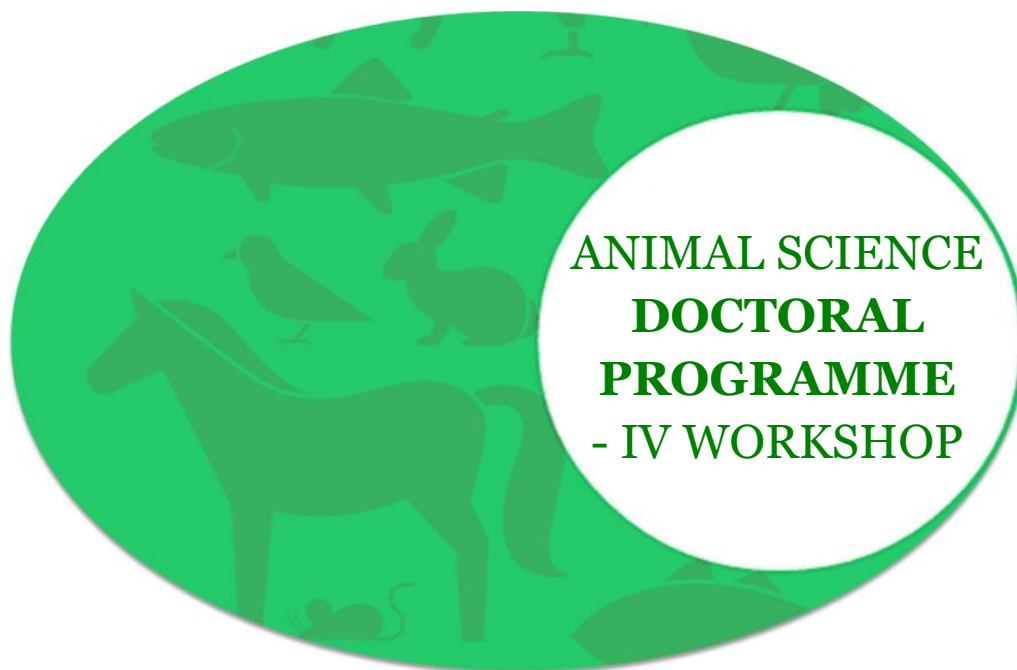




14th SEPTEMBER 2017

Salão Nobre, ICBAS-UP

Rua de Jorge Viterbo Ferreira n.º 228

**Porto,
PORTUGAL**

ANIMAL SCIENCE DOCTORAL PROGRAMME

- IV WORKSHOP

14th September 2017

Organization

António Mira Fonseca, *REQUIMTE & ICBAS*

Luísa Maria Pinheiro Valente, *CIIMAR & ICBAS*

Margarida Duarte Cerqueira Martins de Araújo, *ICBAS-UP*

Ana Margarida Batista Pereira, *SORGAL, Alltech, REQUIMTE & ICBAS-UP*

Inês Gomes Campos, *SORGAL, CIIMAR & ICBAS-UP*

Secretariat

Ana Margarida Batista Pereira, *SORGAL, Alltech, REQUIMTE & ICBAS-UP*

Inês Gomes Campos, *SORGAL, CIIMAR & ICBAS-UP*

Renato Barbosa Ferraz, *CIIMAR & ICBAS-UP*

Alexandra Marques, *CIIMAR*

Conference Chairman

Cláudia Serra, *CIIMAR*

Sónia Batista, *CIIMAR*

António Luis Mittermayer Madureira Rodrigues Rocha, *ICBAS-UP*

Sara Magalhães, *SORGAL & ICBAS-UP*

Jorge Dias, *SPAROS*

Jaime Piçarra, *IACA*

Personnel

Filipa Rocha, *CIIMAR*

Francisca Brito, *Algaplus, CIIMAR & ICBAS-UP*

Ana Basto, *CIIMAR*

Sara Moutinho, *CIIMAR*

Lourenço Ramos Pinto, *SPAROS, CIIMAR & ICBAS-UP*

João Albuquerque, *PREMIX, REQUIMTE & ICBAS-UP*

Luís Baião, *Sensetest, CIIMAR & ICBAS-UP*

Tiago Sá, *CIIMAR*

AGENDA

10:00 Participants reception

10:30 Workshop opening: Presentation of the 2017 topics for Animal Science Doctoral Programmes SANFEED (ICBAS) and AniSCI (ICBAS/UTAD)

Participants: **SANFEED Scientific Committee** (Luísa Valente, CIIMAR & ICBAS-UP; Rita Cabrita, REQUIMTE & ICBAS-UP; Júlio Carneiro, CIBIO & ICBAS-UP), **SANFEED Advisory Board President** (Jorge Dias, SPAROS), **AniSCI Programme Coordinator** (Rita Payan, CECAV-UTAD) & **PhD Student** (Inês Campos, SORGAL, CIIMAR & ICBAS-UP)

Session I – Chairmen: *Cláudia Serra* (CIIMAR) & *Sónia Batista* (CIIMAR)

11:00 Plenary session: Measuring farm animal welfare; What affects farm animal welfare (Luísa Valente, ICBAS-UP)

11:30 Essential trace element content of commercially available dry dog foods (Ana Margarida Pereira, SORGAL, Alltech, REQUIMTE & ICBAS-UP)

11:45 Non-invasive monitoring of long-term adrenocortical activity in the Iberian lynx (*Lynx pardinus*) (Alexandre Azevedo, IZW & ICBAS-UP)

12:00 Unique teleost marine catfish *Plotosus lineatus* osmoregulation strategy (Salman Malakpour, CIIMAR & ICBAS-UP)

12:15 Dietary inclusion of the histidine, threonine and tryptophan in fishmeal-free diets, improves immune status in gilthead seabream (*Sparus aurata*) juveniles at a short-term feed (Lourenço Pinto, SPAROS, CIIMAR & ICBAS-UP)

12:30 LUNCH & POSTER SESSION

Session II – Chairmen: *António Rocha* (ICBAS) & *Sara Magalhães* (SORGAL & ICBAS-UP)

14:15 Plenary session: Sustainability: Challenges and Opportunities for European Feed Industry (Jaime Piçarra, IACA)

14:45 Characterization of the Bísaro pig production system (Gustavo Paixão, CECAV-UTAD)

15:00 Replacing dietary fish oil with poultry fat in European seabass (*Dicentrarchus labrax*) juveniles: growth performance, tissue composition and lipid metabolism (Inês Campos, SORGAL, CIIMAR & ICBAS-UP)

15:15 Lipid nanoparticles: lysine carriers to bypass ruminal digestion in dairy cows (João Albuquerque, PREMIX, REQUIMTE & ICBAS-UP)

15:30 Inorganic and organic dietary trace elements: effect on *Dicentrarchus labrax* larvae growth and bone status (Michael Viegas, SPAROS, INVIVONSA & ICBAS-UP)

15:45 Round table discussion

Chairmen: *Luís Conceição* (SPAROS) & *Jaime Piçarra* (IACA)

Participants: *Suzanne Held*, *Cláudia Serra*, *António Rocha*, *Ingrid Van Dorpe* (PREMIX), *Ana Margarida Pereira* & *Denis Meehan* (REQUIMTE & ICBAS-UP PhD student)

16:30 Best poster and oral presentation awards

Jury: *José Costa Lima* (REQUIMTE, FFUP), *Maria Teresa Dinis* (CCMAR) & *Margarida Araújo* (ICBAS-UP)

16:45 PORT OF HONOR & WORKSHOP CLOSING SESSION

POSTERS

Ammonia and carbon dioxide concentrations in dairy-cattle houses of northwest Portugal, Ana Raquel Rodrigues (AGROS, CAVC, REQUIMTE & ICBAS-UP)

Health status in sole *Solea senegalensis* juveniles after supplementation with *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*, Bruno Reis (SPAROS, CIIMAR & ICBAS-UP)

Early life traits and growth variability of flounder in the Lima nursery (NW Portugal), Cláudia Mendes (CIIMAR & ICBAS-UP)

Effect of incremental levels of three microalgae (*Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp.) on rumen methane production and fermentation parameters using the in-vitro 24 hour batch technique, Denis Meehan (ICBAS-UP student)

Effects of rearing conditions on the composition and bioactive content of seaweed: potential applications as dietary supplements in the gilthead seabream (*Sparus aurata*), Francisca Brito (Algaplus, CIIMAR & ICBAS-UP student)

Development of a web-based tool to evaluate the income over feed cost on North-West Portuguese dairy farms, Isabel Santos (CAVC, REQUIMTE & ICBAS-UP)

Assessment of the best protein/lipid ratio in diets for *Paracentrotus lividus*, Luís Baião (Sensetest, CIIMAR & ICBAS-UP)

Effects of dietary supplementation of *Gracilaria* extraction fractions on growth performance, fillet quality, oxidative stress and immune responses in European seabass (*Dicentrarchus labrax*), Maria João Peixoto (CIIMAR & ICBAS-UP)

Improving growth performance of fish larvae through early nutrition, Maria João Xavier (SPAROS, CIIMAR & ICBAS-UP)

Methionine supplementation improves european seabass (*Dicentrarchus labrax*) inflammatory response and disease resistance against *Photobacterium damsela piscicida*, Marina Casimiro (SPAROS, CIIMAR & ICBAS-UP)

Isolation and characterization of elovl5 with c18 and c20 elongase activity in tambaqui (*Colossoma macropomum*), Renato Ferraz (CIIMAR & ICBAS-UP)

Microbiome in poultry: influence of nutritional factors, Vítor Silva (ICBAS-UP, FCUP & UTAD)

Effects of solid-state fermentation with Basidiomycetes fungi on grape stalks, Valéria Costa-Silva (UTAD-CECAV)

INVITED SPEAKERS

Luísa Valente – *Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto*



Luísa Maria Pinheiro Valente got a degree in Biology in the University of Porto in 1990, and a PhD in Biological Sciences in the Universidade de Trás-os-Montes e Alto Douro in 1999. She is an Associated Professor in ICBAS – University of Porto since 2003. She directs the Laboratory of Nutrition, Growth and Quality (www.ciimar.up.pt/lanuce) and is the scientific director of the Animal Science Doctoral programme in an Industrial setting (SANFEED - Sustainable Animal Nutrition and Feeding). She participated in many research projects (national/EU), including Co-operative projects (QREN/CRAFT), and has a close collaboration with industrial partners. She supervises and has supervised several Doctoral theses, mostly in collaboration with international partners. With over 100 publications in international journals (>1800 citations; h-index: 25) she is also a reviewer of several international journals. Main research fields: Animal Nutrition; Sustainable Aquaculture; Environmental impact; Muscle growth regulation and flesh quality; Sensorial analysis and omega-3 levels for human consumption; Epigenetics.

Jaime Piçarra – *Associação Portuguesa dos Industriais de Alimentos Compostos para Animais*



Jaime Piçarra, Agronomic Engineer specialized in Economical Agriculture, started working in 1985 on the Portuguese Ministry of Agriculture on Agriculture Information and Markets Analyses (SIMA) and on the Portuguese delegation to negotiate second step of our integration process in the EEC now EU. He followed all CAP reforms signed by Portuguese Presidency since 1992, having 30 years of expertise on Agriculture Markets and Livestock Sector. Jaime Piçarra is the Secretary General of IACA, Portuguese Feed Industry Association. He is the Vice-President of the Industrial Compound Feed Production Committee of FEFAC (European Feed Compounder Industry Association), coordinator of the FEFAC Task Force on CAP Reform and FEFAC representative in Civil Dialogue Group on Arable Crops of the European Commission and at International Aspects on Agriculture in DG AGRI. He also follows sustainable issues, in particular FEFAC strategy on Sustainable Soybean. He is the coordinator of the working group on Agriculture Policy and External Relations of FIPA (Portuguese Food and Drink Industry Federation) and FIPA delegate at the Competitiveness Committee in FoodDrinkEurope. He is the Chairman of the General Meeting of the Information Center on Biotechnology. He authored several articles on GMO, agricultural markets and Common Agriculture Policy and participant, as moderator or speaker in Conferences in Portugal, and an international level, related to these issues. FEFAC Honorary Member since April 2016.

ORAL COMMUNICATIONS

ANIMAL WELFARE

Luísa M. P. Valente*^{1,2}

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

² CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Av. General Norton de Matos s/n, 4050-208 Matosinhos, Portugal.

lvalente@icbas.up.pt

In recent years increased wariness concerning animal welfare has challenged the scientific community to improve the understanding of animal behaviour. If it is clear that there is a global need to increase food production driven by both the increasing world population and by the increasing consumption of animal proteins, it is also true that interest in veganism is raising. At the same time a number of animal welfare activists brings focus on important issues arising from animal use in all its forms: conditions of animals kept in captivity, wild species conservation and use of animals for production, scientific purpose and company.

EU animal welfare legislation (Directive 98/58/EU) establishes minimal criteria for protection of animals in captivity. EU legislation also sets welfare standards for their transport and conditions at the time of stunning and slaughter. In Portugal, and in particular in the University of Porto, besides EU legislation, local ethic committees provide guidance and established specific standards concerning the use of animals for academic and scientific purposes always following the 3Rs policy. All PhD students using animals have to follow a course on the use of animal for laboratorial purposes.

The evaluation of animal behavior has largely evolved in the last years and new methods are being considered to help improve animal living standards.

ESSENTIAL TRACE ELEMENT CONTENT OF COMMERCIALY AVAILABLE DRY DOG FOODS

Pereira AMB^{*1}, Matos E², Castanheira F³, Almeida A⁴ Baptista CS⁵, Segundo MA⁴,
Cabrita ARJ¹

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

²SORGAL, Sociedade de Óleos e Rações, Estrada Nacional 109 Lugar da Pardala, 3880-728 S. João OVR, Portugal

³Alltechaditivos – Alimentação Animal Lda., Parque de Monserrate – Av. D- Luis Sá nº9 – Arm. A, 2710-089 Abrunheira, Portugal

⁴UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 4050-313 Porto, Portugal

⁵CECA - ICETA, Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira, 4050-313 Porto, Portugal

anamargaridabp@hotmail.com

Abstract

Essential trace elements are found in minor concentrations in foods. However, their essentiality determines that their insufficient intake results on the occurrence of clinical symptoms. In order to meet dog nutritional requirements (NRC, 2006) and avoid illnesses, pet food is supplemented with trace element sources in amounts allowed by the EU regulation (EC No 1831/2003).

This study aimed to determine the content of essential trace elements (Fe, Cu, Mn, Zn and Se) in commercially available dry dog foods to verify the compliance with the nutritional requirements and with the maximum legal limits.

Twenty adult dog feeds from different brands, representing low, medium, premium and super premium market segments were mineralized through microwave assisted acid digestion. Afterwards, Cu, Mn, Zn and Se were determined by inductively coupled plasma spectrometry, while Fe concentration was measured using flame atomic absorption spectroscopy. The daily intake of each essential trace element was calculated using its determined food content and the daily food allowance recommended by the manufacturer. The percentage of trace element supplied as additives was based on the mandatory information provided by the manufacturer in the label (EC No 767/2009).

The results showed that, except for Se, in which 25% of the diets failed to ensure the dog requirements, all the other essential trace elements were provided in amounts above the nutritional requirements. Regarding the compliance with legal limits, 75% of the

analysed diets had a content of Cu, Zn and Fe above the maximum permitted level. The results also showed that the average percentage of trace elements supplied as food additives was $45 \pm 26\%$ for Fe, $53 \pm 13\%$ for Cu, $41 \pm 28\%$ for Mn, $49 \pm 16\%$ for Zn and $45 \pm 24\%$ for Se total content.

The excessive content of Cu, Zn and Mn with respect to the dog requirements suggests that supplementation is done regardless of the mineral content of the ingredients. Results also showed the non-compliance with the maximum permitted level of Zn and Cu. The measurement of the mineral profile of raw ingredients will be advisable to better comply with nutritional requirements and legal limits, to reduce production costs and, mostly important, to ensure the best trace element nutrition to dogs.

References

European Council. 2003. "Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition." In 1831/2003, edited by European Parliament and of the Council. Official Journal of the European Union.

European Council. 2009. "Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed." In, edited by European Parliament and of the Council.

NRC. 2006. Nutrient Requirements of Dogs and Cats (National Academies Press: Washington DC).

Acknowledgments

This work was financed by Project MinDog, funded by Portugal 2020, financed by the European Regional Development Fund (FEDER) through the Operational Competitiveness Program (COMPETE) - reference number 017616. Financial support from FEDER funds POCI/01/0145/FEDER/007265 and POCI/01/0145/FEDER/007728 and National Funds (FCT/MEC) under the Partnership Agreements PT2020 UID/QUI/50006/2013 and UID/MULTI/04378/2013 is also acknowledged. AM Pereira also thanks FCT, SANFEED Doctoral Programme, Soja de Portugal and Alltech for her PhD grant PD/BDE/114427/2016.

NON-INVASIVE MONITORING OF LONG-TERM ADRENOCORTICAL ACTIVITY IN THE IBERIAN LYNX (*LYNX PARDINUS*)

Alexandre C. Azevedo^{1,2*}, Liliana De Sousa¹, Katarina Jewgenow²

¹ ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira, 4050-313 Porto, Portugal

² IZW, Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

E-mail: ax.cazevedo@gmail.com

In 2002 the Iberian lynx (*Lynx pardinus*) population was reduced to less than 100 individuals in Spain, and extinct in Portugal. *In-situ* and *ex-situ* conservation projects have successfully initiated its recovery, but reintroductions, intensive management and monitoring of the species' and its habitat are still necessary to keep it from extinction. All of these actions can cause stress to both captive and wild animals, potentially influencing conservation success. The neuroendocrine stress response allows animals to cope with environmental change, but can also lead to pathological consequences. Cortisol is one of the most studied mediators of this response and has been used as an indicator of stress in many wild species. However, cortisol measurement in blood, saliva, urine or faeces offers only point values, and is subject to confounding by restraint and sampling methods. Following the recent demonstration of an increase in hair cortisol in Canada lynx (*Lynx canadensis*) after repeated ACTH injections (Terwissen et al., 2013) this study aims to validate a method to measure hair cortisol in the Iberian lynx. Hair cortisol measurement offers an integrated value of adrenocortical activity over a period of weeks to months and avoids confounding from sampling and restraint. However, in addition to the physiological validation obtained from the Canada lynx, this requires *a*) a species-specific analytical validation, *b*) the determination of normative patterns of variation within the species, and *c*) biological validation by assessing how hair cortisol varies in response to known stressors. Preliminary results of hair cortisol measurement in *n*=14 Iberian lynx indicate that hair cortisol levels in captive individuals may reflect an effect of captive management but more data and further validation are necessary to confirm this. Once validated, the combination of hair cortisol data with demographic and ecological datasets will help measure reintroduction success and the impact of conservation actions and environmental disturbance on reintroduced and wild Iberian lynx.

Terwissen CV, Mastromonaco GF & Murray DL (2013): Influence of adrenocorticotrophin hormone challenge and external factors (age, sex, and body region) on hair cortisol concentration in Canada lynx (*Lynx canadensis*). Gen Comp Endo, 194, 162-167.

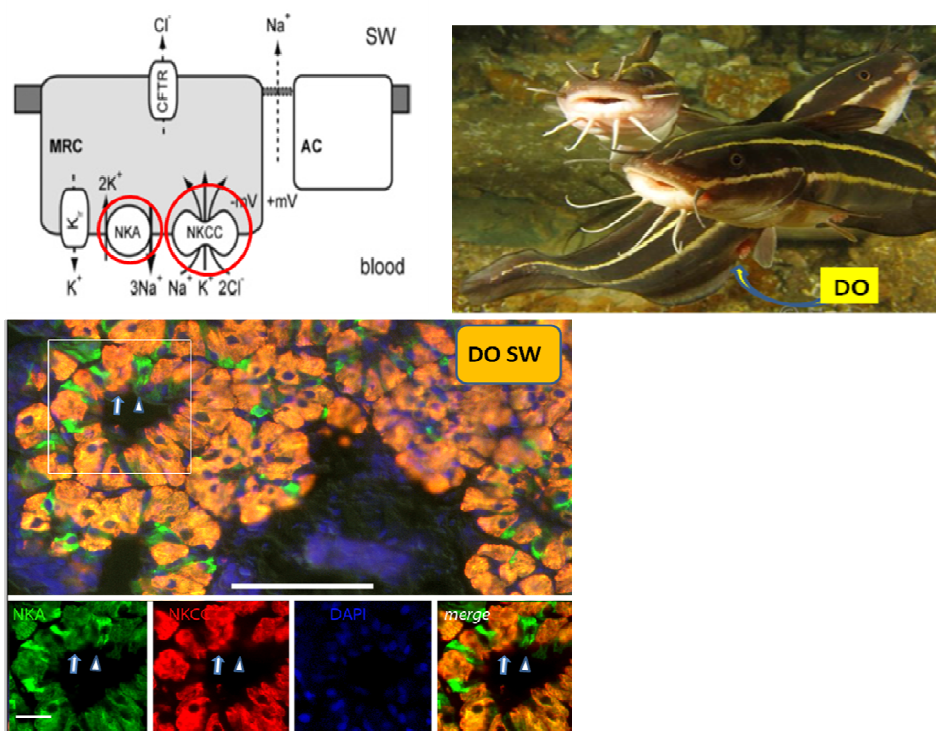
UNIQUE TELEOST MARINE CATFISH *PLOTOSUS LINEATUS* OSMOREGULATION STRATEGY

Salman Malakpour Kolbadinezhad* (CIIMAR, Porto, Portugal) and Jonathan Mark Wilson (CIIMAR, Porto, Portugal)
s.malakpour@gmail.com

Abstract

Unique amongst the teleosts euryhaline marine catfish *Plotosus lineatus* possesses the dendritic organ (DO) a specialized salt secreting organ, whereas other marine teleosts rely on their gill ionocytes. In the present study, the effects of different salinities [brackishwater (BW) 3‰, seawater (SW-control) 34‰, hypersaline water (HSW) 60‰] on Na^+/K^+ -ATPase (NKA) activity and $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ cotransporter (NKCC1) expression and DO ligation were investigated. At all of salinities, our results showed DO NKA activity was significantly higher than in gill, kidney; however, only the kidney showed elevated NKA activity with HSW. BW acclimation resulted in lower NKA activity in gill, kidney and DO. NKCC1 expression was high in DO but not detectable by immunoblot or immunohistochemistry in gill, kidney. Overall capacity (DO/body weight percentage $\times$ NKA activity) increased with salinity and strong NKA/NKCC1 immunolocalization was observed which was absent in gill ionocytes. In SW, DO ligation did not alter gill or kidney NKA expression. In BW, DO ligation only lowered gill NKA activity. In summary high NKA activity in DO elucidated the importance of the DO in ion-regulation while the compensatory responses of the gill and kidney were not detected in DO ligation. The convergent evolution of salt secreting organs in vertebrates by indicating a conservation of rather similar mechanisms of ion transporting via DO might be predictable by the observation of co-expressed NKA/NKCC1.

NaCl secretion by gill ionocytes



DIETARY INCLUSION OF THE HISTIDINE, THREONINE AND TRYPTOPHAN IN FISHMEAL-FREE DIETS, IMPROVES IMMUNE STATUS IN GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES AT A SHORT-TERM FEED.

L. Ramos-Pinto^{1,2,3*}, M. Machado^{1,2}, R. Azeredo^{1,4}, B. Reis^{1,2,3}, S. Fernandez-Boo¹, J. Pérez-Sánchez⁵, J. Caldach-Giner⁵, J. A. Martos-Sitcha⁵, R. Colen^{6,7}, D. Scharma^{6,7}, S. Engrola⁶, L.E.C. Conceição³, J. Dias³, A. Afonso^{1,2}, T. S. Silva³, B. Costas¹

¹Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.

E-mail: lourenco.pinto@ciimar.up.pt

²Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal.

³SPAROS Lda., Área Empresarial de Marim, Lote C, Olhão, Portugal.

⁴Departamento de Biologia, Faculdade de Ciência da Universidade do Porto (FCUP), 4169-007 Porto, Portugal.

⁵Institute of Aquaculture Torre de la Sal (IATS, CSIC), Ribera de Cabanes, Castellón, Spain.

⁶Centro de Ciências do Mar do Algarve (CCMAR), Ed. 7, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

⁷Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Introduction

In the last decade, many studies have focused on improving aquafeeds with the purpose of reducing the level of fishmeal (FM), replacing it with vegetable proteins. A major concern is that this can lead to feeds with imbalanced amino acid (AA) profiles, impairing feed conversion rates and immune response of farmed fish (Conceição et al. 2012). These deficiencies, often marginal, may be restored by supplementing feeds with crystalline AA to achieve a desirable metabolic efficiency, growth performance, and/or health status (Li et al., 2009). Histidine and its derivatives act as antioxidants or can mitigate the impact of oxidative stress (Bellia et al., 2008). Threonine is involved in many physiological and biochemical processes, including immune functions. Tryptophan is a versatile AA being a precursor to produce bioactive molecules and neurotransmitters such as serotonin, kynurenine and melatonin (Li et al., 2009), which affect the immune system. The present study aimed to explore the effects of the inclusion of a combination of these three essential amino acids on gilthead seabream growth and immune status, in the context of a challenging fishmeal-free basal formulation.

Materials and methods

Triplicate groups of 150 gilthead seabream (initial body weight: 13.3 ± 0.3 g) were randomly distributed in 1000-L tanks. Seawater was supplied at 2 L min^{-1} (temperature $23 \pm 2.6^\circ\text{C}$ and salinity 34 ± 0.7) in a flow-through system with artificial aeration (mean dissolved oxygen above 5 mg L^{-1}). Fish were hand-fed *ad libitum* twice a day to satiation for 92 days: CTRL, a control diet formulated in the context of an extreme formulation (0% fishmeal), meeting estimated AA requirements; HTW11, the CTRL diet with a 10% surplus of three essential AA that usually are not supplemented in practical diets (histidine, threonine and tryptophan); and HTW15, the CTRL diet with a 50% surplus of histidine, threonine and tryptophan. After 2 and 12 weeks of feeding, fish were sacrificed with a Tricaine methanesulfonate lethal dose. Samples of head-kidney and liver were collected and snap frozen in liquid nitrogen. The simultaneous profiling of a panel of thirty-two (in the liver) or twenty-nine (in the head-kidney) genes, considered as biomarkers of fish growth and health performance, were analysed using the Seabream PCR-array platform of Nutrigenomics group (<http://nutrigrp-iats.org>).

Results

There were no differences in final body weight (FBW) among treatments. However, there is a tendency for lower growth after 12 weeks in fish fed HTW15 diet, which may be linked to an increased feed conversion ratio (FCR) at 2 weeks in this same group. After 2 weeks of feeding, the hepatic gene expression of insulin-like growth factor-binding protein 1 (*IGFBP1*) was up-regulated in fish fed HTW15 compared to the CTRL group, which is in accordance with the growth performance trend in this treatment; while insulin-like growth factor 1 and 2 (*IGFR1* and *IGFR2*) were down-regulated. Moreover, calpain 1 (*CAPN1*) and *IGFBP2* decreased in fish fed HTW11, whereas insulin receptor (*INSR*) was up-regulated. In contrast, only three out of thirty-two genes analysed were modulated by supplemented feeds in fish fed for 12 weeks: *CTSB*, which plays a part in protein degradation/turnover and antigen presentation/processing is significantly up-regulated in fish fed HTW15; *PGC1a* and *UCP1*, both involved in mitochondrial activity, cellular energy needs/energy demands had two different expressions patterns, while *PGC1a* was down-regulated and *UCP1* was up-regulated in diet HTW15. Regarding head-kidney gene expression, several genes were up-regulated after 2 weeks of feeding, both interleukin 6 and 10 (*IL6* and *IL10*), cluster of differentiation 3 (*CD3X*) and the toll-like receptors 2, 5 and 9 (*TLR2*, *TLR5* and *TLR9*) transcripts increased in HTW15. Even if there were no differences in FBW and growth rate between treatments, there was a tendency of increasing FCR in fish fed HTW15.

Multivariate analysis of the gene expression patterns in liver and head-kidney confirms a consistent effect due to the different dietary treatments and, particularly in the case of head-kidney. The expression of the same set of genes after 12 weeks, revealed a down-regulation of all genes that vary significantly: *IL10* an anti-inflammatory cytokine, membrane *IgM* and *TLR1* in fish fed diet HTW15.

Discussion and conclusions

Analysis of growth and immune status biomarkers at an early stage (2 weeks after starting with the feeding trial) suggests that HTW15 may impair growth performance, even when this higher AA supplementation reveal an immune-stimulation. Therefore, the use of synthetic AA to balance diets with high or total FM replacement can be a promising approach to promote fish immune status. However, these beneficial properties appear to be reverted in a long-term basis. Although, the absence of an immune system challenge, such as a pathogen infection, may enlighten and reinforce the known AA capabilities of empowering fish immune system when subjected to a pathogen. Further innate immune parameters will be analysed from plasma and mucus samples, collected after the two sampling points, these data could clarify the role of these AA supplementation.

Acknowledgements

This work was supported by Project ALISSA (reference ALG-01-0247-FEDER-3520), financed by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020, and through the COMPETE and Operational Human Potential Programmes and national funds through FCT (Portugal). L. Ramos-Pinto, M. Machado, R. Azeredo, B. Reis, S. Fernández-Boo, S. Engrola and B. Costas benefited were supported by FCT, Portugal (PD/BDE/114436/2016, SFRH/BD/108243/2015, SFRH/BD/89457/2012, PD/BDE/129262/2017, INSEAFood/BPD/2016-028, IF/00482/2014/CP1217/CT0005 and IF/00197/2015, respectively).

References

- Bellia F, Amorini AM, La Mendola D, Vecchio G, Tavazzi B, Giardina B, Di Pietro V, Lazzarino G, Rizzarelli E (2008) New glycosidic derivatives of histidine-containing dipeptides with antioxidant properties and resistant to carnosinase activity. *European Journal of Medicinal Chemistry*, 43, 373-380.
- Conceição, LEC, Aragão, C, Dias, J, Costas, B, Terova, G, Martins, C, Tort, L. 2012. Dietary nitrogen and fish welfare. *Fish Physiology and Biochemistry* 38: 119-141.
- Li P, Mai K, Trushenski J, Wu G (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37, 43-53.

CHALLENGES AND OPPORTUNITIES FOR EUROPEAN FEED INDUSTRY

Piçarra, Jaime*¹

¹IACA, Associação Portuguesa dos Industriais de Alimentos Compostos para Animais, Av. 5 de Outubro 21 - 2º Esq, 1050-047 Lisboa, Portugal

jaime.picarra@iaca.pt

Abstract

Content of the Presentation

I want to present FEFAC and our organization in a few words and enter into our Challenges and Opportunities, regarding the evolution of livestock production in Europe and around the world, and Society demand and expectations. Today, all the issues are in discussion in the media, social network and scrutinized by everybody.

So, I'll take FEFAC Vision 2030 outline to 'categorise' the (future) innovation and Sustainable contributions. Innovation in animal nutrition science is the 'classic' innovation topic, but we have other contributions, to have a safer and sustainable feed. So, I'll focus the following issues:

- **Animal nutrition science**

- using nutrients more efficiently, thus reducing the environmental impact of livestock production, while improving the nutritional profile of livestock products to human consumers and safety status of feed to food producing farm animals and the ultimate consumers;
- enhancing the animal health and welfare status, thereby reducing the need for therapeutic treatment through preventive action

- **Sustainability**

- Resource efficiency indicators
- Environmental footprinting
- Responsible sourcing (Soy FEFAC Guidelines)

- **Feed safety management**

- Capacity building
- Optimisation of risk management in the feed chain
- Co-operation with authorities (QUALIACA in Portugal is a good example)

- **Processing technology**

- Will establish closer links to suppliers to optimise the information system on the safety, sustainability and nutritional value of feed ingredients;
- Will be able to integrate sustainability parameters in formulation;
- Will adapt techniques to check the integrity of deliveries;
- Will be able to adapt more instantly the composition of the diet taking into account the variability of composition of the feed material used (variability intra and inter lots);
- Will establish an interface for data communication with farms on different parameters measured on the farm (health status, temperatures, breed, technical performance, etc.) in order to develop customised feeding strategies based for example on animal health status and optimise the delivery of nutrients to animals.

At the end, I'll conclude: **we must build our case, our data, our work, together as a chain. Nobody will do it for us!**

Original Research Article

“CHARACTERIZATION OF THE BÍSARO PIG PRODUCTION SYSTEM”

Gustavo Paixão^{1,*}
Alexandra Esteves¹
Rita Payan-Carreira¹

¹*Animal and Veterinary Research Centre (CECAV), Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal*

*Corresponding author. Gustavo Paixão. E-mail address: gus.paixao@utad.pt

Introduction

Bísaro Pig (BP) is a native Portuguese breed known by its excellent meat quality. BP recovered from almost extinction over the last two decades and became a major trend amongst traditional pig producers. While BP production demand increases, little is known about its current production system. To enhance BP production capacity, maximizing profitability, there is a need to characterize the production system.

Material and Methods

Between February and April 2017, 194 questionnaires were sent, as a disclosed identity mail survey, to all active registered producers. The questionnaire consisted of six pages, covering five main topics: (1) Farm Manager/Owner Profile; (2) Farm Profile; (3) Feeding; (4) Housing; (5) Reproduction. It totaled 40 questions comprising short closed (n = 25) and semi-closed (n = 15) questions in a simple, clear format.

Descriptive statistical analysis was firstly performed. Subsequent comparative analysis was performed at farm category level, according to their exploratory regime of livestock activity: type-III (T-III) and type-II farms (T-II). Group differences were identified using the chi-squared test for categorical variables and ANOVA/Wilcoxon rank test for continuous variables.

Results

A 31% response rate was obtained. Piglets for slaughter represented the major production output (91%). Only few farmers (5%) claimed using solely commercial ration, and feeding is generally complemented or exclusively done with own farm crops: cereals (93%), vegetables (91%), tubers (89%) and fruits (52%). The majority of producers claimed differentiate feed per production group (79%) and allow grazing (74%). Traditional housing system was still the most common (53%) but free-range camping systems had a large expression (40%). All year-around farrowing system (91%) with no oestrous detection (65%) and scarce use of AI (13%) characterized the reproduction management. Males are normally castrated at 29 days (IQR: 9 - 35), before weaning at 35 days (IQR: 30 - 45). Significant differences were found between farm types: T-III farms (n = 33) resembled smallholdings, belonging to a single producer (p = 0.0351) and having family based work (p = 0.006), automation is absent (p = 0.0002) and management is predominantly less strict with some sows benefiting from voluntary waiting period (p = 0.0308), and castrations being done later (p = 0.0366); while T-II farms (n = 26) are commercial based farms, technological more advanced, having larger production (p = 0.0001) and greater farm areas (p < 0.0001).

Discussion

Young, less experienced and more educated farmers resumed an unexpected BP producers' demography. These trends could be partly explained by last decades' EU financial aid for young farmers to establish new holdings. General perception indicated that Bísaro was being produced mainly for cured meat production. Our findings, however, showed a considerable higher number of animals sold for roast piglet, usually after weaning, compared to animals sold for slaughter for fresh or cured meat. In fact, our estimate of BP meat production per year follows the same trend where piglets represented the most representative carcass weight produced comparing to other classes for slaughter. Farrow-to-finish systems still represent the majority of BP producers but a considerable proportion of producers exclusively breed BP for selling piglets. Traditional feeding where commercial ration is largely completed with own farm crops and products is still present on the majority of farms. Housing, however, seem to have changed over the last two decades and almost half of the producers have now their animals in permanent outdoor camping systems. Moreover, nearly all the producers have stated their animals have access to outside areas. Some of the practices though, revealed noncompliance with EU regulations, specifically those related with the age of castrations and feeding the animals with leftovers. Collected data evidenced some important differences between T-III and T-II, yet production traits and practices varied much between farms. This fact suggests a lack of organization between producers and might be in the origin of inconsistent products revealing an important weakness of BP production system.

Conclusions

Although the survey demonstrated individual variability amongst BP producers, Bísaro production system was characterized and two different types of farms were identified. This study provided the tools needed to discuss and revise some management practices in order to enhance consistency of Bísaro products.

REPLACING DIETARY FISH OIL WITH POULTRY FAT IN EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*) JUVENILES: GROWTH PERFORMANCE, TISSUE COMPOSITION AND LIPID METABOLISM

I. Campos*^{ab}, E. Matos^c, M.R.G. Maia^{de}, A. Marques^a, Luisa M.P. Valente^{ab}

^a CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208, Matosinhos, Portugal;

^b ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal;

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

^d REQUIMTE, LAQV, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal;

^e REQUIMTE, LAQV, DGAOT, Faculdade de Ciências, Universidade do Porto, Rua de Campo Alegre, S/N, 4169-007 Porto, Portugal.

Email: ines.campos@ciimar.up.pt

Introduction

Animal by-products can be processed into fats that are sustainable alternatives to fish oil (FO), due to their large availability and low price. When compared to FO, poultry fat (PF), obtained from poultry by-products, is richer in monounsaturated (MUFA) and saturated fatty acids (SFA) and poorer in polyunsaturated fatty acids (PUFA). This different fatty acid profile can impair digestibility (Campos et al. 2016), growth performance and final product quality. However, the high SFA and low PUFA content makes PF more resistant to auto-oxidation than FO (Watanabe, 1989) and can increase the shelf life of the fish fillet (Ahmad *et al.*, 2013). PF could be a novel and more sustainable lipid source for fish feed, reducing fishing pressure, the need for imported feedstuffs and contributing to a circular economy, reducing the economic and environmental burdens of aquafeeds.

The present study was conducted to assess the effects of replacing FO with PF on the nutrient utilization, growth performance, tissue composition and lipid metabolism in European seabass (*Dicentrarchus labrax*) juveniles. The inclusion of an emulsifying agent can help prevent impaired digestibility when using animal fats, so the addition of soy lecithin was also tested.

Materials and Methods

The PF used was obtained by rendering poultry by-products at SAVINOR (Portugal). Six extruded isoproteic (52% dry matter, DM) and isolipidic (19% DM) diets were formulated: a control, fish oil-based diet (FO), four diets with increasing levels of PF to replace FO – 25PF, 50PF, 75PF, 100PF (25, 50, 75, 100% replacement), and a diet with total FO replacement and 1% soy lecithin added as an emulsifying agent (100PFL).

Eighteen groups of 20 fish (initial weight 19.1 ± 2.6 g per fish) were kept in a recirculating saltwater system (salinity 35 ‰, 21 ± 1 °C) with a 12-h light/12-h dark photoperiod regime in 55 L fiberglass tanks. The experimental diets were randomly assigned to the tanks, in triplicate, and fish were fed to apparent satiety three times a day by automatic feeders for 16 weeks. At the end of the growth trial, fish were fasted for 12 h and individually weighed and measured. Plasma was collected from 6 fish per treatment at 2, 6, 12 and 24 h post-feeding to analyze the content of several metabolites (glucose, cholesterol, triacylglycerol and non-esterified fatty acids). Five fish from each tank were sampled for whole body composition. Muscle, liver, heart and viscera (intestine + visceral fat) were sampled from 15 fish of each treatment to analyze the total lipid content and fatty acid profile of these tissues. A portion of the collected livers was fixed and processed for histological analysis. The apparent digestibility coefficients of the experimental diets were also determined, according to Cho & Slinger (1979), after including 1% chromic oxide as inert marker.

Results

The experimental diets were well digested by fish and the inclusion of PF did not affect the digestibility of nutrients among diets ($p \geq 0.05$). Fish fed 50PF had the highest final body weight, but this was not significantly different from final body weight of fish fed FO ($p \geq 0.05$). No significant differences were obtained in feed intake, feed conversion ratio and protein efficiency ratio among experimental diets. Whole-body composition remained similar among dietary treatments: 34-35% DM, 17-18% protein, 13-14% fat and 9 kJ g⁻¹ energy. Nutrient gain and retention efficiencies were also similar among dietary treatments. The hepatosomatic index was highest in fish fed 100PF ($p < 0.05$); the liver of this fish also had the highest total lipid content and vacuolization degree (Fig 1). Liver glycogen content, however, decreased in fish fed 50PF, 75PF and 100PF in relation to those fed FO. Including soy lecithin had no effect on nutrient utilization, but significantly attenuated liver lipid content in relation to fish fed 100PF (26 vs 35%). The total lipid content of the dorsal muscle varied from 1.3 to 1.8% wet weight, and was significantly higher in the fish fed 100PF than in those fed FO or 25PF ($p < 0.05$). The fatty acid profiles of the analyzed tissues generally reflected dietary PF inclusion, leading to increased MUFA and decreased PUFA contents in dorsal muscle of fish fed 75PF and 100PF, and in ventral muscle and liver of fish fed 50PF, 75PF and 100PF. Heart fatty acid profile was less affected: higher MUFA content was observed in fish fed 100PF, and lower PUFA in those fed 75PF and 100PF, compared to fish fed FO. Plasma metabolites were similar for all treatments at 24 hours post-feeding.

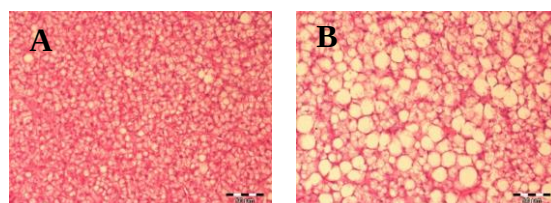


Figure 1 Liver sections of seabass either fed FO (A) or 100PF (B); H&E, 40x.

Discussion and Conclusion

This study shows that it is possible to totally replace FO by PF without impairing European seabass juveniles' growth performance and nutrient gain. However, total replacement of FO by PF clearly increased the deposition of lipids in liver. The inclusion of soy lecithin did not improve growth, but decreased liver fat content. The use of PF had an impact on tissue fatty acid profile but the dorsal muscle of fish fed up to 50% PF still met the recommended levels of EPA and DHA for human consumption. Higher replacement levels (75 and 100%), however, compromised flesh quality. A finishing FO-based diet would be recommended for a better final fillet quality.

Acknowledgements

This work was subsidized by Project ANIMAL4AQUA, funded by Portugal 2020, financed by the European Regional Development Fund (FEDER) through the Operational Competitiveness Program (COMPETE) - reference number 017610. I. Campos was financially supported by Fundação para a Ciência e Tecnologia, Portugal, and Soja de Portugal, through the grant PDE/BDE/113668/2015.

References

- Ahmad, W., D. A. J. Stone and K. A. Schuller (2013). "Dietary fish oil replacement with palm or poultry oil increases fillet oxidative stability and decreases liver glutathione peroxidase activity in barramundi (*Lates calcarifer*)."
Fish Physiology and Biochemistry **39**(6): 1631-1640.
- Campos, I., E. Matos, Luisa M.P. Valente, 2016. Fatty acids apparent digestibility of different animal fat sources in European seabass (*Dicentrarchus labrax*). AQUACULTURE EUROPE 16, Edinburgh, Scotland, 20 – 23 September 2016
- Watanabe, T. (1989). "Nutritive value of animal and plant lipid sources for fish." In: *Progress in Fish Nutrition*. Proc. Fish Nutrition Symp. (Ed.) Shiau, S.Y. Marine Food Science Series, 9:151-166.

LIPID NANOPARTICLES: LYSINE CARRIERS TO BYPASS RUMINAL DIGESTION IN DAIRY COWS

João Albuquerque ^{*1,2}, Ingrid V. Dorpe ³, Ana R. J. Cabrita ⁴, Salette Reis ¹, Susana Casal ⁵, Ana R. Neves ¹

¹ UCIBIO, REQUIMTE, Department of Chemical Sciences, FFUP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

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

³ PREMIX-Especialidades Agrícolas e Pecuárias. Lda, Parque Industrial II – Neiva, 4935.232, Viana do Castelo, Portugal

⁴ LAQV, REQUIMTE, ICBAS, UP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

⁵ LAQV, REQUIMTE, Laboratory of Bromatology and Hydrology, FFUP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

*joao.albuquerque.costa@gmail.com

Introduction

Feed digestion in ruminant animals largely relies on rumen microbial fermentation. The microbiota of the rumen extensively degrades dietary protein, the resulting products being incorporated into their own microbial proteins, while lipids pass through relatively unscathed. Since microbial protein digested in the small intestine is not sufficient to meet the amino acids requirement of high producing animals, an effective protection of feed N from rumen fermentation is needed. In the present study, we propose a new approach that takes advantage of nanotechnology to bypass the microbial fermentation in the rumen, but also to increase the absorption of the nutrients into the bloodstream, relying on lipid nanoparticles (NPs).

Results

A wide variety of different formulations were proposed and tested, consisting of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and multiple lipid nanoparticles (MLN). All these formulations were characterized in terms of size, polydispersity index and zeta potential and their stability in rumen inoculum assessed.

Results showed that only SLN formulations composed of stearic acid and arachidic acid could resist digestion in the rumen inoculum, maintaining their size range of 300-500 nm after incubation and with a highly negative surface charge of around -35 mV. Transmission electron microscopy images confirmed that both SLN could resist digestion and maintain both their size and spherical morphology.

The NPs were loaded with lysine and an adequate quantification method, based on high performance liquid chromatography (HPLC) with fluorescence detection of dansylated derivatives, was developed. Using this method, the encapsulation efficiency of the formulations was determined, rendering values of up-to 70%.

Conclusions

This preliminary study showed that SLN could be synthesized, with adequate physical properties, and with the ability to resist digestion in the rumen, retaining their size, zeta potential and morphology. They were also capable of being loaded with a highly hydrophilic molecule with high efficiency. The proposed formulations are, therefore, promising candidates for future rumen-bypass applications in ruminant nutrition and may help to surpass the current limitations of the existing technologies and products.

Acknowledgments

This work received financial support from FCT (Fundação para a Ciência e a Tecnologia) and FEDER funds under Program PT2020 (project 007728 -UID/QUI/04378/2013). JA thanks FCT, SANFEED Doctoral Programme and PREMIX® for his PhD grant ref. PD/BDE/114426/2016. ARN also thanks her Post-Doc grant under the project NORTE-01-0145-FEDER-000011.

Figures

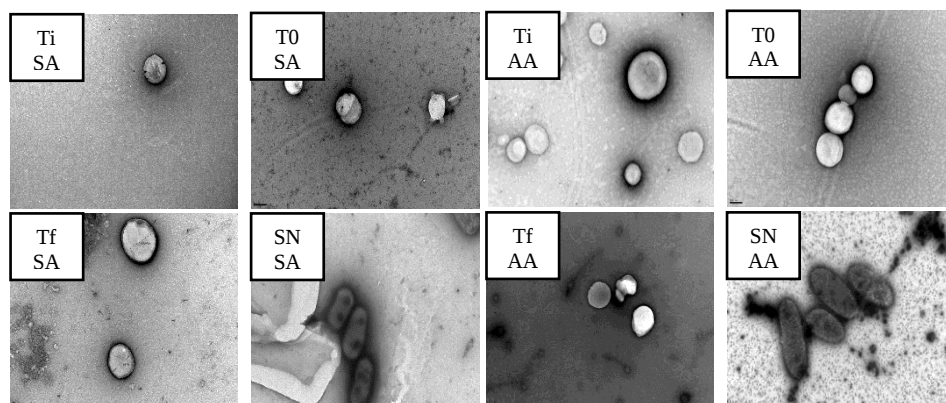


Figure 1 - Transmission electron micrographs of Stearic Acid (SA) and Arachidic acid (AA) NPs after synthesis (Ti), after contact with rumen inoculum (T0) and after 24h incubation at 39 °C in rumen inoculum (Tf). Supernatant (SN) after NPs separation from the rumen inoculum is also shown.

INORGANIC AND ORGANIC DIETARY TRACE ELEMENTS: EFFECT ON *DICENTRARCHUS LABRAX* LARVAE GROWTH AND BONE STATUS

Viegas, MN^{123*}, Aguiar, C², Salgado, MA³, Pinto, W¹, Gavaia, P⁴, Castanho, S⁵, Pousão, P⁵, Dias, J¹

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

² INVIVONSA Portugal S.A., Zona Industrial de Murte, 3060-372 Cantanhede, Portugal.

³ CIIMAR, ICBAS, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

⁴ CCMAR, Universidade do Algarve, Campus Gambelas, 8005-139, Faro, Portugal.

⁵ EPPO-IPMA, Aquaculture Research Station, Olhão, Portugal.

Contact: up201409164@icbas.up.pt

Abstract

In fish larvae and post-larvae dietary trace elements critically influence a wide range of physiological, metabolic and hormonal processes, such as survival, growth, feed utilization, skeletal formation, immune response and susceptibility to pathologies. Dietary trace element bioavailability is influenced by their chemical forms and the presence of anti-nutritional factors. Studies show that organically bound minerals may be more bioavailable than inorganic minerals for certain animal species. A 52-day feeding trial was undertaken with European sea bass (*Dicentrarchus labrax*) larvae to determine whether the inclusion of organically bound copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn) could improve early-stage fish survival, overall growth and incidence of commercially severe malformations.

Four dietary treatments were tested in this experiment. All dietary treatments were formulated to be isoproteic and isolipidic. One dietary treatment was formulated without a mineral-vitamin premix (PM Ø) and another containing a commercial mineral-vitamin premix (PM B). The two remaining diets were formulated with a mineral-vitamin premix (based on the composition of copepods) containing either the inorganic (PM I) or organic (PM O) form of Cu, Fe, Se, Mn and Zn.

European sea bass larvae with 18 days after hatching (DAH) originating from MARESA (Ayamonte, Spain) were randomly distributed into 300L cylindroconical tanks, at an initial density of 9 larvae/L, in triplicate, at EPPO-IPMA facilities (Olhão, Portugal). Larvae were co-fed using *Artemia* and experimental diets from 18 to 30DAH after which feeding was done solely with experimental feeds. Dry feed distribution was performed using automatic feeders. System water parameters were measured daily and adjusted to maintain temperature at 21±1 °C, oxygen saturation level above 90% and salinity at 36 g. L⁻¹.

European sea bass larvae were sampled at 50 and 70DAH to determine total length (TL), dry weight (DW), relative growth rate (RGR) and feed conversion rate (FCR). Incidence of commercially severe malformations (assessed by X-ray) and survival in each treatment was determined at the end of the experiment.

Results show no significant differences between dietary treatments for DW, TL, RGR or FCR. Regarding commercially severe malformations, fish fed the PM Ø diet presented a higher level of incidence (62%) when compared to fish fed diets containing mineral-vitamin premixes (32±1.5%). Expression of bone and immunity related gene markers were also assessed by qPCR.

Acknowledgments: MNV is supported by a PhD grant from FCT (PDE/BDE/113627/2015). This work is part of project 17925_LARVAMIX supported by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

ROUND TABLE

Round Table on
The Challenges of Sustainability on Nutrition in Animal Production
15h45 – 16h30

Members of the Discussion Panel

Chairman:

Luis Conceição (SPAROS)

Jaime Piçarra (IACA)

Participants:

Luisa Valente (CIIMAR & ICBAS-UP)

Cláudia Serra (CIIMAR)

António Rocha (ICBAS-UP)

Ingrid Van Dorpe (PREMIX)

Denis Meehan (REQUIMTE & ICBAS-UP PhD student)

Ana Margarida Pereira (SORGAL, REQUIMTE & ICBAS-UP PhD student)

1. Contextualization

- Luis Conceição, as moderator, agreed that the challenges of sustainability were mirrored in the plenary session by Jaime Piçarra and in the works presented by the animal science PhD students. A variety of sustainability challenges were presented, including environmental impact, replacement of antibiotics, determination of trace elements in animal feeds, improvement of production efficiency, among others. The interconnecting of the challenges was also highlighted.

2. Brainstorming

- Luis Conceição started by querying Ingrid Van Dorpe about the challenges in terms of micronutrients raised by the replacement of animal sources in swines and fishes.
- Ingrid Van Dorpe agreed that there are several paths for mineral supplementation and one of them are the choice of the sources: inorganic elements vs organic ones. The use of more bioavailable minerals sources benefit the animal (avoid metabolic overload) and the environment (lower excretion). But there are other strategies such as the use of ingredients with higher digestibility, which decreases the feces excretion (environmental benefit) and increases growth rates, reducing the use of antibiotics with a concomitant improvement of animals handling and increased sustainability.
- Luis Conceição queried Jaime Piçarra about the importance of providing a flattering picture of the feed industry, which includes not being associated with wastage.

- Jaime Piçarra highlighted the importance of economic sustainability (companies's viability) along with the already mentioned environmental sustainability. He mentioned the existence of agricultural funds available only to farmers who respect the law, which allow to implement standards of quality in the production and thus, the improvement of the offer. He agreed that consumers care about the image of the industry and demand sustainable production and high quality products. However, he also called everyone's attention to the trouble raised by the increased demanding of, for example, the so-called biological products, that may not be compatible with the most important requirement: the low price. He highlighted the importance of adequating the consumer's expectations and the trends of main supermarkets to what the industry can really offer. He suggested that one solution may be to let big distributions chains know the constraints that the industry is facing and therefore, slow down and give it time to adapt to new market challenges (namely demand of biologic products). The discrepancy between the quality standards of the European market vs other important markets was also referred as it may preclude the product trading. He also mentioned the importance of using science and nutrition precision to comply with market needs.
- Ingrid Van Dorpe highlighted that the increase of protein demand (associated with an increased money power) leads the markets to lower the price of protein sources to attract consumers, in such way that nowadays meat is even cheaper than bread, which has a much shorter life cycle.
- Jaime Piçarra agreed and added that this urges for stricter regulations for agro-food producers within the EU trading chain, and that countries should only be able to trade goods if they obey these regulations, or at least be taxed to compensate the environmental, animal welfare and food safety flaws they may commit.
- Luis Conceição added that, hopefully, Asian markets understood there are niche markets that demand the production of more sustainable products and are nowadays willing to adopt the standards of EU in order access these markets, which is beneficial for trading. He raised the discussion with the audience about the substitution of animal protein without competing with Human feeding, including the use of algae and microalgae or by-products and insect protein to feed animals in terms of regulation and EU policies, which also contributes to a functional circular economy.
- Sara Magalhães in the audience asked about the support EU is giving to producing local protein crops as alternative to animal protein and if in a real scenario it would be more profitable to invest in such crops or in sustainable soya production policies.
- Jaime Piçarra answered that EU supports the production of alternatives to meat protein, however he highlighted the importance of addressing the lack and the inconsistency of some policies, in order to establish the necessary conditions for more sustainable production at several levels. He mentioned that producing sustainable soya would, at least at short term, still be the safest bet, while the production of local protein crops must still be encouraged but more as a niche market, not to replace soya but to add value to certain products.
- Luisa Valente commented that the life cycle of soya includes long journeys from the place where it is produced (Brazil) to its destination (where it will be used as a feed ingredient), highlighting the massive ecological footprint. In that sense, she stresses the importance of looking to small/local productions and integrate them as a more sustainable alternatives. She also highlighted the importance of providing consumers with information regarding the ecological footprint of the products allowing them to have a conscious choice (protein from animal vs protein from soya).

- Ingrid Van Dorpe added the fact that planting protein crops also helps increase the environment sustainability as these plants help in nitrogen fixating, reducing the environment pollution.
- António Rocha highlighted the progresses science has made to value potential alternative sources of protein, for example, by producing ingredients without naturally-occurring anti-nutritional compounds.
- Denis Meehan agreed that it is necessary to copy with economic sustainability and added that the value of a product is defined by comparison with homonyms, thus, product prices influence each other. So if soya prices keep increasing as it has been happening, its substitution for microalgae protein may be possible in the future.

POSTERS

AMMONIA AND CARBON DIOXIDE CONCENTRATIONS IN DAIRY-CATTLE HOUSES OF NORTHWEST PORTUGAL

Ana Raquel F. Rodrigues^{*,a,d,e} Ana Gomes^b, Luís Ferreira^c, Ana R.J. Cabrita^a, Henrique Trindade^d, António J.M. Fonseca^a, José L.S. Pereira^{d,e}

^aREQUIMTE, LAQV, ICBAS, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 4050-313 Porto, Portugal

^bCooperativa Agrícola de Vila do Conde, Rua da Lapa, 4480-757 Vila do Conde, Portugal

^cAGROS UCRL, Lugar de Cassapos, 4490-258 Argivai, Portugal

^dCITAB, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5001-801 Vila Real, Portugal

^eESAV, Instituto Politécnico de Viseu, Quinta da Alagoa, 3500-606 Viseu, Portugal

Email: anaferodrigues@gmail.com

Greenhouse gas (GHG) and ammonia (NH₃) emissions from dairy-cattle production are a significant source of environment impacts (Milani et al., 2011). Environmental problems resulting from this activity are due to manure anaerobic decomposition, slurry storage and slurry spread in fields, from fertilisers and from ruminal enteric fermentation (Gerber et al., 2013). Besides, NH₃ and CO₂ exposure severely compromise animal health, namely ocular and respiratory problems. The recommended limits of prolonged exposure for animals are 7.6 mg m⁻³ (10 ppm) for NH₃ concentration and 4912 mg m⁻³ (2500 ppm) for CO₂ (CIGR, 2014). The Northwest (NW) Portugal is the largest dairy-cattle production region in mainland being responsible for more than 50% of total Portuguese milk production (Pereira and Trindade, 2015). Several strategies have been developed to minimise GHG and NH₃ emissions from cattle production and in order to achieve the Portuguese environmental limits and European directives it urges to evaluate the on-farm GHG and NH₃ emissions of dairy farms (Eckard et al., 2010, Joo et al., 2015, Mendes et al., 2017). The present study aims to evaluate the NH₃ and CO₂ concentrations in three naturally ventilated dairy buildings, located at NW Portugal and during the winter (December-2016) and spring (April-2017) seasons. Measurements were carried with photoacoustic infrared multigas monitor (INNOVA 1412) and air samples collected, in sequence (2 minutes intervals), through 6 sampling points located indoor by a multipoint sampler (INNOVA 1409). The NH₃ and CO₂ concentrations observed during winter and spring seasons are presented in Table 1. Ammonia and CO₂ concentrations in dairy-cattle farm 1 were lower during winter than during spring season, with a maximum of 39.4 mg m⁻³ and 3654 mg m⁻³ during spring and 8.7 mg m⁻³ and 1781 mg m⁻³ during winter, respectively. Ammonia concentration in dairy-cattle farm 2 had similar behaviour during winter and spring increasing in the first days of measurements, increasing in the middle and decreasing again at the final. At farm 2, the maximum concentration for NH₃ and CO₂ during winter was 12.6 mg m⁻³ and 2371 mg m⁻³ and during spring was 12.6 mg m⁻³ and 3891 mg m⁻³, respectively. Ammonia concentration in dairy-cattle farm 3 was lower during half time of measurements during spring compared to winter, increasing in the second half of measurements. Maximum value of NH₃ concentration during winter was 52.2 mg m⁻³ and during spring 14.2 mg m⁻³. Carbon dioxide concentrations were quite similar during the two seasons, with a maximum value <3200 mg m⁻³. The average NH₃ concentrations of dairy-cattle farm 1 and 2 were below to limits recommended for a prolonged exposure of animals, in

average dairy-cattle farm 3 had values above those recommended. Hence, for a good indoor air quality, the study suggests the use of mitigating measures for maintaining NH_3 concentrations below 7.6 mg m^{-3} . Concerning to CO_2 concentrations all dairy-cattle farms had values below the limits recommended.

Table 1 - Average, standard deviation, maximum and minimum of ammonia and carbon dioxide (mg m^{-3}) concentrations during winter and spring measurements.

		Ammonia (mg m ⁻³)			Carbon dioxide (mg m ⁻³)		
		Dairy-cattle farm					
		1	2	3	1	2	3
Winter	Average	0.4	2.3	2.1	910	1160	1601
	Standard deviation	0.4	1.5	1.3	80.1	205	212
	Maximum	8.7	12.6	52.2	1781	2371	3198
	Minimum	0.0	0.1	0.5	744	715	1004
Spring	Average	0.9	2.4	1.9	1076	1225	1317
	Standard deviation	0.9	1.4	1.2	198	302	234
	Maximum	39.4	12.6	14.2	3654	3891	3160
	Minimum	0.1	0.1	0.2	725	723	803

This work received financial support from the European Union (FEDER funds POCI/01/0145/FEDER/007265) and National funds (Fundação para a Ciência e a Tecnologia/Ministério da Educação e Ciência, FCT/MEC) and co-financed by FEDER (UID/ QUI/50006/2013 – NORTE-01-0145-FEDER-00011) under the Partnership Agreement PT2020. PhD grant of ARFR (PDE/BDE/114434/2016) is greatly acknowledge to FCT/MEC and POPH (Programa Operacional Potencial Humano).

References

- CIGR. 2014. The Design of Dairy Cow and Replacement Heifer Housing. Report of the CIGR Section II Working Group N° 14 Cattle Housing. International Commission of Agricultural and Biosystems Engineering (CIGR). ISBN 978-2-9552352-0-1, 60 pp.
- Eckard, R. J., C. Grainger, and C. A. M. d. Klein. 2010. Options for the abatement of methane and nitrous oxide from ruminant production: A review. *Livestock Science* 130:47-56.
- Gerber, P. J., A. N. Hristov, B. Henderson, H. Makkar, J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, A. T. Adesogan, W. Z. Yang, J. M. Tricarico, E. Kebreab, G. Waghorn, J. Dijkstra, and S. Oosting. 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. *Animal* 7 Suppl 2:220-234.
- Joo, H. S., P. M. Ndegwa, A. J. Heber, J. Q. Ni, B. W. Bogan, J. C. Ramirez-Dorronsoro, and E. Cortus. 2015. Greenhouse gas emissions from naturally ventilated freestall dairy barns. *Atmospheric Environment* 102:384-392.
- Mendes, L. B., J. G. Pieters, D. Snoek, N. W. M. Ogink, E. Brusselman, and P. Demeyer. 2017. Reduction of ammonia emissions from dairy cattle cubicle houses via improved management- or design-based strategies: A modeling approach. *Science of The Total Environment* 574:520-531.
- Milani, F. X., D. Nutter, and G. Thoma. 2011. Invited review: Environmental impacts of dairy processing and products: a review. *Journal of Dairy Science* 94(9):4243-4254.
- Pereira, J. and H. Trindade. 2015. Impact of the intensity of milk production on ammonia and greenhouse gas emissions in Portuguese cattle farms. *Spanish Journal of Agricultural Research* 13(4):e06SC05.

HEALTH STATUS IN SOLE *Solea senegalensis* JUVENILES AFTER SUPPLEMENTATION WITH *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*

B. Reis^{1,2,3*}, L. Ramos-Pinto^{1,2,3}, R. Colen⁴, S. Engrola⁴, L.E.C. Conceição¹, T. Silva¹, J. Dias¹, B. Costas^{2,3}

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

E-mail: breis@ciimar.up.pt

²Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.

³Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal.

⁴Centro de Ciências do Mar do Algarve (CCMAR), Universidade do Algarve, 8005-139 Faro, Portugal.

Introduction

Under intensive culture conditions, fish are potentially subjected to increased stress owing to environmental factors (water quality and hypoxia) and health conditions (parasites and infectious diseases). All these factors may have negative impacts on fish welfare and overall performance (Reverter et al., 2014). A strategy to improve fish disease resistance, is to supplement feeds with functional ingredients to achieve intended growth performance and improved health status (Chakraborty and Hancz, 2011). Both *Phaeodactylum tricornutum* and *Nannochloropsis gaditana* are microalgae rich in bioactive compounds such as fucoxanthin, astaxanthin, carotene and other valuable pigments but also polyunsaturated fatty acids (PUFA) particularly eicosapentaenoic acid (EPA) (Cerezuela et al., 2012; Gilbert-López et al., 2014). These compounds are paramount to promote optimal growth and health of farmed fish (Sargent, 2002). This study intends to evaluate the effects of dietary supplementation with *P. tricornutum* and *N. gaditana* incorporated in fish feed by two different processes, either as whole cells (integral cell wall) or broken cells (disrupted cell wall) on fish health status.

Materials and methods

Four isonitrogenous (52% crude protein) and isolipidic (15% crude fat) diets were formulated and supplemented with 1% (DM basis) inclusion of either *P. tricornutum* (diets A and B) or *N. gaditana* (diets C and D): diet A and C supplemented with microalgae whole cells and diet B and D using broken cells. Biomasses of *P. tricornutum* and *N. gaditana* used were produced by Fitoplancton Marino SL (El Puerto de Santa Martia, Spain) in photo bioreactors and processed at standard conditions of FITMAR. Diets were randomly assigned to duplicate groups of 18 Senegalese sole juveniles (initial body weight: 18.8 ± 1.1 g) that were fed to satiation twice a day for 128 days. Fish were assigned to raceway tanks (70×30×10 cm) in a seawater recirculation system. Seawater flow was kept at $2.7 \text{ L} \cdot \text{min}^{-1}$ (temperature $20.9^\circ\text{C} \pm 0.9$; salinity $34.6 \pm 1.2\text{‰}$) and dissolved oxygen always above $6.4 \text{ mg} \cdot \text{L}^{-1}$. At the end of the experiment, 4 fish per tank were sampled. The remaining fish were subjected to an inflammatory insult by intraperitoneally injecting Chinese ink and sampled following 4 and 24 h post-injection. Peripheral cell counts and several plasma immune parameters were determined during feeding and inflammation trials.

Results

Innate immune parameters such as peroxidase activity decreased from 0 to 24 h post-injection, whereas lysozyme activity showed no differences between dietary treatments regardless of sampling time. Nonetheless, protease and anti-protease activity varied between dietary treatments. Fish fed Diets A and B showed higher protease activity than fish fed Diet C. Fish fed Diet A showed the highest antiprotease activity compared to all other dietary treatments, in contrast to Diet D, which displayed the lowest levels.

Discussion and conclusions

Overall results showed an effect over time on peroxidase in plasma, with activity decreasing from 0 to 24h, which could be related to a decrease in circulating neutrophil numbers after migration to the inflammatory site (peritoneal cavity). Regarding fish plasma protease and antiprotease activity results showed a slight immune-modulatory effect exerted by *P. tricornutum* compared to *N. gaditana* supplemented diets. Nonetheless, further analyses are currently being carried out to ascertain a clearer and broader effect on fish immune response attributable to the different dietary treatments. Analyses of mucus, liver, head-kidney and haematology are crucial to obtain an overall picture of fish innate immune response to an inflammatory insult.

Acknowledgements

This work was partially supported by EU MIRACLES project (7th Framework Program - Grant Agreement No. 613588) and by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme, Operational Programme Human Potential and national funds through FCT – Foundation for Science and Technology, under the project UID/Multi/04423/2013. BR, LRP, SE and BC were supported by FCT (PD/BDE/129262/2017, PD/BDE/114436/2016, IF/00482/2014 and IF/00197/2015, respectively).

References

- Chakraborty, S.B., Hancz, C., 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture* 3: 103-119.
- Cerezuela, R., Guardiola, F., Meseguer, J., Esteban, M.A., 2012. Enrichment of gilthead seabream (*Sparus aurata* L.) diet with microalgae: effects on the immune system. *Fish Physiology and Biochemistry* 38(6): 1729-1739.
- Gilbert-López, B., Barranco, A., Herrero, M., Cifuentes, A., Ibáñez, E., 2016. Development of new green processes for the recovery of bioactives from *Phaeodactylum tricornutum*. *Food Research International* (In press). <http://dx.doi.org/10.1016/j.foodres.2016.04.022>
- Machado, M., Azeredo, R., Díaz-Rosales, P., Afonso, A., Peres, H., Oliva-Teles, A., Costas, B., 2015. Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. *Fish & Shellfish Immunology* 42: 353-36
- Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture* 433: 50-61.

EARLY LIFE TRAITS AND GROWTH VARIABILITY OF FLOUNDER IN THE LIMA NURSERY (NW PORTUGAL)

Mendes, C.^{1,2,3*}, Ramos, S.^{2,3}, Elliott, M.³, and Bordalo, A.A.^{1,2}

1 - Laboratory of Hydrobiology and Ecology, Institute of Biomedical Sciences, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal claudiavrmendes@gmail.com; Tel: (+351) 22 042 819 5

2 - Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Novo Edifício do Terminal de Cruzeiros do Porto de Leixões

Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

3 - Institute of Estuarine and Coastal Studies, University of Hull, Hull HU6 7RX, UK

The understanding of early life history traits is fundamental for fisheries management; those traits are major determinants of flatfish recruitment strength. Here, hatching, settlement, and growth patterns of 0-group flounder were investigated in the Lima Estuary, Portugal. The 0-group flounder were collected between June and October and major early life events as hatching and settlement dates, as well as the duration of pelagic and metamorphic stages were determined based on otolith microstructure analysis. The growth of the post settlement stage, and recent growth at the time of capture were estimated based on otolith increment widths. Results showed 0-group flounder hatched from March to June, with a peak in April and May. Settlement was from April to July, reaching a peak in May and June. The mean pelagic larval duration and metamorphosis duration were of 33 ± 3 and 15 ± 4 days, respectively, indicating that settlement occurred between 1.5 to 2 months after hatching. And mean otolith post-settlement and recent growth were 10.5 ± 2.0 and 10.4 ± 2.7 $\mu\text{m. day}^{-1}$, respectively. These growth rates indicated the high nursery quality of the Lima estuary and so this study helps to understand how early life characteristics can impact growth dynamics in nursery habitats, and hence recruitment variability.

EFFECT OF INCREMENTAL LEVELS OF THREE MICROALGAE (*Chlorella vulgaris*, *Nannochloropsis oceanica* AND *Tetraselmis* sp.) ON *IN-VITRO* RUMEN FERMENTATION

Denis J. Meehan¹, Margarida R.G. Maia¹, Ana R. J. Cabrita¹, António J. M. Fonseca¹

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

E-mail: den2mee2@gmail.com

Introduction

Microalgae are unicellular microorganisms (prokaryotic and eukaryotic) of varied size (typically 3-10 μm) which grow in salt or fresh water and account for 40% of global photosynthesis⁽¹⁾. Interest in the application of microalgae has ranged from their potential use in biodiesel production⁽²⁾ to being a source of bioactive compounds⁽³⁾ such as phycobiliproteins, long-chain polyunsaturated fatty acids and carotenoids, some of which have shown antimicrobial, antioxidant and anti-inflammatory capacities. Microalgae inclusion in diets for ruminant animals has been studied⁽⁴⁾, but more research is needed to evaluate the effects of different algae species, inclusion levels and interaction with the basal diet on rumen fermentation efficiency and animal performance. The objectives of this work were to examine the inclusion of three microalgae species (*Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp.) at four inclusion levels (0, 2.5%, 5% and 10% of substrate, dry matter, DM, basis) on: 1) the response in rumen methane production and fermentation parameters; and 2) the extent to which this response is influenced by basal substrate (hay silage or maize silage).

Materials and Methods:

All three microalgae (*C. vulgaris*, *N. oceanica* and *Tetraselmis* sp.) were sourced in Portugal (Allmicroalgae, Pataias, Portugal) and stored at room temperature under light-free conditions. Hay silage and maize silage were used as basal substrates in the *in-vitro* incubations. Substrates were dried at 65 °C for 48 h and ground to pass a 1 mm screen.

Rumen inoculum was obtained from one dry Holstein Friesian cow fitted with a rumen cannula (10 cm diameter, Bar Diamond Inc, Parma, ID, USA) and housed at the Vairão Agricultural Campus (Vila do Conte, Portugal). The cow was fed three different total mixed rations (TMR) comprising concentrate and different forages (maize silage plus cereal straw, hay silage, and a mixture of maize and hay silages). Each diet was fed over a 4 week period (fed twice daily at 0930 and 1700 h) with rumen inoculum collected on the last day. After each inoculum collection, the diet was switched and a new 4 week period continued until the next inoculum collection. Clean drinking water was provided at all times. Rumen incubations were made within 60 min of rumen inoculum collection.

Rumen fermentation parameters (methane production, total volatile fatty acid production and fermentation efficiency) were evaluated in short-term (24 h) batch incubations. Two hundred and fifty mg DM of each basal substrate (hay silage and maize silage) were added to 0% (control), 2.5%, 5% or 10% (DM basis) of each microalgae species, or a methane inhibitor (5 mM sodium 2-bromoethanesulfonate, BES; Sigma-Aldrich, St. Louis, MI, USA), resulting in a total of 11 dietary treatments. All treatments were incubated in triplicate in each experimental period. Each dietary treatment was anaerobically inoculated with 50 mL of buffered rumen inocula⁽⁵⁾ and incubated at 39 °C for 24 h. All 24 h fermentations were halted by cooling in an ice-water bath. Sampling and analysis were performed as described by Maia et al.⁽⁶⁾. Data were analysed using the MIXED procedure SAS software program (2002; version 9.1, SAS Institute Inc., Carry, NC). The model included the fixed effect of substrates (maize silage and hay silage), dietary treatments, and the interaction between substrates and dietary treatments, the random effect of trial and the random residual error.

Results:

The interaction between substrate and algae inclusion significantly affected almost all measured fermentation parameters. When incubated with hay silage, *C. vulgaris* did not affect gas production (mL) relative to control, independently of the inclusion level, while gas production increased when *N. oceanica* and *Tetraselmis* sp. were incubated regardless of the dose. With maize silage, algae inclusion did not affect gas production relative to the control, the highest values being observed for *C. vulgaris* included at 5 and 10% and *N. oceanica* at 5%.

When incubated with hay silage, and comparing with the control (0% algae), *C. vulgaris* (at all levels) and *Tetraselmis* sp. at 10% did not affect methane production (mL/g DM), the other treatments significantly increasing methane production. With maize silage, *C. vulgaris* (at all levels) did not affect methane production and *N. oceanica* at 5 and 10% while *Tetraselmis* sp. at 10% decreased it when compared to the 0% inclusion (control). Algae inclusion decreased total VFA production when incubated with hay silage, especially *C. vulgaris*, but with maize silage, *N. oceanica* at 2.5% was the only treatment significantly decreasing total VFA production. Acetic acid (%) was significantly higher for hay silage based treatments and lower with *C. vulgaris* (at all levels) and *N. oceanica* at 2.5 and 5%, regardless of the substrate. Propionic acid (%) was not affected by algae incubated with hay silage but *Tetraselmis* sp. incubated at 10% with maize silage significantly increased this VFA. Fermentation efficiency was not affected by algae inclusion.

The results showed that the studied microalgae have distinct effects on rumen fermentation and can play an important role in mitigating enteric methane production. However, selection of algae and inclusion level is clearly dependent on the substrate used.

Acknowledgement:

The authors would like to thank Allmicroalgae (Portugal) for their kind donation of the microalgae species.

References

1. Moreno-Garrido I (2008) Microalgae immobilisation: current techniques and uses. *Bioresource Technology* **99**, 3949-3964.
2. Yen F, Hu IC, Chen, CY (2013) Microalgae-based biorefinery from biofuels to natural products. *Bioresource Technology* **135**, 166-174.
3. de Moraes MG, Vaz BS, de Moraes EG, Viera Costa JA (2015) Biologically active metabolites synthesised by microalgae. *BioMed Research International*, 835761.
4. Costa DA, Quigley SP, Isherwood P, McLennan SR, Poppi DP (2016) Supplementation of cattle fed tropical grasses with microalgae increases microbial protein production and average daily gain. *Journal Animal Science* **94**, 2047-2058.
5. Mould FL, Morgan R, Kliem KE, Krystallidou E (2005). A review and simplification of the *in vitro* incubation medium. *Animal Feed Science and Technology* **123–124**, 155-172.
6. Maia MRG, Fonseca AJM, Oliveira HM, Mendonça C, Cabrita ARJ (2016). The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. *Scientific Reports* **6**, 32321.

Effects of rearing conditions on the composition and bioactive content of seaweed: Potential applications as dietary supplements in the Gilthead seabream (*Sparus aurata*)

Francisca Silva-Brito ^{1,2*}; Leonardo Magnoni ²; Anake Kijjoa ¹; Rui Pereira ^{2,3}; Helena Abreu ^{2,3}

¹ – ICBAS-Abel Salazar Institute of Biomedical Sciences, Universidade de Porto

² – CIIMAR- Interdisciplinary Centre of Marine and Environmental Research

³ – ALGAplus

*fbrito@ciimar.up.pt

The aquaculture industry has grown rapidly in recent years, thanks to an increase of intensive farming worldwide. The adaptation of fish to intensive rearing conditions is a critical factor, which may involve feeding targeted species suitable diets to attain enhanced growth, with improved health and welfare status [1]. In this sense micronutrients, namely mineral and vitamin, play a central role on fish homeostasis improving fish growth and their resistance to stress and diseases [2].

Commercial mineral and vitamin premixes are incorporated into aquafeed formulations to provide adequate levels of micronutrients for optimal growth and health [3]. However, the use of natural sources of minerals and vitamins that can be used to partially replace commercial premixes remain poorly explored in fish. This strategy may promote the sustainability of aquaculture, while promoting growth, health and improved welfare in fish [4].

Seaweeds and seaweed by-products (e.g. from phycocolloid extraction process) are a potentially abundant source of minerals and vitamins that can be used to improve aquaculture sustainability when incorporated in aquafeeds [3]. Also the inclusion of seaweed or their by-products in the fish diets could be an important source of bioactive compounds such as polyphenols and flavonoids as well as pigments to improve antioxidant defence and immune response [5]. These properties are related with mechanisms developed by seaweeds to protect themselves from environmental stresses, such as high UV radiation, increasing temperatures or air exposure.

Thus, for a given seaweed species, composition may depend on several factors, including the location and season [6]. In particular, cultured seaweed may display changes in composition, including content in micronutrients, depending on the rearing and processing methods [7]. Integrated multitrophic aquaculture (IMTA) systems use seaweeds as “biofilters” to remove organic and inorganic matter from fish farm effluents. Seaweed produced in these systems could prove to be an important source of micronutrients that could be incorporated into the fish dietary formulation [8].

Nevertheless, it remains unclear how the interaction of different environmental conditions, under in IMTA systems, could affect the micronutrient content and bioactive compounds of seaweeds. Furthermore, the potential use of seaweed extracts as sources of micronutrients and bioactive compounds when supplemented into a fish diet remained poorly explored, in particular in terms of their potential to improve antioxidant and immune responses in fish.

The gilthead seabream (*Sparus aurata*) is an ideal experimental model for studying the possible positive effects of dietary seaweed inclusion on growth, stress tolerance and resistance to fish diseases. This species is farmed on a large scale in Southern Europe and consumes seaweeds as part of its natural diet.

Thus, we propose to study:

- The effects of tank dimension and desiccation on the yield and the biochemical composition of the seaweed *Ulva lactuca*;
- The effects of tank dimension, crop density and desiccation on the yield and the biochemical composition of the seaweed *Gracilaria vermiculophylla*;
- The use of *U. lactuca* as a source of micronutrients in seabream diets and the potential effects on growth, digestibility, immune and antioxidant responses on this fish species;
- The effects of *Gracilaria vermiculophylla* extracts included in seabream diets on

growth, immune and antioxidant responses on this fish species;

The first aim of this study is to investigate the effects of the dimension of the tank and air exposure (desiccation) on biomass yield and biochemical composition of *U. lactuca* cultured in a IMTA system. Culture conditions will be applied in an outdoor seawater recirculation system tanks at the ALGAplus and tested in triplicate. Culture in tanks with three different depths (50, 100 and 150 cm) will be applied and the seaweeds will be sampled over 16 days to calculate biomass yield and to analyse composition (protein, lipid and carbohydrate, mineral, vitamin and pigment contents). As for the desiccation trial, seaweeds will be exposed to air for 3 h and sampled 1 and 3 h after the treatment. Changes in seaweed composition will be analysed.

The second aim of this study is to investigate the effect of depths (50, 100 and 150 cm), crop density (0.1, 0.3 and 0.5, g fresh weight l⁻¹), and desiccation on biomass yield and biochemical composition of *G. vermiculophylla* cultured in an IMTA system. Experiments will be carried out under continuous outdoor IMTA conditions at ALGAplus and tested in triplicate over 16 days. At the end of this trial, biomass yield will be calculated and changes in composition will be analysed.

The third aim is to evaluate the effect of the *U. lactuca* as a source of micronutrients to partially replace mineral-vitamin premix in seabream diets. Thus three different diets, including a control diet, will be formulated containing different proportions of premix replaced by *U. lactuca*. The experimental diet will be tested using 6 replicas of each experimental diet for 14 days. Digestibility of the experimental diets will be evaluated, as well as potential changes in seabream's flesh quality. Additionally, the response of fish to a stressor (crowding) will be investigated in seabream fed the experimental diets.

The fourth aim is to study changes in the antioxidant capacity and immune response of seabream fed diets supplemented with *G. vermiculophylla* extracts. For this objective, five experimental diets will be tested, including control or supplemented diets with seaweed extracts. Seabream will be fed the experimental diets for 60 days. After the growth trial two groups from each experimental diet will be subjected to a biotic challenge (bacterial exposure below the LD50) including proper controls. Immune and antioxidant responses will be studied in seabream.

In conclusion, this work intends to investigate the nutritional value of seaweeds and seaweed by-products (extracts) as potential sources of micronutrients and functional ingredients when included into fish diets to enhance the sustainability and quality of aquatic products.

References:

1. Ashley, P.J., *Fish welfare: Current issues in aquaculture*. Applied Animal Behaviour Science, 2007. **104**(3–4): p. 199-235.
2. Oliva-Teles, A., *Nutrition and health of aquaculture fish*. Journal of Fish Diseases, 2012. **35**(2): p. 83-108.
3. Lim, C. and C.D. Webster, *Nutrition and fish health*. 2001: Food Products Press New York.
4. Conte, F., et al., *Consumers' attitude towards fish meat*. Italian Journal of Food Safety, 2014. **3**(3).
5. Devi, G.K., et al., *In vitro antioxidant activities of selected seaweeds from Southeast coast of India*. Asian Pacific journal of tropical medicine, 2011. **4**(3): p. 205-211.
6. Lordan, S., R.P. Ross, and C. Stanton, *Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases*. Marine drugs, 2011. **9**(6): p. 1056-1100.
7. Sharma, R., G.P. Singh, and V.K. Sharma, *Effects of culture conditions on growth and biochemical profile of *Chlorella vulgaris**. Journal of Plant Pathology & Microbiology, 2012. **2012**.
8. Abreu, M.H., et al., *IMTA with *Gracillaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system*. Aquaculture, 2011. **312**: p. 77-87.

DEVELOPMENT OF A WEB-BASED TOOL TO EVALUATE THE INCOME OVER FEED COST ON NORTH-WEST PORTUGUESE DAIRY FARMS

Isabel M. L. Santos^{1, 2*}, Isabel C. Ramos², Ana C.M. Gomes², Joaquim P. Nunes², Ana R. J Cabrita¹, António J. M. Fonseca¹

¹ REQUIMTE/LAQV, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira, n° 228, 4050-313 Porto, Portugal.

² CAVC, Cooperativa Agrícola de Vila do Conde, Rua da Lapa, 293, 4480-757, Vila do Conde, Portugal.

E-mail: isabelmlsantos@outlook.pt

Generally, milk price has been falling in the EU, and particularly in Portugal, due to the end of the EU milk quota regime (31st March 2015), the Russia embargo on Western food imports, the reduction of milk consumption and the growth of the world dairy production. In this context, it is essential to monitor indicators of the farms profit, such as income over feed costs (IOFC), as feed comprises the greatest expense for milk production.

In Portuguese Norwest farms feed costs represent 40-60% of the total production costs, so, to ensure farm profitability, producers need to know their actual feed costs in order to better define strategies to adapt their feed program in response to market volatility.

The majority of the producers focus on increasing total milk production, because this is reflected in an increased total milk income for farmer. With the volatility in feed and milk markets, income over feed cost (IOFC) is a more advantageous measure of profit than simply feed cost per cow (Buza, 2014). The income over feed costs is scientifically recognised as a good indicator of a dairy's farm profitability. It reflects the money that is left, after accounting for lactating cows feed costs, to pay the other farm expenses.

Contrary to other countries, the use of benchmarking is not usual in the Portuguese dairy system. However, this tool aim to improve the decision-making process on the farm, which influences directly farm profitability, namely at the feed component level. To help our farmers monitor their IOFC in a monthly basis, we propose a web-based tool to determine actual feed cost per cow and their IOFC, and compare it to its own farm in the past two years and with other farms of the group, as a benchmarking system. This provides a guide of how efficiently the feeding program is working for the amount of milk produced. Producers can use this information to set strategies and goals and monitor if a management decision implemented has positive results or not. The system also gives a benchmark range to each farm and for each month, using a high and low limit, calculated from the farm average production and feed costs, as recognised by the Pennstate extension group. If IOFC consistently falls below the low range, producers should examine the nutrition and feeding management of the dairy operation. Some areas to investigate are dry matter intake efficiency, use of excessive feed additives, and level of concentrate feeding, just to name

a few. Conversely, if IOFC is consistently at or above the high range, the operation is doing a good job of converting feed to milk (Bailey, 2009).



Figure 1: Web-based tool of IOFC benchmarking system in Cooperativa Agrícola de Vila do Conde farms.

Conclusion

The development of a web-based tool to dairy farmers, that allows them to benchmark their results against other farms, is very important for the future sustainability of dairy production. Getting farmers to know their feed costs and their margin results every month, can significantly affect their profitability results, and support their day to day management decisions, adapted to the reality of price volatility on the dairy feed market. This web based tool is now being developed on other management areas of the dairy farms, such as reproduction and animal health.

References

- Buza, M.H.et al.: Evaluating the effect of ration composition on income over feed cost and milk yield. In Journal of Dairy Science, Volume 97, Issue 5, 3073 – 3080. 2014
- Bailey K. et al, Dairy Risk-Management Education: Managing Income Over Feed Costs; The Pennsylvania State University, 2009.

ASSESSMENT OF THE BEST PROTEIN/LIPID RATIO IN DIETS FOR *PARACENTROTUS LIVIDUS*

Luís Baião^{1,2*}, Filipa Rocha¹, Tiago Sá¹, Luísa M.P. Valente^{1,2}

¹ ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

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

*corresponding author: lbaiao@ciimar.up.pt

Keywords: sea urchin, gonads, diets, characterization

Abstract: In the Mediterranean sea, the sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to its flavor and texture. The increasing market demand for sea urchin roe has resulted in the depletion of wild stocks (Sartori *et al.*, 2016). For this reason, the interest in sea urchin aquaculture has widened over the last decades. This study was designed in order to identify a diet able to promote sea urchin growth whilst assuring gonad development under a recirculating aquaculture system (RAS). Four extruded diets were formulated with two protein and two lipid levels: high protein (51%, HP) and low protein, (30%, LP), and high lipid (11%, HL) or low lipid (6%, LL) levels. The growth trial was conducted in the Fish Culture Experimental Unit of CIIMAR, Porto, Portugal with adults of *P. lividus*, collected in an intertidal zone along the Praia Norte in Viana do Castelo, Portugal. After acclimatization, sea urchins were individually weighed (g) and measured (test diameter, cm) and twelve homogeneous groups of 15 individuals (initial weight 34.92 ± 0.66 g per urchin; diameter 4.48 ± 0.02 cm) were randomly distributed by plastic mesh cages (45 urchins/m²) within 250 L tanks in a saltwater recirculation system (salinity 35‰, 18°C water temperature) with a 12 h light:12 h dark photoperiod regime. Each one of the four experimental diets were randomly assigned to triplicate tanks and fed every two days. The uneaten feed was carefully collected and weighed. The experiment was conducted for 15 weeks.

At the end of the trial all sea urchins were weighed and measured. Final body weight remained similar among dietary treatments: 42-43 g. No significant differences were obtained in specific growth rate, protein intake and protein efficiency ratio among experimental diets. Increasing dietary protein level significantly decreased dry matter and energy intake, and increased feed conversion ratio (FCR). The gonadal somatic index (GSI) was generally high (around 20%) and remained similar among dietary treatments.

This study shows that is possible to grow sea urchins in a RAS at 18°C. The growth was similar between dietary treatments, however, the HP diet presented lower FCR. Future studies are required to evaluate the nutrient utilisation in the tested diets in order to identify the most adequate protein and lipid level for this species. It is also known that the economic viability and market acceptability strongly depends on the development of high-quality sea urchin gonads. So, it will be also important to evaluate gonad quality to understand the feasibility of this diets to produce gonads that are well accepted by consumers.

Acknowledgements: This work was supported by the Structured R&D&I Project INNOVMAR - Innovation and Sustainability in the Management and Exploitation of Marine Resources (ref. NORTE-01-0145-FEDER-000035) within the research line "INSEAFOOD - Innovation and valorization of seafood products: meeting local challenges and opportunities", founded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF).

References:

Sartori, D., Pellegrini, D., Macchia, S. & Gaion, A. (2016). Can echinoculture be a feasible and effective activity? Analysis of fast reliable breeding conditions to promote gonadal growth and sexual maturation in *Paracentrotus lividus*. *Aquaculture* 451: 39-46.

EFFECTS OF DIETARY SUPPLEMENTATION OF *GRACILARIA* EXTRACTION FRACTIONS ON GROWTH PERFORMANCE, FILLET QUALITY, OXIDATIVE STRESS AND IMMUNE RESPONSES IN EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*)

Maria J. Peixoto^{1,4}, Leonardo J. Magnoni^{1,2}, José F. M. Gonçalves^{1,4}, Robert H. Twijnstra³, Anake Kijoa^{1,4}, Rui Pereira⁵, Arjan P. Palstra^{3,6}, Rodrigo O. A. Ozório^{1,4*}

¹CIIMAR, 4450-208, Matosinhos, Portugal; ²IIB-INTECH, 7310 Chascomús, Argentina; ³WMR, Wageningen University, 4401 NT Yerseke, The Netherlands; ⁴ICBAS, Universidade do Porto, 4050-313 Porto, Portugal; ⁵ALGAPLUS, Lda., 3830-196 Ílhavo, Portugal; ⁶WLR, Wageningen University, 6708 WD Wageningen, The Netherlands. E-mail: maria.peixoto@ciimar.up.pt

Introduction

The by-products from seaweed (SW) industry generated from the extraction processes have potential application as feed supplements for aquafeeds. SW extracts contain natural pigments and compounds with antioxidant and immune-stimulant properties. In spite of their beneficial effects, SW extracts may contain compounds with anti-nutritional effects when supplemented in diets, negatively affecting fish growth performance, welfare and ultimately fillet quality. The current study appraised the effects of two methanolic fractions extracted from the red seaweed (*Gracilaria* sp.), supplemented in European seabass (*Dicentrarchus labrax*) diets. Three experimental diets were prepared: (i) a control diet without supplementation (CTRL), (ii) a control diet supplemented with the *Gracilaria* methanolic extract (GE, 0.5%), or (iii) a control diet supplemented with the insoluble residue of the extraction (GR, 4.5%). After 42 days of feeding, the growth performance, digestive enzymes activities, fillet pH and color, skin color, and immune and oxidative stress responses were analyzed.

Material and Methods

The red seaweed *Gracilaria* sp. was produced and supplied by ALGAPLUS (Ílhavo, Portugal). *Gracilaria* sp. was previously washed, dried and the resulting powder material was percolated with methanol, at room temperature, until exhaustion. The methanolic solution was evaporated, under reduced pressure, to give a crude methanol extract.

Diets were isoproteic (50% crude protein), isolipidic (20% crude lipid) and isoenergetic (23 KJ.g⁻¹ gross energy). Seabass were daily fed for 42 days and the feeding schedule was daily adjusted for expected growth and detected mortality, with a feeding level set at 2% (DM) of average body weight. The physical-chemical parameters of the rearing water were set to: O₂ level (85±10 % saturation), pH (7.9±0.6), temperature (20.4±0.6 °C) and salinity (30 ppm). Total ammonium and nitrates maintained at residual levels. The photoperiod was set for 12 h light:12 h dark.

At the end of the feeding trial, fish were weighted and blood, liver and intestine were sampled. Fillet were sampled for color and pH measurements. Several fish performance parameters were calculated (Weight gain, feed conversion ratio, hepatosomatic index). Specific activity of the digestive enzymes α -amylase, lipase, trypsin and chymotrypsin were measured in whole intestine homogenates. Fillet quality analysis was performed based on pH and flesh color measurements, once per day for a period of seven consecutive days. Additionally, at day seven, skin color measurements were performed based on the same methodology as fillet color. Oxidative stress biomarkers were determined in liver homogenates. Immune status was assessed through the determination of key innate immune parameters, namely lysozyme, peroxidase and the alternative complement pathway levels.

All data was tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). The analysis of variance was performed applying One-Way-ANOVA test using the dietary treatments as dependent variable. Significant differences were considered when $P < 0.05$. As for fillet characteristics, Mann-Whitney U tests were used for the statistical analysis of the L*, a* and b* color measurements and ANOVA for differences in pH.

Results

Juvenile seabass grew up to 2.5-fold from initial weight in 42 days of feeding trial. Weight gain (WG: 22.2-24.7 g), daily growth index (DGI: 2.0-2.1% BW day⁻¹), voluntary feed intake (VFI: 1.9-2.0% BW day⁻¹) and feed conversion ratio (FCR: 0.8-0.9), showed no differences between groups, regardless of the dietary treatment.

Seabass showed no apparent differences in amylase, lipase, trypsin and chymotrypsin activities between the dietary treatments ($P > 0.05$).

No significant differences were observed on fillet pH and L* value between the dietary groups. Fish fed GE diet showed significant differences in b* value ($P = 0.028$). The fillet a* value ($P = 0.026$) of the anterior measurement differed between GE and CTRL groups. GR group had a* value of posterior measurement significantly different ($P = 0.018$) from CTRL group on day 3. Skin color measurements on day 7 revealed significantly higher a* ($P = 0.012$) and L* ($P = 0.020$) values in fish fed GE diet when compared with fish fed the CTRL diet. Fish fed GR diet had a significant higher a* ($P = 0.035$) when compared to fish fed CTRL diet.

Lipid peroxidation levels, catalase, glutathione peroxidase, glutathione reductase and total glutathione did not vary between the dietary treatments ($P < 0.05$).

Seabass fed GE diet showed higher alternative complement activity (ACH50) when compared to the CTRL diet ($P = 0.042$). Plasma peroxidase and lysozyme did not vary among the dietary treatments.

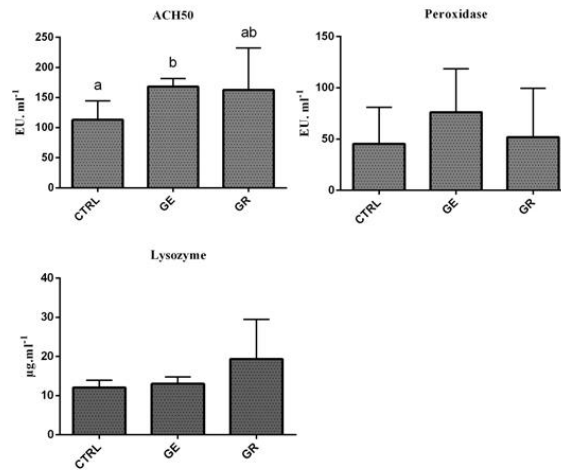


Fig. 1. Alternative complement pathway (ACH50), peroxidase and lysozyme activities analyzed in plasma from seabass fed the experimental diets for 42 days. Presence of letters indicates significant differences between treatments ($P < 0.05$) for $N = 9$ fish per treatment.

Discussion

Results revealed that both GE and GR had no apparent negative effect when supplemented in seabass diets, as no differences for growth performance, digestive enzyme activities, fillet pH, and oxidative stress biomarkers are observed after a 42-day feeding trial. Therefore, this study showed that seabass can be fed diets including *Gracilaria* sp. methanolic extraction products with no negative impacts on zootechnical, biochemical or quality parameters. However, dietary supplementation with SW extraction products produces significant differences in the fillet color. In addition, changes in the alternative complement pathway may suggest that innate immune parameters may be stimulated by dietary supplementation with SW extraction products.

METHIONINE SUPPLEMENTATION IMPROVES EUROPEAN SEABASS (*Dicentrarchus labrax*) INFLAMMATORY RESPONSE AND DISEASE RESISTANCE AGAINST *Photobacterium damsela* piscicida

M. Machado^{1,2,3*}, F. Fontinha^{1,3}, R. Azeredo^{1,5}, S. Fernandez-Boo¹, L. Ramos-Pinto^{1,3,4}, L.E.C. Conceição⁴, J. Dias⁴, N.M.S. dos Santos^{2,6} and B. Costas^{1,3}

¹ Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal. E-mail: mcasimiro@ciimar.up.pt

² Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.

³ Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal.

⁴ Sparos Lda, Area Empresarial de Marim, Lote C, Olhão, Portugal.

⁵ Departamento de Biologia, Faculdade de Ciências da Universidade do Porto (FCUP), 4169-007 Porto, Portugal.

⁶ Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.

Introduction

A dependency of the immune system upon the availability of amino acids (AA) is associated to their role as signalling molecules essential for cellular function (Li et. al., 2009). Methionine is an example of an indispensable AA with recognized roles in the immune system, in the methylation of DNA and proteins, in the synthesis of the polyamines, and also participates in processes involved in the control of inflammation. Therefore, the potential immunomodulatory role of methionine during infection deserves further attention, and more in-depth studies are needed to understand the immune mechanisms that this particular AA is activating. From a practical perspective, the role of methionine as functional additive in aquafeeds still needs to be explored and several studies have reported that AA deficiency may compromise the immune system repertoire (Li et. al., 2007). Moreover, methionine supplementation was able to increase the European seabass (*Dicentrarchus labrax*) cellular innate immune status and inflammatory response (Machado et. al., 2015). Accordingly, the present study aimed to evaluate the effects of both dietary methionine deficiency and supplementation on the European seabass inflammatory response and disease resistance.

Material and methods

European seabass weighing 8.5 ± 0.7 g were maintained in a recirculated seawater system (Temperature: 24 ± 0.5 °C; Salinity: 35; Photoperiod: 10h dark: 14h light). Fish were distributed into 12 tanks (200 l; n=50) and triplicate groups of these fish were fed four experimental diets: a control diet (CTRL) formulated to meet the European seabass AA requirements, and two other diets, identical to the CTRL but supplemented with methionine in 0.5 or 1 % (dry matter basis, respectively: diets Met0.5 and Met1). A negative control diet (NCTRL) was also formulated to be deficient in methionine. After a four weeks feeding period, fish were i.p. injected with *Photobacterium damsela* piscicida (Phdp, 5×10^4 cfu / ml). Six fish per tank were sampled after 4, 24 and 48 hours, whereas the remaining fish were maintained during 3 more weeks and mortality was recorded. For all sampling times fish were sacrificed by anaesthetic overdose with 2-phenoxyethanol, blood was collected for haematological profile, peripheral cell dynamics and plasma humoral immune parameters assessment (Machado et al., 2015). Leucocyte migration to the inflammatory focus was also evaluated.

Results and discussion

The present study shows that methionine modulates immune response in seabass, what is in line with previous studies in fish. Once a fish is subjected to an inflammatory agent, the immune response is boosted (Machado et. al, 2015). In accordance, fish fed all dietary treatments showed an increase of leucocyte concentration and lysozyme activity with time in response to the bacterial infection. The leucocyte response was higher in fish fed MET0.5 and a clear monocytosis and neutrophilia together with the increase of bactericidal capacity was observed in fish fed MET1 compared to fish fed NCTRL. Fish fed MET1 also displayed higher levels of total leucocytes, macrophages and neutrophils, in the inflamed peritoneal cavity, compared to fish fed the remaining diets regardless time and in particular at 48h. The role of methionine on cell proliferation supports the previously described results. Underpinning the proposed beneficial effect of dietary methionine supplementation, fish fed MET1 increased disease resistance whereas both NCTRL and MET0.5 displayed similar mortality records. In contrast, fish fed the CTRL diet exhibited a higher susceptibility to the pathogen with the highest cumulative mortality. Head-kidney immune-related genes are being assessed and may bring further insights on the effects of dietary methionine supplementation and deficiency in the immune response mechanism and methionine metabolic pathways.

Acknowledgements

This work was supported by Project ALISSA (reference ALG-01-0247-FEDER-3520), financed by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020, and through the COMPETE and Operational Human Potential Programmes and national funds through FCT (Portugal). M. Machado, R. Azeredo, L. Ramos-Pinto and B. Costas were supported by FCT, Portugal (SFRH/BD/108243/2015, SFRH/BD/89457/2012, PD/BDE/114436/2016 and IF/00197/2015, respectively).

References

- Li, P., Mai, K.S., Trushenski, J., and Wu, G.Y., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37(1):43-53.
- Li, P., Yin, Y.L., Li, D., Kim, S.W., and Wu, G.Y., 2007. Amino acids and immune function. *British Journal of Nutrition*, 98(2):237-252.
- Machado, M., Azeredo, R., Díaz-Rosales, P., Afonso, A., Peres, H., Oliva-Teles, A., Costas, B., 2015. Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. *Fish & Shellfish Immunology*, 42:353-362.

ISOLATION AND CHARACTERIZATION OF ELOVL5 WITH C18 AND C20 ELONGASE ACTIVITY IN TAMBAQUI (*COLOSSOMA MACROPOMUM*)

R. B. Ferraz^{*,1,2}; N. Kabeya^{3,4}; R. A. Ribeiro⁵; M. Lopes-Marques¹; R. Ozório¹; L. F. Castro^{†,1,6}; O. Monroig^{†,3}.

1-CIIMAR - Animal Genetics and Evolution Research Group. U. Porto, Portugal; 2- ICBAS - Institute of Biomedical Sciences Abel Salazar, U. Porto; 3- Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling; 4- Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo; 5- Universidade Federal do Acre, Brazil; 6- Department of Biology, Faculty of Sciences, U. Porto, Portugal.
([†]joint last authors), * Presenting author, email: renatobarbosaferraz@gmail.com

Introduction

Tambaqui (*Colossoma macropomum*) is a native fish species of the Amazon basin, being one of the main species in Brazilian aquaculture with a production of approximately 134 Tm in 2014. Yet for this species little is known regarding the requirements of essential nutrients such as long-chain ($\geq C_{20}$) polyunsaturated fatty acid (LC-PUFA), namely arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) genously produced (biosynthesis) through the combined action of two enzymes, namely fatty acid desaturase (Fads) and elongation of very long chain fatty acids protein (Elovl) (Castro, Tocher et al. 2016). These enzymes have been extensively studied in several aquacultured species in an effort to understand the ability of fish species to utilize dietary vegetable oils (VO) replacing fish oils (FO) in current aquafeed formulations. Elovl catalyze the rate-limiting step of the elongation reaction that results in net 2 C elongation of pre-existing fatty acyl chains. The biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) is particularly relevant in fish (Nugteren 1965). Generally, Elovl2, Elovl4 and Elovl5 are regarded as critical enzymes in the elongation of PUFA (Jakobsson, Westerberg et al. 2006). Elovl5 involved in the pathway have been investigated in various fish species. Here we have set out to isolate and functionally characterize Elovl5 enzyme from Tambaqui, contributing towards the understanding of the nutritional requirements of this species.

Materials and methods

Total RNA extracted from tambaqui liver was used for cDNA synthesis. Molecular cloning of the tambaqui *elovl5* cDNA was performed by polymerase chain reaction (PCR) methodologies. The full-length open reading frame (ORF) was amplified using gene specific primers containing appropriate restriction sites for further cloning into the yeast expression vector pYES2. Tambaqui Elovl5 was functionally characterized by heterologous expression in yeast. Briefly, transgenic yeast expressing the Tambaqui Elovl5 ORF were grown in the presence of one of the following substrates: 18:3n-6, 18:4n-3, 20:4n-6 and 20:5n-3. After 48 h of incubation, yeast were harvested and washed. Total lipid extracted from yeast were used to prepare fatty acyl methyl esters (FAME) that were analyzed by gas chromatography. Fatty acid elongation efficiencies from exogenously added substrates were calculated by the proportion of substrate fatty acid converted to an elongated product as (product area/(product area + substrate area)) x 100.

Results

Control yeast transformed with the empty vector pYES2 did not show activity towards any of the substrates tested. The yeast transformed with the tambaqui *elovl5* ORF showed additional peaks when grown in the presence of 18:3n-6, 18:4n-3, 20:4n-6 and 20:5n-3 (Fig. 1). The elongation activity was particularly high towards C18 substrates, since 18:3n-6 and 18:4n-3 were converted to 20:3n-6 (78.7 % conversion) and 20:4n-3

(77.9 % conversion), respectively (Fig. 1; A, B). The elongation activity towards C20 substrates was lower than towards C18 substrates since 20:4n-6 and 20:5n-3 were converted to 22:4n-6 (33.4 % conversion) and 22:5n-3 (53.1 % conversion), respectively (Fig. 1; C, D).

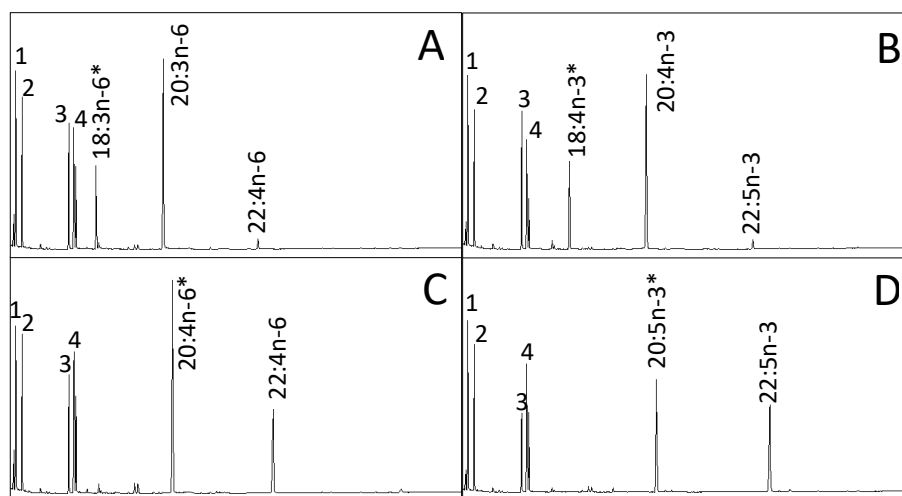


Fig. 1. Functional characterization of *C. macropomum* Elovl5 in yeast. Exogenously added substrates (*). Peaks 1–4 in all panels correspond to yeast endogenous FA, namely 1 - (16:0), 2 - (16:1n-7), 3 - (18:0) and 4 - (18:1n-9). FA derived from the exogenously added substrates or elongation products are indicated accordingly in each panel above the corresponding product.

Discussion and conclusion

Our data confirmed that the cloned Tambaqui Elovl5 demonstrated the ability to elongate C18 and C20 PUFA substrates. The similar pattern has been observed in Elovl5 isolated from several other teleost including zebrafish (Agaba, Tocher et al. 2005). Given that the Fads2 isolated from this species showed an enzyme with both $\Delta 6$ and $\Delta 5$ desaturase activities (Ferraz et al. unpublished), our results strongly suggest that this species can efficiently utilize dietary VO containing C₁₈ precursors LA (18:2n-6) and ALA (18:3n-3) to guarantee its essential LC-PUFA (ARA, EPA and DHA) requirements. This is in agreement with the freshwater species have greater LC-PUFA biosynthetic ability than marine fish as a result to their adaptation a restricted dietary input of these essential nutrients. Overall, the herein results point out tambaqui as an interesting species for sustainable development of Brazilian aquaculture with species that can efficiently utilize alternative sources to FO.

Acknowledgment

This study was supported by CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil.

References

- Agaba, M. K., D. R. Tocher, X. Zheng, C. A. Dickson, J. R. Dick and A. J. Teale (2005). "Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish." *Comp Biochem Physiol B Biochem Mol Biol* 142(3): 342-352.
- Castro, L. F., D. R. Tocher and O. Monroig (2016). "Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire." *Prog Lipid Res* 62: 25-40.
- Jakobsson, A., R. Westerberg and A. Jakobsson (2006). "Fatty acid elongases in mammals: their regulation and roles in metabolism." *Prog Lipid Res* 45(3): 237-249.
- Nugteren, D. H. (1965). "The enzymatic chain elongation of fatty acids by rat-liver microsomes." *Biochimica et Biophysica* 106: 280-290.

EFFECTS OF SOLID-STATE FERMENTATION WITH BASIDIOMYCETES FUNGI ON GRAPE STALKS

Valéria Costa-Silva* ^a, Mariana Anunciação ^a, Luís Ferreira ^a, Miguel Rodrigues ^a and Guilhermina Marques^b

^aThe Animal and Veterinary Research Centre, University of Trás-os-Montes and Alto Douro, (UTAD-CECAV), Department of Animal Science, Quinta de Prados, 5001-801, Vila Real, Portugal, ^b Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, (UTAD-CITAB), Quinta de Prados, 5000-801 Vila Real, Portugal.

Valéria Regina da Costa e Silva
Rua Padre Azevedo de Ribeirão nº7 4740-165 Esposende
valeriasilva@utad.pt

Abstract

Over the years, there has been an increasing trend toward the most efficient utilization of agricultural by-products. The insufficient storage and improper disposal of these wastes may generate serious pollution problems and a significant loss of biomass (Dulf *et al.*, 2016) that include plant bagasse, husk and fruit seeds. These wastes are constituted by cellulose, which represents potential source of sugars and energy (Quiroz *et al.*, 2014) which could potentially be used to animal nutrition.

White-rot fungi (WRF) have been studied due to their potential to produce ligninolytic enzymes for biotechnological applications such as delignification of wood and other lignocellulosic materials or bioremediation of contaminated sites and the selective degradation of lignin by WRF makes these organisms highly useful for pre-treatment of lignocellulosic biomass (Pinto *et al.*, 2012, Kuijk *et al.*, 2014). The fungal pre-treatment of the agro-industrial wastes using the solid-state fermentation (SSF) and filamentous fungal strains may give an added value to agricultural wastes, through bioconversions (Quiroz *et al.*, 2014). SSF is defined as a microbial culture that develops on moist substrates in the absence of free water but with sufficient moisture to support microorganism growth (Correia *et al.*, 2004, Oliveira *et al.*, 2016).

The objective of this study was to investigate the effect of basidiomycete's fungi on grape stalks, by-products of the wine production industry. Three species of fungi were used: *Lentinula edodes*, *Pleurotus eryngii*, and *Pleurotus citrinopileatus*. Substrates were colonized with fungal spawn for 0, 7, 14, 21, 28, 35 and 42 days in controlled conditions of temperature and humidity. After each time, the samples were dried to constant weight in an air-forced oven at 45°C and ground over a 1 mm screen for subsequent analysis.

Some of the most important analyses in the chemical evaluation have already been performed. Some of these quantifications include: fractions of the vegetal wall, protein, fat, phenolic compounds, digestibility *in vitro*, among others.

Analyzes such as the percentage of protein or the decrease of lignin have already shown some advantages in the colonization of fungi in grape stalks. The fungi that provides more advantages in some aspects, until now, was *Lentinula edodes*.

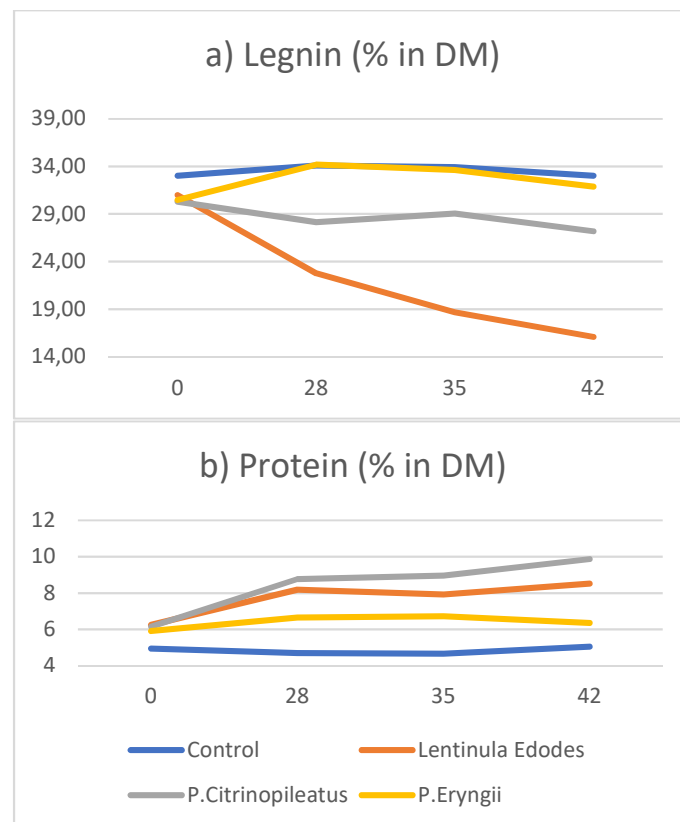


Figure1- Quantification of the percentage of lignin (a) and protein (b) in the dry matter throughout the incubation times.

The next steps to be performed in this study are to select the most appropriate fungi and colonize large amounts of substrate. After this colonization will be defined percentages to include in animal diets. These diets may be part of the feeding of ruminant animals such as cows or even herbivorous animals such as rabbits.

After choosing the percentages and the animal species to be used, the *in vivo* study starts. This study will be well thought considering all the aspects that we want to analyse in the future like weight gain, mortality, food intake, and others.

There are many aspects that will have to be studied to find out if the colonization of fungi in by-products of the agricultural industry is a viable measure for the valorization of these products. And if it is a possible measure would be very advantageous, it would recycle waste material and would be used in animal feed, as would reduce pollution charge.

[1]Correia, R.T.P.; McCueb, P.; Magalhães, M.M.A.; Macedo, G.R.; Shetty,K.; 2004. Production of phenolic antioxidants by the solid-state bioconversion of pineapple waste mixed with soy flour using *Rhizopus oligosporus*. *Process Biochemistry* 39, 2167–2172. [2] Dulf, F. V.; Vodnar, D. C.; Socaciu, C., 2016. Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. *Food Chemistry* 209, 27–36. [3] Kuijk, S. J. A.; Sonnenberg, A. S. M.;Baars, J.J.P.; Hendriks, W.H.; Cone, J.W., 2015. Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review. *Biotechnology Advances* 33, 191–202. [4] Pinto, P.A.; Dias, A.A.; Fraga, I.; Marques, G.; Rodrigues, M.A.M.; Colaço, J.; Sampaio, A.; Bezerra, R. M. F.;, 2012. Influence of ligninolytic enzymes on straw saccharification during fungal pretreatment. *Bioresource Technology* 111, 261–267. [5] Quiroz, R. C.; Roussos, S.; Hernández, D.; Rodríguez, R.; Castillo, F.; and Aguilar, C. N., 2014. Challenges and opportunities of the biopesticides production by solid-state fermentation: filamentous fungi as a model. *Critical Reviews in Biotechnology* 35:3, 326-333.

MICROBIOME IN POULTRY: INFLUENCE OF NUTRITIONAL FACTORS

VITOR SILVA^{*1,2}, JOSÉ LUIS MOURÃO³, JOSÉ VIEIRA⁴, ANTÓNIO MIRA DA FONSECA², AGOSTINHO ANTUNES^{1,5}

1- CIIMAR- Interdisciplinary Centre of Marine and Environmental Research

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

3- UTAD- Universidade de Trás-os-Montes e Alto Douro

4-SORGAL- Sociedade de Óleos e Rações de Portugal

5-Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

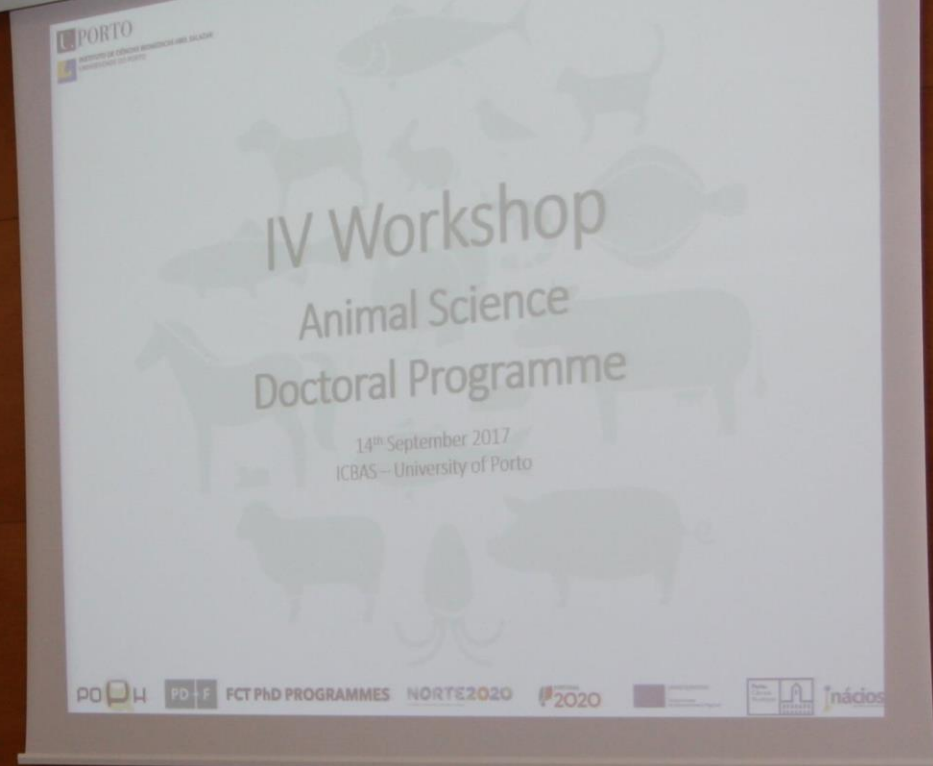
vsilva.1991@gmail.com

Poultry has a major relevance for human feeding, being one of the most important protein sources. Over the past century poultry has transitioned from predominantly breeding layers to breeding a mixture of both layers and broilers, based on the consumers demand evolution. Chicken's digestive system is adapted to extract energy and nutrients from difficult to digest food sources, which requires a interplay between the chicken and the microbiota present within the gastrointestinal tract (GIT). While supplementing the chicken's diet with prebiotics and probiotics, it is expected that the zootechnical performance is improved through positive selection of the GIT microbiota. Recent advances in culture-independent techniques allowed the identification of new taxa within the microbiota. The chicken GIT harbors a very diverse microbiota that aids in the breakdown and digestion of food and comprises over 900 species of bacteria, which contain both human and animal pathogens. Unlike mammals, caecum has an important role in birds' digestion, being a fermentation powerhouse due to the presence of a dense microbial community. The main objective of this work is to obtain information regarding the chicken GIT microbiome and selectively increase the chicken growth process performance.

LIST OF PARTICIPANTS

Alessandra Alves da Silva (ICBAS-UP)	Daniela Resende (FEUP)	João Rito (Universidade de Coimbra)	Pedro Nuno Figueiredo (ICBAS-UP)
Alexandra Marques (CIIMAR)	David Fernandes (ICBAS-UP)	José António Rodrigues (FCUP / REQUIMTE)	Regina Santos (ICBAS-UP)
Alexandra Ribeiro (SenseTest Lda)	Delvan Alves da Silva (ICBAS)	José Costa Lima (REQUIMTE, FFUP)	Renata Maria da Silva (UNL-INPA)
Alexandre Azevedo (ICBAS-UP)	Denis Meehan (ICBAS-UP)	Júlio Carvalheira (ICBAS-UP)	Renato Ferraz (ICBAS-UP/CIIMAR)
Aline Rocha (ICBAS-UP)	Diana Almeida (ICBAS-UP)	Leonardo J. Magnoni (CIIMAR)	Rita Cabrita (ICBAS-UP)
Aline Marculino de Alcântara (UNL-INPA)	Dionísio Sousa (ICBAS-UP)	Liliana Cordeiro (FCUP)	Rita Payan (UTAD)
Álvaro Sousa (ICBAS-UP)	Eliane Silva (ICBAS-UP)	Lourenço Pinto (SPAROS, CIIMAR & ICBAS-UP)	Rute Isabel Correia Martins (FFUP)
Ana Barreira (ICBAS-UP)	Elisabete Carneiro (Invivonsa Portugal, S.A.)	Luís Baião (ICBAS-UP)	Salman Malakpour (CIIMAR)
Ana Basto (CIIMAR)	Elisabete Matos (Soja de Portugal)	Luís Conceição (SPAROS Lda)	Sandia Machado (FFUP)
Ana Catarina Costa Fernandes (FCUP)	Fábio Rangel (FCUP)	Luisa Barreiros (ICETA/REQUIMTE/FFUP)	Sandra Silva (ICBAS-UP)
Ana Cristina da Silveira de Moura (FCUP)	Filipa Fontinha (ICBAS-UP/CIIMAR)	Luísa Valente (ICBAS-UP)	Sara Magalhães (SORGAL/ICBAS-UP)
Ana da Conceição Moreira Gomes (CAVC)	Filipa Rocha (CIIMAR)	Luzia Teixeira (ICBAS-UP/UMIB)	Sara Marques (UCIBIO-REQUIMTE/FFUP)
Ana Ferreira (FCUP)	Francisca Isabel da Silva Brito (ICBAS-UP)	Manuel Vilanova (ICBAS-UP)	Sara Marques (ICBAS-UP)
Ana Isabel Barbosa (FFUP)	Francisco Castanheira (Alltech)	Madalena Real (ICBAS-UP)	Sara Moutinho (CIIMAR)
Ana Margarida Pereira (ICBAS-UP)	Francisco Guardiola (CIIMAR)	Marcela Segundo (UCIBIO, REQUIMTE, FFUP)	Sara Regina Rodrigues Fernandes (FFUP)
Ana Raquel Rodrigues (REQUIMTE/LAQV/ICBAS-UP)	Francisco Rocha (FCUP)	Margarida Araújo (ICBAS-UP)	Sílvia Antunes de Azevedo (ICBAS-UP)
Ana Rita Oliveira Macedo Pinto (UCIBIO/REQUIMTE)	Gabriela Sousa (FCUP)	Margarida Guedes (ICBAS-UP)	Sofia Abrunheiro (ICBAS-UP)
Andreia Granja (UCIBIO-REQUIMTE)	Gonçalo Pinto (ICBAS-UP)	Margarida Lopes (FCUP)	Sofia Lima (REQUIMTE)
António Isidoro (Soja De Portugal)	Gustavo Paixão (CECAV-UTAD)	Margarida Maia (REQUIMTE, LAQV, ICBAS-UP)	Sónia Batista (CIIMAR)
António Rocha (ICBAS-UP)	Inês Campos (ICBAS-UP/CIIMAR)	Maria Antónia Salgado (ICBAS-UP)	Sónia Rocha (FFUP)
Bárbara Oliveira (ICBAS-UP)	Inês Honrado (ICBAS-UP)	Maria João Xavier (ICBAS-UP)	Tiago Aires (Soja de Portugal)
Bárbara Sousa (ICBAS)	Inês Ramos (FFUP)	Maria Teresa Dinis (CCMAR)	Tiago Leal Ferreira de Sá (CIIMAR)
Carla Aguiar (INVIVONSA PORTUGAL)	Inês Valente (REQUIMTE/LAQV)	Mariana Mendes Moreira (Universidade do Minho)	Vasco Capela (ICBAS-UP)
Carolina Figueiredo (SORGAL, S.A.)	Ingrid Van Dorpe (Premix Lda.)	Marta P. Monteiro (FCUP/CIIMAR)	Vera Filipe de Sousa Figueiredo (ICBAS-UP)
Catarina Pedro Fernandes (FMUL)	Irene González (CIIMAR)	Michael Viegas (ICBAS-UP/SPAROS)	Vitor Silva (ICBAS-UP)
Cláudia Baptista (ICBAS-UP)	Isidro Blanquet (SEA8)	Nayara Netto dos Santos (ICBAS-UP)	
Cláudia Mendes (ICBAS-UP)	Jaime Piçarra (IACA)	Nuno Leite (CIIMAR/UP)	
Cláudia Serra (CIIMAR)	Joana Pais (Alltech)	Nuno Maurício (Soja de Portugal)	
Daniela Lopes (FFUP)	Joana Ribeiro (ICBAS-UP)	Paolla Mara Faria (ICBAS-UP)	
	João Albuquerque (ICBAS-UP)	Patrícia Peixoto (FFUP)	

PHOTO GALERY



Júlio Carneiro (CIBIO & ICBAS-UP), Rita Cabrita (REQUIMTE & ICBAS-UP), Rita Payen (CECAV-UTAD), Luísa Valente (ICBAS-UP), Luís Conceição (SPAROS) and Inês Campos (SORGAL & ICBAS-UP student)



Salão Nobre ICBAS-UP/FFUP



Luísa Valente (ICBAS-UP) on Animal Welfare (1st Plenary Session)



Student presentations (Session I)

Luis Baião^{1,2*}
Filipa Rocha²
Tiago Sá²
Luísa M.P. Valente^{1,2}

*lbaião@cimar.up.pt

ASSESSMENT OF THE BEST PROTEIN/LIPID RATIO IN DIETS FOR SEA URCHIN (*Paracentrotus lividus*)

INTRODUCTION

In the Mediterranean sea, the sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to its flavor and texture. The increasing market demand for sea urchin roe has resulted in the depletion of wild stocks. For this reason, the interest in sea urchin aquaculture has widened over the last decades. This study was designed in order to identify a diet able to promote sea urchin growth whilst assuring gonad development under a recirculating aquaculture system (RAS).

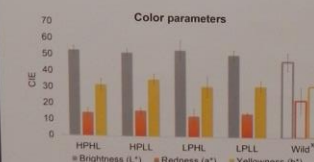
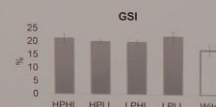
MATERIAL AND METHODS

Dietary treatments	Protein levels		Lipid levels	
	High (HP) 51 (% DM)	Low (LP) 30 (% DM)	High (HL) 11 (% DM)	Low (LL) 6 (% DM)
HPHL	x		x	
HPLL	x			x
LPHL		x	x	
LPLL		x		x

- 45 urchins/m² within 250 L tanks in a saltwater recirculation system.
- 18 °C water temperature, 12 L:12 D
- Treatments tested in triplicates
- hand-fed every 2 days

RESULTS

Growth	HP		LP	
	HL	LL	HL	LL
Final body weight (g)	43.0 ± 0.7	42.4 ± 2.4	42.4 ± 1.3	43.2 ± 1.9
Weight gain (g)	8.2 ± 0.2	8.7 ± 1.9	6.9 ± 1.2	8.5 ± 1.2
FCR	1.8 ± 0.1*	1.6 ± 0.3*	3.4 ± 0.5*	2.9 ± 0.6*



- All diets were well accepted by the sea urchins and induced similar growth and nutrient utilization, however, the HP diets presented lower feed conversion ratio (FCR) than the LP diets.
- The gonadal somatic index (GSI) was generally high and similar among dietary treatments (<21%), but higher than average values observed in wild specimens (11%). The diet with lowest protein and lowest lipid levels (LP LL) resulted in the highest gonad yield and highest GSI.
- Total carotenoid concentrations were similar in all diets and always highest in females. The tested diets did not affect gonad texture (firmness and resilience) and color, but redness value seems higher in wild urchins.
- The LP LL seems to be able to supply sea urchins nutritional needs, but future studies are required to further evaluate the dietary impact on gonad acceptance by consumers.

ISOLATION AND CHARACTERIZATION OF ELOVL5 WITH C18 AND C20 ELONGASE ACTIVITY IN TAMBAQUI (COLOSSOMA MACROPOMUM)

R. B. Ferraz^{1,2}
N. Kabejya^{3,4}
R. A. Ribeiro⁵
M. Lopes-Marques¹
R. Ozono^{1,2}
L. F. Castro^{1,6}
O. Monroig⁷
renatobarbosaferaz@gmail.com

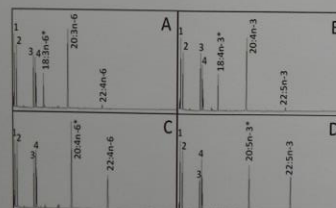
INTRODUCTION

- Tambaqui is a native fish species of the Amazon basin, being one of the main species in Brazilian aquaculture.
- Aquaculture sector is currently replacing fish oil by vegetable oil to enable sustainable fish production.
- Long-chain polyunsaturated fatty acid (LC-PUFA) can be endogenously produced (biosynthesis) through the combined action of two enzymes, i.e. fatty acyl elongases (Elovl) and desaturases (Fads).
- Aim: Cloning and functional characterization of a Elovl5 involved in the LC-PUFA biosynthetic pathways.

MATERIAL AND METHODS

Tambaqui Elovl5 was functionally characterized by heterologous expression in yeast. Briefly, transgenic yeast expressing the Tambaqui Elovl5 ORF were grown in the presence of one of the following substrates: 18:3n-6, 18:4n-3, 20:4n-6 and 20:5n-3. After 48 h of incubation, yeast were harvested and washed. Total lipid extracted from yeast were used to prepare fatty acyl methyl esters (FAME) that were analyzed by gas chromatography.

RESULTS



FA substrate	FA product	Conversion (%)
18:3n-3	20:3n-3	14,282
18:2n-6	20:2n-6	6,974
18:4n-3	20:4n-3	25,964
18:3n-6	20:3n-6	23,93
20:5n-3	22:5n-3	17,159
20:4n-6	22:4n-6	11,141
22:5n-3	24:5n-3	Nd
22:4n-6	24:4n-6	Nd

Tab. 1. The elongation activity was particularly high towards C18 substrates, since 18:3n-6 and 18:4n-3 were converted to 20:3n-6 (78.7%) and 20:4n-3 (77.9%). The elongation activity towards C20 substrates was lower than towards C18 substrates since 20:4n-6 and 20:5n-3 were converted to 22:4n-6 (33.4%) and 22:5n-3 (53.1%).

Fig. 1. Functional characterization of *C. macropomum* Elovl5 in yeast. Exogenously added substrates (*). Peaks 1-4 in all panels correspond to yeast endogenous FA, namely 1- (16:0), 2- (16:1n-7), 3- (18:0) and 4- (18:1n-9). FA derived from the exogenously added substrates or elongation products are indicated accordingly in each panel above the corresponding product.

DISCUSSION AND CONCLUSION

- Cloned tambaqui Elovl5 demonstrated the ability to elongate C18 and C20 PUFA substrates.
- Given that the Fads2 isolated from this species showed an enzyme with both Δ6 and Δ5 desaturase activities (Ferraz, R. et al. unpublished).
- Thus, tambaqui is an interesting fish model for studies on the use of vegetable oil sources rich in LA (18:2n-6) and ALA (18:3n-3) as a replacement of fish oils, to fulfill the essential LC-PUFA (ARA, EPA and DHA) requirements.
- The current results may enable a sustainable development of tambaqui aquaculture in Brazil.

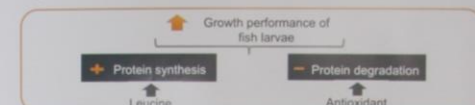
Acknowledgements
This study was supported by CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil.

IMPROVING GROWTH PERFORMANCE OF FISH LARVAE THROUGH EARLY NUTRITION

Maria Xavier^{1,2,3,4}
Luís E.C. Conceição³
Luísa M.P. Valente^{1,2}
Sofia Engrola⁴

Introduction:

Muscle growth and atrophy are dependent on the net balance between protein synthesis and degradation. L-leucine it is well known to stimulate protein synthesis in muscle tissues, through mTOR signaling pathway. The inclusion of antioxidant molecules in fish diets might be expected to reduce protein degradation and potentially enhance growth rates.



Objectives:

To increase the knowledge regarding the effects of nutritional strategies at very early stage of development in order to improve protein deposition, growth performance in fish larvae.

Task 1

Anti-oxidants as modulators of protein synthesis in fish larvae:

The experiment will include 8 treatments:



To be assessed at the end of the experiment:

- Growth performance
- Metabolic plasticity with radiolabeled tracers
- Oxidative status
- Muscle cellularity
- Expression of candidate genes related to muscle growth and protein turnover

Task 2

Amino acids as promoters of protein retention in fish larvae:

Based on the best results of task 1 on the effects of supplemental BCAAs/ antioxidants will be fed since mouth opening as long-term modulators of protein accretion in fish larvae

Task 3

Marine biomass based formulation to improve retention in marine fish larvae:

Based on the best results of tasks 1 and 2, validate the concept that nutrition may mitigate adverse environmental impacts in marine fish larvae using practical feed formulations

To estimate the safe level of antioxidant to include in the sole diets:

Toxicological test was performed in <i>Artemia salina</i> prior to the growth trial	
NOEC	ACI
No Observed Effect Concentration = Highest conc. of the treatment at which the toxicity is not sig. different (statistically) from the control	Acceptable Daily Intake = NOEL/Safe Factor (mg/kg bodywt)
Results:	
GT	NOEC: 1.700 (mg/kgbw)
GS	NOEC: 207.70 (mg/kgbw)
CC	NOEC: 97.74 (mg/kgbw)
	ADT: 4.15 (mg/kgbw)
	ADT: 1.85 (mg/kgbw)
	ADT: 0.2 (mg/kgbw)
	ADT: 0.25 (mg/kgbw)

Due to low solubility of curcumin in water the results of this antioxidant were discarded. Therefore the safe level of this antioxidant was assessed through HPLC/MS/MS

CIIMAR
Centro Interdisciplinar de Investigação Marinha e Ambiental
Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

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

FEUP
Faculdade de Engenharia da Universidade do Porto, Portugal

SPAROS Lda.
Rua Empresarial de Oitões, Lote C, E700-221, Oitões, Portugal

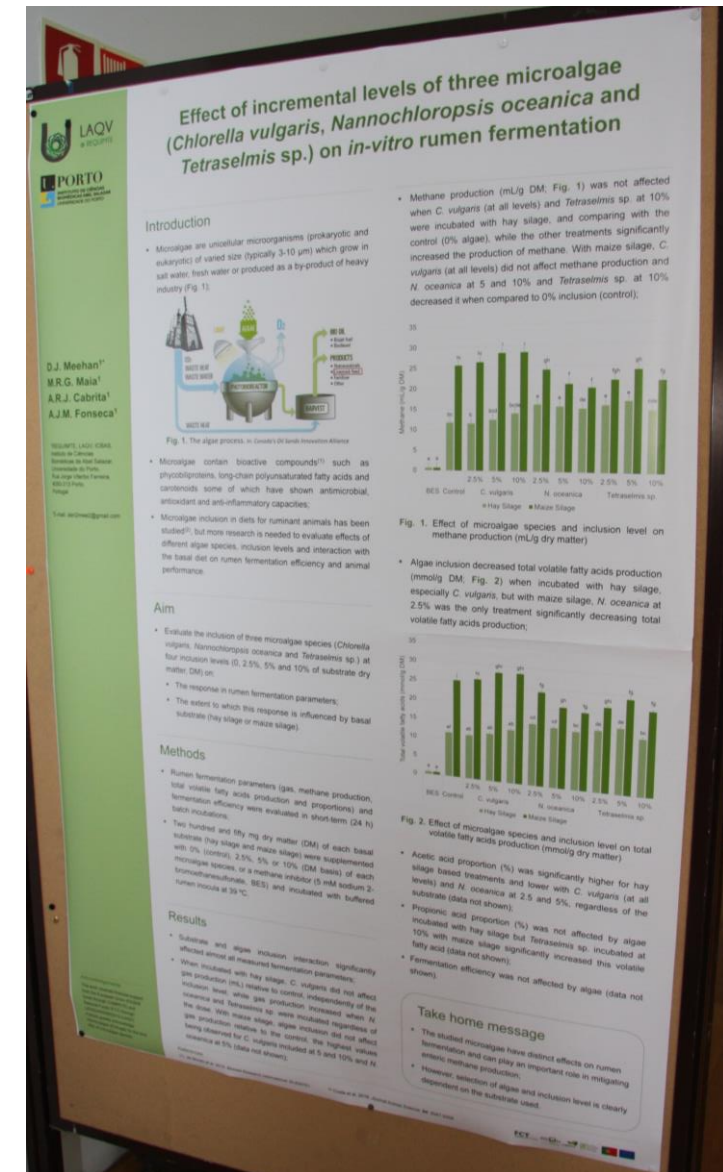
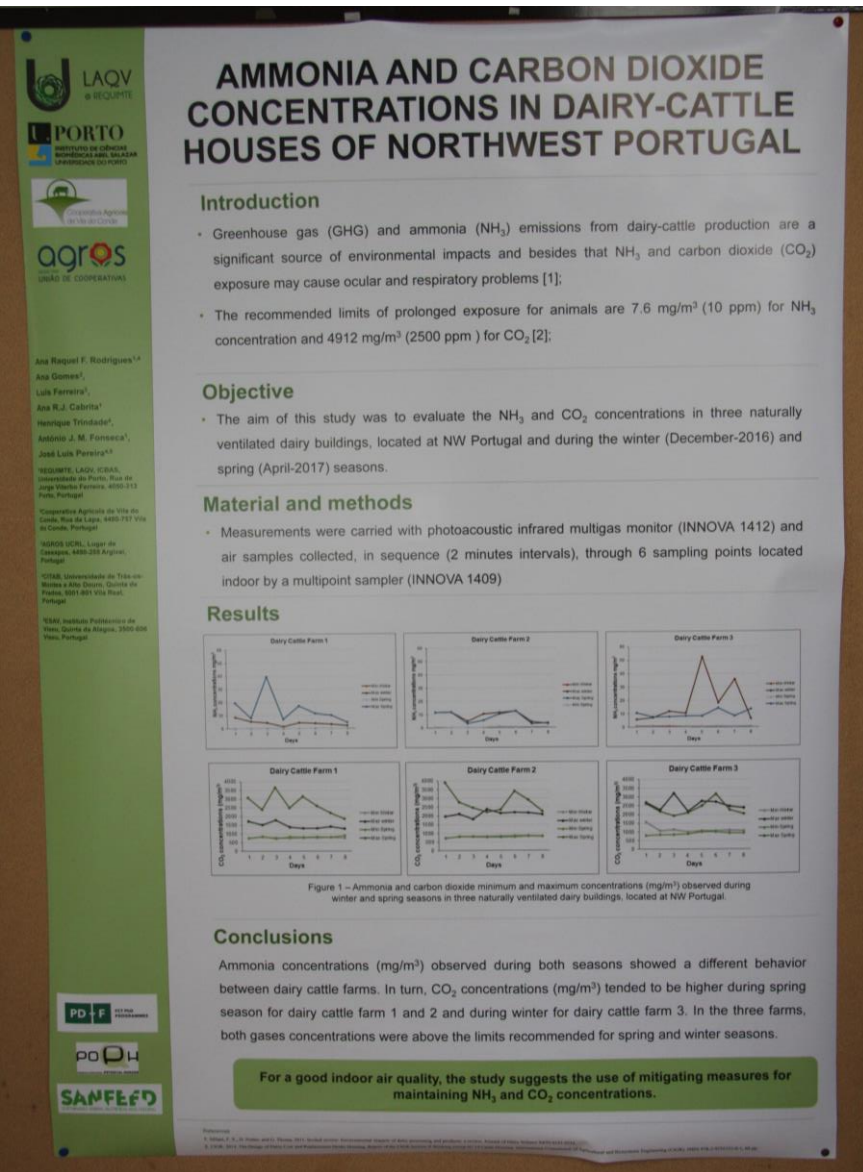
CCMAR
Centro de Ciências do Mar do Algarve, Univ. Algarve, Campus de Gambelas, Faro, Portugal

25 DNA Senegalese Sole
Recirculation system
During 35 days

GT:
Density: 20 Artemia/m²
Seven concentrations (0, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 mg/kg, w/w) of the GT.
CC: GS tested (mg/kgbw)
Artemia survival was registered at day 5.

GS:
Density: 20 Artemia/m²
Seven concentrations (0, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 mg/kg, w/w) of the GS.
CC: GS tested (mg/kgbw)
Artemia survival was registered at day 5.

Acknowledgements
FCT, PO, LI, FCT PIG PROGRAMMES



Lunch and poster session

Effects of solid-state fermentation with white-rot fungi on grape stalks nutritive value

Carla Silva, V. Almeida, M. Almeida, E. Ferreira, L. Rodrigues, M. Marques, G. Almeida

Abstract: The aim of this study was to evaluate the effect of solid-state fermentation with white-rot fungi on the nutritive value of grape stalks. The study was conducted in a laboratory setting, using a factorial design with two factors: Fungus (F1, F2, F3) and Time (T1, T2, T3). The results showed that the nutritive value of grape stalks increased significantly with the use of white-rot fungi and with the increase in fermentation time.

Introduction

Grape stalks are a by-product of the wine industry, representing a significant portion of the total waste generated. The use of white-rot fungi for the degradation of lignocellulosic materials has been widely studied, showing promising results in terms of nutrient release and biodegradation efficiency.

Objective

To evaluate the effect of solid-state fermentation with white-rot fungi on the nutritive value of grape stalks.

Methods

The study was conducted in a laboratory setting, using a factorial design with two factors: Fungus (F1, F2, F3) and Time (T1, T2, T3). The results showed that the nutritive value of grape stalks increased significantly with the use of white-rot fungi and with the increase in fermentation time.

Results

The results showed that the nutritive value of grape stalks increased significantly with the use of white-rot fungi and with the increase in fermentation time.

Conclusions

The use of white-rot fungi for the degradation of lignocellulosic materials has been widely studied, showing promising results in terms of nutrient release and biodegradation efficiency.

Acknowledgments

The authors thank the funding agencies for their support in this research.

References

Almeida, M., Almeida, V., Ferreira, L., Rodrigues, M., Marques, G., Almeida, C. (2020). Effects of solid-state fermentation with white-rot fungi on grape stalks nutritive value. *Journal of Agricultural Science*, 155(1), 1-10.

CECAV NORTE2020

2020

MICROBIOME IN POULTRY: INFLUENCE OF NUTRITIONAL FACTORS

Vitor Silva^{1,2}, Ana Rita Silva³, José Luís Mourão⁴, José Vieira⁵, Elisabete Matos⁶, António Mira da Fonseca⁶, Agostinho Antunes^{1,2}

1- CIMAR- Interdisciplinary Centre of Marine and Environmental Research
2- ICBAS-UP- Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto
3- UTAD- Universidade de Trás-os-Montes e Alto Douro
4- SCRSAL- Sociedade de Crustáceos e Rãs, S.A. Guimarães
5- Departamento de Biologia, Faculdade de Ciências, Universidade do Porto
6- Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

Abstract

The microbiome of poultry is a complex system, influenced by various factors, including nutrition. The aim of this study was to evaluate the effect of different nutritional factors on the microbiome of poultry.

Introduction

The microbiome of poultry is a complex system, influenced by various factors, including nutrition. The aim of this study was to evaluate the effect of different nutritional factors on the microbiome of poultry.

Methods

The study was conducted in a laboratory setting, using a factorial design with two factors: Diet (D1, D2, D3) and Time (T1, T2, T3). The results showed that the microbiome of poultry changed significantly with the use of different diets and with the increase in time.

Results

The results showed that the microbiome of poultry changed significantly with the use of different diets and with the increase in time.

Conclusions

The use of different nutritional factors can significantly influence the microbiome of poultry.

Acknowledgments

The authors thank the funding agencies for their support in this research.

References

Silva, V., Silva, A., Mourão, J., Vieira, J., Matos, E., Mira da Fonseca, A., Antunes, A. (2020). Microbiome in poultry: influence of nutritional factors. *Journal of Agricultural Science*, 155(1), 1-10.

CECAV NORTE2020

2020

FCT

SANFED

SOJA DE PORTUGAL

Early life traits and growth variability of flounder in the Lima nursery (NW Portugal)

Mendes, C.^{1,2,3}, Ramos, S.^{1,2,3}, Elliott, M.^{1,2,3}, Bortado, A.A.^{1,2,3}

1- Laboratory of Pathology and Ecology, Institute of Biomedical Sciences, University of Porto (ICBAS-UP), Rua Jorge Balsemão, 228, 4150-015 Porto, Portugal
2- Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Terminal da Cova da Moura de Lisboa, Av. General Norton de Matos 141, 1440-208 Lisboa, Portugal
3- Institute of Oceanography and Coastal Studies, University of Trás-os-Montes e Alto Douro, 5200-900 Vila Real, Portugal

1. Introduction

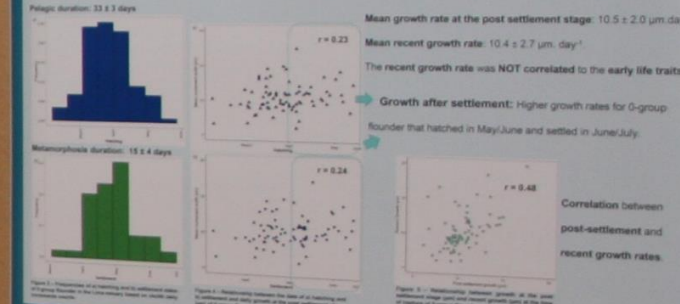
Early life traits are major determinants of fish recruitment strength and understanding its impact on growth is essential to fisheries management. The European flounder, *Platichthys flesus*, typically spawns in offshore areas and the planktonic larvae migrate to nurseries where settlement marks the transition to the juvenile benthic stage.

Aim: Investigate hatching and settlement patterns, and the relationship to growth dynamics of 0-group flounder in an estuarine nursery.

2. Methodology



3. Results



4. Conclusions

The growth of 0-group flounder after the settlement period reflected early life history traits, in particular the time of hatching and settlement. The study highlighted the importance of integrating different stages of the flounder life cycle to understand growth dynamics and ultimately recruitment patterns to adult populations.



Lunch and poster session



Exhibition area (SANFEED Industrial Partners)



António Isidoro (Soja de Portugal) and Luísa Valente (ICBAS-UP)



Jaime Piçarra (IACA) on Sustainability in the European Feed Industry (2nd Plenary Session)



Student presentations (Session II)

IV Workshop Animal Science Doctoral Programme

14th September 2017
ICBAS – University of Porto



FCT PhD PROGRAMMES

NORTE2020

2020



nádeos



Round table discussion

(Luísa Valente, Jaime Piçarra, Ana Margarida Pereira, Luís Conceição, Ingrid Van Dorpe, Denis Meehan, Cláudia Serra and António Rocha)



Best oral communication and best poster awards jury (Maria Teresa Dinis, Margarida Araújo and José Costa Lima) and winners (Inês Campos and Ana Raquel Rodrigues)



Port of Honor