-3 and CLA $c9t11$ was higher for the P milk than for the C milk ($P < 0.001$), in spite of their lower relative release ($P < 0.05$). In relation to gastric lipid oxidation, both milks showed a linear increase on markers of PUFA oxidative degradation over simulated human gastric digestion.

In conclusion, our data highlights that cow's milks with different fat composition in C16:0 (FA with mixed origin) present different gastric behaviour, because differences concerning individual FA relative release were observed.

Keywords

Dairy management, milk fatty acid profile, human simulated gastric digestion, free fatty acid, conjugated dienes/trienes

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. 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 COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation (POCI), and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia in the framework of the project PTDC/SAU-NUT/30322/2017.

References

1. MacGibbon, A. K. H., & Taylor, M. W. (2006). Composition and Structure of Bovine Milk Lipids. In *Advanced Dairy Chemistry Volume 2 Lipids*, Fox, P. F.; McSweeney, P. L. H., Eds. Springer US: Boston, MA, pp 1-42. doi:10.1007/0-387-28813-9_1
2. Tzompa-Sosa, D. A.; van Aken, G. A.; van Hooijdonk, A. C.; & van Valenberg, H. J. (2014). Influence of C16:0 and long-chain saturated fatty acids on normal variation of bovine milk fat triacylglycerol structure. *Journal of Dairy Science*, 97, 4542. doi:10.3168/jds.2014-7937
3. Bauer, E.; Jakob, S.; & Mosenthin, R. (2005). Principles of physiology of lipid digestion. *Asian-Australian Journal of Animal Sciences*, 18, 282. doi:10.5713/ajas.2005.282
4. Michalski, M.-C. (2009). Specific molecular and colloidal structures of milk fat affecting lipolysis, absorption and postprandial lipemia. *European Journal of Lipid Science and Technology*, 111, 413. doi: 10.1002/ejlt.200800254
5. Mulet-Cabero, A. I.; Egger, L.; Portmann, R.; Menard, O.; Marze, S.; Minekus, M.; Le Feunteun, S.; Sarkar, A.; Grundy, M. M.; Carriere, F.; Golding, M.; Dupont, D.; Recio, I.; Brodkorb, A.; & Mackie, A. (2020). A standardised semi-dynamic in vitro digestion method suitable for food - an international consensus. *Food & Function*, 11, 1702. doi:10.1039/c9fo01293a

Novel applications: hair glucocorticoids in a mammalian range expansion

Alexandre Azevedo^{1,2*}, Liam Bailey¹, Victor Bandeira³, Jella Wauters¹, Katarina Jewgenow¹

¹Leibniz-Institute for Zoo- and Wildlife Research, Berlin, Germany

²Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal

³Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

*Presenting author: ax.c.azevedo@gmail.com

Abstract

The distribution of wild species is changing at an unprecedented rate as a consequence of human-induced environmental degradation (Pimm *et al.*, 2014). When species declines or distribution changes are detected, it is often too late or resource-demanding to intervene. However, sub-lethal physiological effects occur in individual animals due to environmental change, often long before population declines, range shifts or extinctions are detectable. The discipline of macrophysiology aims to assess the large scale temporal and spatial variation in physiological traits, and could provide more timely information on the effects of anthropogenic change on wild animals (Chown and Gaston, 2008).

Amidst the general context of wildlife decline, some species have increased their abundance and range, apparently unaffected by human pressure. Understanding how these species thrive while others dwindle could help us manage our impacts on wildlife. Over the past 40 years, the Egyptian mongoose (*Herpestes ichneumon*) has continuously expanded its distribution in Portugal (Barros *et al.*, 2015). Once confined to the south of the Tagus River, the species has spread northward, now occurring almost throughout the country.

In this study we quantified hair glucocorticoids (hGC) in Egyptian mongoose hair samples (N=236), collected between 2008 and 2014 from its entire distribution in Portugal. Next, we assessed the spatial variation of hGC by comparing values between the historic and expansion distribution and by exploring hGC variation within the expansion area. In order to adequately estimate the spatial variation of hGC, we accounted for expected variations with age, sex, season, body condition, body size, storage time, and environmental variables.

Our study is the first to assess glucocorticoid variation in a mammalian expansion. The analyses showed that hGCs decrease toward the expansion front, as the distance from the historic area increases. We found little evidence to support an effect of human population density on mongoose hGCs. Together, these results suggest the expansion of Egyptian mongoose is unlikely to be limited by effects related to the physiological stress response.

Keywords:

Hair glucocorticoids, range expansion, *Herpestes ichneumon*

Acknowledgements:

We thank Mareen Albrecht, Katrin Paschmionka for technical assistance.

References

Barros T, Carvalho J, Pereira MJR, Ferreira JP, Fonseca C (2015) Following The Trail: Factors Underlying the Sudden Expansion of the Egyptian Mongoose (*Herpestes ichneumon*) in Portugal. *PLOS ONE* 10: e0133768.

Chown SL, Gaston KJ (2008) Macrophysiology for a changing world. *Proceedings of the Royal Society B: Biological Sciences* 275: 1469–1478.

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM, Sexton JO (2014) The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344: 1246752–1246752.

Inclusion of macro- and microalgae in European seabass (*Dicentrarchus labrax*) diets: effects on intestinal microbiota

Mariana Ferreira^{1,2*}, Yousri Abdelhafiz³, Luisa M.P. Valente^{1,2}, Viswanath Kiron³

¹ CIIMAR, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal

² ICBAS, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

³ Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

*Presenter author: marianaipf@gmail.com

Introduction: Nutritional manipulation of the intestinal microbiota can have a positive impact on fish health. The seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica* have been recently explored by the feed industry, but the impact of the inclusion of such products on the gut microbiota of European seabass (*Dicentrarchus labrax*) is poorly understood. We evaluated the impact of *G. gracilis* and *N. oceanica*, single or blended, on the composition of microbial community in the intestine of European seabass.

Materials and Methods: European seabass were fed four diets for 106 days: a commercial-based diet (CTRL) and three experimental diets with the inclusion of 8 % *G. gracilis* (GRA), 8 % *N. oceanica* (NAN), or a blend of 4 % of each alga (NANGRA), at the expense of fish meal and wheat meal. At the end of the trial, posterior intestine mucus was collected; V3-V4 region of the 16S rRNA was amplified and sequenced using Illumina® MiSeq platform. After quality filtering, taxonomic assignment of the representative bacterial ASVs was performed using RDP classifier. R packages “iNEXT” and “phyloseq” were used to calculate alpha diversity and Kruskal-Wallis followed by Dunn’s test was employed to detect significant differences. The packages “microbiome” and “DESeq2” were employed, respectively, to determine relative abundance of core taxa and to identify OTUs that were differently abundant in the study groups.

Results and Discussion: The inclusion of *G. gracilis* and *N. oceanica* had a significant impact on the alpha diversity: species richness was significantly lower in GRA fish compared to CTRL; while Shannon and the Simpson diversity were significantly reduced in fish fed both GRA and NAN diets compared to CTRL. When algae were included in a blend (NANGRA diet), the alpha diversity measures remained similar to those observed for the CTRL fish. Core microbial taxa were composed of the genera *Flavobacterium*, *Parcubacteria* and *Lactobacillus*. The most abundant phyla in the posterior intestine were Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. Compared to CTRL fish, the inclusion of the tested algae, single or blended, led to a decrease or increase of specific groups of bacteria.

Conclusion: Overall, results indicate that the inclusion of *G. Gracilis* and *N. oceanica* leads to a reduction of the gut microbial alpha diversity, while the blend attenuates these effects. Further clarification on the function of each specific group of bacteria affected by these dietary treatments will allow for a better understanding on the impact of the tested algae, single or in combination, on the gut microbiota of European seabass.

Keywords:

Seaweeds; Microalgae; Gut Microbiota; European seabass

Acknowledgements:

This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). M. Ferreira acknowledge

Fundação para a Ciência e a Tecnologia (FCT) for grant SFRH/BD/144843/2019 (FCT/FSE). The support received from Bisa Saraswathy, Researcher, Nord University, for data analyses is acknowledged.

SHORT ORAL COMMUNICATIONS

Search for biomarkers of resistance in *R. decussatus* to *P. olseni* parasite infection by shotgun proteomics

João Estêvão¹, Hugo Osório², Benjamin Costas¹, Andreia Cruz³, Sergio Fernández-Boo¹

¹ Animal Health and Aquaculture (A2S), CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Porto, Portugal.

² i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal.

³ Oceano Fresco S.A, Nazaré, Portugal.

* Presenting author email: jestevao@ciimar.up.pt

Abstract

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-seller¹. A decline on the species happened since the 90's due to biotic and abiotic factors, such as parasite infection and degradation of the environment. Infection by *Perkinsus olseni* parasite is the main biotic factor that causes decrease of populations. It was first diagnosed in the 1980s, after introduction of the invasive species *Ruditapes philippinarum* from Asia for intensive production in Europe². It has been observed that *Perkinsus* causes changes in humoral and cellular responses and rise of mortality in infected individuals³.

Several studies focused on host-parasite early interaction and markers of resistance^{4,5}, while none 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 haemolymph's proteome profile of tolerant individuals in comparison with susceptible ones.

Materials and Methods

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

After *Perkinsus* diagnosis and haemolymph extraction, 5 adult individuals (>40 mm) highly-infected (susceptible) and 5 individuals non/low-infected (tolerant) were chosen at each population for a proteomics analysis by LC-MS. Three analyses were considered to identify markers of tolerance/susceptibility: i) Tolerant vs. Susceptible (in all populations); ii) Tolerant (in all populations) vs. Control (Noia population); iii) Tolerant vs. Susceptible (at each population). Expressed and exclusive proteins were identified according to Osório *et al.*⁷.

Results and discussion

A substantial number of expressed proteins was identified (771, Abundance Ratio>1.5), being 206 found in tolerant (t) individuals and 565 on susceptible (s) ones. Moreover, 395 exclusive proteins were identified, from which t=87 and s=308. Functions related to metabolism were most represented in susceptible than in tolerant individuals suggesting a higher production of energy to counteract the infection. Also, six proteins were differentially expressed in all tolerant individuals and seems to be possible markers of tolerance to *P. olseni* infection.

These results points towards a host generated tolerance suggesting an existence of adaptation mechanisms to parasite.

Keywords

R. decussatus; *P. olseni*; host-parasite interaction; proteomics; innate immunity

Acknowledgements

This research was supported by the project “Tools4Breed – Challenge test and genetic markers for *Perkinsus* as a tool for *Ruditapes decussatus*’ selective breeding” with reference FA_05_2017_025 financed by Fundo Azul and República Portuguesa and by the FCT through national funds and strategic project UID/Multi/04423/2019. João Estêvão was supported by FA (FA_05_2017_025) and by FCT (UI/BD/150906/2021) grants.

References

1. FAO. (FAO, 2020). *The State of World Fisheries and Aquaculture 2020*. The State of World Fisheries and Aquaculture 2020 doi:10.4060/ca9229en.
2. Azevedo, C. (1989). Fine structure of *Perkinsus atlanticus* n. sp. (Apicomplexa, Perkinsea) parasite of the clam *Ruditapes decussatus* from Portugal. *The Journal of parasitology*, 75, 627–635.
3. Villalba, A., Reece, K. S., Camino Ordás, M., Casas, S. M. & Figueras, A. (2004). Perkinsosis in molluscs: A review. *Aquatic Living Resources*, 17, 411–432.
4. Fernández-Boo, S., Villalba, A. & Cao, A. (2016). Protein expression profiling in haemocytes and plasma of the Manila clam *Ruditapes philippinarum* in response to infection with *Perkinsus olseni*. *Journal of Fish Diseases*, 39, 1369–1385.
5. Hasanuzzaman, A. F. M. *et al.* (2020). New insights into the Manila clam – *Perkinsus olseni* interaction based on gene expression analysis of clam hemocytes and parasite trophozoites through in vitro challenges. *International Journal for Parasitology*, 50, 195–208.
6. Leite, R. B. *et al.* (2013). mRNA-Seq and microarray development for the Grooved carpet shell clam, *Ruditapes decussatus*: A functional approach to unravel host -parasite interaction. *BMC Genomics*, 14, 741.
7. Osório, H. *et al.* (2021). Proteomics Analysis of Gastric Cancer Patients with Diabetes Mellitus. *Journal of Clinical Medicine*, 10, 407.

Immune and intestinal benefits of krill meal and krill oil in Pacific white shrimp (*Litopenaeus vannamei*) diets

Teixeira C.^{1,2,3*}, Serra C.R.¹, Dias J.³, Rema P.⁴ and Costas B.^{1,2}

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research. Terminal de Cruzeiros de Leixões. Portugal.

²ICBAS - Abel Salazar Biomedical Sciences Institute. Portugal.

³SPAROS Lda. - I&D Nutrition in Aquaculture. Portugal.

⁴UTAD - University of Trás-os-Montes e Alto Douro. Portugal.

*Presenting author: cteixeira@ciimar.up.pt

Abstract

A high digestible protein and an accurate lipid content are indispensable to achieve maximum growth and maintain shrimp health. Fish meal (FM) and fish oil (FO) are the major sources of protein and lipids in shrimp aquaculture, but the global demand for these two ingredients are exceeding the available supplies and their prices are escalating. Because of this, it is extremely important to find suitable FM and FO substitutes in aquafeeds. In this context, krill meal (KM) and krill oil (KO) have been studied as a FM and FO replacement but also as added value to enhance feed intake and promote growth. However, little is known regarding KM and KO health promoting properties. The main goal of the study was to investigate the immunomodulatory effects of KM and KO in diets for Pacific white shrimp (*Litopenaeus vannamei*). Juvenile shrimps, weighing 1.15 ± 0.0 g, were randomly distributed among 10 tanks of 60 L of water capacity (45 shrimp each tank), in a saltwater recirculating system, at UTAD (University of Trás-os-Montes e Alto Douro) facilities. All animals were acclimatized to the experimental conditions and fed with a control diet (CTRL), formulated with high levels of FM and FO, during 10 days. The shrimps were fed 3 times a day, at 6 % of body weight and, when necessary, adjustments were made in the feed quantity. Thereafter, shrimps were fed with the experimental diets, in 5 replicate tanks of each diet. The experimental diets consisted of a negative control diet (NC), formulated with low FM and high SM, and, the krill diet (KRILL), formulated similarly to NC diet with and inclusion of 2.5 % of KM and 0.5 % of KO. The feeding trial lasted for 35 days. At the end of the trial, 8 shrimps were randomly selected from each tank, weighed and sampled to evaluate plasma immune parameters, intestinal gene expression, intestinal histology and intestinal microbiota. Although shrimps fed with the KRILL diet (FBW = 2.4 ± 1.1 g) presented a slower growth performance than those fed with the NC diet (FBW = 2.8 ± 0.9 g), the immune system seems to be stimulated by this diet. Anti-protease activity, lysozyme and anti-bacterial activity in plasma tended to increase in the shrimps fed with the KRILL diet. Intestinal histology was not affected by dietary treatments. Further analyses will be conducted to evaluate intestinal gene expression and microbiota.

Keywords: Immunity,

Krill meal, Krill oil, *Litopenaeus vannamei*

Acknowledgements

J. Couto (SPAROS, Portugal) is acknowledged for technical assistance. C. Teixeira and B. Costas benefited from grants by FCT (PD/BDE/135541/2018 and IF/00197/2015, respectively).

References

1. Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, 285(1-4), 146-158. doi:10.1016/j.aquaculture.2008.08.015
2. Nunes, A. J. P., SÁ, M. V. C., & Sabry-Neto, H. (2011). Growth performance of the white shrimp, *Litopenaeus vannamei*, fed on practical diets with increasing levels of the Antarctic krill meal, *Euphausia*

superba, reared in clear- versus green-water culture tanks. *Aquaculture Nutrition*, 17(2), e511-e520.
doi:10.1111/j.1365-2095.2010.00791.x

POSTERS

Evaluation of different Nile tilapia body composition models

A. I. G. Raposo^{*1,2}, F. Soares², L. E. C. Conceição³, L.M.P. Valente^{1,3}, T. S. Silva²

¹ICBAS - Abel Salazar Biomedical Sciences Institute - University of Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto

²SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal.

³CIIMAR, UP, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos S/N, 4450-208, Matosinhos, Portugal

E-mail: andreiraposo@sparos.pt

Abstract:

Using mathematical models to estimate the body composition of fish can be an interesting alternative to conventional methods (e.g. AOAC methods). The cost associated with the analysis is lower, there is no need to use toxic chemicals, it is not time consuming and fish is not sacrificed. Thus, body composition models can be useful in the estimation of fish nutrient retention and nutritional requirements, in the prediction of the environmental impact of fish farms and used in the development of dynamic fish growth models.

The objective of this work was to develop, calibrate and evaluate different types of models for the prediction of Nile tilapia body composition, and then to compare the best of these against other published models using an independent validation dataset, to obtain a Nile tilapia body composition model with a demonstrable high predictive capacity.

Tilapia whole-body composition and respective whole-body weight data were collected from 142 publications, covering fish from 0.01 g to 1470 g. Regression analyses were performed for each body component using 10 models with different calibration methods, which resulted in 23 “model + calibration method” combinations. The combinations were evaluated with qualitative (residual analysis) and quantitative methods (RMSE, MAPE and CRM). Cross-validation methods (leave-one-out cross-validation, repeated k-fold cross-validation with k=10, k=5 and k=2) were used to objectively evaluate and select models with the best predictive capacity. Such models were calibrated using all data and then compared against published tilapia body composition models, using an independent validation dataset.

Results show that model predictions are greatly affected not only by the type of model, but also by the calibration method used. In particular, models calibrated under the assumption of multiplicative noise, seem to have a better prediction capability than those where noise was assumed to be additive. This suggests that performing the regression in “log space” is not only convenient, but advisable, even for isometric models. The models with the best predictive capability are the allometric model (assuming multiplicative noise) and hybrid model in its robust form, with both having good prediction capability when compared with models from other authors.

Concluding, highly-predictive models were developed in this study and the predictions of those models are coherent with the validation data and the predictions of other published models. Furthermore, this study shows that the use of objective criteria to test body composition models is important and necessary to help us answer scientific and technical questions.

Keywords:

Mathematical models, Body composition, Nile tilapia, Regression analysis, Prediction tools

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.

Improving animal health and aquaculture sustainability through green and circular economy concepts

A. Garcia ^{1,2*}, S. Fernández-Boo ², K. Viswanath ³, B. Costas ^{1,2}

¹ Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal

² Interdisciplinary Centre for Marine and Environmental Research, Matosinhos, Portugal

³ Faculty of Biosciences and Aquaculture, Nord University, Norway

*Presenter author: afgarcia@ciimar.up.pt

Abstract

Aquaculture is one of the fastest growing food production industries and faces a huge commitment on sustaining fish production demands¹. One of the major challenges of modern aquaculture is to combine strategies which allow to achieve sustainability. In fact, aquaculture faces an emergent need to find alternative ingredients to replace fish feeds, which are currently based on fishmeal and fish oil derived from capture fisheries. The replacement of fish meal by plant protein sources has been explored and proved to be a competitive option². Moreover, recent studies have also explored the potential of halophytes to be included on fish diets. The latter synergy can be further explored in aquaculture since organic nutrients resulted from aquaculture effluents could serve as a fertilizer for these plants thus contributing to a circular economy concept^{3,4}. However, studies approaching the impact of halophytes on metabolic and immune responses and its eventual improvement of fish susceptibility to diseases are still scarce. The present project will explore both a green fractionation process and the concept biorefineries from halophytic biomass to further explore the effect of halophytes in diets for European seabass (*Dicentrarchus labrax*). The work plan will be divided in two main parts: *in vivo* and *in vitro* studies. *Salicornia ramosissima* protein fractions will be selected for *in vivo* studies through incorporation on fish diets. After 2 months of feeding, fish will be challenged with pathogenic bacteria in order to understand the possible protective effect of the green fraction. The intestine health status of fish fed with halophytes-based diets and the gut microbiome will be evaluated. Gene expression analysis of key genes involved in metabolic and immune pathways of interest will also be assessed. Besides *Salicornia ramosissima*, bioactive fractions from three other halophytes: *Tripolium pannonicum*, *Salicornia europaea*, *Crithmum maritimum*, will be incorporated *in vitro* studies. The bioactive activity of these plants will be tested against several bacterial pathogens from aquaculture and effects of halophytes on fish cells will also be assessed by *in vitro* studies of host/pathogen interaction.

Keywords:

Aquaculture, aquafeeds, halophytes

Acknowledgements:

This project is supported by national funds through FCT – Foundation for Science and Technology, within the scope of UIDB/0443/2020 and UIDP/04423/2020 and by European Union's Horizon 2020 research and innovation programme under Grant Agreement No 862834. Ana Garcia is supported by a BYT-FCT PhD Grant (UI/BD/150907/2021)

Bibliography:

1. Piazzon MC, Caldach-Giner JA, Fouz B, et al. Under control: how a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegetable diets. *Microbiome*. 2017;5(1):164. doi:10.1186/s40168-017-0390-3
2. Kaushik SJ, Covès D, Dutto G, Blanc D. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture*. 2004;230(1-4):391-404. doi:10.1016/S0044-8486(03)00422-8

3. Ventura Y, Wuddineh WA, Shpigel M, et al. Effects of day length on flowering and yield production of *Salicornia* and *Sarcocornia* species. *Sci Hort* (Amsterdam). 2011;130(3):510-516. doi:10.1016/j.scienta.2011.08.008
4. Turcios AE, Papenbrock J. Sustainable treatment of aquaculture effluents-What can we learn from the past for the future? *Sustain*. 2014;6(2):836-856. doi:10.3390/su6020836

Acute inflammation response of European seabass (*Dicentrarchus labrax*) juveniles fed tryptophan supplemented diets under crowding conditions

Diogo Peixoto^{1,2,3*}, M. Machado¹, P. Santos^{1,4}, A. Ricardo^{1,2}, I. Duarte^{1,2}, R. Azeredo¹, B. Costas^{1,2}

¹ CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal.

² ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

³ Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Instituto Universitario de Investigación Marina (INMAR), CEIMAR-Universidad de Cádiz, 11510, Puerto Real, Cádiz, Spain.

⁴ MARE-Politécnico de Leiria – Centro de Ciências do Mar e do Ambiente, Peniche, Portugal.

*Presenter author: dpeixoto@ciimar.up.pt

Abstract

Tryptophan is an essential amino acid (AA) with important roles on the modulation of stress response, antioxidant system, behaviour and immunity. In an attempt to explore tryptophan potential as a functional AA, this study aimed to assess the immunological condition, inflammatory response and disease resistance of European seabass (*Dicentrarchus labrax*) fed dietary tryptophan supplementation under stressful conditions followed by a bacterial challenge. Seabass juveniles were randomly distributed in 16 tanks in two recirculated seawater systems with a density of 5kg/m³. By lowering the water level in one of the systems, fish were kept at higher density (8 tanks with a density of 10kg/m³) and consequently, under stressful crowding conditions. Two dietary treatments were evaluated: a control diet (CTRL) meeting the indispensable AA profile established for seabass and a CTRL-based diet supplemented with tryptophan (0.3% DM basis; TRP). Sampling at 15 days (0 h, n=10) allowed the collection of data regarding haematological profile, plasma cortisol and immune parameters, as well as immune response and oxidative stress biomarkers in gut. The remaining fish were intraperitoneally-injected with 100 µL of *Photobacterium damsela piscicida* (5×10⁷ cfu/mL) and sampled after 4, 24, 48 and 72 h. Cortisol levels tended to increase in unstressed fish fed CTRL and TRP until 24 h post-infection and decrease after 48 and 72 h. Differently, unstressed fish fed CTRL presented higher cortisol levels at 4 h post-infection and a decrease after 72 h. No significant differences were observed in cortisol levels from 0 to 72 h post-infection in stressed fish fed TRP. Still, cortisol levels were lower in stressed fish fed TRP at 4 h post-infection compared to their counterparts fed CTRL. Lymphocytes in stressed fish fed CTRL increased from 4 to 48 h post-infection and the same occurred in stressed fish fed TRP from 4 to 72 h post-infection. Regarding gut total glutathione content, a decreased with time was observed in unstressed fish fed CTRL and in stressed fish fed TRP. Our results showed that immune and antioxidants parameters in plasma and gut were not significantly modulated by tryptophan after 15 days of feeding. However, cortisol levels dropped in stressed fish fed TRP following the inflammatory insult, suggesting that this AA might counteract stress-induced cortisol production, thereby potentially reverting cortisol-mediated immunosuppressive effects. Preliminary data from the present study point to dietary tryptophan supplementation as a nutritional strategy to modulate fish endocrine responses during acute inflammation and stressful conditions.

Keywords

Aquaculture; tryptophan; inflammation; stress

Acknowledgements

This work was supported by project INFLAMMAA (PTDC/CVT-CVT/32349/2017), financed by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020, and through national funds through Fundação para a Ciência e a Tecnologia (FCT, Portugal). Diogo Peixoto was supported by FCT, Portugal (UI/BD/150900/2021).

Local and systemic immune response of gilthead seabream *Sparus aurata* juveniles fed microalgae-derived β -glucans

B. Reis^{1,2,3,4,*}, A. Gonçalves¹, P. Santos², M. Sardinha¹, L.E.C. Conceição¹, R. Serradeiro⁵, J. Pérez-Sánchez⁶, J. Caldach-Giner⁶, U. Schmid-Staiger⁷, K. Frick⁷, J. Dias¹, B. Costas^{2,3}

¹SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal.

²Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Terminal de Cruzeiros de Leixões. Av. General Norton de Matos s/n 4450-208 Matosinhos, Portugal.

³Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.

⁴Sorgal S.A., Estrada Nacional 109, Lugar da Pardala 3880-728, São João de Ovar, Portugal.

⁵Riasearch, Rua do Farol, 131, Torrão do Lameiro 3880-394 Ovar, Portugal.

⁶Nutrigenomics and Fish Growth Endocrinology Group, Institute of Aquaculture Torre de la Sal, IATS-CSIC, 12595, Castellón, Spain

⁷Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Innovation Field Algae Biotechnology - Development, Nobelstrasse 12, 70569 Stuttgart, Germany

*E-mail: breis@ciimar.up.pt

Introduction

Animal health related issues are nowadays the major constraint for aquaculture expansion and sustainability (Adams 2019). Besides vaccination, prophylactic measures such as the incorporation of immunostimulants and prebiotics in feeds (Meena et al. 2013; Song et al. 2014), have also been used to prevent disease outbreaks. To enhance fish disease resistance and general health, diets are often supplemented with β -glucans, which are compounds with known beneficial effects in fish innate immune response (Guzmán-Villanueva et al., 2014). β -glucans show repeating patterns on their structure that are recognized in the gut by cell pattern recognition receptors (PRR), leading to the activation of the host's innate immune cells enhancing its immune response (Dalmo et al., 2008). The present work aimed to evaluate the effects of both short- and mid-term feeding diets supplemented with microalgae (*Phaeodactylum tricornutum*) extracted β -glucans on gene expression, oxidative stress biomarkers and plasma immune parameters in gilthead seabream (*Sparus aurata*) juveniles.

A practical commercial-like diet was used as control (CTRL), whereas 3 others based on CTRL were further supplemented with a constant concentration of β -glucans, derived from *Saccharomyces cerevisiae* (diet MG1000) and different extracts of *P. tricornutum* (diets Phaeo21 and Phaeo37). Diets were randomly assigned to quadruplicate groups of 95 gilthead seabream (initial body weight: 4.1 ± 0.1 g) that were fed to satiation three times a day for 8 weeks in a pulse feeding regimen. Therefore, fish were fed the different experimental diets intercalated with the CTRL dietary treatment every 2 weeks. After 2 and 8 weeks of feeding, 3 fish/tank were sampled for blood and tissues collection.

All groups showed equal growth performance and no significant changes in plasma innate immune status. Nonetheless, seabream fed β -glucans supplemented diets showed an improved anti-oxidant status compared to those fed CTRL at both sampling points. Furthermore, diet Phaeo37 seems to induce an immune tolerance effect in the gilthead seabream gut, causing a general down-regulation of immune related genes, without compromising systemic immune response. In conclusion, results suggest that the dietary administration of a *P. tricornutum* 37% enriched- β -glucans extract might be relevant in a context of gut inflammation due to its immune tolerant and anti-oxidative effects.

Acknowledgements

This work has received funding from the Bio Based Industries Joint Undertaking (BBI JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 745754 (project MAGNIFICENT). This output reflects the views only of the author(s), and the European Union

and BBI JU cannot be held responsible for any use which may be made of the information contained therein. BR and BC were supported by FCT - Foundation for Science and Technology (PD/BDE/129262/2017 and IF/00197/2015, respectively).

References

Adams A (2019) Progress, challenges and opportunities in fish vaccine development. *Fish Shellfish Immunol* 90:210-214.

Dalmo, R.A., Bogwald, J., 2008. Beta-glucans as conductors of immune symphonies. *Fish & Shellfish Immunology*, 25, 384–396.

Guzmán-Villanueva, L. T., Tovar-Ramírez, D., Gisbert, E., Cordero, H., Guardiola, F. A., Cuesta, A., Meseguer, J., Ascencio-Valle, F., Esteban, M. A., 2014. Dietary administration of β -1,3/1,6-glucan and probiotic strain *Shewanella putrefaciens*, single or combined, on gilthead seabream growth, immune responses and gene expression. *Fish & Shellfish Immunology*, 39, 34-41.

Meena DK, Das P, Kumar S, Mandal SC, Prusty AK, Singh SK, Akhtar MS, Behera BK, Kumar K, Pal AK, Mukherjee SC (2013) Beta-glucan: an ideal immunostimulant in aquaculture (a review). *Fish Physiol Biochem* 39 (3):431-457.

Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E (2014) Prebiotics as immunostimulants in aquaculture: A review. *Fish Shellfish Immunol* 40 (1):40-48.

Microplastic occurrence in European seabass *Dicentrarchus labrax* produced in a recirculation aquaculture system (RAS): a case study

Ricardo S. Matias^{1,2}, Sónia Gomes^{1,2}, Luís G.A. Barboza^{1,2}, Lúcia Guilhermino^{1,2}, Luísa M.P. Valente^{1,2}

¹ICBAS, Abel Salazar Biomedical Sciences Institute, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

²CIIMAR, Interdisciplinary Centre of Marine and Environment Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Nórton de Matos, S/N, 4450-208 Matosinhos, Portugal

*Presenter author: ricardomatias.bio@gmail.com

Abstract

Over the past decades, plastic has been widely introduced in aquatic ecosystems, threatening their integrity. Among those, microplastics (MPs) were identified as an important emerging threat. MPs are defined as micro-sized plastic particles (sizes ranging from 1µm to 5mm), consisting of synthetic polymers either directly released into ecosystems or resultant from the fragmentation larger plastic debris. Thus, MPs are now ubiquitous in ecosystems and available for aquatic biota, which can be accidentally ingested and translocate across biological membranes. MPs ingestion may lead to gut damage and/or blockage and consequent deterioration of fitness level. The consumption and translocation of MPs into fish muscle has, therefore, supported the growing concern regarding the potential risk to consumers. Also, hazard risks associated with interaction between MPs and other contaminants may affect their uptake and combined toxicity.

Although it may operate in different production systems, aquaculture is still susceptible to both endo- and exogenous plastic contamination. Moreover, a considerable amount of MPs can enter in production systems via supplying water bodies and fish feed, which may facilitate MPs uptake in fish through the gut and gills. Indeed, MPs contamination in cultured seafood has been reported before and has prompted growing concerns due to aquaculture's importance on global food security.

In this project, MP contamination levels were evaluated in 52 European seabass *Dicentrarchus labrax*. Individuals (~250g) produced in a recirculation aquaculture system (RAS). Water and aquafeed samples were also collected. MPs were extracted from tissues – gut, gills, liver, muscle – and aquafeed, following the methodology described in Barboza et al. (2020). MPs were quantified and characterized (size, shape, colour) under a stereomicroscope. Recovered items were chemically identified by Fourier Transform Infrared Spectroscopy (FTIR) and the spectra compared with reference databases.

From all samples, gut and muscle are expected to present the highest and lowest levels of particles found, respectively. So far, 262 MPs/suspect items were recovered from muscle (0.79±0.50 items/g) however, item chemical composition has yet to be verified by FTIR. Such results will allow the calculation of human exposure scenarios and risk hazard of MP ingestion based on European Food Safety Authority (EFSA) recommendations regarding fish consumption.

Keywords:

Microplastics, European seabass, FTIR, RAS, One Health

Acknowledgements:

This work is a result of the project ATLANTIDA (NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).

References:

Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., Guilhermino, L., 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Science of the Total Environment* 717, 134625. <https://doi.org/10.1016/j.scitotenv.2019.134625>

Studying the prokaryotic interactions in a recirculating aquaculture unit of sole (*Solea senegalensis*)

Diana Bastos Almeida^{1,2,3}, *Miguel Semedo*², *Catarina Magalhães*^{2,4,5}, *Isidro Blanquet*⁵, *Ana Paula Mucha*^{2,4}

¹ ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

² CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

³ SEA8 - Safiestela Sustainable Aquafarming Investments, Lda.

⁴ FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

⁵ School of Science, University of Waikato, Hamilton, New Zealand

*Presenter author: diana.almeida@ciimar.up.pt

Recirculating aquaculture systems (RAS) allow water reuse by managing waste and nutrient recycling, consequently making intensive fish production compatible with environmental sustainability, and improving fish welfare. A key aspect of these systems is the beneficial bacterial community of the biofilter that improves water quality, for example through nitrification and/or pathogen resistance. Biotic relationships between microorganisms present in these communities may play a crucial role in driving their functional potential. However, these interactions are often neglected in engineered environments. The aim of this work is to detect prokaryotic interactions in RAS, through the definition of relevant taxa, and with cluster identification using co-variance and co-occurrence tools. Taxa were obtained by 16S rRNA gene sequencing using Illumina MiSeq® and the DADA2 pipeline with the SILVA database. The selection was based on the taxonomic attribution and includes potentially pathogenic bacteria, belonging to either the *Tenacibaculum* or *Vibrio* genus, and nitrifying bacteria, those belonging to the Nitrosococcaceae, Nitrosococcaceae or Nitrospinaceae family and the Nitrososphaeria class. Other taxa that have significant interactions with the groups selected were also included. Two independent systems of an aquaculture facility were studied (Pre-Ongrowing and Weaning). In the Pre-Ongrowing, nine subcommunities were identified in the correlation network, while 19 were found in the Weaning. Correlations between nitrifying taxa were found in both systems. Correlations between *Tenacibaculum* and *Vibrio* were found only in the Weaning system. Cases of individual nitrifying taxa were found, as well as *Nitrospira* taxa interacting with *Nitrosomonas*. Since *Nitrospira* are commonly associated with nitrite oxidation and *Nitrosomonas* with ammonia oxidation, the later result is to be expected. As for the first, this could be an indication of some taxa that perform the complete nitrification process. Future work will include an elucidation into the individualist/communitarian behaviour of these groups, links with habitat (biofilm or water) and phylogeny.

Keywords:

Network, Correlation, Microbiome, Recirculating Aquaculture Systems

Acknowledgements:

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for the PhD fellowship ref. PD/BDE/135542/2018 and Safiestela Sustainable Aquafarming Investments, S. A.. This research was supported by the project 39948_FeedMi, supported by Portugal and the European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020; and by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

Finding biomarkers for subacute ruminal acidosis in dairy cows by *in vitro* studies

Liliana Cordeiro^{1,3,4*}, Hugo M. Oliveira², Margarida R. G. Maia¹, Luís Ferreira³, Francisco Castanheira⁴, José A. Rodrigues⁵, António J. M. Fonseca¹, Ana Rita J., Cabrita¹, Inês M. Valente^{1,5}

¹REQUIMTE/LAQV, ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal

²INL - International Iberian Nanotechnology Laboratory, Avenida Mestre José Veiga s/n, 4710-057 Braga, Portugal

³AGROS UCRL, Lugar de Cassapos, 4490-258 Argivai, Portugal

⁴Alltechaditivos - Alimentação Animal Lda, Parque de Monserrate Av. D. Luís Sá nº9 Armazém A, 2710-089 Abrunheira, Portugal

⁵REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre nº 687, 4169-007 Porto, Portugal.

*lilianacordeiro@sapo.pt

The energetic demands for milk production, especially in the beginning of the lactation, represents an important metabolic challenge for dairy cows. In order to meet this high energy requirement, formulation strategies are commonly based on feeding large quantities of highly fermentable carbohydrates, with low physically effective fibre content, which generate large quantities of volatile fatty acids (VFA) and consequently a decline in ruminal pH and an increasing risk of subacute ruminal acidosis (SARA) [1]. SARA is one of the most important metabolic diseases in the dairy industry sector that impacts cow health and performance, even in well-managed high producing dairy cows [2-5]. The VFA profile of the rumen liquor is the commonly biomarker for SARA diagnosis, as in response to changes in ruminal pH, rumen bacteria shift their pathway for VFA production, reducing the synthesis of acetic acid and increasing the propionic and butyric acids production. Despite the extensive knowledge of digestive mechanisms, there is still insufficient knowledge about for SARA's biomarkers [6], and for this reason, other compounds in the rumen liquor might be identified as potential SARA biomarkers. This study aimed to characterize the profile of fermentation media from *in vitro* incubations simulating acidosis, towards the identification of new potential SARA biomarkers to be further evaluated in rumen, milk, urine and faeces samples from living animals diagnosed with ruminal acidosis. Rumen content was collected from 3 rumen-fistulated Holstein cows and incubated anaerobically at 39 °C in a buffer medium containing 1 g of each of the six experimental treatments: corn starch, wheat starch, cellulose, and mixtures of cellulose and corn or wheat starch (50:50 w/w). At 0, 2, 4, 6, 8, 10, 12 and 24 h, pH, NH₃-N content, total gas and methane production and yield, total production and VFA profile, lactate and organic compounds concentrations were measured. The experiment was replicated three times. The results showed that acidotic conditions were achieved for incubations with both corn and wheat starch, confirmed by a significant decrease of the medium pH and an increase of lactic acid concentration at 24 h of incubation. At the same incubation time, it was also observed an increased content of 3-methylbutanal and *trans*-2-pentenal. Although no report of presence of 3-methylbutanal in the rumen was found in the literature, the formation of this aldehyde is known to be related to the degradation of amino acids, namely leucine, as a result of the activity of lactic acid bacteria [7]. The origin of *trans*-2-pentenal is still unknown.

Keywords:

Biomarkers, Dairy cows, Rumen content, SARA

Acknowledgments

The work was supported through the project UIDB/50006/2020 | UIDP/50006/2020, funded by FCT/MCTES through national funds. LEC also thanks FCT, SANFEED Doctoral Programme, AGROS UCRL and Alltech for her PhD grant PD/BDE/136294/2018. IMV and MRGM acknowledge the funding program (DL 57/2016 – Norma transitória) supported by Fundação para a Ciência e a Tecnologia I.P.

References

1. Morgante, M., et al., Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *J Anim Physiol Anim Nutr (Berl)*, 2007. 91(5-6): p. 226-34.
2. Villot, C., et al., Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *animal*, 2018. 12(3): p. 481-490.
3. Neubauer, V., et al., Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J Anim Physiol Anim Nutr (Berl)*, 2018. 102(1): p. 343-349.
4. Humer, E., et al., Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J Anim Physiol Anim Nutr (Berl)*, 2017.
5. Plaizier, J.C., et al., Changes in Microbiota in Rumen Digesta and Feces Due to a Grain-Based Subacute Ruminal Acidosis (SARA) Challenge. *Microb Ecol*, 2017. 74(2): p. 485-495.
6. Celi, P., et al., Biomarkers of gastrointestinal functionality in animal nutrition and health. *Animal Feed Science and Technology*, 2019. 250: p. 9-31.
7. Smit, G., B.A. Smit, and W.J. Engels, Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiol Rev*, 2005. 29(3): p. 591-610.

LIST OF PARTICIPANTS

Adejola Yusuf Adewale	Benjamin Costas	Eduardo Costa
Adhemir Valera Andrade	Bora Bölükbaş	Elisabete Matos
Alexandra Correia	Bruna Carolina Ulsenheimer	Fernando Giovanni Lino
Alexandra Marques	Bruna Isabel Correia Pedras da Silva Lopes	Filipa
Alexandre Azevedo	Bruno Reis	Filipa Lopes
Alice Ramos	Carla Teixeira	Filipa Soares
Ana Basto	Catarina Abrantes	Filipa Sofia Jardim Barros
Ana Garcia	Catarina Brígida da Silva Augusto	Foivos Zisis
Ana Gomes	Catarina de Sousa Gomes	Francisco Castanheira
Ana Gomes	Cátia Costa	Frohn
Ana Gonçalves	Cátia Mota	Geofrey Omondi Otieno
Ana Isabel Barbosa	Chrisalyn Gonzalez	Hugo Santos
Ana Pedro	Cristian Andres Machuca Valverde	Inês Abegão
Ana Raquel Ferreira Rodrigues	Cristina Velasco	Inês Beatriz Barrigas Queiroga
Ana Sofia Carvalho Soares	Daniel Pinho Bastos Rainho	Inês Carvalho
Ana Varela	Daniela	Inês Ferreira
Analu Ramos	Daniela Leal	Inês Garrido
Andreia Ovelheiro	Daniela Resende	Iqraanwer
Andreia Raposo	Denis Meehan	Isidro Blanquet
Antoni Malachowski	Diana Almeida	Jacob Matovu
Barbara Oliveira	Diogo Peixoto	Joana
Beatriz Prata e Castro de Abreu Amorim	Duarte Dias	Joana Fernandes
Begoña Perez	Ebtissem Brahmi	Joana Pedro

Joanna Maciak
João Albuquerque
João Estêvão
Joaquina Pinheiro
José F. M. Gonçalves
Juan Carlos Capaz
Juliana Calheiros
Juliana Chagas De Menezes
Juliana Menezes
Karthik
Kennia Brum Doncato
Khalid shahin
Leonor Gomes Fernandes
Leonor Pereira
Liliana Cordeiro
Luciano Vílchez-Gómez
Ludgero Tavares
Luis Artur Soares Ferreira
Luis Conceicao
Luisa Valente
Luzia Teixeira
Mabel
Manuel Sardinha
Manuel Vilanova
Marcela Segundo
Margarida Maia

Maria da Costa
Maria de Fátima da Silva Piloto
Maria Pinheiro Franco Spínola
Mariana Ferreira
Mariana Hinzmann
Mariana Hinzmann
Mariana Marrana
Mariana Rebelo
Marina Machado
Marisa Monteiro
Marta Carvalho
Marta Laranjo
Matilde Lages
Micael Costa
Mohamed
Ms.Atshaya.S
Muhammad khubaib
Naqash
Olga Oliveira
Olivia OConnor
Panagiotis Alex Kappos
Patrícia Canhão
Patrícia Sousa
Paul Nathaniel T
Paulo Aranha
Paulo Aranha

Paulo Manuel Rodrigues Martins da Costa
Pedro Henrique Gradeço Félix
Pedro Parreira
Pedro Santos
Prasanta Jana
Renata Maria da Silva
Ricardo Azevedo
Ricardo Jorge Silva Pereira
Ricardo Passos
Ricardo Silva Matias
Rita Azeredo
Rita Cabrita
Robert Mutunga
Roger
Roxana Bertha Inohuye Rivera
Rui Alves
Sandra Nascimento
Sara Marques
Sílvia Azevedo
Sofia Lima
Sónia Catarina de Sousa Mergulhão Mota
d'Almeida
Sónia Gomes
Susana M F Ferreira
Susana Pereira
Susana Pinho

Tania Zenteno-Savín
Telmo Fernandes
Tiago Pereira
Tiago Sá
Tome Silva
Vera Filipe Magalhães de Sousa Figueiredo
Victor Bandeira
Vikash Kumar

THANK YOU!
SEE YOU IN THE NEXT EDITION OF
THE ANIMAL SCIENCE DOCTORAL
PROGRAMME WORKSHOP