18th International Symposium on Fish Nutrition and Feeding
40 Years of Research in Fish Nutrition
Las Palmas de Gran Canaria, Spain
June 3rd - 7th, 2018

Integrative omics approaches in gilthead sea bream (Sparus aurata): from nutrients to metabolites

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The production of environmentally sustainable aquaculture feeds starts with the selection of high-quality raw materials that may need to be supplemented with specific nutrients. Nevertheless, different competences for developing tools and methodologies are needed to assess and predict nutrient requirements or status of fish, going further than just identifying differences in growth parameters. Thus, the challenge is to screen and make the best use of a given tool, but also to integrate new knowledge arising from transcriptomics, proteomics, metagenomics and metabolomics in order to define a reliable healthy fish phenotype. Of particular value are biomarkers that precede the onset of metabolic disturbances or those that predict the capacity to cope with dietary, environmental and age-related stresses. Hence, changes in the intestinal transcriptome, integrity of intestinal barrier, intestinal mucus proteome and gut microbiota mostly reflect a pro-inflammatory condition in fish fed plant-based diets, but dietary butyrate helps to restore the wild phenotype resulting in improved diseases outcomes in fish challenged with bacteria and enteric parasites. Currently, a new promising omics approach is metabolomics, and more than 15,000 m/z ions have been detected in the serum metabolome of juvenile fish with around 850 highly discriminant features between fed and short-term fasted fish, using ultra-high performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry (HRMS). When comparing the serum metabolome of fish fed marine and plant-based diets, several lipid related compounds, including phosphocholines, lysophosphocholines and sphingolipids raised as highly discriminatory compounds. A number of exogenous compounds (cysteinolic acid, tauropine, trimethylamine N-oxide, arsenobetaine, hercynin) also contributed to discriminate fish with different nutritional backgrounds. However, from a functional point of view, it is of special relevance that the abundance of N-aryl amino acids, with repair properties in epithelial mucosa, is consistently reduced in fish fed plant-based diets. Conversely, circulating levels of pyrimidine and related nucleosides, especially markers of DNA degradation (deoxycytidine) and methylation (methylcytosine) were increased by plant-based diets, which is viewed as part of a pro-inflammatory condition and/or overall cellular DNA instability. This opens new research issues to alleviate or mitigate the drawback effects of plant based diets in marine carnivorous farmed fish.

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The effect of season on the gilthead seabream liver metabolome: from FT-IR fingerprints to interpretable metabolic profiles.

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Fourier-transformed infrared (FT-IR) spectroscopy has shown good potential for the characterization of metabolomes, given its low cost and high-throughput. On the other hand, due to its low resolution (compared to approaches based on NMR or HPLC-MS), results are usually expressed as low-dimensional fingerprints (often obtained using matrix factorization methods such as PCA) which, though informative, may not be directly interpretable in terms of specific metabolites. In this presentation, we explore the use of matrix factorization methods that respect the nonnegative nature of absorbance spectra, allowing unmixing of spectral data into its components using a purely data-driven approach, and seek to demonstrate how this can be useful in a practical sense.

For this study, we have analysed FT-IR spectroscopy measurements of gilthead seabream hepatic tissue from three distinct growth trials, having used both NMU (nonnegative matrix underapproximation) and NMF (nonnegative matrix factorization) to recover the spectral signatures of the individual components present in the samples, and have compared this approach with a more traditional one (PCA). Results show that, despite the very different dietary treatments tested in each of the three trials, a very clear and consistent pattern can be seen which consists of high carbohydrate (i.e. glycogen) content in the liver of gilthead seabream during summer months, regardless of diet and fish size, and a lower carbohydrate content during winter months. This implies, on one hand, the need to have into account the “season” (or temperature) factor, when interpreting seabream liver metabolome data and, on the other hand, suggests that the inclusion of highly-available carbohydrate energy sources in seabream diets (e.g. glycerol, gelatinized starch) might be more relevant during the summer, when glycogen depletion is observed due to high energy demands, rather than during winter months.

To conclude, using nonnegative matrix factorization methods on FT-IR spectroscopy data of fish tissues extends the usefulness of this technique, by reducing the need to manually segregate and annotate observed signals, and enables the recovery of metabolic profiles based on metabolic fingerprint data. As such, it allows the inference of metabolic patterns from FT-IR spectroscopy data with a minimum number of prior assumptions.

The circadian transcriptome of marine fish (Sparus aurata) larvae: synchrony matters

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Growth and health in farmed fish may rely on the synchronization among endogenous rhythms, which are affected by feeding and other clues imposed by production practices. It is known that activity of nutrient-sensitive clock proteins impacts nutrient and energy utilization in other animals. Thus, we hypothesized that the metabolism of fish larvae has a synchronized time-based organization to allow timely nutrient metabolism and fast growth. To test this hypothesis, a high-density oligonucleotide microarray was used to examine the daily expression of 13,939 unique genes in whole gilthead sea bream (Sparus aurata) larvae kept under a light/dark cycle with continuous feed availability. Up to 2,229 genes were differentially expressed, and the first two components of Principal Component Analysis explained more than 81% of the total variance. Clustering analysis of differentially expressed genes identified 4 major clusters that were triggered sequentially, with a maximum expression at 0 h, 3 h, 9-15 h and 18-21 h zeitgeber time. Various core clock genes (per1, per2, per3, bmal1, cry1, cry2, clock) were identified in clusters 1-3, and their expression was correlated with several genes in each cluster. Functional analysis of these clusters revealed a consecutive activation of molecular pathways related to phototransduction, intermediary metabolism, development, chromatin remodeling, and cell cycle regulation. This circadian synchrony of key biological processes in fish larvae can be used as an indicator of larvae quality. This can be the basis of new molecular tools for evaluating larvae rearing protocols, the efficacy of nutritional interventions and for larvae batches certification. As several sampling points are not practical under aquaculture operations, we propose the use of a single sampling point to assess the expression of subsets of genes from antiphase clusters to find algorithm indicators of synchrony. Alternatively, expression ratios of candidate genes at two different times may be informative about the scope of processes such as neuromuscular development and oxidative capacity, which are also considered highly informative of larval growth and later performance of juvenile fish. Such approaches can contribute to improve the quality of farmed fish providing new criteria and insights for fish quality certification.

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EPA and LA affect adipogenesis and lipid metabolism-related genes expression in in vitro and in vivo models of rainbow trout (Oncorhynchus mykiss)

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In this work, the effect on adipogenesis and lipid metabolism-related genes expression of two different fatty acids, eicosapentaenoic (EPA) and linoleic (LA) acids, a n-3 common in fish oil and a n-6 mainly present in vegetable oils such as soy, respectively, was studied in rainbow trout (Oncorhynchus mykiss) using in vitro and in vivo approaches. Day 7 cultured pre-adipocytes were incubated with the fatty acids for either 48 h to quantify lipid accumulation or 6 h to determine effects in gene expression. Both, EPA and LA showed a significant potential to activate adipogenesis, increasing the levels of expression of the principal genes involved in this process, such as peroxisome proliferator-activated receptor gamma (pparg) and CCAAT/enhancer-binding protein alpha (cebpa). Furthermore, the two fatty acids up-regulated the expression of the fatty acid transporter (fatp1), the fatty acid binding protein (fabp11b), and the lipases, lipoprotein lipase (lpl), hormone-sensitive lipase (hsl) and adipose triglyceride lipase (atgl), suggesting general activation of the mature adipocyte machinery, but only LA showed a significant increase in lipid accumulation in vitro. Next, EPA or LA (20 mg/kg) or vehicle solution as a control were administered by gavage to approximately 100 g trout previously fasted for 24 h. At 6 h after injection, blood, liver and adipose tissue samples were collected. Glucose, triglycerides, NEFAs and glycerol levels were analyzed in plasma, while tissue gene expression analyses are currently ongoing. In plasma, LA triggered an increase in glycerol levels suggesting an effect in the lipid turnover, and EPA provoked a decrease in circulating glucose, indicating that this fatty acid could be involved in carbohydrate metabolism as it has been seen in other fish species. Overall, although fatty acids effects on liver and adipose tissue metabolic function in vivo are still to be determined, the in vitro results have demonstrated that both fatty acids can similarly induce adipogenesis and modulate adipocyte function. Thus, suggesting that a substitution of dietary fish oil by vegetable oils would not compromise the quality of the fish in terms of adipose tissue metabolism and fat accumulation.

Mir-34 and sirt1/foxo1: insights of hepatic glycolipid metabolism in Megalobrama amblycephala

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To study the regulatory effect of miR-34a on glycolipid metabolism in Megalobrama amblycephala, M. amblycephala fish were fed either a control or a high glucose diet for eight weeks. Metabolic models of high glucose group, high glucose inhibition group, normal glucose group; and normal glucose overexpression group were constructed by miRNA interference technique of miR-34a silencing and overexpression in M. amblycephala. The results showed that the hepatic expression of SIRT1 gene was decreased by the high glucose diet after eight weeks, which promoted the expression of foxo1a. Additionally, hepatic phosphoenolpyruvate carboxykinase (pepck) and glucose 6-phosphatedehydrogenase expression were upregulated, thereby promoting gluconeogenesis. The upregulation of PPARα increased the expression of lipid metabolic enzymes such as FAS, lipase, and ACC; therefore, excessive glycogen was deposited as lipid in the adipose tissue. Furthermore, by measuring protein and mRNA expression levels of SIRT1, FoxO1a, glucose metabolic enzymes, and lipases following miR-34a inhibitor injection, we found that SIRT1 expression increased, activating FoxO1 expression levels, reducing hepatic gluconeogenesis, decreasing hepatic glycogen accumulation, and accelerating hepatic fat decomposition and fatty acid synthesis. In a nutrient metabolic homeostatic state, injection of miR-34a mimics promoted the expression of FoxO1a and PEPCK, enhanced fat metabolism and fatty acid synthesis, and inhibited SIRT1 protein expression. These results revealed that miR-34a regulates hepatic glycolipid metabolism induced by SIRT1 as the core regulator in high glucose or metabolic homeostatic states in M. amblycephala.

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EX VIVO CHARACTERIZATION OF METHIONINE ABSORPTION IN THE INTESTINAL TRACT OF RAINBOW TROUT (Oncorhynchus mykiss) USING 14C RADIOLABELED METHIONINE FLUX AND GENE EXPRESSION

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Introduction: Methionine (Met) is commonly shown to be the first limiting amino acid in the diets of farmed fish including salmonids. Supplementation of Met in these diets is typically achieved using DL-Met. In animal intestine, Met absorption is typically mediated by specialized proteins also known as solute carrier transporters. The transporters are sodium dependent and/or sodium independent located in the apical (mucosal) and basolateral (serosal) membranes of intestinal epithelial cells.

Objective: The present study was conducted to characterize DL-Met transporters in rainbow trout intestine (pyloric caeca, midgut and hindgut) employing Ussing chamber and RT-qPCR.

Methodology: Ussing chamber technique was used to carry out unidirectional flux assays measuring radioisotope movement of 14C methionine from the mucosal bath to the serosal bath (Jms). Trout weighing ~100 grams each were sacrificed and gastrointestinal tracts were immediately excised, dissected and placed in a Ussing chamber. The fluxes were performed at 12°C in sodium containing buffer to initially characterize the Na-dependent transporters. RT-qPCR confirmed the expression of predicted transporters determined from kinetic analysis. Expression was compared against house-keeping gene EFα1.

Results: Results demonstrated that at concentration lower than 200 µM, Na-dependent DL-Met uptake could be described by saturable process, which obeyed Michaelis-Menten kinetic. There were no significant differences in affinity (Km) among three intestinal segments ($P > 0.05$). Capacity of Met absorption,(Vmax) was similar in both pyloric caeca (0.035 ± 0.005 µM/cm2/hr; n= 17) and midgut (0.032 ± 0.004 µM/cm2/hr; n= 21) segments, while the hindgut showed a significantly lower value (0.011 ± 0.005 µM/cm2/hr; n=18). These data were in line with the expression of Met transport genes in these segments: dominant expression of rBAT (SLC3A1), a high affinity low capacity Na-independent transporter and a reduced expression of y+LAT1 (SLC7A7), a high affinity low capacity Na-dependent transporter in the hindgut segment relative to other regions.

Conclusion: From these initial experiments, we conclude that the pyloric caeca and midgut had a higher capacity to absorb Met over the hindgut at concentration less than 200 uM.

The study of the fish microbiome: the story behind the results

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The study of the fish microbiome is one of the most rapidly growing fields in aquaculture. The high interest in this topic is due to the increased understanding of the importance of the microbes for the host health and productivity of animals. However, today's microbiome profiling data produced by high-throughput sequencing, has a potential high variation due mainly to inter-individual variation as well as technical noise introduced during the complex multi-step procedure used to obtain the final data. Awareness of these challenges is growing among researchers studying the microbiome in humans and land animals, but appear unrecognized in fish microbiome research.

In order to find the optimal microbial biomarkers that may be useful for improvement of nutritional performance and health status in fish, the different technical challenges need to be considered and approached. It is imperative to develop reference samples and guidelines adapted to fish conditions, regarding sampling collection and storage, DNA extraction, library preparation and data analysis. Refinement and improvement of the techniques used for the study of the fish microbiome will improve the scientific results that can be applied to improve the welfare and production of farmed fish.

The presentation will give a summary of different technical challenges and strategies used during several Atlantic salmon projects aiming to evaluate the gut microbiota under farming conditions. We also present the results of a technical study evaluating important factors reported as sources of variation in microbiota studies such as DNA extraction protocols, primer selection and bioinformatics pipelines in reference samples, as well as variation between intestinal sampling sites. Finally, we will highlight the importance of using appropriate quality controls such as a mock community of known composition, as well as positive and negative controls during different steps to monitor the robustness of the methodologies and decrease technical variation and bias during conduction fish microbiota studies.

Precision cut liver slice culture as a platform for studying lipid metabolism in Atlantic salmon

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The necessary introduction of cheap and sustainable vegetable oil based feeds has reduced the omega-3 long chain polyunsaturated fatty acid (n3-LC-PUFA) content in Atlantic salmon. Although salmon are capable of synthesizing n3-LC-PUFAs from shorter chain precursors, the level to which synthesis occurs is the product of complex interactions between fish and feed. Interrogation of these interactions using traditional feeding trials is both costly and time consuming, especially for data intensive applications such as metabolic modeling. To overcome this, we have developed an in vitro system using precision cut liver slices (PCLS) to test the effect of altered dietary fatty acid composition on hepatic lipid metabolism in a relatively high throughput manner. With our PCLS system, dozens of dietary combinations can be tested simultaneously on a single fish in less than one week. Additionally, PCLS maintain the complex three-dimensional structure of the liver with multiple interacting cell types, making it superior to traditionally used hepatocyte culture. Our goal is to characterize the effect of culture time, fatty acid supplementation, and insulin on the hepatic transcriptome, especially regarding lipid metabolism, as a first step towards building a high-throughput system for investigating feed-fish interactions.

We observed a time dependent drift in expression similarity between whole liver and liver slices, with high similarity maintained through five days in culture. Meanwhile, gene expression in major metabolic pathways including protein, lipid, carbohydrate, and vitamin metabolism stabilized after three days. Supplementation with alpha linolenic acid (ALA, 18:3n-3), the precursor for n3-LC-PUFA biosynthesis, had an overall positive effect on fatty acid metabolism related gene expression, activating PUFA biosynthesis and beta-oxidation genes. Insulin, an important hormonal regulator of metabolism, had a more anabolic effect, deactivating genes related to fatty acid breakdown and activating genes related to fatty acid biosynthesis. We also observed an interaction between insulin and ALA, where several genes that were induced by both insulin and ALA alone, were highly downregulated when insulin and ALA were combined. This work highlights the utility of PCLS as a tool for investigating lipid metabolism and underscores the complex interactions between hormonal and nutritional inputs governing metabolic gene expression.

Effect of low fish meal and fish oil diet on growth performance, hepatic fatty acid composition and fads2 expression of juvenile from nutritional programmed broodstock

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Introduction

Terrestrial vegetable ingredients are used for replacing dietary fish meal (FM) and fish oil (FO). However, the usage of terrestrial vegetable ingredients will still lead to some deleterious effect of the growth of marine species. Nutritional programming means during some critical stages of development, the induce of nutritional stimuli will lead to the permanent changes to physiology or metabolism of the organ. In our previous studies, we have demonstrated that the offspring of gilthead sea bream nutritional programmed by VO could utilize low FM and FO diets better, by feeding their parents with specific low FO. The present study aims to determine the growth and gene expression of the progeny of the gilthead sea bream nutritional programmed by FM and/or FO.

Material and methods

1800 experimental juveniles (mean weight: 3.36 ± 0.48 g), produced from nutritional programmed broodstock (FMFO: 100% FO and FM, VMFO: 60% VM and 100%FO and VMVO: 60% VM and 75%VO) were randomly assigned to 18 250L tanks. Each group of progenies were fed with control (20% FM and 6% FO) and challenge diet (5% FM and 3% FO) for 45 days.

Result and discussion

Within the comparison in groups that were fed with same experimental diet but from different broodstock group, negative effect by the dietary FM and FO replacement of broodstock was observed in juvenile growth performance. However, no significantly difference was found between the juvenile for VMFO and VMVO group. In the analysis of fatty acid composition of liver, most of hepatic fatty acid reflexed the fatty acid in diet, whereas within the juveniles that were fed with challenge diet, the composition of the products of Δ6 desaturase were higher in the offspring of VMVO group. The expression of hepatic fads2 of juvenile from the VMVO group was significantly higher than the other two group that were fed with challenge diet. In conclusion, the nutritional programming on broodstock by vegetable meal in this experimental level level harmed the growth performance of the progeny, while the replacement of FO by VO in broodstock diet might enhance the utility of dietary vegetable oil of the offspring.

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Integrative 1H-NMR metabolomic investigation of the effect of alternative diets on rainbow trout plasma

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Fish farming needs high quality feeds to support the growing global demand for fish. Over the last two decades, the development of sustainable feeds based on plant feedstuffs has strongly reduced the use of marine resources. However, full plant-based feeds still reduce growth performances in carnivorous species. Thus, active research programmes are conducted on alternative feedstuffs to provide sustainable feeds for aquaculture and especially trout farming.

Our project aims to establish a link between feed and plasma metabolome of rainbow trout (Oncorhynchus mykiss) through i) the characterization of alternative diets devoid of marine resources substituted by insect, micro-algae or yeast products, and ii) the analysis of trout plasma metabolome fed these alternative diets.

Fish were fed ad libitum for three months with four, iso-proteic and iso-energetic experimental diets: a control diet based on plant feedstuffs (PB) and three diets containing 15% of either insects (INS), micro-algae (SPI) or yeast (YST). Ethanolic extracts of diets were prepared and analysed. Blood was collected 48 h after feeding on anesthetized fish and centrifuged to get plasma. The 1D 1H-NMR profiles of both diet extracts and plasmas were acquired on a 500 MHz NMR spectrometer. Spectra were processed and buckets or spectral regions of interest were integrated with NMRProcFlow (nmrprocflow.org) webtool and used for statistical analyses with BioStatFlow (biostatflow.org).

Analysis of diet extract 1H-NMR spectra showed qualitative and quantitative differences in compound signals for INS and SPI diets compared to PB one. Branched-chains amino acid signals were more than twice higher in these diets. Additionally, several specific compounds were related to alternative feedstuff. Multivariate analysis on plasma spectra showed a discrimination of individuals fed INS diet from those fed PB diet despite high intra-group variability. Univariate analyses on plasma revealed a few metabolites representative of fish fed each of the experimental diets.

Integrative metabolomics combining diet and trout plasma characterization revealed a clear distinction between alternative feeds and specific effects of the diets on plasma.

This research was funded by FUI 2014 (NINAQUA with Le Gouessant, COPALIS, Algae Natural Food and Phileo Lesaffre Animal Care), ANRT (CIFRE 2016/0775) and MetaboHUB (ANR-11-INBS-0010).

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Empowering health and defenses in Atlantic salmon with functional supplements: a comparative analysis between pre- and probiotic effects on intestinal function, metabolism and immune response

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Functional feeds are used in animal livestock productions as a feeding strategy to boost health, help fish regain homeostatic balance after a challenging episode and increase disease resistance. In aquaculture, pre- and probiotics have been studied for their effects in several species with positive effects in growth and overall health, supporting the strategic usage of these supplements. However, there is a knowledge gap on how these supplements can aid fish under challenging conditions, thus the aim of this study was to perform a comparative analysis between the effects of a prebiotic - mannanoligosaccharide - and a live probiotic - Saccharomyces cerevisiae - in Atlantic salmon facing an acute stress and a bacterial infection.

Atlantic salmon (Salmo salar, 85gr) were fed 1.8% bw daily a commercial diet as a control, a prebiotic or probiotic supplemented diet (0.6 and 0.5% respectively) for 30 days. Then fish intestinal homeostasis was disrupted by an acute stress, and 48h later fish were all challenged intraperitoneally with the pathogenic bacteria Piscirickettsia salmonis at 1.0x108 cfu uL-1. Sampling occurred before and 13 days after bacterial challenge in both stressed and non-stressed groups, with the collection of intestines, liver and head kidney in RNALater for gene expression assessment and serum for biochemical analysis. The expression of a comprehensive panel of genes was evaluated by qPCR including genes related to intestinal functioning (permeability, integrity and digestion), metabolic and immune response, and showed a differential response dependent of challenge and on the functional supplement.

Overall, fish fed functional diets presented better condition by the end of the 30 days, and responded better to the acute stress. However, while facing a pathogen the response was different. Fish fed prebiotic despite presenting immunostimulation and stable intestinal functions during prophylactic phase, responded to the bacterial infection in a less intense manner than fish fed probiotic, presenting gene expressions indicative of instability and difficulty in coping. In the later, intestinal functions were stabilized during the challenges, and the response to the bacteria was strong, delaying mortality in this group including in previously stressed fish. This study highlights the potential of functional diets to aid homeostatic