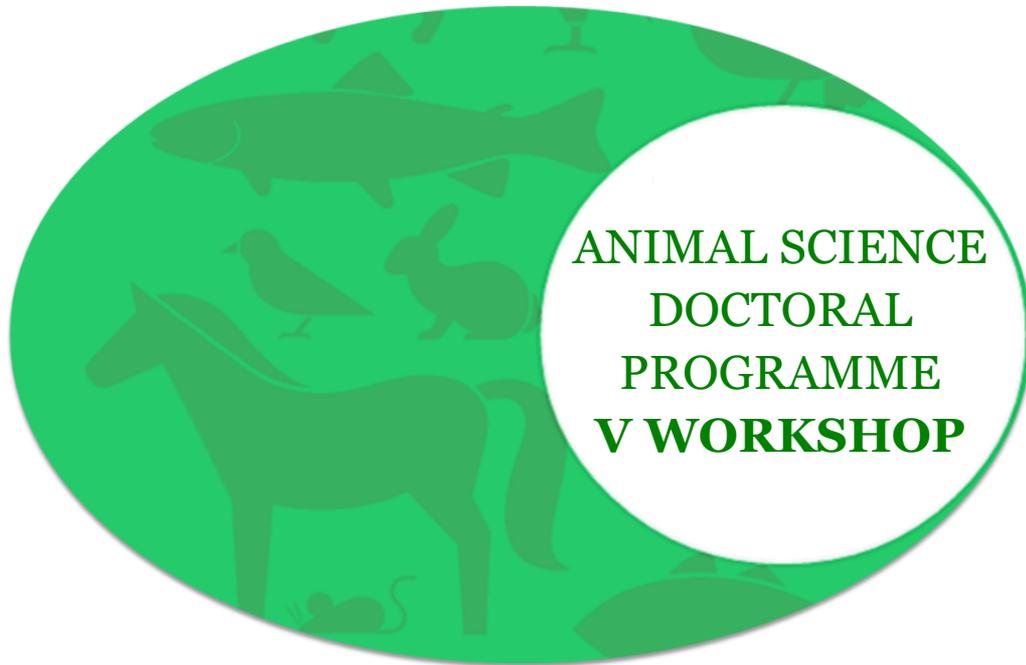


U. PORTO



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO



18th SEPTEMBER 2018

Salão Nobre, ICBAS-UP

Rua de Jorge Viterbo Ferreira No 228

Porto, Portugal

ANIMAL SCIENCE DOCTORAL PROGRAMME

- V WORKSHOP

18th September 2018

Organization

António Mira Fonseca, *REQUIMTE & ICBAS*

Lúisa Maria Pinheiro Valente, *CIIMAR & ICBAS*

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

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

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Liliana Cordeiro, *REQUIMTE, ICBAS-UP, Alltech & Agros*

Conference Chairpersons

Inês Valente, *REQUIMTE*

José António Rodrigues, *REQUIMTE & FCUP*

Ana Paula Mucha, *CIIMAR & FCUP*

Elisabete Matos, *SORGAL*

Jorge Dias, *SPAROS*

Fausto Freire, *ADAI-LAETA & U.Coimbra*

Personnel

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

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

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

Vitor Silva, *CIIMAR, ICBAS-UP & SORGAL*

Diana Almeida, *CIIMAR, ICBAS-UP & Sea8*

Michael Viegas, *CIIMAR, ICBAS-UP, SPAROS, INVIVONSA*

INVITED SPEAKERS



Hugo Miguel Rodrigues Cunha Oliveira

Hugo Oliveira graduated in Pharmaceutical Sciences from the University of Porto in 2003. He completed his MSc in Environmental Analytical Chemistry in 2005, in a joint program between the University of Porto and University of Aberdeen (UK). In 2010, Hugo received his European Ph.D. degree in Analytical Chemistry by the University of Porto, which consisted of the development of automatic sample preparation protocols for liquid chromatography, applied to food and environmental analysis. After the end of his Ph.D., Hugo integrated the trace metals team from the University of Hawaii that took part in the research cruise CLIVAR S4P in Antarctica, dedicated to climate variability. Then, he was a post-doctoral fellow at REQUIMTE/LAQV (University of Porto), where his research comprised the development of analytical methodologies for environmental and feedstuff monitoring, with an emphasis on (bio)chemical markers of methane emission of ruminants. Hugo is currently a Staff Researcher at INL. His research is focused on the development of analytical solutions based on integrated optical sensors for application in agricultural and environmental fields.



Joana Gabriela Laranjeira da Silva

Joana Laranjeira Silva has a B.Sc. in Microbiology by Porto Catholic University; an M.Sc. in Cellular Biology by Coimbra University and a PhD in Biotechnology and Food Safety by Porto Catholic University. Joana L. Silva is responsible for the R&D development of Allmicroalgae, Leiria Pataias. Currently, she has two H2020 projects and four P2020 projects under execution. Joana Laranjeira Silva has an *h-index* =24.0 with more than 80 scientific papers with peer revision. She already participated, as a researcher, in more than 20 projects and co-orientated more than 30 master students and 6 PhD students.



Fausto Miguel Cereja Seixas Freire

Fausto Freire is an assistant Professor with tenure at the University of Coimbra, Portugal, with research and teaching on the emerging field of Industrial Ecology, focusing on developing and implementing Life Cycle based approaches. He coordinates the Center for Industrial Ecology and is a member of the coordination committee of the Energy for Sustainability initiative at the University of Coimbra. Faculty at the MIT-Portugal program and MIT visiting Scholar (several months, from 2011-2018). Fulbright Scholar at the University of Michigan in 2008. Member of the International Society for Industrial Ecology. Member of the Ecoinvent Editorial Board. Member of the executive committee of the Ibero-American Life-Cycle network (Portugal representative). Prof. Freire's research and teaching focuses on the development and application of life-cycle models to enhance the sustainability of products and systems. He has developed and implemented ecodesign tools and a novel approach (LCAA) integrating LCA and economic optimization models. He has studied several products and systems, including bioenergy, buildings, industrial, food and agricultural systems, urban waste, etc. Prof Freire has coordinated several large projects, supervised about twelve Postdoctoral researchers, twenty PhD students and many MSc thesis and young researchers. He has received several awards and grants. Senior Member and Energy expert of the Portuguese Engineers Association (Ordem dos Engenheiros). Prof. Freire is co-author of more than 250 publications (book chapters, ISI articles and conference papers, H scopus index of 18).

AGENDA

10:30 • WORKSHOP OPENING

Scientific Committee Doctoral of the Programme in Animal Science:

António Mira da Fonseca (REQUIMTE/ICBAS-UP)

Luísa Valente (CIIMAR/ICBAS-UP)

Ana Rita Cabrita (REQUIMTE/ICBAS-UP)

SANFEED Advisory Board President: *Jorge Dias (SPAROS)*

PhD Student: *Ana Margarida Pereira (REQUIMTE/ ICBAS-UP/ SORGAL/ Alltech)*

SESSION I

Chairpersons: *José António Rodrigues (REQUIMTE) & FCUP & Inês Valente (REQUIMTE)*

10:45 • Plenary lecture: Livestock 4.0: the potential role and impact of (bio)chemical markers and sensors in the next generation of livestock farming systems, *Hugo Oliveira, (INL)*

11:15 • Effects of the nutritive value of base forage on milk production and income over feed costs, *(Isabel Santos (REQUIMTE/ICBAS-UP/CAVC))*

11:30 • Genetic parameters for reproductive and longevity traits in Bísaro pigs, *Gustavo Paixão (CECAV/UTAD)*

11:45 • Methane concentrations in dairy-cattle buildings of northwest Portugal, *Ana Raquel Rodrigues (REQUIMTE/ ICBAS-UP/ CAVC/AGROS)*

12:00 • Use of infrared thermography imaging to monitor stress-related responses by lambs fed with *Lupinus* diet, *Mariana Almeida (CECAV/UTAD)*

12:15 • The effect of organic and inorganic selenium supplementation in puppies' growth – preliminary results, *Ana Margarida Pereira (REQUIMTE/ ICBAS-UP/ SORGAL/ Alltech)*

12:30 • LUNCH & POSTER SESSION

SESSION II

Chairpersons: *Ana Paula Mucha (CIIMAR/FCUP) & Elisabete Matos (SORGAL)*

14:15 • Plenary lecture: Microalgae for animal feed: an overview *Joana L. Silva (Allmicroalgae)*

14:45 • Potential application of agar waste from *Gracilaria* sp., *Francisca Silva-Brito (CIIMAR/ICBAS-UP/ALGA+)*

15:00 • Volatile composition and sensory characteristics of *Paracentrotus lividus*, *Luis Baião (CIIMAR/ICBAS-UP/ SenseTest)*

15:15 • Improving growth performance of fish larvae through early nutrition, *Maria Xavier (CIIMAR/ICBAS-UP/ CCMAR/ SPAROS)*

15:30 • Modulation of growth and bone status by dietary trace mineral supplementation in early stage *Solea senegalensis* (*Michael Viegas, CIIMAR/ICBAS-UP/SPAROS/ INVIVONSA*)

15:45 • Life cycle assessment of animal feed ingredients produced from poultry by-products, *Inês Campos (CIIMAR/ ICBAS-UP/SORGAL)*

16:00 • COFFEE-BREAK & POSTER SESSION

16:20 • ROUND TABLE ‘Circular Economy’

Chairpersons: *Jorge Dias (SPAROS) & Fausto Freire (ADAI-LAETA/ U.Coimbra)*

Introduction: *Fausto Freire (ADAI-LAETA/ U.Coimbra)*

Participants:

Hugo Oliveira (INL)

Inês Valente (REQUIMTE)

Ana Paula Mucha (CIIMAR/FCUP)

Francisca Silva-Brito (CIIMAR/ICBAS-UP/ALGA+)

Ana Raquel Rodrigues (REQUIMTE/ICBAS-UP/CAVC/AGROS)

17:20 • BEST POSTER AND ORAL PRESENTATION AWARDS

Jury:

Paula Ferreira (ICBAS-UP)

Lúcia Saraiva (REQUIMTE/ FFUP)

Ingrid Van Dorpe (Premix)

17:30 • WORKSHOP CLOSING

PLENARY SESSIONS

LIVESTOCK 4.0: THE POTENTIAL ROLE AND IMPACT OF (BIO)CHEMICAL SENSING IN THE NEXT GENERATION OF LIVESTOCK FARMING SYSTEMS

Hugo M. Oliveira¹

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The livestock sector is a key element in the global economy. Regarding the growing concerns about climate change and food security, the generation and management of information related with the production systems will have an essential role in the definition of the next generation of livestock farming systems. This communication intends to identify and discuss the current path towards Livestock 4.0. Firstly, it will identify some of the major features of Industry 4.0 and how these new advances have been applied to the development of innovative farming methods using some selected examples. Secondly, it will explore the advances in new sources of (bio)chemical information by chemical sensors and its respective role in new ways of managing nutrition, health, and environmental issues in the farm. Finally, it will discuss the challenges and opportunities of this fast-growing field towards more sustainable livestock systems.

MICROALGAE FOR ANIMAL FEED: AN OVERVIEW

Joana L. Silva¹

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The present work aims to discuss the potential of microalgae within Animal Feed applications.

Microalgae are a natural source of: - Essential Micronutrients *i.e.* minerals, vitamins, aminoacids and essential fatty acids (omega 3 & omega 6); - Natural Pigments for feed coloring (*i.e.* ornamental fish + farmed fish + petfoods); - Functionality & Bioactivity especially in delicate and sensitive stages of the animals' lifecycle and, - Technical performance as ingredient binding agent, natural colorant, palatability enhancer; among others.

Microalgae can be cultivated in auto, mixo and heterotrophic conditions. The nutritional and biochemical profile of the microalgae biomass varies among the produced/commercialized specie/strain.

Concerning the industrial microalgae production and animal feed, several constraints are imposed namely: the scale and the associated production costs, the price of the ingredient/food, the added value and the proof of the concept and the market that is still immature.

To achieve the bulk feeds, Allmicroalgae is working on the scalability and the versatility of the bioprocesses. A combination of different reactors *i.e.* "Greenwalls", "Photobioreactors" and "Fermentation" are available. The algae can be deliver as powder, fresh paste and frozen paste. A Taylor made production might be also requested by the client.

For feed an animal nutrition the most required microalgae strains are: *Nannocloropsis* sp. (rich in carotenoids and lipids *i.e.* EPA and ARA); *Phaeodactylum tricornutum* (rich in EPA and fucoxantin); *Schyzochytrium* (rich in DHA) and *Chlorella* sp. (rich in protein and carotenoids).

Allmicroalgae defends the synergy between R&D centers, the producers and the consumers/end-users. Thereby, several research innovation projects have been proposed with the involvement of Portuguese universities and research centers. The main idea is to conduct local farming assays with the incorporation of microalgae in real feed formulations. Algavalor is a case study that will be briefly presented.

ORAL COMMUNICATIONS

EFFECTS OF THE NUTRITIVE VALUE OF BASE FORAGE ON MILK PRODUCTION AND INCOME OVER FEED COSTS

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

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Milk production per cow has increased tremendously in the last years through a combination of genetic selection for milk yield and improvements in nutrition and management (Potts et al., 2017). Dairy cow nutrition management is extremely important when assessing environmental sustainability of dairy farming, but it also impair economic results due to the huge weight of feed costs on total costs of production of dairy farms (Buza et al., 2014) (de Ondarza and Tricarico, 2017). To reasonable define dairy efficiency and production goals for each farm, several aspects have to be evaluated as animal requirements, and feed composition and availability. (de Ondarza and Tricarico, 2017). From an economic point of view, Income Over Feed Cost (IOFC) is recognised as a proven way to evaluate dairy's profitability (Buza et al., 2014). Reducing dry matter and nutrient losses can significantly improve total dairy enterprise efficiency, by inducing significant changes on the need of purchased compound feeds (Borreani et al., 2018). Additionally, controlled variation in the delivery of nutrients to the animal by appropriate feed and forage selection is essential to achieve nutritional efficiency goals (Bill Weiss, 2007) (de Ondarza and Tricarico, 2017).

The present study aimed to evaluate the effects of the nutritive value of maize silage (MS) and the inclusion of grass-silage (GS) on MS based diets, on feed costs, milk production, and IOFC. Fifty-four commercial dairy farms associated to the Cooperativa Agrícola de Vila do Conde, Portugal, representing different herd sizes and feeding strategies, were used to monthly determine, for three years (2015-2017), the purchased compound feed costs, total feed costs (market values were used for the home-raised forages), milk yield and quality, and IOFC. The data were analyzed using a general linear model comprising the effect of quartile of dry matter (DM), starch, neutral detergent fiber (NDF), and nonstructural carbohydrates (NSC) contents of maize silages, year and their interaction. A similar model was used to evaluate the effect of grass silage (GS) inclusion.

Daily milk yield averaged 30.8 kg/cow (29.3 kg energy-corrected milk (ECM)/cow), and IOFC ranged from 1.10 to 8.44 €/cow/day, with a mean value of $4.08 \pm 1.087\text{€}$. Total feed costs and purchased feed costs averaged 4.80 (3.01-6.49) and 2.99 (1.33-4.70) €/cow /day, respectively. In all the studied years, the lowest milk production was found for the first quartile of DM content (25.7-31.3%), IOFC being higher on the third (33.0-34.8%) and fourth (34.9-49.7%) quartiles. As starch content of MS increases, the total and purchased feed costs decreases. The dietary inclusion of GS reduced the purchased feed costs and milk urea nitrogen (MUN) ($p < 0.05$) on the three studied years, but reduced milk production (Kg and ECM) and IOFC. The nutritive value of the MS used significantly affected the feed costs, milk production and quality, and IOFC; the interaction between year and chemical composition of MS having a huge impact in the results. Indeed, since the end of milk quota in the dairy sector within the European Union, Portuguese farmers are currently have been facing many challenges that include production limitations by industry, that affects farmers management decisions to adapt their production levels each year. In this scenario, appropriate feed formulation and production of forages with high nutritive value are essential to achieve high efficiency and profitability of dairy farms.

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GENETIC PARAMETERS FOR REPRODUCTIVE AND LONGEVITY TRAITS IN BÍSARO PIGS

Gustavo Paixão^{1*}, Alexandra Esteves¹, Rita Payan-Carreira¹ e Nuno Carolino²

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The Bísaro pig has gained popularity in recent years reflecting the success of the conservation program. Nevertheless, no data is available for animal genetic evaluation in this breed. Therefore, this study aimed to estimate genetic parameters and trends for reproduction related traits in Bísaro pigs.

Through a residual maximum likelihood procedure applied to mixed linear models, 27844 farrowing records, from 1995 to 2017, were used to analyse the following traits: total number of pigs born per litter (NBT), number of pigs born alive (NBA), number of stillborn (NSB), number of pigs weaned per litter (NBW), age at first farrowing (AFF), farrowing interval (FIT), length of productive life (LPL), lifetime number of litters (LNL), lifetime pig production (LTP) and lifetime efficiency (LTP365). The fixed effects included in the animal and repeatability models were the herd-year, season and linear and quadratic age. Average litters are constituted by 9.3 piglets, 8.9 of those born alive and 0.4 are stillbirths. 7.4 piglets survive the lactating period and are weaned, resulting in a mean pre-weaning mortality of 16.4%. Usually, the first farrowing happens just after the sow completes one year of age and subsequent litters after six months. The typical Bísaro sow is kept in production for two years with an average number of 2.6 litters. The heritability estimates for litter size traits (NBT, NBA and NBW) were low and ranged from 0.007±0.004 to 0.015±0.006. Differently, the heritabilities for traits related to longevity and lifetime production traits (LPL, LNL, LTP and LTP365) were moderate (0.078±0.026 to 0.121±0.030). AFF registered the highest value of heritability (0.345±0.028). Contrarily, NSB and FIT presented the lowest values of heritability (0.007±0.004 and 0.002±0.005), contrasting with high coefficient of additive genetic variation (0.177 and 0.271), indicating that selection of these traits are feasible. Permanent environment effects accounted for 9%, 2%, 10% and 6% of the variation for NBT, NSB, NBA and NBW, respectively. Genetic correlations between NBT and NBA (0.968), NBW and NBT (0.974), and NBW and NBA (0.945) were positive and very high. Undesirable genetic correlations were found between both NBT and NSB (0.352) and between NBA and NSB (0.107). This finding, along with the positive relation between NBA and NBW demonstrates that NBA should be preferred as a selection trait rather than NBT. Traits of longevity, productivity and productive efficiency showed high positive genetic correlations (0.811-0.969) and moderate to high phenotypic correlations (0.266-0.946). No significant genetic trends were registered over time for most of the analysed traits, except for AFF and LPL, having registered an overall decreased of mean estimated breeding values (21.3 and 17.5) and negative genetic trends of -0.6 and -0.4 (P<0.001), respectively.

Acknowledgements

This study was sponsored by the Portuguese Science and Technology Foundation (FCT) under Projects UID/CVT/00772/2013 and UID/CVT/00772/2016. GP holds a PhD grant from the Animal Science Doctoral Program (AniSci), operation number NORTE-08-5369-FSE-000040, co-funded by the European Social Fund and through the National funds NORTE 2020 and Regional Operational Programme of North 2014/2020.

METHANE CONCENTRATIONS IN DAIRY-CATTLE BUILDINGS OF NORTHWEST PORTUGAL

Ana Raquel F. Rodrigues^{*1,4,5}, Ana Gomes², Luís Ferreira³, Ana R.J. Cabrita¹, Henrique Trindade⁴, António J.M. Fonseca¹, José L.S. Pereira^{4,5}

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Increased concentration of greenhouse gases (GHG) in atmosphere contributes to global warming due to their relatively high thermal absorption capacities. Methane (CH₄) has a half-life of 12.4 years with a global warming potential in relation to CO₂ of 28, being the second most important gas in volume after carbon dioxide (CO₂) (Barbera et al., 2018; Hill et al., 2016). Ruminant production is responsible for 80 % of the livestock sector GHG emissions, with CH₄ from enteric fermentation being the largest source (Opio et al., 2013). CH₄ released to the atmosphere is a serious environmental problem and, from an animal perspective, represents a loss of energy (Eckert et al., 2018). Diet composition, feed intake and feeding strategy and frequency can promote enteric CH₄ variations (Bell et al., 2018; Ngwabie et al., 2011). Several methods are used to quantify dairy cattle CH₄ emissions, varying from respiration chambers to sulphur hexafluoride (SF₆) technique or even non-invasive approaches allowing inventory reports. These, in turn, allow for delineation of strategies to reduce CH₄ emissions from dairy cows whilst guarantee levels of milk production as an adoption of Paris Agreement (Eckard et al., 2010; unfccc.int., 2015).

The aim of the present study was to evaluate CH₄ concentrations in three naturally ventilated dairy cattle buildings, with different feed and milking systems, located at NorthWest Portugal during the winter 2016, spring 2017, summer 2017, autumn 2017 and winter 2017 seasons. Data from dairy cattle farms were collected in all seasons. Measurements were carried with a photoacoustic infrared multigas monitor (INNOVA 1412) and air samples collected, in sequence (2 minutes' intervals) by a multipoint sampler (INNOVA 1409), through 5 sampling points located indoor.

During winter 2016, the average CH₄ concentration (mg m⁻³) was higher during the day in two of the dairy cattle buildings studied, with opposite behaviour in the following four measurements (from spring 2017 to winter 2017), for all dairy cattle buildings (the CH₄ concentrations observed in one of the cattle buildings is presented in Fig. 1). These results might be explained by high indoor values of temperature (° C) and relative humidity (RH, %) during 2017. All dairy cattle buildings had average indoor temperatures of 14 °C and average indoor HR between 79-85 % by night and average temperatures between 16-18 °C and average HR between 70-76 % by day. Obtained values of temperature (° C) and humidity (%) indicate a possible change in feed intake pattern, with higher feed intake by night, when temperatures slightly decrease resulting in high production of CH₄ (mg m⁻³). The present work is part of a 2-year study that aimed at monitoring CH₄. CH₄ concentration (mg m⁻³) obtained will be evaluated according to each dairy farm management practices, chemical analysis of diet, most notably, fibre content.

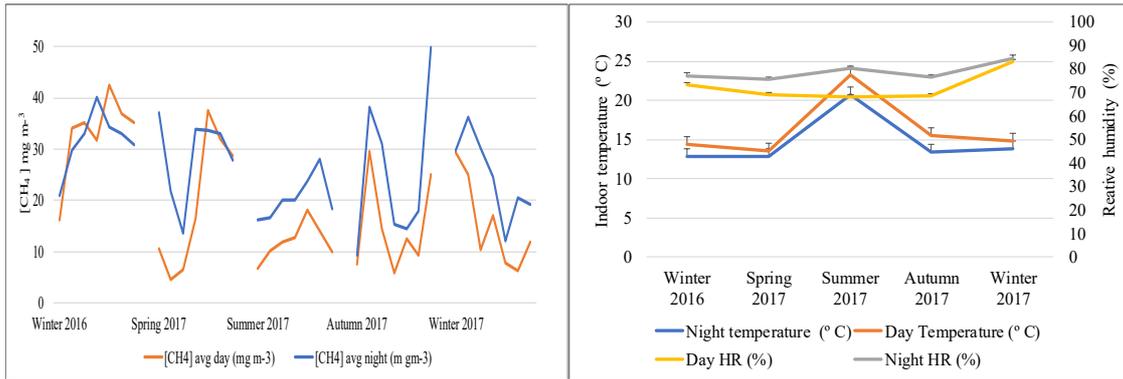


Figure 1- Comparison between night (20-08 H) and day (08-20 H) of average concentrations of CH₄, in one of the dairy cattle buildings during winter (2016 and 2017), spring, summer and autumn seasons (2017); Standard deviation of [CH₄] not represented for clearer representation, always below 41 mg m⁻³.

Acknowledgments

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).

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USE OF INFRARED THERMOGRAPHY IMAGING TO MONITOR STRESS-RELATED RESPONSES BY LAMBS FED WITH LUPINUS DIETS

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Introduction

Sheep are generally limited to a reduced number of feeds, such as low-diversity pastures or concentrated feeds. New feeds, with different flavours, can cause specific sensory responses such as aversions, which is stressful and may reduce the welfare of sheep. On the other hand, in recent years some non-invasive techniques have been developed to monitor welfare animal-based indicators such as body temperature. Infrared thermography imaging (IRT) is a tool that has been used in a very promising way to assess stress of farm species subjected to several management practices like tail docking, foot trimming or dehorning, since it allows a remote reading of temperature, with no physical contact with the animal. However, little attention has been given to the use of IRT to assess stress during the intake of new feeds. With IRT it is possible to identify changes in peripheral temperature which results from changes in blood flow as a stress-induced response. In this sense, this work aims to monitor stress-related responses using IRT of lambs being fed *Lupinus* diets. *Lupinus* species have been known to have antinutritional traits, such as the presence of alkaloids which can also interfere with feed palatability. It is expected that any aversion to a certain feed translated into discomfort which might be perceived through body temperature.

Materials and Methods

The research was conducted at Universidade de Trás-os-Montes e Alto Douro in the Unidade Experimental Arnaldo Dias-da-Silva. Sixteen Churra da Terra Quente lambs, three months old, were divided into three groups and fed three different diets. Animals in the control group (n=4) were fed a mixture of soybean meal and wheat while in the other two soybean meal was partially substituted by *Lupinus*. LL group (n=4) was fed soybean meal, wheat and *Lupinus luteus* and LA group (n=8) animals ate soybean meal, wheat and *Lupinus albus*. The animals were fed every day at the same time. Concentrated feeds were offered in individual buckets in the morning but hay and water was provided *ad libitum* for the rest of the day. Thermographic images were taken twice a week during feeding period using an infrared camera FLIR F4 with resolution 60 x 80 pixels, spectral range 7.5–13 µm (FLIR Systems AB, Sweden). Since the animals were kept within an enclosed stable any effect of environmental factors that might have influenced the thermographic readings was minimized. Thermographic images were obtained on the left eye. Temperature data was extracted from thermal images using the FLIR Tools+ software. Data were analyzed using a two-way ANOVA considering diet and week as factors. Least significant difference Student's t-test was used to compare means. All statistical analyses were performed using the JMP-SAS software (Version 13, SAS Institute Inc. Cary, NC, USA).

Results and Conclusions

Lambs fed with the Control diet had higher eye temperature than the ones in LL and LA groups (37.59 vs. 37.14 and 37.17 °C, respectively; P<0.05). Even though there are statistical differences between lambs fed with a control diet and the ones fed with lupine seeds these are very small differences, inferior to 1°C. Due to a small *n* it cannot be affirmed that these variations are due to the introduction of the legume grains to

the lamb diet. Since lupines are known to have antinutritional traits, this could cause a small aversion to the feed. No effect of time ($P=0.063$) was observed on the variation of IRT eye of lambs. The lack of change in eye temperature during the experiment indicates that environmental or external factors did not interfere since they would have a direct impact on the animal's behaviour, subsequently manifesting in the results.

THE EFFECT OF ORGANIC AND INORGANIC SELENIUM SUPPLEMENTATION IN PUPPIES' GROWTH – PRELIMINARY RESULTS

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Selenium is an essential trace element, with multiple body functions. According to the current knowledge, it should be provided in dog's complete diets to ensure the daily requirement of 100 µg per 1000 kcal of feed metabolizable energy (ME), without surpassing the legal limit of 56.8 mg kg⁻¹ diet dry matter.¹ It has been demonstrated in several species that the source of selenium affects its utilization, which is most probably a consequence of the distinct bioavailability of selenium forms.² A study was conducted aiming to evaluate the influence of inorganic selenium (Na₂SeO₃) and selenium yeast (mostly composed of selenomethionine) in puppies' growth. Both forms of selenium were incorporated in two identical complete dry dog foods (treatments) and fed to puppies from 10 weeks until adulthood. The study was designed as a randomized block design. Twelve dogs (6 males and 6 females) from 3 litters were distributed into 6 blocks according to body weight (BW), gender and litter. Dogs were randomly allocated to one of the dietary treatments. Daily food allowance was weekly adjusted according to body weight to ensure ME requirements ($ME, kcal = 130 \times BW^{0.75} \times 3.2 \times [e^{(-0.87p)} - 0.1]$; $p = \frac{BW \text{ at time of evaluation}}{\text{expected mature BW}}$).³ Data from 10 to 30 weeks of age was analysed according to a mixed model including diet, gender, age, and their interactions as fixed effects, age within dog as a repeated measure, block as a random effect, and the random residual error.

Dogs fed with the organic supplemented diet presented significantly higher feed intake and weight gain ($p < .0001$). The interaction between diet and gender significantly affected weight gain, with organic selenium supplemented diet promoting a higher response on body weight gain of males than inorganic selenium supplemented diet ($p = 0.001$), suggesting that selenium requirements may be gender-dependent. These preliminary results support the hypothesis that organic selenium enhances the growth and performance of dogs. The remaining growth period, as well as general health indicators and specific selenium biomarkers, will be further analyzed in order to fully evaluate the bioavailability of inorganic and yeast selenium and therefore, support the selection of sources and levels of selenium inclusion in pet foods.

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This work received financial support from the European Union (FEDER funds through the Operational Competitiveness Program (COMPETE) POCI-01-0247-FEDER-017616 – Project MinDog). Ana Margarida Pereira also thanks FCT, SANFEED Doctoral Programme, Soja de Portugal, and Alltech for her Ph.D. grant ref. PD/BDE/114427/2016.

POTENTIAL APPLICATION OF AGAR WASTE FROM *Gracilaria* sp.

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Gracilaria sp. (GRA) is used for agar extraction, generating significant amounts of agar waste (AW). The reintroduction of AW into the production cycle has generated great interest as a means of improving the sustainability of industries. AW from agar industries may contain several antioxidant compounds. To test this assumption, we analysed indicators of antioxidant capacity in AW, extracts (E) and compared to synthetic antioxidants [butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), Vitamin C and Vitamin E]. GRA extractions were carried out in water at 95°C or at 25°C for 1h. Then the waste generated by each process was reextracted with ethanol (96%) at 60°C for 3h (AW₉₅ and AW₂₅, respectively). In parallel, GRA was directly extracted with ethanol (96%) at 25°C or 60°C, for 3h and for 6h (E_{25/3}, E_{60/3}, E_{25/6} and E_{60/6}).

Total phenolic content (TPC, mg phloroglucinol equivalent g⁻¹ DM) of both E_{25/3} (33.07 ± 4.04) and AW₉₅ (31.76 ± 5.16) were ~4 times higher than those in the E_{60/3} (7.52 ± 3.32; *P*=0.00) and E_{60/6} (7.08 ± 3.20; *P*=0.00). Also, TPC of both E_{25/3} and AW₉₅ were ~1.5 times higher than those in the E_{25/6} (25.02 ± 7.21; *P*=0.00 and *P*=0.028, respectively) and AW₂₅ (20.53 ± 1.47; *P*=0.00).

The free radical scavenging capacity of AW and E was tested using α , α -diphenyl- β -picrylhydrazyl assay (DPPH, IC₅₀ in μ g ml⁻¹). The lower IC₅₀ value, the higher antioxidant capacity. Low IC₅₀ value was detected on E_{25/3} (1212.52 ± 114.42) which was similar to AW₉₅ (1670.04 ± 180.47) and AW₂₅ (838.49 ± 95.57). In contrast, high IC₅₀ value was detected in both E_{60/6} (2497.22 ± 486.57) and E_{60/3} (2920.66 ± 837) indicating a negative effect of high extraction temperature on antiradical activity. Finally, all the AW and E presented higher IC₅₀ values than those measured in the Vit. C (5.08 ± 0.34), Vit. E (10.11 ± 0.92) and BHA (13.10 ± 5.73) and (5.08 ± 0.14).

Thiobarbituric acid reactive substances (TBARS, mM g⁻¹ tissue) was quantified in fish liver homogenates in the presence of E and EW to test lipid peroxidation inhibition. Both synthetic antioxidants, BHA and BHT, exhibited high lipid peroxidation inhibition (1.84 ± 0.22 and 2.29 ± 0.41, respectively). Substantial lipid peroxidation inhibition was observed in EW₉₅ (6.98 ± 0.22) and EW₂₅ (7.01 ± 0.18) at 50 mg ml⁻¹ (*P*<0.000), which was comparable to the effect of Vit. E (6.99 ± 0.22) at 0.75 mg ml⁻¹. In addition, lipid peroxidation inhibition of E_{60/6} (8.03 ± 0.27), E_{60/3} (8.06 ± 0.17), E_{25/6} (8.53 ± 0.11) and E_{25/3} (8.10 ± 0.21) at 50 mg ml⁻¹ was similar to the effect of Vit. C (8.46 ± 0.22) at 0.75 mg ml⁻¹ (*P*>0.05).

In conclusion, EW presented analogous antioxidant capacities to those quantified in synthetic antioxidants if are included at higher concentrations. Considering extraction conditions of GRA, both high temperature (60°C) and extended time (6h) negatively affect the TPC and the free radical scavenging capacity. This study suggests that EW from *Gracilaria* sp. represents a natural and sustainable source of compounds with antioxidant capacities that may potentially be used to supplement animal feeds.

VOLATILE COMPOSITION AND SENSORY CHARACTERISTICS OF *PARACENTROTUS LIVIDUS*

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The edible part of the sea urchin is the gonads, which is one of the world's costliest seafood products. Due to its scarcity and seasonality, the quality of the sea urchin gonads is often lacking. There is a shortage of information relatively to this subject and this study emerges with the purpose to characterize the sensory features and odour active volatile compounds (VOC) profile of *P. lividus*, taking into consideration the gender and harvest location (North and the South of Portugal). Sensory evaluation was performed by an experienced panel through the application of Projective Mapping paired with Ultra Flash Profile. VOC were determined by headspace solid-phase microextraction coupled to gas chromatography mass spectrometry (HS-SPME/GC-MS).

The Principal Component Analysis (PCA) has shown that gender influenced the sensory properties of sea urchin roe, while the volatile profile of sea urchin roe was affected by gender and harvest location. The perceptual map built from General Procrustes Analysis (GPA) showed that at both locations, males tended to be sweeter, softer and with a pale colour, while females revealed an intense sweet tropical taste and a bright-orange colour. Regarding VOC, PCA unveiled variations in the levels of p-xylene, 1,2,4-trimethylbenzene and m-xylene between locations, whereas levels of β -pinene, 2,4-dimethyl-1-heptene, D-limonene and heptane discriminate the gender. A considerable number of monoterpenes were only identified in the Southern group, while some benzene derivatives compounds were only detected in Northern samples. Male gonads from the North presented volatile compounds that were absent in the female group.

This study contributes to a better characterization of the sensory profile of sea urchin gonads aiming the selection of premium gonads. Furthermore, these results together with consumers' preference data can be used to improve the gonads quality through dietary modulation of sea urchin in captivity aiming the regular production of high quality gonads.

Keywords: sea urchin, projective mapping, odour, quality

Acknowledgments: Luís F. Baião was financially supported by Fundação para a Ciência e Tecnologia, Portugal, and Sense Test, Lda, through the grant PD/BDE/129043/2017. Work supported by Project INNOVMAR (NORTE-01-0145-FEDER-000035) within the line "INSEAFood - Innovation and valorization of seafood products", founded by NORTE2020 through the ERDF.

IMPROVING GROWTH PERFORMANCE OF FISH LARVAE THROUGH EARLY NUTRITION

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Introduction

Senegalese sole (*Solea senegalensis*) is a highly valuable flatfish species for aquaculture diversification in Southern-European countries. However, only recently optimised sole weaning protocols have been developed, with a high impact on larval survival and growth rates [1].

Fish larvae have a tremendous growth potential, displaying growth rates that may exceed 70% a day [2]. This brings a high cellular metabolism of fish larvae, giving rise to formation of reactive oxygen species (ROS). Excessive ROS may interact with all types of biomolecules, including proteins, enzymes and amino acids, causing oxidative damage [3], what leads to increased protein degradation. To maintain homeostasis and prevent oxidative stress, living organisms have evolved antioxidant defence mechanisms that include both enzymatic and non-enzymatic components. Several antioxidants, herbs and extracts rich in polyphenols, have been proven to improve growth performance, antioxidant defences, and immune response in fish [4,5]. Therefore, a suitable intake of antioxidant nutrients and additives may be a useful strategy for fish larviculture.

Objectives

This study aimed to evaluate the different antioxidant supplements as modulators of oxidative status, in order to enhance growth performance in fish larvae.

Material and Methods

Senegalese sole post-larvae, with 45 days after hatching (DAH), were reared in a recirculation aquaculture system and fed with experimental inert diets during 25 days. Sole was fed with one of the eight diets: a commercial diet (Control treatment) and seven experimental diets supplemented with different antioxidants (products A, B and C). The experiment was done in triplicates and abiotic conditions were maintained at optimum values for maximum sole growth.

At two sampling points (60 DAH and 70 DAH) larvae were collected to determine growth performance, muscle cellularity, oxidative status, as well as, expression of candidate genes related to muscle growth, protein turnover and oxidative status. At the end of growth trial, the post-larvae were submitted to a thermal shock for one week. The system water temperature was raised from 21 to 25°C. Fish were sampled at 24h, three and seven days after the temperature shock for analyses of oxidative status and gene expression.

Results

Sole fed with the diet A 0.5 showed a significant increase of 17% (192.2±39.4mg) when compared to fish from the CTRL group (163.8±40.2mg). Moreover, post-larvae from the treatment B 0.1 were also larger than Sole from the CTRL, with a final weight of 182.8±44.8mg (Fig. 1A). At the end of the trial, the A 0.5 group had the best performance (39.1±2.7mm) as well as B 0.1 and C 0.1 in contrast with the CTRL group that had the lowest length (34.96±2.9mm) (Fig. 1B). Food conversion ratio for all diets were less than 1, with an average of 0.5±0.1. No differences between diets were observed between 60-70 DAH. In the period

60-70 DAH, relative growth rate (RGR) was similar between treatments, ranging between 3.7-4.5 %/day, with no statistical differences between diets ($P>0.05$). Furthermore, survival did not differ among treatments, showing values greater than 97% in all treatments. Although the condition factor of the Sole from CTRL and C 0.2 (1.8 ± 0.3 ; 1.7 ± 0.2) were significantly higher than fish from remaining treatments, the mean values were very similar between treatments.

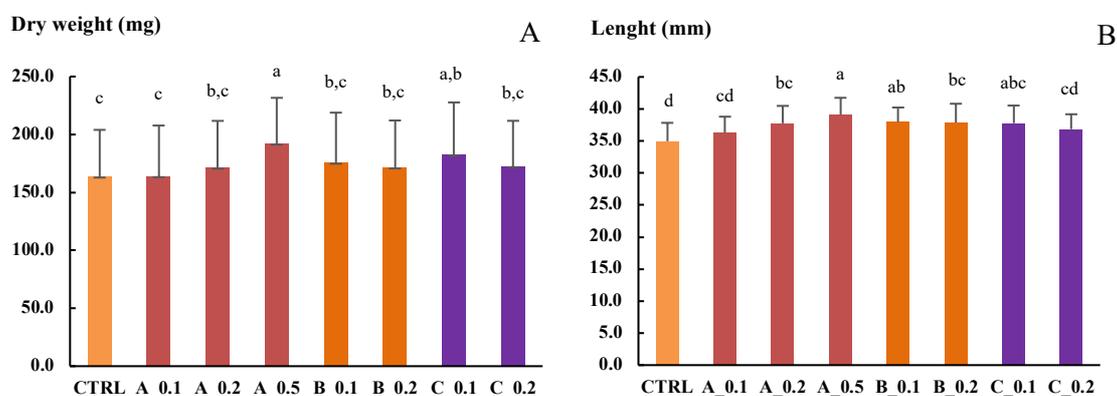


Fig. 1A Dry weight (mg) of Senegalese sole at 70 days after hatching (DAH) of the dietary treatments (CTRL, A_0.5; A_0.2; A_0.2; B_0.2; B_0.1; C_0.2 and C_0.1); Fig. 1B Standard length (mm) of Senegalese sole at 70 DAH of the several treatments (CTRL, A_0.1; A_0.2; A_0.5; B_0.1; B_0.2; C_0.1 and C_0.2); values are mean \pm SD (N = 3/treatment). Different superscript letters at sole age indicate significant differences (One-way ANOVA, $P=0.00$; followed by Tukey test).

Conclusion

Two Sole diets that were supplemented with antioxidants improved fish growth performance. Antioxidants supplementation doses is a critical step to positively influence growth performance in the long-term.

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MODULATION OF GROWTH AND BONE STATUS BY DIETARY TRACE MINERAL SUPPLEMENTATION IN EARLY STAGE *SOLEA SENEGALENSIS*

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Essential dietary trace elements, such as zinc (Zn) and manganese (Mn), critically influence a wide range of physiological, metabolic and hormonal processes in fish larvae and post-larvae. Despite their importance for normal fish growth and skeletal development, trace mineral nutrition has not been extensively studied in early stage fish.

To determine whether the supplementation of a combination of two doses of organically bound Mn and Zn (B-TRAXIM 2C) could improve early-stage survival, growth, mineral deposition rates, and bone status of Senegalese sole (*Solea senegalensis*) post-larvae, triplicate tanks were fed, for 39 days, with one of four dietary treatments, in which an identical basal formulation was concomitantly supplemented with two levels of zinc (25 and 50mg/kg) and two levels of Mn (50 and 100mg/kg). An additional feed formulated without mineral-vitamin supplementation was also tested. The incidence of vertebral anomalies was also assessed by x-ray analysis.

Results show that dry weight, FCR, vertebral bone density and the retention rate of manganese in fish fed a diet supplemented with 50mg Zn/kg diet were lower ($P < 0.005$) than those found in fish fed a diet supplemented with 25mg Zn/kg diet. Fish fed diets supplemented with 100mg Mn/kg diet, presented higher whole fish and vertebral bone manganese content and manganese retention rates ($P < 0.005$), when compared to fish fed 50mg Mn/kg diet. The absence of mineral-vitamin supplementation was shown to have a detrimental effect on overall performance and survival. A preliminary analysis on vertebral malformation incidence indicates that a supplementation level of 50mg Zn/kg feed decreased vertebral bone malformations, when compared to fish fed 25mg Zn/kg feed. Senegalese sole post-larvae fed the different dietary treatments presented no significant differences ($P > 0.05$) regarding total length, RGR and vertebral apposition rates.

A mineral-vitamin supplementation to the diet of early stage Senegalese sole is essential for optimal larval development and a supplementation level of 50mg Zn/kg and 50mg Mn/kg feed appears to be sufficient for normal Senegalese sole growth and may help reduce the incidence of vertebral malformations. Data generated in this trial provides new knowledge in trace mineral nutrition of early stage marine fish.

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.

LIFE CYCLE ASSESSMENT OF ANIMAL FEED INGREDIENTS PRODUCED FROM POULTRY BY-PRODUCTS

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The production of poultry for human consumption leads to large amounts of by-products (about 25% of each chicken is not used for direct human consumption). Rendering these poultry by-products leads to the production of hydrolyzed feather meal (HF), poultry by-product meal (PBM) and poultry fat (PF), which can be interesting protein (HF and PBM) and lipid sources (PF) for animal feeds, due to their high availability and low price. This could reduce the need for imported feedstuffs and the environmental impacts of animal feeds. This study used the Life Cycle Assessment (LCA) methodology to evaluate the environmental impacts of producing HF, PBM and PF, providing relevant information to feed manufacturers regarding the environmental sustainability of these novel feedstuffs, while also showing the main contributions to impacts in the production processes towards providing recommendations to reduce the impacts of HF, PM and PF production.

Information regarding HF and PBM and PF production chains were collected directly from two by-product rendering units in Portugal, AVICASAL (HF) and SAVINOR (PBM, PF). Data were used to create a life cycle inventory and model for each of the production systems, including chicken production and transport of the final products to the feed manufacturer (decision point for the feed producers). Life cycle impacts of each production system were calculated using the CML 2 baseline 2000 method. Four impact categories were chosen, following the most currently used in aquaculture and aquafeeds LCAs: abiotic depletion, acidification, eutrophication and global warming.

The impacts obtained for each production system show that acidification and eutrophication are mainly influenced by the chicken production phase, while abiotic depletion and global warming impacts are mainly due to the rendering process of the by-products. A sensitivity analysis was conducted to see if changes in the fuels used to generate heat during the rendering could decrease the impacts associated with this phase. This analysis showed that using exclusively wood pellets could reduce all the impact categories considered except eutrophication. Overall, the production of these ingredients from poultry by-products has relatively low impacts for the categories analyzed and could therefore be used as environmentally sustainable feedstuffs in animal feeds.

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.

POSTERS

AGE BUT NOT SEX AFFECTS HAIR CORTISOL LEVELS IN A FREE-RANGING POPULATION OF EGYPTIAN MONGOOSE (*HERPESTES ICHNEUMON*)

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Cortisol is a key mediator of the physiological response to stress, and its measurement in hair is increasingly used to understand the effect of natural and anthropogenic stressors on wild animals. In order to do this, it is first necessary to understand how hair cortisol varies naturally in each species. In this study we collected hair samples from 294 wild caught Egyptian mongoose specimens and assessed how hair cortisol varied with sex, age and season within this population. We found a significant effect of age, with higher levels of hair cortisol in the two juvenile age classes, when compared to adults and sub-adults. Additionally, although the results were not statistically significant, hair cortisol tended to be higher in samples collected during summer in comparison to those collected in autumn, winter or spring. Similarly, non-breeding females tended to have higher hair cortisol levels than lactating and pregnant females. These results add to the limited number of studies measuring hair cortisol variation in wild populations. They also add to the inconsistency of the trends in cortisol variation with sex, age and season between mammal species, thus highlighting the importance of accounting for these factors when using hair cortisol to assess the influence of stressors.

SHORT-TERM EFFECTS OF FUNCTIONAL ADDITIVES IN OXIDATIVE STATUS AND INNATE IMMUNITY OF GILTHEAD SEABREAM JUVENILES

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Balanced diets are paramount to maintain fish homeostasis and health status, a fact particularly important under farming conditions, where fish are often subjected to stressful situations. To enhance fish disease resistance and immunity, feeds are often supplemented with health promoting additives reducing the need to use chemotherapeutics. Several compounds with proven antioxidant and/or immunostimulating effects in fish can be found in readily available commercial additives like beta-glucans, fructooligosaccharides (FOS), bioactive peptides and polyphenols amongst others.

The present work aims to evaluate the effects of a short-term dietary supplementation with different commercial additives not only in plasma metabolites and innate immune parameters in gilthead seabream (*Sparus aurata*) juveniles but also in liver oxidative stress defenses and metabolic biomarkers.

Five isonitrogenous (45% protein) and isolipidic (18% fat) diets were formulated. One of them was formulated to be similar to current commercial seabream diets (10% FM) and was regarded as control (CTRL). The CTRL diet was then supplemented with 4 different additives: BG (0.1% beta-glucans), ART (0.6% artichoke extract), SH (3% marine protein hydrolysate) and PRE (0.1% prebiotic). Diets were assigned to triplicate groups of 20 gilthead seabream (IBW: 88.7 ± 2.3g). After 12 and 26 days of feeding 4 fish per tank were euthanized. Blood was collected for haematological procedures and innate immune parameters were analysed in plasma to evaluate fish immune status. Oxidative stress defenses and metabolic biomarkers were also analysed in liver to evaluate overall health status.

Total white blood cells, mean corpuscular haemoglobin (MCH) and haemoglobin concentration remained unchanged among different dietary treatments. Fish fed PRE diet showed lower total red blood cells (RBC) compared to CTRL fed fish at 26 days. Cell blood differential count revealed an absence of dietary effects for monocyte and neutrophil concentration. In contrast, peripheral lymphocyte concentration decreased whereas circulating thrombocytes increased from 12 to 26 days. Fish fed PRE diet presented a thrombocytosis compared to ART fed fish at 12 days. Plasma protease activity decreased from 12 to 26 days regardless of dietary treatment. Fish fed PRE diet showed higher plasma anti-protease activity than CTRL fed fish at 12 days, while CTRL and BG fed fish had this activity enhanced at 26 days of feeding compared to PRE and ART groups. Plasma bactericidal activity increased in BG fed fish compared to ART, regardless of time. Alternative complement pathway activity (presented as ACH₅₀) increased from 12 to 26 days regardless of dietary treatment. Furthermore, SH fed fish showed higher ACH₅₀ values than PRE group at 26 days of feeding. Plasma triglycerides (TAG) and lactate were not affected by dietary treatments, while glucose levels increased and total protein decreased from 12 to 26 days. Hepatic catalase activity and metabolites (i.e. glycogen and TAG) increased from 12 to 26 days regardless of dietary treatments, whereas liver total glutathione and lipid peroxidation were similar over time and were not affected by the dietary treatments.

Overall results show an effect over time on haematological, innate immunity and liver metabolism and oxidative stress defenses in consequence of fish normal growth and development. Nonetheless, different dietary treatments affected fish haematology with PRE diet leading to lower RBC counts after 26 days of

feeding. Also, this same diet showed an early effect (12 days) perceived by the increase in anti-protease activity of fish. Yet, it was not possible to ascertain a clear effect of the dietary treatments on other innate immune parameters evaluated to date. Liver homogenates were prepared for enzymology and gut samples were collected for histology, and both are currently being analysed to obtain a comprehensive assessment of fish liver and intestinal health status.

GUT HEALTH IMPROVEMENT IN FISH AND SHRIMP THROUGH FORTIFIED DIETS: FOCUS ON INFLAMMATORY CONDITION, IMMUNE STATUS AND MICROBIOTA DIVERSITY

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The concept of maintaining animal health through the best possible nutrition is well-accepted in modern animal farming. Particularly for fish and shrimp production, there has been a compelling need to increase the dietary use of alternative protein sources, including by-products from agriculture, fisheries or from slaughter of terrestrial farm animals. Plant proteins are currently the most important alternative to fish meal, given their high availability and good price. However, a recognized disadvantage in most plant-derived nutrient sources is the presence of heat stable anti-nutritional factors that may condition its nutritional value by altering digestion and nutrient utilization, promoting gut inflammation (enteritis) and compromising fish health.

Similarly, to humans and terrestrial animals, short-chain fatty acids, prebiotics and probiotics have been successfully used in aquaculture species. It is nowadays acknowledged that gut microbiota composition and equilibrium is modulated by dietary characteristics, and that an imbalance on this equilibrium, known as “dysbiosis”, is the cause of different inflammatory diseases. Thus, understanding diet-gut interactions and intestinal homeostasis in farmed fish and shrimp is crucial to maximize performance.

The main goal of this work is to develop a standardized dysbiosis model for fish and shrimp and to provide a better understanding of the influence of short-chain fatty acids, prebiotics and probiotics supplementation of extreme diets on the restoration of gut integrity, microbiota diversity, immune mechanisms (including inflammation) and disease resistance in fish and shrimp. This project should allow the development of fortified commercial diets with specific knowledge-based formulations, hence supporting better farming results in terms of growth and disease susceptibility of aquatic animals. Using a holistic approach (including metagenomics, transcriptomics, immunohistochemistry, histology, plasma immune parameters, nutrient digestibility and growth/challenge trials), we expect to understand the mechanisms of action of three particular additives (e.g. short-chain fatty acids, prebiotics and/or probiotics) during dysbiosis and infection. The ultimate goal is to contribute to health enhancement and disease resistance of aquaculture fish and shrimp through fortified diets, thus allowing a shift away from chemotherapeutic and antibiotic treatments.

EFFECTS OF MICROALGAE SPECIES AND PRODUCTION LOT ON *IN VITRO* RUMEN FERMENTATION PARAMETERS

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Microalgae are microscopic single-cell photosynthetic organisms, found in freshwater or marine environments. These sunlight-driven “green” cell factories are rich sources of proteins, lipids, minerals, vitamins and bioactive molecules with antimicrobial, antioxidant and immunomodulatory effects [1]. However, the content of each nutrient and bioactive compound varies among species [2] and within species depending on growth conditions and growth stages [1]. Interest in microalgae as a novel feed has emerged in recent decades but its retail price has limited its inclusion in farm animal diets, being almost exclusively used in aquaculture [3]. This context is expected to change in the near future with the projected scale up of Portuguese microalgae farm production and consequent price decrease.

Ruminant production is estimated to be responsible for nearly 9% of human-induced greenhouse gas emissions [4] with methane being the main gas. Methane is produced during rumen fermentation of feeds and is not only a potent greenhouse gas but represents a 2 to 12% loss of digestible energy to the animal [5]. Dietary modulation has been successfully used to reduce methanogenesis. Thus, supplementation with “green” feed ingredients such as microalgae may have positive impacts for the environment and on feed utilization, leading to more sustainable ruminant farming systems.

This work aimed to evaluate the effects of different production lots of three microalgae species (*Chlorella vulgaris*, *Nannochloropsis oceanica*, and *Tetraselmis* sp.) on rumen fermentation parameters in 48-h *in vitro* incubations with rumen inocula. The effect of microalgae supplementation to different basal substrates was also evaluated.

Material and methods

Five lots of *C. vulgaris*, *N. oceanica* and *Tetraselmis* sp. were provided as gently spray-dried powder and kept at room temperature protected from light. Basal substrates (wheat straw and maize silage) were dried at 65 °C for 24 and 48 h respectively, then ground to pass a 1-mm screen and stored at room temperature. Two non-lactating adult Holstein dairy cows, fitted with a rumen cannula (10 cm diameter, Bar Diamond Inc., Parma, ID, USA) were used as rumen inocula donors. Cows were housed at the Vairão Agricultural Campus of ICBAS-UP (Vila do Conde, Portugal), managed according to good animal practices (Directive 2010/63/EU) under procedures and methodologies approved by ORBEA and licensed by DGAV (permit #FT2014DGV 046412 ICB). Cows were fed a maize silage- and a wheat straw-based diets at 9:30 h and 17:30 h, and had continuous access to water. After 30 days of adaptation to the diet, rumen contents were collected before the morning feed, strained and used as inocula.

Each basal substrate supplemented with 10% of each microalgae species and lot was incubated with buffered rumen fluid (one part strained rumen fluid and two parts of Mould buffer solution) at 39 °C for 48 h. After 48 h of incubation, fermentation was stopped and the rumen fermentation parameters (gas and methane production, pH, total volatile fatty acids content and profile) were evaluated.

Data were analysed using the MIXED procedure of SAS (2002, version 9.1, SAS Institute Inc., Cary, NC, USA) with the fixed effects of substrate, microalgae, lot nested within microalgae and all interactions, the random effect of trial and the random residual error. When interactions were not significant ($P > 0.05$) they were removed from the model.

Results

Basal substrate affected most fermentation parameters. When compared to wheat straw, maize silage promoted the overall fermentation as suggested by the higher gas, methane and volatile fatty acids production, which is agreement with the highest fermentability of this substrate. Among microalgae species, *Tetraselmis* sp. led to lower gas and methane production than *C. vulgaris* and *N. oceanica* but no differences were observed in total volatile fatty acids production or profile. These results suggest that *Tetraselmis* supplementation might reduce methanogenesis without negatively affecting overall rumen fermentation. The effect of lot within microalgae species affected the production of gas and total volatile fatty acids, and acetic, *iso*-butyric, *iso*-valeric, valeric and *iso*-caproic acids proportion. No effect was observed on methane production. Although no information of production lots were provided, we may speculate that these effects reflect differences in the chemical composition of the different lots, namely in protein content. Further research on dry matter and organic matter digestibility might provide further insights into the effect of microalgae species and production lot on rumen fermentation.

Acknowledgments

R.P. (PD/BDE/135540/2018), M.R.G.M. (SFRH/BPD/70176/2010) and D.J.M. (UTAD/2/2017) greatly acknowledge the financial support of FCT and the European Regional Development Fund (FEDER) through NORTE 2020, respectively.

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MODELLING BIOFILM MICROBIAL COMMUNITY TO OPTIMIZE WATER QUALITY AND FISH HEALTH IN A MARINE RECIRCULATING AQUACULTURE SYSTEM

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Recirculating aquaculture systems (RASs) provide opportunities to reduce water usage and to improve waste management and nutrient recycling, making intensive fish production compatible with environmental sustainability. Managing disease outbreaks pose specific challenges in RAS in which a healthy microbial community contributes to water purification and water quality. Minerals, drug residues, hazardous feed compounds and metabolites may accumulate in the system and affect fish health, quality and safety. How the different factors interact and influence the fish and the various purification reactors are still poorly understood. The main objective of this proposal is to model the microbial communities of a marine RAS, with special emphasis on the biofilter, in order to control water quality and bacterial infections throughout the production cycle. The work will be developed in a sole hatchery, and will start with a comprehensive characterization of the microbial communities from all RAS compartments (biofilters, water, biofilms and microbiome of different fish stages). In this phase, several relevant water quality parameters will be monitored in the different compartments, covering not only physicochemical variables but also pathogenic bacteria, with special focus on *Tenacibaculum maritimum*. In addition, several experiments will be set up in the laboratory in order to model the biofilter microbial communities for their effectiveness in the control of water quality and the presence of pathogenic bacteria. At a later stage, the modelling process will be optimized at laboratory trials using fish, water and biofilm inoculum from the hatchery, and finally implemented in the real system in order to validate its efficiency.

DEMOGRAPHIC STRUCTURE AND GENETIC DIVERSITY OF THE ENDANGERED BÍSARO PIG: EVOLUTION AND CURRENT STATUS

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Sustainable use of genetic resources, in adaptive breeding and conservational programs, depends on good management of genetic diversity. The Bísaro pig is a native Portuguese breed, descended from the Celtic line, known for its highly valued cured products. Bísaro pig farms are concentrated in the north of Portugal, comprehending small producers in traditional systems and medium-sized farms. The study aimed to evaluate the demographic and genetic variability of the Bísaro pig population, allowing to establish an effective breeding program and to adjust the current conservation program. The complete Bísaro pig pedigree information was used in this study, including 206507 birth records from January 1994 to June 2017.

Since the breed's foundation, the number of registered animals, producers, and farrowing records has steadily increased. The mean progeny size was 113.30 ± 211.42 and 19.01 ± 15.30 for sires and sows, respectively. Nevertheless, the majority of boars only had between 11 to 50 descendants with a limited contribution of 9.20% of total births registered. Contrarily, 2.92% of the breeding males originated 25.94% of all registered births showing a marked unbalanced use of certain sires. The generation intervals, defined as the average age of parents at the birth of their progeny kept for reproduction or not, were 1.92 ± 1.12 and 2.19 ± 1.26 years, respectively. The mean calculated equivalent generations was 4.45, and 97.8% of all the animals had known parents, indicating a good degree of pedigree completion and depth.

The average inbreeding coefficient was 10.27%, higher than most of those calculated for other local and commercial pig breeds worldwide. In line with this results, 54.06% of animals were inbred, this high value suggests that inbred animals have particularly high values of inbreeding coefficient, which is supported by the high frequency of matings between close relatives. A low f_e/f_a ratio was obtained (1.04), showing a well-balanced founder/ancestor contribution, and subsequent genetic transfer from generation to generation. However, the f_e/f ratio was particularly low (0.02) when compared with other native breeds, suggesting a history of high selection or the frequent use of particular animals. Furthermore, the number of founders accounting for 50% of the population genes was 43, meaning that fairly 0.02% of all registered animals accounted for half of the population genetic diversity, and meaning that much of inbreeding is caused by the abusive use of certain founders through their descendants. The estimated loss of genetic diversity due to unequal founder contribution was 0.43% and represented a higher relative proportion when compared to random genetic drift. The effective population size ranged from 15.42 to 68.54, and the genetic conservation index steadily grew over the years with a mean value of 6.63 ± 5.09 .

The estimated parameters indicate that Bísaro pig meets the requirements to implement an effective selection program. The moderate genetic variability be taken into account given the elevated inbreeding coefficient and the low effective population size. Thus, encouraging producers to reduce matings between relatives and to limit the use of certain sires should be highlighted, to keep a reliable, healthy genetic pool.

Acknowledgements

This study was sponsored by the Portuguese Science and Technology Foundation (FCT) under Projects UID/CVT/00772/2013 and UID/CVT/00772/2016. GP holds a PhD grant from the Animal Science Doctoral Program (AniSci), operation number NORTE-08-5369-FSE-000040, co-funded by the European Social Fund and through the National funds NORTE 2020 and Regional Operational Programme of North 2014/2020.

USING LIPID NANOPARTICLES TO DELIVER LYSINE ACROSS THE DIGESTIVE TRACT OF THE DAIRY COW

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Introduction

In high producing animals, the amino acids from microbial protein synthesized in the rumen that are absorbed in the small intestine are insufficient to satisfy the amino acids requirements. To increase the amount of amino acids absorbed by the animal, protein sources that bypass rumen fermentation comprise an effective strategy. However, for an efficient conversion of dietary protein to milk protein, the balance of amino acids absorbed should be considered. In common protein sources and microbial protein synthesized in the rumen, lysine is commonly the first limiting amino acid. Indeed, several commercial rumen-protected lysine are available and routinely used. However, the results often suggest that the rumen protection of these products is unsatisfactory. In this work, a novel approach is proposed towards the rumen bypass of nanoparticles for lysine (Lys) delivery, as well as the remaining organs of the bovine digestive tract until it reaches the blood stream.

Methods

Several types of lipid nanoparticles (NPs), composed of a wide variety of lipids and surfactants, were considered and assessed as potential rumen-bypass options: solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and multiple lipid nanoparticles (MLNs). All formulations were characterized in terms of average size, size distribution profile, zeta potential and encapsulated Lys. Their ability to bypass ruminal digestion was also determined. The selected formulations were then optimized using a Box-Behnken mathematical design to maximize Lys content. The resulting formulations were then assessed for their stability in the abomasum, small intestine and in the blood stream.

Results

The results showed that only SLNs composed by arachidic acid or stearic acid, and using tween 60 as a surfactant, were able to resist ruminal digestion. These two formulations were then optimized, rendering encapsulation efficiencies of 65% and 60%, for arachidic and stearic acid SLNs, respectively. Both formulations appeared to be able to resist digestion in both the abomasum and in the small intestine.

Conclusions

The proposed NP formulations showed interesting properties and were shown to be able to resist digestion in the rumen, the abomasum and in the small intestine. They were also loaded with a high amount of Lys. To conclude, the studied NPs are promising candidates for future Lys delivery applications in dairy cow nutrition and could help to overcome the current limits in the field.

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. The authors would also like to thank Silvia Azevedo (ICBAS, UP) for her assistance with the rumen resistance assays.

IDENTIFICATION AND DETECTION OF BIOMARKERS FOR CARBOHYDRATES AND PROTEINS FERMENTATION PATTERN IN RUMINANT ANIMALS

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The livestock sector has a high economic importance worldwide. Being a sector highly dependent on efficiency and productivity, non-healthy animals can represent significant costs. For this reason, disease control and balanced diets are one of the main sector's priorities [1].

The nutritional balance and interactions between ruminal microorganisms and diet are essential for a healthy metabolism of ruminants. For instance, several studies have demonstrated that feeding diets with high levels of fermentable carbohydrates and low levels of physical effective fibre decrease rumen pH through accumulation of organic acids, such as volatile fatty acids (VFA) and lactic acid, increasing the risk of subacute ruminal acidosis (SARA) [2-3]. SARA is presently recognized as one of the most important digestive disorder, due to its negative economic impact on livestock sector. There is not yet a consensual definition of SARA but it's generally agreed that occurs when ruminal pH decrease to non-physiological levels for several hours a day. This disorder is also associated with several other pathologies, such as laminitis, ruminal ulceration, hepatic abscesses and displacement of the abomasum [4-5].

In recent years, research on diagnosis and monitoring of cattle using non-invasive methods has gained importance. Milk and breath analysis are beginning to be considered as diagnostic sources and some volatile compound identified in these samples have been already pointed as potential biomarkers [6-7].

The present PhD project aim to detect and identify compounds that can be used as biomarkers for diagnosis of certain pathologies in cattle. This project will mainly focus on the identification and characterization of products from the metabolism of proteins and carbohydrates that could constitute biomarkers for the early diagnosis of digestive and metabolic disorders, with a view to their application in the livestock sector.

The work plan is divided into 4 steps:

1. Validation of protocols of analysis of volatile metabolites on biological samples;
2. Identification of volatile metabolites in ruminal fluid from *in vitro* assays;
3. Development of pH monitoring system for the early detection of SARA;
4. Clinical case studies.

Acknowledgments

This work was financed by project NORTE-01-0145-FEDER-0001, co-financed by the Northern Regional Operational Program (NORTE 2020), through Portugal 2020 and the European Regional Development Fund (FEDER) and received financial support from the European Union (POCI/01/0145/FEDER/007265) and from national funds (FCT/MEC, Foundation for Science and Technology and Ministry of Education and Science) under the agreement PT2020 UID/QUI/50006/2013. LEC also thanks FCT, SANFEED Doctoral Programme, AGROS UCRL and Alltech for her PhD grant PD/BDE/136294/2018. IMV

(SFRH/BPD/111181/2015) also acknowledge her post-doctoral grant to FCT/MEC and the European Social Fund within the 2014–2020 Strategic Framework.

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SHORT-TERM FEEDING WITH ARGININE AND CITRULLINE IMPROVES THE GILTHEAD SEABREAM (*SPARUS AURATA*) IMMUNE STATUS

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Several amino acids (AA) are known to regulate key metabolic pathways that are crucial for immune response. In particular, arginine appears to have important roles regarding immune modulation since it is required for macrophage responses and lymphocyte development. Moreover, citrulline is a precursor of arginine, and it was reported as an alternative to arginine for improving macrophage function in mammals. The present study aimed to explore the effects of dietary arginine and citrulline supplementation on the gilthead seabream immune status. Triplicate groups of fish (23.1 ± 0.4 g) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of arginine or citrulline (0.5% and 1% of feed), ARG1 and CIT1 and ARG2 and CIT2, respectively. After 2 weeks of feeding, fish were euthanized and blood was collected for blood smears, and plasma for humoral immune parameters. A significant increase of antiprotease activity was observed in fish fed ARG2 compared to fish fed the CTRL diet. A significant increase in monocytes was also observed for the relative proportion of peripheral blood leucocytes in fish fed diets with the highest supplementation level (i.e. ARG2 and CIT2), compared to their counterparts fed the lower supplementation level. Peripheral monocyte numbers also correlated positively with nitric oxide, which showed an increasing trend in a dose dependent manner. These results appear to enlighten that dietary supplementation with arginine or its precursor (citrulline) have an immunostimulatory effect after 2 weeks of feeding.

DIETARY TRYPTOPHAN DEFICIENCY AND SUPPLEMENTATION COMPROMISES EUROPEAN SEABASS IMMUNE STATUS, INFLAMMATORY MECHANISMS AND DISEASE RESISTANCE - A MOLECULAR APPROACH

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Introduction

Tryptophan catabolism plays a critical role in the immune mechanisms in macrophages mediating anti-microbial effects, regulating T-cell function and modulating cell oxidative status, favouring immune suppression and tolerance. However, the potential benefit of tryptophan supplementation for animal health management is not fully demonstrated. Therefore, the present study aimed to evaluate the effects of dietary tryptophan deficiency and supplementation on the European seabass immune status and inflammatory response through the evaluation of immune and tryptophan catabolism related genes and disease resistance to *Photobacterium damsela* piscicida (*Phdp*).

Material and methods

Seabass juveniles were fed four experimental diets in triplicate tanks: control diet (CTRL); the CTRL diet supplemented with 15 and 30 % above L-Tryptophan requirement (TRP15 and TRP30, respectively) and a negative control diet (NTRP), deficient in tryptophan. Fish were sampled after 2 and 4 weeks and the remaining fish were intraperitoneally injected with *Phdp* (5×10^3 cfu / fish) and sampled after 4, 24, 48 and 72 hours. Head-kidney was sampled for mRNA gene expression. The mortality of the remaining fish was recorded during 21 days.

Main results and discussion

In a tryptophan deficiency scenario, neither immune status nor the response to an inflammatory agent were altered, while disease resistance to *Phdp* was weakened. On the other hand, when dietary tryptophan is offer 30% above requirement level and in response to *Phdp* a decrease in several pro-inflammatory genes were observed, eventually compromising at some level fish disease resistance.

In conclusion, results suggest that both dietary tryptophan deficiency and supplementation may compromise the inflammatory mechanisms and disease resistance.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS) IN *COLOSSOMA MACROPOMUM*

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Long-chain polyunsaturated fatty acids (LC-PUFA) are critical biological molecules and play a paramount role in fish health, growth and development. Extensive studies have addressed the nutritional requirements of these molecules particularly in production contexts. Tambaqui (*Colossoma macropomum*) is a native fish species of the Amazon basin, being one of the top aquaculture-produced species in Brazil. We have previously shown the molecular capacity of this species to biosynthesize LC-PUFAs. Here, we address critical regulatory module of fatty acid metabolism, PPAR signalling pathway. Fatty acids and their derivatives are known to directly regulate the activities of a variety of transcription factor such as nuclear receptors (e.g. PPARs), whose functions involve the control of genes responsible for fatty acid, cholesterol, and carbohydrate metabolism. In particular, it is known that the homeostasis of lipid metabolic molecular modules is mostly under the control of the PPAR signaling pathway. PPARs act as transcription factors and regulate multiple endocrine aspects. Moreover, in teleosts PPARs have been shown to regulate the endogenous LC-PUFA biosynthesis in relation to dietary inputs. In this study, we identified the full repertoire of PPAR genes: PPAR α A, PPAR α B, PPAR β A, PPAR β B and PPAR γ , through the use of a *de novo* assembly of the liver transcriptome of the tambaqui. The expression of these transcription factors was addressed under a nutritional assay. In general, our results further elucidate on the endogenous ability of tambaqui to satisfy its LC-PUFA requirements.

NUTRITION AND THE INNATE IMMUNE SYSTEM: MODULATION OF BOVINE DENDRITIC CELLS AND MACROPHAGES IMMUNE RESPONSE BY DIETARY SUPPLEMENTS OF MICROBIAL ORIGIN

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Background

The high mortality and morbidity rates in calves due to infectious disease, predominantly prior to weaning, is still a matter of concern in cattle herds. Proper management and animal welfare have been the focus of farmers and technicians, mainly in dairy production. Nonetheless, gastrointestinal infections with subsequent diarrhoea and dehydration, and respiratory syndromes are the main health problems of calves until weaning and the most important cause of calf mortality (McGuirk 2008). Strategies that reinforce immunity of newborns may decrease the incidence of these disease and improve calves' health with subsequent long-term positive effects on their growth, welfare and performance (Kim et al. 2011).

Several studies have suggested that dietary supplements could improve immune function through “trained immunity”, a recently proposed mechanism that allows innate immune cells such as monocytes, dendritic cells and natural killer cells to show enhanced activity after a first exposure to pathogens (Saeed et al. 2014, Quintin et al. 2012). Thus, our aim was to evaluate the ability of supplements of microbial-origin, including yeast-cell wall derived products, to induce *in vitro* innate immune memory on bovine dendritic cells and macrophages.

Material and methods

Bovine peripheral blood mononuclear cells were isolated by gradient centrifugation, and monocytes (CD14⁺ cells) were sorted using magnetic sorting. Microbial cells and products, namely yeast derived beta-glucans as well as pre-ruminant digestion products of microalgae, were used to stimulate *in vitro* bovine monocyte derived dendritic cells and macrophages. The digestion products were obtained by an enzymatic method simulating the gastric and intestinal digestion (Boisen and Fernández 1997).

Apoptosis/necrosis induction was evaluated by flow cytometry. Upon a resting period, cells previously stimulated with biocompatible supplements were challenged with several Toll-like Receptor (TLR) and C-type Lectin Receptor (CLR) agonists, molecules capable of inducing an immune response. The resulting cytokine and reactive oxygen species production was evaluated by real time-polymerase chain reaction and/or enzyme-linked immunosorbent assay and by flow cytometry, respectively.

Results

Pre-treatment of monocyte derived dendritic cells and macrophages with some of the used dietary supplements of microbial origin, including particulate yeast derived beta-glucans, led to increased pro-inflammatory cytokine production in response to TLR and CLR agonists such as lipopolysaccharide, a synthetic triacylated lipopeptide (Pam3CSK4), and zymosan.

Conclusions

Our results show that dietary supplements of microbial-origin may induce innate cell memory. These products are thus promising feed supplements in calves' nutrition and may represent an alternative to in-feed antibiotics.

Acknowledgements

RP, TL, MM, and AC were supported by FCT grants PD/BDE/135540/2018, PD/BD/128393, SFRH/BPD/70176/2010 and SFRH/BPD/91623/2012, respectively.

Authors thank Allmicroalgae - Natural Products for the kind offer of microalgae.

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MODULATING THE PROTEIN PROFILE AND TECHNOLOGICAL VALUE OF MILK THROUGH A NUTRITIONAL APPROACH

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Milk proteins are categorized into three major groups: caseins (about 80%), whey proteins (approximately 19%), and milk fat globule membrane proteins (thereabout 1%) (O'Mahony and Fox 2014). Caseins are the main contributors of the cheese structure and the proportion of caseins in relation to milk crude protein is a key factor for cheese-making efficiency (Coulon et al. 1998).

The influence of nutrition on milk crude protein has been intensely studied and, in general, the nutritional strategies used to increase milk crude protein lead to a proportional increase of the casein content (Coulon et al. 1998). However, over the last years, novel feeding strategies have been developed to reduce nitrogen excretion through urine and feces in the environment. Offering lower protein diets is an effective strategy in terms of nitrogen losses to the environment (Powell and Rotz 2015), but it is associated to reductions in milk protein production and lower casein number (Coulon et al. 1998). The supplementation of low protein diets with bypass protein supplements has been recommended to maintain the productive performance of ruminants (Abbasi et al. 2018), nevertheless, Tacoma and others (2017) observed that replacing soybean meal by a bypass protein supplement decrease casein yield, in spite of do not affect milk true protein yield and milk yield.

In this project, it will be assessed the influence of the following strategies related with nitrogen efficiency on casein yield and milk true protein profile:

- Reducing dietary soluble protein;
- Increasing the starch content of low protein diets, and;
- Supplementation of low protein diets with synthetic rumen-protected amino acids.

In addition, *in situ* experiments will be conducted to compare the ruminal disappearance of different additives and ingredients.

To summarize, the present thesis project has as main goals a better understanding of the influence of novel feeding strategies related with efficiency of utilization of dietary nitrogen on casein yield and milk protein profile.

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MICROBIOME IN POULTRY: INFLUENCE OF NUTRITIONAL FACTORS

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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 an interplay between the chicken and the microbiota present within the gastrointestinal tract (GIT). While supplementing the chicken's diet with prebiotics and probiotics, it is expected that the zootechnical performance is improved through positive selection of the GIT microbiota. Recent advances in culture-independent techniques allowed the identification of new taxa within the microbiota. The chicken GIT harbors a very diverse microbiota that aids in the breakdown and digestion of food and comprises over 900 species of bacteria, which contain both human and animal pathogens. Unlike mammals, caecum has an important role in birds' digestion, being a fermentation powerhouse due to the presence of a dense microbial community. The main objective of this work is to obtain information regarding the chicken GIT microbiome and selectively increase the chicken growth process performance. Histological samples will be scanned to evaluate possible morphological changes regarding the different feedings. Villi height and quantity was assessed as well as the different cell types present. Combined with the different microbiome analysis results, it will be possible to infer which supplements will have a positive effect on chickens' growing and nutrient absorption as well as improved wellbeing and reducing the stress as well as the cost of production.

ROUND TABLE

“CIRCULAR ECONOMY”

The Round Table kick-started with an introduction by Professor Fausto Freire, who told the audience about his expertise in Life Cycle Analysis (LCA), emphasizing the multidisciplinary of it through the presentation of a field diversity of projects in which LCA has been applied. He also enlightened concepts such as “product circularity” and “trade-off”.

The discussion started with a question to the audience. Jorge Dias, asked Tiago Aires, about his opinion regarding ingredients that will continue in the market and the emergent that will become important in the next 20 years in the field of aquaculture.

Tiago Aires highlighted the need for fast growth, locally produced ingredients, giving the example of the microorganisms (protein from bacteria or microalgae, among others). According to Tiago Aires, fish meal protein which is the major ingredient in aquaculture feed has the potential to be replaced by other sources and that its level of incorporation has actually been reduced to one third. However, in a 20-year scenario, the growth of the aquaculture industry cannot only rely on ingredients of plant origin, like soybean meal, which is harvested in tropical countries, linked to deforestation, and whose long journeys from where it is produced, have been leaving behind a massive footprint.

Jorge Dias agreed and added that the need to continue the intensification of animal production goes beyond concepts such as “optimization” and “nutrition of precision”. According to him, it is mandatory to find new and sustainable ingredients. Jorge Dias also highlighted that research on ingredients such as protein from microalgae must continue despite its association with high energetic costs of production, since they may be sustainable alternatives in the future.

Pedro Félix, a student in the audience, asked about the use of agri-food by-products as a substrate to grow insects for potential use as a protein source.

Elisabete Matos commented that the production of protein from insects has a huge potential for both feed and food. However, there are a lot of uncertainties associated with their nutritional value, since there are around one million species, which can originate substrates with different nutritional values. Advantages of the production of protein from insects are the suitability for local production and compliance with product circularity. Elisabete Matos believes that soon, the use of insect meal for poultry feed will be legislated, and therefore authorized in the European Union.

Ingrid Van Dorpe, in the audience, added that insect protein production is still insignificant and it is important to continue the research on other alternative proteins, giving the example of bacteria in glass stored waters. In relation to novel food proteins, she highlighted the importance to recover gastronomic traditions such as, eating pigeons and snails, whose feed conversion ratio is 1, once for the occidental population is still uncommon to eat insects. According to her, genetic improvement will continue to play an important role in the growth of animal production.

Luísa Valente, in the audience, commented that the high economic costs associated with the product circularity, such as growing insects, use of agri-food subproducts and microalgae protein production, are an obstacle for companies to invest on product circularity. In her opinion, the government should support the companies’ investment in this field.

Ingrid Van Dorpe disagreed, because in Portugal all agri-food byproducts are processed and utilized by feed industry, such as bakery byproducts, spent brewery grains, tomato peel, and seed by-products.

Jorge Dias queried Hugo Oliveira about the possibility of integrating the Livestock 4.0 technologies in our concept of sustainability.

Hugo Oliveira answered that the potential impact of scale manufacturing of sensors in the environment depends on how the sensors will be eliminated, like the batteries of the electrical cars. According to him, Livestock 4.0 technologies are important for nutrition of precision, however, the direct impact on the increase of animal protein production will be minimal. The intensification of animal production will continue to be sustained by genetic improvement, while diet will still be crucial to fulfil the greatest genetic potential.

Jorge Dias agreed and asked the opinion of the researchers Ana Paula Mucha and Inês Valente about the applicability of sustainability in their work.

Ana Paula Mucha highlighted the importance of clarifying the consumer about the concept of “green” ingredient because in some situations the energetic costs related to the product circularity are quite high. Ana Paula Mucha also highlighted that the main focus of sustainability should be reducing the environmental impact of the processes/methodologies and their waste.

Inês Valente commented that she attended a doctoral program in Sustainable Chemistry and so, this scientific concept encompasses the manufacture and use of more environmentally friendly reagents and methodologies. Inês Valente also commented that multidisciplinary groups are the key to achieve the goal of simultaneously to protect the human and animal health and the environment.

Jorge Dias thanked and asked Ana Raquel Rodrigues how her Ph.D. project entitled, “Methane concentrations in dairy-cattle buildings of northwest Portugal”, has influenced her opinion about the utilization of natural resources.

Ana Raquel Rodrigues answered that she aims to quantify the gases emissions from dairy-cattle facilities in order to define a plan to reduce those emissions taking into account the characteristics of dairy-cattle facilities of northwest Portugal. She added that European Commission has elaborated the green paper “European Strategy on Plastic Waste in the Environment” and also that in Portugal there is already both work plan and European Funds to implement “Circular Economy” approaches. However, in her opinion, the concept “product circularity” is based on the principle of disengagement of the company that produces the by-product. Ana Raquel Rodrigues also refers that society lives in a period of programmed obsolescence, being very easy to buy new products.

Jorge Dias agreed and added that each of us, as part of a society, should make better use of our material assets and asked Francisca Silva-Brito about her opinion.

Francisca Silva-Brito answered that there is not a single solution, however, the dissemination of information is of most importance as a mean to raise awareness, train people and ultimately change our society convictions’. For example, occidental society should be sensitized for insects as food, once traditional animal proteins like poultry are not enough for the growing population in the world.

Ingrid Van Dorpe shared the concern of Ana Raquel Rodrigues about the programmed obsolescence, namely the no longer existence of habits of reparation when equipment is damaged.

Jorge Dias concluded that we are facing very important challenges and each of us must be more critical about the concepts that should be applied to nutrition. However, he agreed that this might be a hard process given the high costs related to product circularity which leads to a disengagement from industry sector.

Fausto Freire finished the session leaving as take home message, that the concept of “circular economy” was inspired on the mechanisms of the natural systems and that there are no single or easy solutions.

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Beatriz Maria Dias Ramos	Gustavo Paixão	Luís Ferreira	Pedro Campelos	

THANK YOU!

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