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Rua de Jorge Viterbo Ferreira n.º 228

Porto,
PORTUGAL
ANIMAL SCIENCE DOCTORAL PROGRAMME

III WORKSHOP

15th September 2016

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António Afonso, CIIMAR & ICBAS- UP
Rui Pereira, ALGAplus
PROGRAM

14:00 – Registration
14:15 – Opening Session and presentation of SANFEED Programme Topics 2016/2017: Scientific Committee of the ANIMAL SCIENCE DOCTORAL PROGRAMME
   António Mira da Fonseca, REQUIMTE, LAQV & ICBAS-UP
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Jorge Dias, SPAROS

Coordinator of the AniSci PROGRAMME, NORTE-08- 5369-FSE-000040
Rita Payan Carreira, UTAD

Session I: Chairperson: Luís Ferreira, AGROS & Hugo Oliveira, REQUIMTE, LAQV & ICBAS-UP

14:30 – Plenary session: Methane mitigation in ruminants – a microbes perspective
   Sinead Leahy, AgResearch, New Zealand

15:00 – Alkaloids in the valorization of european Lupinus spp. seeds crop
   Sara Magalhães, REQUIMTE, LAQV & ICBAS-UP PhD student

15:15 – Impact of shortening the voluntary waiting period on the net profit of a dairy farm
   Isabel Santos, CAVC, REQUIMTE, LAQV & ICBAS-UP PhD student

15:30 – Lipid nanoparticles as a novel rumen-bypass technology towards the delivery of lysine in dairy cow nutrition
   João Albuquerque, PREMIX, REQUIMTE, LAQV & ICBAS-UP PhD student

15:45 – Coffee Break & Posters session

Session II: Chairperson: António Afonso, CIIMAR & ICBAS-UP & Rui Pereira, ALGAplus

16:15 – Plenary session: Carcass and meat quality: tendencies and preferences
   Alfredo Teixeira, ESA-IPB

16:45 – Partial and total replacement of fish oil by poultry fat on European seabass (Dicentrarchus labrax) growth and tissue composition
   Inês Campos, SORGAL, CIIMAR & ICBAS-UP PhD student
17:00 – Growth performance, oxidative stress and immune responses in gilthead seabream (Sparus aurata) fed different type and content of seaweed supplementation

Augusto Queiroz, CIIMAR & ICBAS-UP PhD student

17:15 – Oxygen consumption as proxy for metabolic costs in swimming fish

Maria João Peixoto, CIIMAR & ICBAS-UP PhD student

17:30 – Molecular perspective on osmoregulatory challenge of parasitic feeding in the sea lamprey (Petromyzon marinus) and host (Salvelinus fontinalis)

Diogo Martins, CIIMAR & ICBAS-UP PhD student

17:45 – Industrial property- uses and best practices

Vera Gonçalves, Patents

18:00 – Round Table discussion: “Intellectual Property”

Vera Gonçalves, Patents, Sinead Leahy, AgResearch, Alfredo Teixeira, ESA-IPB, Nuno Medina, SAVINOR, João Albuquerque PREMIX, REQUIMTE, LAQV & ICBAS-UP PhD student & Diogo Martins, CIIMAR & ICBAS-UP PhD student

20:00 – Closing Dinner
ORAL COMMUNICATIONS
METHANE MITIGATION IN RUMINANTS – A MICROBES PERSPECTIVE

Sinead Leahy
AgResearch Limited, Grasslands Research Centre, Palmerston North, NZ
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Abstract
Climate change and the need to feed a growing global population are the two greatest challenges facing agriculture. Ruminant animals occupy an important niche in modern agriculture because of their ability to digest plant materials that are of very low nutritional value to humans (and other animals) and convert that feed into high-quality products, such as meat, milk and fibre.

Ruminants contribute significantly to the world’s economy by supporting the livelihoods and food security of over a billion people, including an estimated 70 percent of the world’s rural poor. However, they also represent a significant environmental issue. They release an estimated 125 million tons of methane, a potent greenhouse gas, into the atmosphere annually. The ability of ruminants to digest plant material is made possible by the actions of a specialised community of microbes that live inside an organ called the reticulo-rumen (commonly referred to as the rumen). Some by-products of this digestive process are used by certain microbes (called methanogens) to produce methane, which is belched out into the atmosphere by the animal. Our lack of sufficient understanding of rumen microbes is still considered one of the major knowledge gaps hindering effective enhancement and modification of rumen function. Our quest for knowledge has been hampered by the extreme difficulty of working with rumen microbes (many of these microbes cannot survive in the presence of oxygen) and by the lack of suitable tools to study them. However, the advent of DNA sequencing and the discipline of genomics has heralded a new era of research in rumen microbiology.

Advances in microbial genomics have revolutionised discovery-based research into the rumen ecosystem. This presentation will discuss the role AgResearch-led projects such as the Global Rumen Census, the Hungate1000, Rumen methanogen genomics and Deep sequencing of the rumen microbiome have had in increasing our knowledge of the microbial processes leading to the formation of methane in the rumen and how we can use that knowledge to support the development of practical and safe technologies to mitigate ruminant methane.
CARCASS AND MEAT QUALITY: TENDENCIES AND PREFERENCES

Alfredo Teixeira
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Abstract

Data on the evolution of global meat production and the latest trends and preferences in terms of quality of carcasses produced and meat quality are presented and discussed. With the continuous growth of population by 2050 the World’s population is estimated in 10 billion or so inhabitants. Feeding the World population in 2050 will be a major challenge at the forefront of the global development agenda. In this setting the increasing of meat consumption will entail an increase in production and maintaining an active market. Consequently the factors relating to the quality of carcass and meat globally accepted as carcass weight, degree of fatness, conformation, tissue composition and chemical composition will continue to be a challenge in the near future. In this sense we present the latest trends to find objective and accurate methods for carcass evaluation and classification, as well as to access the key factors on the meat quality characteristics. Results recently published in the successive reports under the action COST Farm Imaging Animal (FAIM) involving 120 researchers from several EU countries, including Portugal, in its working group WG1 Body Composition, tried to optimize non-destructive methods — in vivo and post mortem — for measuring the body composition and meat quality. Among the methods tested the use of computed tomography (CT) showed potential to be considered as a reference method in place of traditional carcass dissection. The use of ultrasound, for its increasing accessibility, remains an effective solution intensively used with promising results in different species, particularly in cattle, swine, sheep and goats. In terms of meat quality the knowledge of meat products from production to consumer (from the farm to the fork) is now an urgent necessity in order of the value and differentiation but also protection from possible frauds. The necessity to assess carcass evaluation the development of effective and rapid systems to monitor production, to evaluate the physical and chemical composition, health and sensory quality, is nowadays a necessity to ensure food quality and safety as well as to give food information to consumers. The near infrared spectroscopic techniques (NIRS) supported by all laboratory analytical methods and sensory analysis are now widely used to find the best models to estimate the different parameters of physical-chemical, microbiological and sensory quality of meat.

Under the already mentioned action COST, FAIM, the group on meat quality reviewed and evaluated the existing methods and developed recommendations for harmonized procedures spectroscopic methods to estimate in vivo and online meat quality. The group produced a document (The Handbook of reference methods for meat quality assessment) reference on methods for assessing the quality of the meat.

Key words: carcass, meat, quality and evaluation
ALKALOIDS IN THE VALORIZATION OF EUROPEAN LUPINUS SPP. SEEDS CROP

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Introduction

Lupins (Lupinus spp.) are low cost and non-genetic modified legume seeds that provide 30-40% dietary protein, ca. 28% fiber, healthy fatty acids, vitamins and minerals Sbihi, et al., 2013. Besides, lupins also contain several phytochemicals that result from the plant secondary metabolism, alkaloids being major compounds. Their levels in the seed must be as low as possible to ensure a safe consumption of lupins Lucas, et al., 2015. Indeed, in feedstuffs, lupins bitter taste, highly related to the seed alkaloids content, may decrease diet palatability, affecting feed intake and body weight gain; teratogenic alkaloids are of major concern for livestock due to death losses and to crooked calf disease in pregnant range cows Pilegaard and Gry, 2009. Although alkaloids may be toxic when ingested at high concentrations, several biological properties were already described for rich-alkaloid lupin extracts, such as antimutagenic, antibacterial, antifungal and anticancer Khan, et al., 2015. As far as we are aware, the anti-inflammatory and antioxidant potential of these lupins secondary compounds has not been studied yet. The present work aimed at determining, in a cell-free system, the anti-inflammatory and antioxidant potential of rich-alkaloids extracts from seeds of European Lupinus species, at concentrations considered non-toxic when consumed, by evaluating the 5-lipoxygenase (LOX) inhibitory capacity and the nitric oxide radical (•NO) scavenging activity, respectively. As the 68th United Nations General Assembly declared 2016 as the International Year of Pulses United Nations, 2014, we consider of interest the study of a major group of phytochemicals in lupins also from a pharmacological perspective.

Material and methods

Eight varieties (included in the European Plant Variety Database PVD, 2015) and one Portuguese ecotype of lupins, corresponding to mature raw seeds of 3 white lupins (L. albus), 2 narrow-leafed lupins (L. angustifolius) and 3 yellow lupins (L. luteus), were analyzed (Table 1). Seeds were dried (65 °C, 24 h) and grounded (1 mm). Alkaloids were extracted as according to Muzquiz, et al., 1994 and Gresta, et al., 2010, with slight modifications. The inhibitory effect on LOX and the antiradical activity of the extracts were assessed according to Pereira, et al., 2015 and Vrchovska, et al., 2007, respectively. In both assays, three experiments were performed in triplicate.

<table>
<thead>
<tr>
<th>Lupin varieties</th>
<th>Origin</th>
<th>Total alkaloids content (g/kg DM)</th>
<th>IC₂₅ for LOX inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>WL Estoril</td>
<td>Portugal</td>
<td>0.19</td>
<td>0.136</td>
</tr>
<tr>
<td>WL Multitalia-IT</td>
<td>Italy</td>
<td>51.69</td>
<td>0.525</td>
</tr>
<tr>
<td>WL Multitalia-PT</td>
<td>Portugal</td>
<td>12.19</td>
<td>0.229</td>
</tr>
<tr>
<td>YL Nacional</td>
<td>Portugal</td>
<td>10.31</td>
<td>0.766</td>
</tr>
<tr>
<td>YL Taper</td>
<td>Poland</td>
<td>0.78</td>
<td>0.104</td>
</tr>
<tr>
<td>YL Dukat</td>
<td>Poland</td>
<td>0.12</td>
<td>0.341</td>
</tr>
<tr>
<td>NLL Azuro</td>
<td>Portugal</td>
<td>24.40</td>
<td>0.416</td>
</tr>
<tr>
<td>NLL Sonet</td>
<td>Poland</td>
<td>0.64</td>
<td>&gt; 0.354</td>
</tr>
</tbody>
</table>
Results and discussion

The rich-alkaloid lupin extracts exhibited a concentration-dependent LOX inhibitory capacity (Figure 1). According to the effect observed (IC$_{25}$), Taper and Nacional were the most and the least potent varieties, respectively (Table 1). For Sonet, 18% of inhibition was noticed for the maximum concentration tested (0.354 mg of dried extract/mL). Pure compounds also inhibited LOX in a concentration-dependent manner, gramine displaying the strongest effect (data not shown). Due to low solubility in the phosphate buffer used in the assay, the highest concentration tested for lupanine, sparteine and angustifoline was 0.077 mg/mL, which corresponded to 13, 18 and 23% inhibition, respectively. Both lupin extracts and pure standards revealed lower inhibitory capacity than quercetin (IC$_{25}=0.00051$), the positive control used.

![Figure 1. 5-LOX inhibitory effect of white, yellow and narrow-leaved lupins’ rich-alkaloid extracts.](image)

The rich-alkaloids extracts studied herein revealed a moderate LOX-inhibitory potential. There was not a direct relation between extracts activity and its total alkaloid content (Table 1) but these compounds contribute to some extent for the extracts activity; indeed, LOX inhibitory activity of Taper may be greatly attributed to gramine’s activity. The results obtained suggest that besides the phenolic compounds previously reported Czubinski, et al., 2012, alkaloids can play a role in LOX inhibition in lupin seeds.

All the extracts and pure compounds displayed weak activity against 'NO, Azuro displaying the best scavenging activity (20% at the highest concentration. Gramine was able to scavenge 'NO up to 34% at the maximum concentration (1 mg gramine/mL). Lupanine (0.238 mg/mL) presented ca. 11% of activity, whereas spartein and angustifoline revealed no activity.

Conclusion

The studied rich-alkaloid lupin extracts showed moderate LOX inhibitory activity, explained, at least partially, by their alkaloid composition, but were weak 'NO scavengers.

References

IMPACT OF SHORTENING THE VOLUNTARY WAITING PERIOD ON THE NET PROFIT OF A DAIRY FARM

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Introduction

Milk sales are the largest source of revenues on dairy farms, thus a good reproduction performance is important to generate a new lactation. Suboptimal reproductive performance can have considerable economic consequences, largely because of decreased milk production per cow per year and a decrease in the number of calves per year (Sørensen and Østergaard, 2003). Achieving a good reproduction performance is probably one of the main challenges faced by the dairy industry. The voluntary waiting period (VWP) is a time period (days or weeks) after which the cow is first eligible for insemination to allow for optimum uterine involution and recovery from negative energy balance. The VWP is quite variable across dairy herds, and in some herds it changes according to parity and production level. The lower reproductive performance of the herd can be originated from factors linked to the cow or due to management decisions of the farmer, or both. This study aimed to evaluate the effect of shortening the voluntary waiting period (from 120 to 90 days) in a Portuguese dairy farm, using a deterministic model approach.

Material and methods

The deterministic model was applied to a farm with 293 animals, from which 153 are milking cows with a mean 305-day lactation yield of 11.431 litres. Heat detection is made through visual observation and the mean calving interval is 422 days, resulting from a VWP of 120 days, with a mean of 2.1 artificial inseminations (AI) per pregnant cow. A partial budgeting model was applied considering the actual VWP of 120 days and the alternative situation of a VWP of 90 days. Partial budgeting is particularly useful for analysing relatively small changes on the farm. The general format for a partial budget is made up of four sections: 1) extra revenues, 2) reduced costs, 3) reduced revenues and 4) extra costs. This partial budget is on an annual basis, and the main goal is to maximize the net profit of the herd over the time. Costs and revenues were considered for the herd, instead of the individual cow. The model inputs were obtained from the scientific literature, mean prices for Portugal and herd records.

Table 1: Partial budgeting results.

<table>
<thead>
<tr>
<th>Imput variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXTRA REVENUES</strong></td>
<td></td>
</tr>
<tr>
<td>Male calves</td>
<td>422.18 €</td>
</tr>
<tr>
<td>Heifer selling</td>
<td>13,099.58 €</td>
</tr>
<tr>
<td>Milk production</td>
<td>689.08 €</td>
</tr>
<tr>
<td><strong>REDUCED COSTS</strong></td>
<td></td>
</tr>
<tr>
<td>Culling cows</td>
<td>-537.03 €</td>
</tr>
<tr>
<td>Replacement costs</td>
<td>8,449.99 €</td>
</tr>
<tr>
<td>Dry off costs</td>
<td>151.20 €</td>
</tr>
<tr>
<td>Labour costs</td>
<td>583.20 €</td>
</tr>
<tr>
<td>Costs with hormonal protocols + extra AI</td>
<td>0.00 €</td>
</tr>
<tr>
<td>Costs with transition diseases</td>
<td>713.76 €</td>
</tr>
<tr>
<td>Feed costs</td>
<td>101.89 €</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>10,537.06 €</td>
</tr>
<tr>
<td><strong>REDUCED REVENUES</strong></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>3,673.78 €</td>
</tr>
</tbody>
</table>
Results and discussion

The net profit from the shortening of the VWP from 120 to 90 days was estimated in 3673.78€ for the whole farm in one year (Table1).

A sensitivity analysis was performed to evaluate how systematic variation to the input variables of the model (assumptions) leads to outcome changes (Dijkhuizen & Morris, 1997). This allowed us to study some effect of uncertainty of our input variables considering other best or worst scenarios. Several different changes on input parameters were simulated with mainly two alternatives for most inputs. The variable inputs tested were heifer selling value, price of milk, male calves value, cost of transition diseases, hormonal protocols and extra AIs, feed price, culling cows rate and the slaughter price per kg (carcass weight).

The variables that had a higher impact on the sensitivity analysis were the heifer selling value and the culling cow rate, where negative values of the net return were found with decreasing the heifer value to 1200€ or increasing the culling cow rate to 17%, instead of reducing 1% with the shorter VWP. All the other variations on the input did not significantly affect the net profit value.

Determining the optimal VWP on a dairy herd is a complex issue, and the multifactorial nature of the variables involved would be better concerned on a stochastic model that considers the uncertainty and variability by using probability distributions. The results would then concern a distribution of potential outcomes and would have time in consideration. Therefore, the application of a stochastic model such as Monte Carlo simulation, would have a greater impact on this results and would add value to our analysis. However, the data needed to conduct such a model with the desirable accuracy were impossible for us to obtain for this farm in this time period. So, with the data available and to answer this decision problem for this farm, we thought that a deterministic model such as partial budget would be indicated and easily understood by the farmer. Although the model is relatively simple, an accurate calculation of the costs and revenues involved in each point isn’t always simple. Although not all the factors were taken into account, we have included the major ones that differ in costs and revenues between the two alternatives.

As the cows show natural heat before the 90 days and have a good nutritional support near calving, it could be assumed that the reproduction problems will not increase.

Conclusions

Using a deterministic model with a herd level of detail we found that the 90 days VWP is more advantageous than 120 days, having a net profit of 3673.78€. The net economic loss of this partial budgeting is mainly affected by heifer selling value reduced to -692.75€ and by increasing the culling rate in 2% to -760.16€. The optimal VWP differs from cow to cow depending on multiple cow factors and market circumstances.

References


LIPID NANOPARTICLES AS A NOVEL RUMEN-BYPASS TECHNOLOGY TOWARDS THE DELIVERY OF LYSINE IN DAIRY COW NUTRITION

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Introduction

Lysine is one of the most important amino acids (AAs) in milk production of dairy cows, being also scarce in typical diets. Considering this, attempts in supplementing diets with lysine have been performed over the last years and the substitution of regular diets to alternative diets with higher lysine contents have been also considered. However, the current methods have not significantly changed the amount of lysine that reach the bloodstream and that is available for milk production. In fact, the rumen (first compartment of the bovine stomach) is full of bacteria and other microorganisms that are responsible for the digestion process. Moreover, regarding protein digestion, these microorganisms are able to degrade most of the proteins present in feeds, incorporating the resulting AAs into their own proteins. After that, the microorganisms pass to the next compartment of the digestive track and are destroyed, supplying AAs and other nutrients to the animal. The microorganisms in the rumen are also capable of AAs conversion and de novo synthesis, which is important to understand why the first attempts of AAs supplementation have had limited success.

In this context, the development of formulations that could bypass rumen digestion is of the utmost importance. Such formulations have been developed and several products have already entered the market, offering rumen-protected lysine. These products have shown some success in bypassing the rumen, although with limited intestinal absorption efficiency, since the currently available products only focus on passing the rumen and reaching the intestine and not in reaching the bloodstream.

Objectives of the work

Considering the limitations of the current technology and products, we propose the application of nanotechnology towards the development of a technology that not only allows rumen-bypass, but also increases absorption at the intestine level and is capable of selectively accumulating near the mammary gland where lysine can be used for milk production. The developed technology is to be used to produce or to incorporate in existing products of PREMIX®.

Ongoing experimental work

After an extensive review of the state of the art and of the rumen digestive track, it was found that lipids, especially saturated fatty acids, are able to pass through the rumen virtually unaffected by the microorganism digestion. Considering this, nanoparticles (NPs) composed of lipids were proposed for the delivery of lysine. Both solid lipid nanoparticles (NPs composed by lipids that are solid at body temperature) and nanostructured lipid carriers (NPs composed of a mixture of lipids that are solid and liquid at body temperature) have been produced with a wide variety of lipids.
NPs formulations were tested in order to see if they were able to withstand digestion in the rumen. This stability was tested in a batch assay with rumen inoculum at 39°C under slow stirring. Fermentations were stopped after 24 h by cooling the bottles in an ice-slurry bath at 4 °C and the NPs were separated from the inoculum by fractioned centrifugation. The formulations were analyzed in three different time points: i) immediately after NPs synthesis; ii) NPs in contact with the rumen inoculum with no incubation time; and iii) after 24h of incubation with the rumen inoculum. Both the supernatant (rumen inoculum) and the deposits (NPs) were characterized using dynamic light scattering and transmission electron microscopy.

The results showed that two of the formulations tested were able to resist digestion in the rumen and are, therefore, promising candidates for the delivery of lysine in a rumen-protected formulation. The selected lipid candidates were stearic acid and arachidic acid, both in the form of solid lipid nanoparticles. These lipids possess melting points of 72.5°C and 75.5°C, respectively, which would be important for withstanding the temperatures achieved in granulators during industrial production. Hence, this preliminary work allowed the synthesis of NPs with sizes ranging from 300 to 500 nm with highly negative surface charge (lower than -35 mV) that appear to able of protecting lysine during its transit in the rumen.

**Future work**

Currently, a high performance liquid chromatography-based method to selectively quantify lysine, both in the particles and after rumen digestion, is being developed and validated. At this stage, the stability of the formulations in the remaining compartments of the cow’s digestive track and also during the industrial production process will have to be assessed before the formulations can be implemented in a final product.
PARTIAL AND TOTAL REPLACEMENT OF FISH OIL BY POULTRY FAT ON EUROPEAN SEABASS (*DICCENTRARCHUS LABRAX*) GROWTH AND TISSUE COMPOSITION

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Introduction

The use of fish oil in fish feeds brings ecological and ethical concerns, and is no longer sustainable, which calls for the identification of sustainable alternatives. Rendered poultry fat, produced from by-products obtained from poultry slaughterhouses, can be an interesting alternative to fish oil, due to its low price and wide availability. However, its fatty acid profile (low polyunsaturated fatty acids (PUFA) and high saturated fatty acids (SFA) content) can lead to impaired digestibility, growth performance and final product quality. On the other hand, this high SFA and low PUFA contents make this ingredient more resistant to auto-oxidation than fish oil (Watanabe, 1989) and can increase the shelf life of the fish fillet (Ahmad et al., 2013). Locally produced poultry fat could be a novel and more sustainable fatty acid source for fish feed, reducing fishing pressure and the carbon footprint of aqua feeds, since these omega-3 fatty acid sources are locally available.

The present study was conducted to evaluate the effects of partial and total replacement of fish oil by poultry fat on the growth of European seabass and muscle, liver, heart and viscera total lipids composition and fatty acid profile. Since the use of soy lecithin as an emulsifier agent can help overcome the impaired digestibility of the animal fat used, the inclusion of this ingredient was also tested.

Materials and Methods

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

Homogeneous groups of 20 fish (initial weight 19.1 ± 2.6 g per fish) were submitted to a 12-hour light/12-hour dark photoperiod regime and were kept in a recirculating saltwater system (salinity 35‰, 21±1°C) in 12 55l fiber glass tanks. The experimental diets were randomly assigned to the tanks, in triplicate, and fed to apparent satiety three times a day by automatic feeders for 16 weeks. Fish were bulk weighed at 8 weeks from the beginning of the growth trial to monitor weight gain and feed consumption. At the end of the growth trial, fish were individually weighed and measured. Five fish from each tank were sampled for whole body composition and muscle, liver, heart and viscera (intestine + visceral fat) were collected from 15 fish from each diet to evaluate the effect of the fish oil replacement on the total lipid content and fatty acid profile of these tissues.

The remaining fish were used to test the Apparent Digestibility Coefficients of the test diets, after including 1% chromic oxide as an inert marker, according to Cho & Slinger (1979).
Results

The growth performance of fish is reported in Fig. 1. No significant differences were registered in the weight gain or the feed intake among dietary treatments (one way ANOVA, p < 0.05). Feed intake, feed conversion ratio, daily growth index and protein efficiency ratio were similar for all diets. However, the hepatosomatic index was higher in fish fed the 100PF and 100PFL diets (ANOVA, p < 0.05), suggesting an increased deposition of lipids in the livers of these fish. Whole-body composition was similar among all dietary treatments: 34-35% dry matter, 17-18% crude protein, 13-14% crude fat and 9 kJ.g⁻¹ gross energy. The total lipid content of the dorsal muscle varied from 1.3 to 1.8% (in wet weight) and it was significantly higher in the fish fed the 100PF diet than in fish fed the FO and 25PF diets (ANOVA, p < 0.05).

Discussion and Conclusion

The present results show no significant differences in growth performance among dietary treatments. This outcome suggests it is possible to totally replace fish oil by poultry fat in European seabass diets without impairing its growth and efficiency. However, the total replacement of FO by PF had an impact on the liver weight, which suggests lipid accumulation in this organ; further evaluations must be conducted in this organ to understand what happened. The inclusion of soy lecithin doesn’t seem to be beneficial, as that diet didn’t produce better results than the diet with the same fish oil replacement level and without soy lecithin. The replacement of fish oil (a rich polyunsaturated fatty acid source) by a fat rich in saturated and monounsaturated fatty acids will probably have a high impact on the muscle composition in long term feeding, which requires further analysis.

Acknowledgements

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GROWTH PERFORMANCE, OXIDATIVE STRESS AND IMMUNE RESPONSES IN GILTHEAD SEABREAM (*Sparus Aurata*) FED DIFFERENT TYPE AND CONTENT OF SEAWEED SUPPLEMENTATION

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**Introduction**

Many circumstances, biotic and abiotic, can model the oxidative status in fish, such as age, phylogenetic position, feeding behavior, environmental factors, toxins present in the water, pathologies and parasites. Aquaculture procedures can also cause stress situations in fish, which are conditioned by the limits of rearing area. These stresses may decrease fish immunological defences and increase the production of reactive oxygen species, which are directly associated with the quality and palatability of the final product.

Oxidative stress condition is described as the state where the antioxidant potential is insufficient to scavenge efficiently pro-oxidant molecules, like free radicals. Some parameters that can be used to measure fish welfare are: oxidative stress through its enzymes activity levels, products and co-factors activities; and innate immunity, through peroxidase, lysozyme and alternative complement system activities.

**Methodology**

Eight isoproteic (45%), isoenergetic (22 KJ·g−1) and isolipidic (19%) diets (SPAROS Lda.) were formulated to contain 0%, 2.5% or 7.5% of each seaweed. Rhodophyta (*Gracilaria* sp.), R2.5 or R7.5; Ochrophyta (*Fucus versiculosos*), O2.5 or O7.5; or Chlorophyta (*Ulva rigida*), C2.5 or C7.5. In addition, a mix diet (Mix) with 2.5% of each seaweed.

Three hundred and sixty fish (body weight: 31.2 ± 0.6 g each) were fed *ad libitum*, twice daily, for 90 days with the 8 experimental diets. Each diet were allocated to triplicate tanks (15 fish·tank−1), 115 L capacity each, connected to a recirculating seawater system, with a 4 L·min−1 water flow renewal. Photoperiod were set to 13 hours light/11 hours dark. Temperature (20.5 ± 0.4 °C), dissolved oxygen (8.13 ± 1.2 mg·L−1), NH3 (0.5 mg·L−1) and NO2 (0.5 mg·L−1) were daily monitored.

**Results**

The present study had no mortality. Final body weight (FBW), daily growth index (DGI), protein efficiency ratio (PER) and feed efficiency (FE) were significantly higher in fish fed Mix and R7.5 diet than fish fed Ctrl diet (P<0.05). Feed intake (FI) decrease in fish fed R7.5 (P<0.05).

Alternative complement system (ACH50) showed an average increase of 64% for all seaweed supplemented treatments, when compared with Ctrl treatment. The ACH50 activities (P<0.01) increase significantly in R2.5, C7.5, R7.5 and Mix. Lysozyme activity (LYS) was significantly higher by Dunnet test in R2.5 and C7.5 treatment than in the Ctrl treatment (P<0.01).

Lipid peroxidation (LPO), total glutathione (TG) and reduced glutathione (GSH) were not affected by dietary seaweed supplementation, whereas oxidized glutathione (GSSG) and glutathione ratio (GSH/GSSG) were significantly influenced by dietary seaweed supplementation. The GSSG content were significantly lower in fish fed seaweed.
supplemented diets than in fish fed Ctrl diet (P<0.01). The ratio GSH/GSSG was significantly higher in fish fed O7.5, followed by R7.5 and O2.5, than in fish fed Ctrl diet (P<0.01).

The average values of glutathione s-transferase (GST), glutathione reductase (GR), catalase (CAT) and glutathione peroxidase (GPX) were 40%, 33%, 28% and 40%, respectively, higher in seaweed supplemented groups than in Ctrl group. Fish fed R7.5 and Mix showed increased GST activity (P<0.01). The GR activity was higher in fish fed Mix than in fish fed Ctrl diet (P<0.05). The CAT activity increased in R7.5, C7.5 and O2.5 treatments (P<0.05). The GPX activity increased in fish fed R7.5, C7.5, O2.5 and Mix diets (P<0.01).

Several studies showed that seaweeds from Rhodophyta, Chlorophyta and Ochrophyta groups have higher active biologically content such as phloroglucinol, mannitol, fatty acids and fucosterol; elemental minerals and carbohydrates; and antimicrobial and antioxidant properties. As it is known, the antioxidant system require minerals and/or substances for its activation. Thus, it is plausible to infer that the varieties of compounds in seaweed may have played a role as co-factor or dependent element over the analysed indicators.

Conclusions

The present study showed that the beneficial effects of dietary seaweed supplementation may vary with the type and the dietary level. The treatments R7.5 and Mix had significant effects on growth performance, immune responses and oxidative status in gilthead seabream when compared to the other treatments.
OXYGEN CONSUMPTION AS PROXY FOR METABOLIC COSTS IN SWIMMING FISH

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Respirometry is used as a tool to estimate energy expenditure and to measure metabolic costs in aquatic animals in diverse biotic conditions (Svendsen et al. 2016). Water temperature, salinity and pH are some of the factors that affect oxygen consumption rates in fish (Fry 1971). In addition to these, when measuring oxygen uptake in order to calculate metabolic indicators, several other constrictions need to be accounted for, including fish behavior in natural conditions. Spontaneous increases in activities such as fin movements or opercula opening due to circadian rhythms alter oxygen uptake (Kramer 1987) and may lead to errors when calculating metabolic rates (Svendsen et al. 2016). Furthermore, various researchers have evidenced constraints when working with each of the three major respirometry techniques: closed-system respirometry, flow-through respirometry or intermittent-flow respirometry (Keys 1930; Steffensen 1989). The main issue associated with the first two techniques is the inability to maintain steady-state conditions throughout the entire measurement period: events of hypoxia and hypercapnia are very common (Svendsen et al. 2016). Even using intermittent-flow respirometry, the errors associated with water to fish volume, as well as the difficulties to feed the fish inside the respirometer chambers, make this technique fallible.

For the aquaculture sector, knowing fish metabolic rates and how they vary with ambient conditions, may help producers to overcome economical losses associated with mass-scale deaths or feed wastes. However, most respirometry set-ups are prepared for a single fish in a resting state, or individual swimming tunnels. These set-ups leave out of the equation the energy fluctuations associated with schooling hierarchies, swimming velocities or feeding behavior, present in fish groups. Hence, to better simulate the aquaculture conditions, tank intermittent-flow respirometry appears as the most suitable solution.

The objective of the present work was to determine and define appropriate conditions to measure routine (RMR) and maximum (MMR) metabolic rates, as well as the associated aerobic metabolic scope (AMS) and standard dynamic action (SDA) of seabass juveniles in aquaculture-like conditions. To achieve that goal, a pre-trial was performed where 50% hypoxia was induced in two types of tanks. 1) Tanks covered with a lid, to better simulate a common respirometer chamber where no diffusion occurs except when the flush pump is connected. 2) Uncovered tanks, where the water surface was in contact with the air. The oxygen diffusion rates were measured and compared between the two conditions. After the tanks returned to normoxic levels (above 80% oxygen saturation), 80 seabass were introduced in a 600 L tank, up to a final biomass of 18 g/L. The oxygen consumption associated with the transport and handling of the fish when being placed in the tank was measured overnight until a baseline oxygen rate was achieved (RMR). On the next morning, fish were fed the equivalent to 3% their body mass, for a period of 4 hours, in order to calculate the SDA associated with that meal. At this point, SDA peak, the cost of digestion and total digested energy were calculated. At the end of the SDA measurements, a chasing protocol was performed, with the objective of accelerating fish metabolism, enabling the calculation of MMR. The AMS was then calculated by the difference between MMR and RMR.
During the trials, differences in tail beat frequencies, swimming speeds and distribution of the fish in the tank were observed. These differences were then studied based on film and coordinates analysis after fish were forced to swim at three different biologically relevant speeds (5, 10 and 20 cm/sec). Interestingly, the majority of the fish selected the middle of the tank, assuming a position within the schooling and escaping the friction of the wall of the tank, as well as the vortex in the center. This organization between the fish caused differences in water velocities in different positions of the tank, which can be observed when determining the tail-beat frequency of fish from different positions within the tank.

In conclusion, tank intermittent-flow respirometry allows inferring about fish oxygen consumption in group making possible to analyze fish metabolic rates in aquaculture conditions. However, the fact that seabass organize themselves into schooling hierarchies needs to be considered. The metabolic rates measured in a fish group most likely will not represent the individual metabolic rates. This becomes even more relevant when considering that when organized, feed intake will also vary. Nevertheless, this respirometry technique seems to have a direct applicability with beneficial economic revenues to the aquaculture sector.


MOLECULAR PERSPECTIVE ON OSMOREGULATORY CHALLENGE OF PARASITIC FEEDING IN THE SEA LAMPREY (PETROMYZON MARINUS) AND HOST (SALVELINUS FONTINALIS).

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Abstract

The sea lamprey, Petromyzon marinus, is an anadromous species in which the freshwater ammocoete (larvae) undergoes metamorphosis and the fully transformed juvenile migrates to seawater where it begins parasitic feeding on fish and marine mammal body fluids. Sea lamprey parasitic feeding poses a physiological challenge for the host by inflicting nonlethal wounds, body fluid depletion and osmoregulatory challenge due to water loss. From the perspective of the sea lamprey, feeding has also impact on osmoregulatory status serving as a source for isosmotic fluid.

In this study the physiological effects of feeding were assessed in both host (brook trout, Salvelinus fontinalis) and parasite focusing on the molecular mechanisms which has not yet been done during the marine life stage of the anadromous sea lamprey. Our results show that in seawater lamprey, ion uptake mechanisms were successfully downregulated (Na⁺:Cl⁻ cotransporter (NCC), epithelial Na⁺ channel (ENaC) and V-ATPase mRNA expression) as well as kidney Na⁺/K⁺-ATPase (NKA) activity. In contrast, ion secretion mechanisms were upregulated [NKA α-subunit (NKAα1a) and Na⁺:K⁺:2Cl⁻ cotransporter 1 (NKCC1) mRNA expression], except in seawater feeding sea lamprey. This together with the lower ion content in plasma suggest that feeding on a isosmotic body fluids from the host helps compensate for osmotic ion gains through the gills and skin. Also, an increase NKA activity in anterior intestine suggests a role in nutrient absorption.

In the brook trout, lamprey’s attacks to feed on its body fluids only occurred in seawater. Ions levels in the plasma in seawater acclimated trout remained fairly unaltered, though when parasitized, [Na⁺] and [Cl⁻] was found higher compared to freshwater kept ones. Nevertheless ion level remained well below those found in the environment suggesting they were successfully osmoregulating. In parasitized brook trout in seawater, a reduction on food intake was observed and animals demonstrated loss of weight and slower growth rates compared to freshwater and seawater controls, which translated in a decrease in their condition factor. Animals demonstrated a lethargic behavior which could be explained by the severe drop in hematocrit % that was observed in parasitized trout which suggest anemia and correlated with the observed increase in lactate dehydrogenase (LDH) levels (although not significant for this last one) in plasma. At the mRNA level, an increase in Na⁺:H⁺ exchanger 2 (NHE2) and NKA α1b was observed in both fasting and parasitized brook trout groups in seawater and NKAα1a expression was found lower in seawater fasting animals both not in the parasitized ones. At the protein level, V-type H⁺-ATPase (V-ATPase) B subunit and NKA α subunit were found lower in parasitized brook trout compared to both salinity controls and only compared to controls in seawater, respectively. The NKA activity increased in seawater control and parasitized groups. This results suggest that brook trout adjust their molecular osmoregulatory mechanism in order to cope with ions fluctuation as result of sea lampreys parasitic feeding.

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POSTERS
EVALUATING THE APPARENT DIGESTIBILITY OF FATTY ACIDS FROM DIFFERENT ANIMAL FAT SOURCES IN EUROPEAN SEABASS (DICENTRARCHUS LABRAX)

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Introduction

Fish oil as fatty acid source in aquafeeds has reached unsustainable levels, raising ecological and ethical concerns. Rendered animal fats, obtained by processing animal by-products from slaughterhouses, can be interesting alternatives to fish oil, due to their low price and wide availability. However, their lower content in polyunsaturated fatty acids (PUFA) and higher content in saturated fatty acids (SFA) can lead to impaired digestibility, growth performance and final product quality. But, on the other hand, the high SFA and low PUFA contents make these ingredients more resistant to auto-oxidation than fish oil (Watanabe 1989).

The present study was conducted to evaluate the effects of two locally produced rendered animal fats in the apparent digestibility of fatty acids in European seabass.

Materials and Methods

Two rendered animal fats, poultry fat (PF) and a mammal fat (MF) mixture (70% lard and 30% beef tallow) were obtained from local slaughterhouses by-products. A reference diet for European seabass was formulated with 14% supplemental fish oil (FO) as main fat source. The two test diets were isoenergetic (23kJ/g) and isonitrogenous (48% crude protein) and contained either poultry fat or the mammal fat mixture to replace the fish oil. All diets were added 0.07% 5α-cholestane as inert digestibility marker.

Homogeneous groups of 15 European seabass (mean initial weight 100g) were subjected to a 12-hour light/12-hour dark photoperiod regime and kept in a recirculating salt water system (salinity 35‰, 20±1°C) designed as described by Cho & Slinger (1979) to collect the feces. Each diet was randomly assigned to two of these tanks, being the experiment divided into two periods of fifteen days, for replication of results (n = 4). The experimental diets were analysed for their content in dry matter (105°C for 24h), and fatty acids (total lipids extracted according to Folch et al. (1957), fatty acid methyl esters (FAME) in lipid extracts were transesterified by acidic methylation (Lepage and Roy, 1984) and then analyzed using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Europe GmbH, Germany)). The Apparent Digestibility Coefficient (ADC) of dry matter was calculated as: 100 – (100 × (% marker in feed / % marker in feces)). The ADC of fatty acids was calculated as: 100 – (100 × (% marker in feed / % marker in feces) × (% FAME in feces / % FAME in feed)).
Results
Total polyunsaturated fatty acid (PUFA) levels were, as expected, higher in fish oil (FO) than in other animal fats, but similar contents were observed in PF. Total monounsaturated fatty acid (MUFA) levels were higher in poultry fat (PF) and in mammal fat (MF) while total saturated fatty acids (SFA) were highest in MF and similar among FO and PF. These two animal fats also have distinct fatty acid profiles from each other, as PF is richer in PUFA and MF is richer in SFA, but their total MUFA content was similar.

The apparent digestibility coefficient of total PUFA was similar among FO and MF diets but higher than in the PF diet, whereas total SFA digestibility was statistically higher in FO diet than in MF diet, but was similar to PF diet (ANOVA, p < 0.05). Total MUFA ADC was similar in all diets. In general, regardless of the fatty acid source, each individual fatty acid was well digested by the European sea bass with ADC values ranging from 97.7-99.8 %. Significantly higher ADC values were observed in important fatty acids such as EPA and DHA in the reference diet (with FO) than in the other test diets. Linoleic and alpha-linoleic acids were also better digested in the FO diet than in the PF diet (ANOVA, p < 0.05).

Discussion and Conclusion
The fatty acid digestibility results obtained in European seabass were high regardless the fatty acid source, but the ADC values of omega-3 fatty acids were highest in the diet with fish oil. The animal fats with highest levels of saturated fatty acids, as predicted, led to a reduced digestibility of polyunsaturated fatty acids, even though this was only significant in the. However, the digestibility of each fatty acid in these animal fat sources were still high (> 97%) regardless of its origin. These results suggest that the different animal sources can all be considered good energy sources and feasible alternatives to fish oil. Nevertheless, the lower ADC values of omega-3 fatty acids may compromise fish flesh quality.

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References
APPLYING NANOTECHNOLOGY TOWARDS THE TARGETED DELIVERY OF LYSINE TO THE MAMMARY GLANDS OF DAIRY COWS

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Context

In order to overcome the limitations of feed ingredients and to optimize milk production, formulations that are able to supply rumen-protected amino acids (AAs) have been developed in dairy cow nutrition. These formulations have already rendered several commercially available products, but whereas it has significant success in delivering methionine the same cannot be said for lysine, one of the most important AAs in milk production. In this context, we propose the application of nanotechnology towards the development of a technology that not only allows rumen-bypass, but also increases absorption at the intestine level and is capable of selectively accumulating near the mammary glands where lysine can be used for milk production. The developed technology intends to be used to produce or to incorporate in existing products of PREMIX®.

Work Planning

In order to achieve this goal, we propose a work plan divided into 4 main sequential steps. The first step will be the synthesis of nanoparticles (NPs) to be loaded with lysine and to function as transporters and protectors of the AA. These nanoparticles will be characterized and optimized in terms of matrix composition, size distribution profile and zeta potential. The capability and efficiency of loading lysine into the NPs will also be determined and their release profile will also be important. Mainly, the selected NPs formulation will have to be stable and resistant to the digestion process inside the rumen. After this first characterization and optimization, the formulations will be assessed in terms of possible industrial up-scale production and processing. The NPs stability and resistance to the temperatures reached during industrial processes as well as other adverse conditions during processing will be of particular interest at this point. The physical form at which the formulations will be incorporated into the feeds of the dairy cattle will also be important: lyophilized formulations (in powder) could be added to the mix with other materials whereas non-lyophilized formulations (in an aqueous suspension) could be pulverized in the mixer resulting in a more uniform distribution. Once this industrial characterization is performed, the formulations will be assessed regarding their stability and release profiles over all the digestive tract until they reach the intestine where their absorption into the bloodstream will also be determined. Finally, and in order to achieve a formulation that can selectively target the mammary glands, specific agents will have to be used to functionalize the NPs conferring targeting capabilities. At this point, the efficacy of functionalized NPs will have to be weighed...
against the economic viability of the functionalization process. The stability of the NPs in the bloodstream should also be determined in order to consider the targeted formulation’s efficacy and viability. In order to assess the absorption and the efficacy of the NPs in vitro studies with relevant cell lines and in vivo assays in whole animals will have to be performed. Once all these steps are performed we will be able to achieve our goal: a product that is able to be ingested by the dairy cow and deliver lysine to its mammary glands where it can be used to improve the milk production.
IMPROVING BIOAVAILABILITY OF TRACE ELEMENTS IN DOG FEED:
THE ROLE OF ORGANIC SOURCES

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Abstract

Trace minerals are involved in several vital metabolic functions. Zinc (Zn) plays a role in enzyme reaction, protein and carbohydrate metabolism, cell replication, skin, and wound healing. Selenium (Se) is a component of glutathione peroxidase that acts in preventing oxidative damage, along with Vitamin E, and contributes to the production of thyroid hormones, which stimulate hair growth. Both Zn and Se can also act as immunomodulators, highlighting their importance in early stages of growth. Deficiency of trace minerals results in light to severe clinical manifestations. Although, the absence of clinical signs does not mean an adequate mineral status. ‘Normal’ growth and fertility may be achieved with mineral nutritional status at 50%, but immune response and metabolism may be affected at a subclinical level in “healthy” animals. Therefore, the optimal trace mineral status to maximize whole animal performance requires a more complex assessment.

Raw materials incorporated into complete feedstuffs for animals have low concentration of trace minerals, thus the addition of premixes in the production process is frequently needed in order to meet the nutritional requirements. Those premixes are mostly composed by minerals in its inorganic form. Free ions from inorganic sources can form complexes with other dietary constituents, resulting in low bioavailability. Minerals in the organic form, mainly soluble salts complexed organic matrices, are protected from reactions with other constituents and, theoretically, have greater absorbability than inorganic sources. However, data on bioavailability trace minerals in dogs is scarce and often extrapolated from other species.

This project intends to evaluate the effects of dietary supplementation with mineral sources of different bioavailability on the dog’s nutritional status, specifically in growth and health parameters.

To accomplish this goal, this research includes in vitro and in vivo studies. The process of fortification used in the animal feed manufacturer at an industrial scale will be simulated in the laboratory. The minerals in test will be added to the matrix and all the constituents rigorously quantified. High-throughput miniaturized spectrophotometric assays under 96-well microplate format will be developed to assess selenium and zinc. Several classical reactions will be tested, and the most suitable will be selected according to the matrix component's interference. The method will be fully validated.

In the initial in vitro experiments, the effect of pH variations within the gastrointestinal tube will be evaluated. Further on, the complexity of the experiments will increase after the addition of enzymes (pepsin in gastric juice; amylase, lipase and bile in intestinal juice) that mimic chemical conditions along the digestive process. Subsequently, a quick methodology to estimate the passage of Zn and Se in organic forms through synthetic biomimetic membranes will be developed and implemented. The developed method will be further validated through comparison to cellular models.
The *in vivo* studies will be performed in both puppies and adult dogs. The effect of feed mineral source (inorganic vs organic) will be evaluated by measuring several parameters, including: growth and development rate, total apparent diet digestibility, stool characteristics, breath odor, hair characteristics in puppies. In adult dogs, the evaluation will be based on physical and clinical parameters of good nutritional status. Mineral status indicators (biomarkers) for routine evaluation in veterinary practice will be developed and validated in both puppies and adult dogs. In the end, the results of this project will contribute to bridge the knowledge gap on bioavailability of different forms of Se and Zn and ultimately, be applied to pet food industry for feedstuffs improvement.
MODELLING MEAT EATING QUALITY TRAITS DURING AGEING AS AFFECTED BY BEEF CARCASS CHARACTERISTICS AND EARLY POST-MORTEM pH DECAY DESCRIPTORS

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Previous work has demonstrated that beef carcasses can be promptly and accurately classified into optimal quality and cold-shortened in accordance to the concept of pH/temperature “ideal window” by using carcass characteristics and early post-mortem pH/temperature decay descriptors. The objective of this study was to assess the combined effects of the aforementioned variables on the two main eating quality attributes of meat – namely, tenderness (measured as shear force) and juiciness (measured as cooking loss) – during chill ageing. pH and temperature in longissimus thoracis muscle of 51 beef carcasses were recorded during 24 h post-mortem, and decay descriptors were then obtained by fitting exponential models. Measures of Warner-Bratzler shear force and cooking loss were obtained from cooked meat after 3, 8 and 13 days of cold ageing. The fitted mixed-effect models revealed that both meat tenderisation and cooking loss increased with ageing (p<0.01) although their rates slowed down in time (p<0.05). Beef carcasses with a higher pH (obtained at different endpoints: 1.5, 3.0, 4.5 or 6.0 h post-mortem) produced aged meat with increased tenderness (p=0.013) and increased water retention during cooking (p=0.016) than those of lower pH. Nonetheless, the slower the pH decay rate, as happens in a cold-shortened carcass, the lower the potential for tenderisation (p=0.038) and water retention (p=0.050) during ageing. Whereas sex affected shear force, with females producing meat of higher tenderness, aged meat of increased water retention was produced by heavier beef carcasses (p<0.001). The good fitting quality of the shear force (R²=0.847) and cooking loss (R²=0.882) models and their similarity among the different endpoints post-mortem indicated that both eating quality attributes can be approached by recording the pH decline of a beef carcass during the first 3.0 hours after slaughter.
DAIRY CATTLE-SLURRY TREATMENT TECHNOLOGIES: TOWARDS AN INTEGRATING APPROACH OF CROP PRODUCTION, ANIMAL FEEDING AND ENVIRONMENTAL IMPACTS

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Abstract

Dairy cattle farms have a well-known environmental impact that affects all ecological compartments: air, soil, water and biosphere [1]. Dairy cattle farming are a significant source of anthropogenic gases from enteric fermentation, manure storage and land application, mainly ammonia (NH₃), nitric oxide (NO), nitrous oxide (N₂O), carbon dioxide (CO₂) and methane (CH₄). The emission of such gases represents not only an environmental problem but also leads to energy and nitrogen (N) losses in ruminant production systems [2-5]. Several efforts are required on the development of new technologies and strategies that mitigate gaseous emissions, N losses and improve the efficiency of the energy and N cycles [6, 7]. In the Northwest of Portugal, dairy cattle production has a major impact on the economy, with strong repercussions at national scale. Therefore, our Ph.D. thesis project aims to:

a) Study natural supplements as additives in the dairy cattle diet towards a decrease in GHG emissions from feeding operations;

b) Compare commercial dairy cattle diets with and without additives on gaseous emissions from manure deposited in a simulated concrete floor;

c) Assess the concentrations and emissions of NH₃ and greenhouse gases from commercial dairy cattle facilities;

d) Evaluate the effects of different additives on lowering gaseous emissions from dairy cattle excreta, using a laboratory system simulating a dairy house concrete floor.

Material and methods

To study feed additives for lowering gas emissions, short-term (24-h) batch incubations (in vitro) will be conducted with rumen digesta used as inocula to evaluate effects of feed additives (source and level) on rumen fermentation. The most promising sources and levels will be further evaluated in in vivo experiments [4].

In commercial dairy farms located at Vila do Conde, will be collected urine and faeces from dairy cows fed with different dietary crude protein levels. The excreta collected from dairy cows fed with different diets will be studied in order assess the effects of different crude protein levels on NH₃, NO, N₂O, CO₂ and CH₄ emissions from a simulated concrete floor. The experiment will be made with small laboratory chambers to measure gaseous emissions over 96-h from applications of constant amounts of urine and faeces to a concrete floor [8]. Ammonia and NO fluxes will be measured,
respectively, with acid traps and chemiluminescent analyser (SIR MODEL S-5012) whereas greenhouse gases will be measured by gas chromatograph (GC-4000A).

The concentrations and emissions of NH₃ and greenhouse gases from dairy cattle facilities will be evaluated in three naturally ventilated dairy-cattle facilities located at Vila do Conde, and will take place between October-2016 and September-2018. Ammonia, N₂O, CO₂ and CH₄ measurements will be made over the 2 years study period [9]. Ammonia and greenhouse gases will be measured with a photoacoustic infrared multigas monitor (INNOVA 1412) and air samples will be collected, in sequence, through ports located indoor and outdoor, by a multipoint sampler (INNOVA 1409).

In order to lowering gaseous emissions from dairy cattle excreta deposited on concrete floors, three different additives (source and level) will be evaluated using a laboratory system [8]. Ammonia, NO, N₂O, CO₂ and CH₄ emissions will be followed over 96-h after application of constant amounts of dairy cattle urine and faeces to a concrete floor.

References

AMINO ACIDS AS KEY MEDIATORS OF IMMUNE STATUS AND NUTRITIONAL CONDITION IN GILTHEAH SEABREAM

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Introduction

Aquaculture and products of fishery industry represent an important contribution to the world’s economy and nutrition (FAO, 2012). Because of their savoury meat, several species of sea bream are farmed worldwide. Among Sparidae, the gilthead seabream, Sparus aurata, has been one of the most cultured species in Mediterranean aquaculture (Colorni & Padrós, 2011). However, regardless of the source, several diseases can affect fish and it is imperative to change the current practices of chemotherapeutic and antibiotic treatments in this industry (Lafferty et al., 2015). Thus the main challenge of farmers is to obtain robust fish that are able to adapt to local environments, contributing to a sustainable and efficient aquaculture. In the last decade, many research has focused on improving aquafeeds, with the purpose of reducing feed conversion rates (Li et al., 2009). This research has led to the definition of two types of concepts, functional aquafeeds and environmentally-oriented aquafeeds. Functional feeds are supplemented with specific ingredients (such as AA) in order to achieve a desirable metabolic efficiency, growth performance, health status and/or compositional traits of farmed fish at various developmental stages, while environmentally-oriented feeds are those modified to minimize negative impacts of environmental changes (e.g. salinity, temperature variations, and stress factors) on growth, health and reproduction of farmed fish (Li et al., 2009). Indispensable AA (IAA) such as arginine, methionine and tryptophan have been reported to play important roles in innate immunity and inflammation, so their potential use in functional aquafeeds seems relevant (Costas et al., 2011; Machado et al., 2015).

Objectives

The main goal of this project is to provide a better understanding of the influence of dietary AA supplementation on nutritional condition, immune mechanisms and disease resistance in fish exposed to environmental stressors. The experiments will be performed with the gilthead seabream (Sparus aurata). In particular, we intend to investigate:

1) To what extent dietary supplementation with AA can modulate the inflammatory response in fish;
2) If IAA have a significant impact on disease resistance and apoptotic mechanisms in fish;
3) To what extent different degrees of supplementation with key AA influence fish nutritional condition.

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Dietary methionine supplementation increases white blood cells numbers whereas its deficiency does not compromise the European seabass *Dicentrarchus labrax* health status

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**Introduction**

The concept of maintaining animal health through the best possible nutrition is well-accepted in modern animal farming. In fact, amino acid (AA) requirements may increase as a direct consequence of metabolic changes associated with inflammation and infection (Panagiotis Sakkas et al., 2013). Methionine for instance is an indispensable AA with recognized roles in the immune system and their dietary supplementation proved to enhance mammalian host immunity. Through the generation of decarboxylated S-adenosylmethionine, methionine is a donor of the methyl group that participates in the methylation of DNA and proteins, the synthesis of the polyamines spermidine and spermine, and regulation of gene expression (Li et al., 2007). Particularly in fish, methionine supplementation was able to increase the European seabass (*Dicentrarchus labrax*) innate immune status as well as the inflammatory response following inoculation with inactivated *Photobacterium damselae* subsp. *piscicida* (Machado et al, 2015). Since several studies have reported that AA deficiency may compromise the immune system repertoire (Li et al., 2007; Wu, 2009), the present study aimed to evaluate the effects of dietary methionine deficiency on the European seabass health status. In contrast, it was also intended to verify whether methionine supplementation can improve some aspects of the European seabass immune system.

**Material and methods**

European seabass weighing 8.5 ± 0.7 g were maintained in a recirculated seawater system (Temperature: 20 ± 0.5 °C; Salinity: 35 ppt; 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 seabass amino acid requirements, and the CTRL diet supplemented with methionine in 0.5 or 1 % (dry matter basis, respectively: diets Met1 and Met2). A negative control diet (NCTRL) was also formulated to be deficient in methionine.

The experiment lasted for 28 days and at the end 3 fish per tank were sacrificed by anaesthetic overdose with 2-phenoxyethanol. Blood was collected for white (WBC) and red (RBC) blood cells counting and for the determination of haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration according to Machado et al. (2015).

**Results and Discussion**

Haematological parameters such as total RBC, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin...
concentration, remained unchanged in fish fed dietary treatments at the end of the feeding trial. Still, an increase in total WBC was observed in fish fed diet Met2 compared to those fed diet CTRL. Interestingly, NCTRL presented a deficiency in methionine which did not translate into lower WBC numbers. Since methionine appears to have an important role on the regulation of gene expression and cell proliferation, it would be important to evaluate the effect of methionine deficiency on host immune mechanisms following stimulation with antigens. Likewise, methionine supplementation requires further investigation in order to be applied in practical diets as an efficient solution to boost the European seabass immune repertoire.

Figure 1. Total white blood cells in European seabass fed dietary treatments during 28 days. Values are means ± SD (n=9). Different letters indicate significant differences among dietary groups (one-way ANOVA; P ≤ 0.05).

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DIETARY TRACE ELEMENTS IN SOLEA SENEGALENSIS: EFFECT OF INORGANIC VS. ORGANIC FORMS ON FISH LARVAE GROWTH

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Abstract

The role of trace elements in biological systems has been described in several fish species. Trace minerals are essential to a wide range of physiological, metabolic and hormonal processes that influence critically survival, growth, feed utilization, skeletal formation, immune response and susceptibility to pathologies of fish larvae and post-larvae. The bioavailability of dietary trace elements is influenced by their chemical forms and the presence of anti-nutritional factors. Available literature indicates that organically bound minerals may be more bioavailable than inorganic minerals for certain animal species. To determine whether the inclusion of organically bound (to amino acids) copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn) could improve early-stage fish survival and overall growth, a 28-day feeding trial was undertaken with Senegalese sole (Solea senegalensis) larvae. Senegalese sole larvae were fed with Artemia nauplii from 20 to 30 days after hatching (DAH) with a subsequent sudden weaning with a basal diet from 31 to 35 DAH. From 36 to 64 DAH fish larvae were fed experimental diets that were formulated to be isoproteic, isolipidic and with identical levels of vitamins and minerals, varying only the chemical form (organic or inorganic) of Cu, Fe, Se, Mn and Zn in the mineral-vitamin premix. Also, for both organic and inorganic forms of minerals, two doses of mineral-vitamin premix were tested, 1 and 2%.

Preliminary results indicate that the use of organically bound minerals does not increase dry weight, length or survival of Senegalese sole larvae significantly but when supplementing feed with a mineral-vitamin premix at 1%, the use of organically bound minerals increased fish relative growth rate (RGR) by 2.5%/day when compared to using inorganic minerals. In regards to the dose of mineral-vitamin premix used, when using inorganic minerals, increasing the dose from 1 to 2% also increased the fish RGR by 2.6%/day.

To infer on the effect that the experimental feeds, used in this experiment, can have on bone status, X-ray analysis will also be performed to assess bone density and possible malformations.

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DO PROTEIN HYDROLYSATES IMPROVE PROTEIN UTILIZATION AND GROWTH IN SENEGALESE SOLE LARVAE?

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Abstract

Acceptance of inert diets at early fish larval stages is still a bottleneck in most marine species. For several altricial species, it has been suggested that the dietary inclusion of protein hydrolysates might influence the maturation of the digestive system by triggering the onset or increasing the activity of some digestive enzymes. As Senegalese sole is an altricial species that undergoes a complex metamorphosis, in the present study we hypothesized that the manipulation of dietary protein complexity may positively affect larvae digestive capacity, and promote somatic growth. Three diets were formulated using approximately the same practical ingredients, but with the inclusion of different molecular weight peptides. The Intact diet protein content was mostly based on intact plant protein where the target peptide molecular weight (MW) would be higher than 70kDa; in the PartH diet the protein fraction was mostly based on a plant protein hydrolysate with a high rate of 5–70 kDa peptides; and in the HighH diet the protein fraction was mostly based on a commercial fish protein hydrolysate with a high inclusion of 5 kDa peptides. Larvae were reared upon a co-feeding regime based on live feed and a cold-extruded inert diet, from mouth-opening until 35 days after hatching (DAH). From 6DAH onwards live feed was gradually substituted and at 31DAH the inert diet constituted more than 75% of the feed supplied (dry matter basis). Post-larvae were completely weaned at 36DAH and fed exclusively upon the inert diet until 60DAH.

The digestive capacity was assessed by a tracer study along with the mRNA expression of genes encoding for the precursors of enzymes enrolled in protein digestion and the enterocyte peptide transporter pepT1. The in vivo method of controlled tube-feeding was used to assess the effect on the larvae capacity to utilize 14C-labelled polypeptides with different MW (1.0 and 7.2kDa) representing the target peptide MW of each of the hydrolysates included in the diets. The PartH diet promoted growth in metamorphosing larvae, whereas the Intact diet promoted growth in post-larvae and juveniles. The Intact diet promoted the larvae absorption capacity for 1.0kDa peptides during the metamorphosis climax (16DAH), which may have contributed for enhanced growth in later stages. The PartH diet promoted the transcription of tryp1c and pept1 at 28DAH, which seemed to reflect on increased post-larvae capacity to retain dietary 7.2kDa polypeptides. The Intact diet has also promoted the transcription of pepsinogenA in 28DAH post-larvae. Regarding the present results, it is suggested that the dietary protein fraction formulation of microdiets for Senegalese sole shall be adapted to each developmental stage and, thus, changed throughout development.

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MARINE CATFISH UNIQUE AMONGST THE TELEOSTS IN HYPOOSMOTIC REGULATION

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Abstract

The euryhaline Plotosidae catfish *Plotosus lineatus* is unique amongst the teleosts in that it possesses a specialized salt secreting organ, the dendritic organ (DO) whereas other marine teleosts rely on their gill ionocytes. In the present study, we investigated the effects of salinity [brackishwater (BW) 3‰, seawater (SW-control) 34‰, hypersaline water (HSW) 60‰] on Na⁺/K⁺-ATPase (NKA) and Na⁺:K⁺:2Cl⁻ cotransporter (NKCC1) expression to elucidate the importance of the DO in ion-regulation, and DO ligation to characterize the compensatory responses of the gill and kidney. Our results show that DO NKA activity was significantly higher than in gill, kidney at all salinities; however, NKA activity only increased with HSW in kidney but not in either gill or DO. 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. DO size increased with salinity and strong NKA/NKCC1 immunolocalization was observed which was absent in gill ionocytes. This latter observation contrasts with practically all other marine teleosts in which gill ionocytes are central to excess ion excretion. In SW, DO ligation did not alter gill or kidney NKA expression. In BW, DO ligation only increased gill NKA activity. In summary NKA activity is high in DO and co-expressed with NKCC which indicates a conservation of rather similar mechanisms of ion transporting in the convergent evolution of salt secreting organs in vertebrates.
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