NEW TOOLS AND STRATEGIES FOR THE METABOLIC PHENOTYPING AT MOLECULAR LEVEL OF MEDITERRANEAN FARmed FISH

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Introduction
Clinical haematology and basic blood biochemistry are common diagnostic tools to assess health and welfare in humans and most livestock production systems, but the use of such analyses as diagnostic tools is poorly established in fish farming due to the paucity of reliable information on reference values. Thus, several attempts have been conducted within the ARRAINA project to compile and generate new data for all fish species in the project, and gilthead sea bream in particular (Ballester-Lozano et al., 2015). Similarly, clinical signs of liver steatosis, accumulation of intestinal lipid droplets or intestine submucosa inflammation are arising from lipid-related metabolic disorders, but a direct link with a specific nutrient or group of nutrients is often lacking for the histopathological scoring. This also applies to molecular approaches and important research efforts were done over the course of the last years to construct and continuously update the sea bream and sea bass CSIC-transcriptomic databases (www.nutrigroup-iats.org/) on actively transcribed genes of larvae, and intestine and immune-relevant tissues of juvenile fish (Calduch-Giner et al., 2013). Examples of use of these on-line searchable databases includes: i) definition of molecular identity and extensive searches of new genes exclusive of fish lineages, ii) reference libraries in proteomics and next generation sequencing studies, and iii) design of specific microarray and PCR-arrays for wide- and pathway-focused gene expression profiling and fingerprinting. The aim of the present overview is to underline how these new molecular tools have contributed to identify and validate new biomarkers and approaches that have specificity, sensitivity and diagnostic/predictive value for the assessment and improvement of nutritional condition in Mediterranean farmed fish.

Materials and methods
Custom high-density oligo-microarray (8 x 15K) from the assembled nucleotide sea bass and sea bream sequences were designed and printed using the eArray web tool (Agilent). Both arrays comprised 60-oligomer probes for more than 14 000-15 000 sequences with a different annotation. For pathway-focused gene expression profiling, we designed and validated three PCR-arrays (growth-chip, lipid-chip and gut-chip), operated by means of a handling robot for the simultaneous and semi-automated gene expression profiling of more than 200 biomarkers. Among others, makers of GH/IGF system, muscle growth and cell differentiation and proliferation, protein breakdown and protein folding and assembly, inflammatory and anti-inflammatory immune responses, energy sensing, OXPHOS and mitochondrial respiration uncoupling, FA synthesis and oxidation, phospholipid/cholesterol/lipoprotein metabolism were included, as well as a wide range of gut markers informative of intestinal architecture and function. Samples used in gene expression analyses were derived from ARRAINA trials with fish fed micro-particulated diets, semi-purified diets formulated for specific nutrient deficiencies or practical diets for juvenile fish with varying inclusion levels of FM and FO (from 40% in control diet to 7.5% in the extreme low FM/FO diet).

Results
Microarray gene expression profiling of sea bream larvae sampled every 3 h through a 24 h period revealed a pronounced circadian rhythm with more than 3 000 unique genes that were differentially expressed. Importantly, the two first components of PCA explained more than 80% of total variance that would drive three major steps: i) the anticipatory food response before the morning onset of lights, ii) the afternoon protein accretion and tissue damage repair, and iii) the night reset/stand-by of biological processes before the onset on a new rhythm of extremely fast grow and high feed intake.

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Microarray gene expression profiling of sea bass and sea bream intestine revealed a highly regulated gut transcriptome from anterior to posterior intestine sections with an over-representation of immune-related processes in the posterior intestine section. This spatial transcriptional regulation also affected the mucosal intestinal chemosensing via G protein-coupled receptors (GPR) with a high abundance of GPRs with a role in intestinal motility and secretion in the anterior intestine section, whereas those related to immune response and inflammation were highly expressed in the posterior section. Experimental evidence also indicated that the gut transcriptome is highly regulated in a seasonal basis in sea bream with changes in the gene expression profile of several thousands of genes, but regardless of season and feed intake ARRAINA feed formulations did not drive massive changes in the gut transcriptome of sea bream. However, the “gut-chip” transcriptomic profiling revealed a slight pro-inflammatory condition in fish fed the extreme low FM/FO diet that was mostly reversed by butyrate feed supplementation.

The “growth-chip” highlighted highly regulated molecular signatures that were specific of tissue and nutrient deficiencies in fish fed diets formulated for specific deficiencies in Met, FAs, PLs, P, minerals and vitamins. The signatures of selected markers of lipid metabolism were also highly regulated in liver, adipose tissue and skeletal muscle during fasting and re-feeding in sea bream and sea bass, and current research is done to establish the link with specific nutrients.

**Discussion and conclusions**

Microarray gene expression profile of whole tissue larvae revealed a pronounced rhythm and key upstream regulators of this biological clock are now emerging as powerful tools to underline the nutritional status and effectiveness of nutritional programing during early life stages. Intestine is now considered a major target tissue in nutritional studies and the establishment of the spatial expression pattern of gut is becoming very useful to assess the effect of new fish feed formulations and additives on intestinal fish health. Lipid-related metabolic disorders are a common nutritional disorder in farmed fish, and the use of molecular tools in combination with other omics approaches has been proven of diagnostic and predictive value, although further research is needed to standardize methods and tools to be applied in a clinical basis.

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**References**
