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Aquaculture: Enhancing Food Security and Nutrition

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Chapter 13
An Overview of Functional Genomics in Aquaculture

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1 Introduction

Functional genomics is a field of molecular biology that attempts to describe gene (and protein) functions and interactions. The term 'genome' itself is more than 75 years old and refers to the entire genetic material of an organism, or its complete set of genes located on chromosomes (Hieter & Boguski, 1997). In 1986, 'genomics' was coined by Thomas Roderick to describe the scientific discipline of mapping, sequencing and analysing genomes. At the inception, it stated, and data was analysed; subsequently, different specific genomic techniques were developed with the rapid advances in technology, particularly the advances in PCR and sequencing technologies, a series of highly efficient approaches for genomic studies were developed. As a result of scientific demand and technological advances, a very specific branch of science evolved that is now called Genomics. In order to understand functional genomics, it is important to first define function. Define function in two possible ways. These are 'selected effect' and 'causal role'. The 'selected effect'

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Table 13.1 Overview of scope of functional genomics in aquaculture

Applications	References
Understanding the genetic basis of desirable traits such as growth rate, disease resistance, feed efficiency	Houston et al. (2014)
Identifying genetic markers for selective breeding programmes	Sonesson et al. (2013)
Impact of genetic modification on aquatic organisms	Pott et al. (2018)
Enhanced growth, improving nutritional efficiency and feed formulation	Houston et al. (2014), Gheyas et al. (2018)
Enhancing stress tolerance and environmental adaptability	MacKenzie et al. (2017)
Developing genomics-informed breeding programmes	Gheyas et al. (2018)
Current Status of Genome Sequence and its Application in Aquaculture	
Genomics breeding programmes for environmental adaptability, sustainability, reducing environmental impact and improving water quality through bioremediation, genetic selection and enhancing biodiversity through genetic conservation	MacKenzie et al. (2017)
Enhancing nutritional value through genomics-informed breeding	Yue & Wang, 2017

function refers to the function for which a trait (DNA, RNA, protein, etc.) is selected for. The 'causal role' function refers to the function that a trait is sufficient and necessary for. Functional genomics usually tests the 'causal role' definition of function.

In aquaculture, functional genomics refers to the study of the structure, function and regulation of genes and their products in aquatic organisms, with the goal of improving aquaculture production, nutrient utilization, disease resistance and sustainability (Gheyas et al., 2018) (Table 13.1).

2 Methodologies in Functional Genomics

2.1 Transcriptomics

Transcriptomics including microarray and RNA-sequencing (RNA-seq) are the cheap, fast and easy methods to detect the differential expressed genes. Transcriptional profiling using microarrays has developed into the most prominent tool for functional genomics because expression microarrays were developed in the 1990s and marked the beginning of the genomic era and has convincingly demonstrated how information from raw sequence data can be converted into a broad understanding of gene function. After the invention of the next-generation sequencing (NGS) method in 2005, it has led to drastic changes in the way RNA-seq data is

generated and analysed. Using the RNA-seq approach, it is easy to quantify the expression of known isoforms or transcripts and for unknown transcripts. Usually, transcriptomics studies provide information about what seems to be happening in an organism under specific biological circumstances (Lowe et al., 2017). The relative abundance of RNA transcripts offers a better view of active cell expression than the genomic approach (Ye et al., 2018).

Microarrays are based on hybridization, whereas RNAseq utilizes new ultrahigh throughput sequencing that became available in the recent years. The hybridization-based approaches typically involve incubating fluorescently-labelled complementary DNA (cDNA) with pre-defined sequences, such as PCR products or long oligonucleotides (mostly 60 mers), densely spotted onto a solid modified glass surface. In contrast to microarray methods, sequence-based approaches determine gene expression levels by directly sequencing cDNAs. Both approaches generate a relative abundance of mRNAs, which reflect gene expression levels. The outcome of transcriptomic studies strongly depends on sequence availability, computational methods for gene annotation and gene set enrichment. The overall goal of transcriptomics is not to identify single genes that may be altered, but to define which biological pathways are being altered in a more holistic approach. Although the technical aspects of both technologies differ considerably (as described below), they both generate lists of differentially expressed genes, and the biological interpretation of these genes is central to the biological interpretation of the experiment.

A microarray slide is typically a glass or silicon chip containing thousands of tiny spots. The microarray slide size is generally (approximately 75 mm 3 26 mm). Each DNA spot in the array contains about 10,212 moles (picomoles) of a single gene or known DNA sequence (uniquely cloned cDNA or oligonucleotide), called an oligo or probe or reporter. Probes are a small fragment of transcripts or genes that are used to hybridize a targeted cDNA sample under high-stringency conditions. Microarray not only shows the order of magnitude of transcripts but also gives the benefit of genes studied that are not influenced, whereas, on the other hand, RT-PCR (reverse transcriptase polymerase chain reaction) and northern technique blot limit the validation of a few genes per experiment (Allanach et al., 2008). TMAs are high-throughput technology that provides a comprehensive assessment of the expression. TMAs are used to analyse the protein expression at the same time in multiple individual cells and tissue samples on a single slide (Govindarajan et al., 2012).

Microarrays were first used in fish studies during the late 1990s (reviewed in Gracey & Cossins, 2003; Goetz & MacKenzie, 2008), based on the sequencing of expressed sequence tags (ESTs). Expressed sequence tags are single-pass sequences of random complementary DNA (cDNA) clones from cDNA libraries. The ESTs were often generated from cDNA libraries that were enriched for genes associated with infection or developmental stages (O'Farrell et al., 2002; Taggart et al., 2008).

The principle of microarray expression profiling study is based on the hybridization of a single-strand nucleic acid fragment to its complementary single strand with high specificity (Schena et al., 1995). The target cDNA is first labelled using fluorescent dyes and then hybridized to the array surface. The retained labelled target

will then be subjected to stringent washes, capture and quantification of the fluorescent signals, followed by the data analysis process. Two platforms that are commonly in use are: cDNA microarrays and oligonucleotide microarrays (Schena et al., 1995). The cDNA microarrays contain a collection of probes generated by PCR amplification from cDNA libraries, expressed sequence tag clones or long genome cloned fragments. Oligonucleotide microarrays commonly contain short oligonucleotides (25–30 nt) or long oligonucleotides (50–80 nt). In both platforms, the short DNA segments are printed onto solid supports, usually microscopic slides, by direct contact mode (mechanical robotic spotting) or non-contact mode (ink jet technique). Different platforms each have their advantages and limitations, but in common, all have been shown reliably to capture gene expression signatures on a genomic scale (Schena et al., 1995).

2.2 Collection and Analysis of Microarray Data

For the collection of data, a microarray scanner is used comprising a computer, a camera and a laser. Microarray technique uses relative quantitation where a laser beam is passed and excites fluorescence, thus emitting the intensity from a particular spot under various conditions, which is matched to the intensity of the same spot. The excited fluorescence generates the signals when the laser beam scans the microarray, the camera captures and records the images (the pattern of fluorescence emission) produced, and this is stored as data in computer and finally analysed. The gene character of each spot is determined by the varying intensities of its colours.

EST analysis is an efficient approach for gene discovery and gene identification. For instance, between 2001 and 2007, catfish ESTs increased from 10,000 to 44,000 and the putative genes number increased from 5905 to 25,000. In the Pacific oyster (*Crassostrea gigas*), 40,845 high-quality ESTs represented 29,745 unique transcribed sequences (Fleury et al., 2009); in gilthead sea bream (*Sparus auratus*), 30,000 ESTs represented 18,196 putative unigenes (Louro et al., 2010). Currently, there are over 180 aquaculture species having more than 100 ESTs in dbEST, with approximately a dozen species having more than 10,000 ESTsPAI.

EST analysis can provide comparisons of gene expression profiling in different tissues and conditions. For instance, in a recent study with rainbow trout (*Oncorhynchus mykiss*), Kondo et al. (2011) sequenced over 30,000 ESTs from rainbow trout adipose tissue. These ESTs were used to search adipokine-related genes. The result showed that none of them encoded adipokine and PPARγgene, which play important roles in mammalian adipocytes. Further qRT-PCR results confirmed EST analysis results, that is, rainbow trout adiponectin transcripts were weakly detected in adipose tissue but strongly detected in muscle, suggesting the difference of energy metabolism between fish and mammals (Kondo et al., 2011). Chini et al. (2008) constructed normalized cDNA libraries from liver, ovary and testis in bluefin tuna (*Thunnus thynnus*), identifying several sequences with known functions in other organisms, but not previously described in this species. Also,

sequences were described being expressed in one, two or more tissue libraries. Similarly, Zou et al. (2011) constructed normalized cDNA libraries from testis, ovary and mixed organs of mud crab (*Scylla paramamosain*). Through EST analysis, sex-specific transcripts were identified.

EST analysis is an effective genomic approach for rapid identification of expressed genes and has been widely used in genome-wide gene expression studies in various tissues, developmental stages and under different environmental conditions. In addition, the availability of cDNA sequences has accelerated further molecular characterization of genes of interest and provided sequence information for microarray construction and genome annotation (Rise et al., 2004).

2.3 RNA-seq Technology

RNA-seq is one of the important applications of NGS technologies to study RNA molecules, such as tRNA (transfer RNA), mRNA (messenger mRNA), rRNA (ribosomal RNA) and ncRNA (noncoding RNA), which are present in the cells. RNA-seq provides information on tens to hundreds of millions of transcripts and data on billions of individual bases of an organism. It is the study of a whole set of RNAs that are transcribed in a cell or tissue and their quantity for certain physiological and developmental stages (Gedil et al., 2016). Using the NGS approach, it is easy to identify the quantity and presence of RNA in a biological sample at a specific condition and found a more sensitive and accurate way to study the differential expression analysis, which drastically overcomes the lacuna of microarray (Voelckel et al., 2017). RNA-seq played the important role in the field of transcriptomes of aquatic animals to identify the specific genes. Moreover, it can be carried out without the prior information of the reference genome and that is very informative for a non-model organism whose genome is not yet sequenced (Voelckel et al., 2017).

RNA is polymeric in nature involving many biological processes of an organism, such as gene expression, regulation of genes, coding and decoding (Yang & Kim, 2015). Traditional methods based on cloning-based techniques, such as suppression subtractive hybridization or cDNA libraries, are more laborious and complex, which need lots of time to do, whereas on the other side RNA-seq is very easy and straight forward. RNA-seq plays an important role in gene expression, coexpression analysis, genic identification, genic variants, alternative splicing and miRNA target identifications (Yang & Kim, 2015) (Fig. 13.1).

3 Bioinformatics Analysis for RNA-seq Technology

A typical RNA-seq data analysis can be summarized as follows: (i) Perform quality control for raw RNA-seq data. Low-quality sequence tags produced from library construction or sequencing process are trimmed away by the software provided by

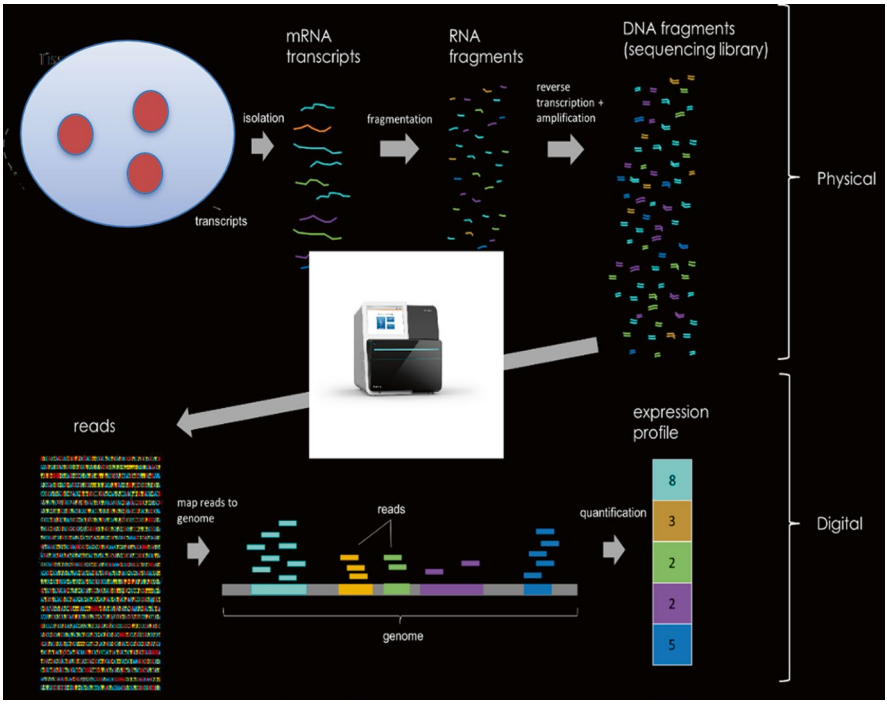

Fig. 13.1 An overview of RNA-sequence

the sequencing platform. (ii) For cases with a reference genome, map millions of short reads to the reference genome, determine the position of each RNA transcript in the genome, calculate the expression level of each transcript, and then find differentially expressed genes across the samples. All the above processes are carried out by corresponding pipelines, including representatives of Bowtie to map reads to the reference genome, Tophat to identify splice junctions, cufflinks to test for differential expression and so on. For cases without a reference genome, de novo transcriptome assembly is performed from short RNA-seq reads and then all assembled contigs are subjected to functional annotation, which requires extensive computer resources. Further specific analysis will be performed to answer certain questions involved in transcriptomics, such as analysis of RNA editing and ncRNAs, discovery of novel transcripts and correlation of transcriptome data to available genomic or epigenetic data.

4 Metabolomics

Metabolomics, a relatively new field introduced by Professor Jeremy Nicholson of Imperial College London in the mid-1990s, emerged from the investigation of the metabolome. The term ‘metabolome’ refers to all small molecules with a molecular

weight less than 1500 Da present during a specific physiological period in a cell, organ, tissue, organism, or non-living things like food and feed (Qiu et al., 2023). Metabolomics involves the qualitative and quantitative, or semi-quantitative, study of these small molecules to examine metabolic pathways and link metabolome changes to physiological processes in living organisms. Metabolomics analysis technology is valuable in nutritional research as it reveals how external substances affect an organism's internal processes, such as growth, development, metabolism and reproduction. Unlike other omics fields, which focus on gene expression, metabolomics studies the end products of these processes, the metabolites generated by metabolic pathways. The results of metabolomics research are closely related to the phenotype of organisms. Fiehn (2002) noted that this research helps us understand how organisms change and respond to environmental stimuli, accurately describing their physiological states. Activities of the cells are reflected by the metabolome changes that can identify complex biologically essential changes. Subtle differences in genome and proteome expression can translate into metabolite differences, making them easier to identify. Unlike genomics and transcriptomics, which require high-throughput sequencing and large databases, metabolome analysis is more intricate yet simpler. Metabolites have significantly lower molecular masses than genes and proteins and are independent of genetics or species (Taylor et al., 2002). It acts as a complementary platform and is often used in accordance with transcriptomics and proteomics since metabolomics is a downstream result of gene and protein expression.

Metabolomics workflow started with the extraction of samples by chemical solvent, solid phase extraction or hydrodistillation method. In aquaculture, metabolomics analyses are sometimes used to describe the effects of fish diet and pollutants on fish (Ussery et al., 2018). In addition, metabolomics analyses are also extended to resolve issues related to fish production, such as in farmed Mandarin fish, *Siniperca chuatsi* (Xiao et al., 2020), as well as post-harvest quality control, such as in the gilthead sea bream, *Sparus aurata* (Melis et al., 2017). Moreover, metabolomics analyses can be used to investigate diseases, such as the metabolic profiling of Atlantic salmon infected with *A. salmonicida*. Metabolomics has also been used to characterize fish feed and is positioned to reveal the physiological effects of dietary manipulations. In order to improve the nutrition and quality of aquaculture goods, metabolomics is utilized to investigate the metabolites that give aquaculture products their flavour and aroma (Diez-Simon et al., 2019). Compared to other omics, researchers and scientists rarely explore the applications of metabolomics in aquaculture research. The temperature (thermal tolerance) has effect on the metabolomics as low temperatures can induce changes in cellular structure, function, metabolism, protein folding, assembly, activity and stability. Elevated temperatures can also depress metabolism, alter oxidative regulation and affect immunity. The effects of chronic elevated temperatures on *O. mykiss* were investigated through ^{1}H-NMR profiling.Metabolomics showed that a higher temperature (20 °C vs. 15 °C) induced thermal stress and downregulated levels of PCr and ATP in muscle, and glycogen levels in the liver. In a comparable study, Kullgren, Jutfelt, Fontanillas, Sundell, Samuelsson, Wiklander, Kling, Koppe, Larsson, Bjornsson and Jonsson

assessed metabolic effects of elevated temperature in *S. salar*. ^{1}H-NMR plasma metabolomics revealed that *S. salar* kept at elevated temperature (8°, 12° and 18 °C) for 3 months had lower levels of glutamine, tyrosine and phenylalanine involved in energy metabolism.

The most common metabolomics tools used are mass spectrometry (MS) and nuclear magnetic resonance (NMR). Furthermore, MS technology is often combined with chromatography such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) or capillary electrophoresis coupled with mass spectrometry (CEMS) for the analysis of metabolites and compounds (Jorge et al., 2016). Although the NMR-based approach has dominated as the analytical platform of metabolomics studies, the number of metabolomics studies using MS-based techniques has been on the rise because of its high sensitivity and accuracy during the detection of metabolites in a sample.

NMR interrogates metabolites based on the behaviour of their atoms (frequently protons [1 H] or carbon [13C]) following exposure of a sample to a strong external magnetic field.Due to differences in molecular composition and conformation of metabolites, the nuclei of their atoms will interact and behave differently with magnetic fields to produce unique signatures that can be detected to enable molecular identification. NMR is an efficient and popular platform for structural assessment and absolute quantification of metabolites.

MS-based platforms employ molecular ionization to detect and identify metabolites in samples. By measuring the mass-to-charge ratio (m/z) of ionized molecules or their fragments, and comparing with a reference library of known compounds, metabolite identity can be established. MS-based platforms characterize the molecular weights of metabolites with high accuracy and can quantify their concentrations in the nanomolar (nM) to picomolar (pM) range. This allows the detection of additional metabolites compared with NMR.37. Metabolomics workflow started with the extraction of samples by chemical solvent, solid phase extraction or hydrodistillation method. The sample was then analysis using selected analytical platform such as NMR or MS tools, before data analysis was conducted by multi-variate analysis and statistical analysis.

5 Proteomics

Proteomics in aquaculture is one of the growing fields over the past decade, contributing to aquaculture towards reaching its primary goal of large-scale production with a quality and sustainable product. Variation in proteomics occurred during the different life stages of fish species, depicting the significance of signalling events that take place during development in a rapidly changing environment. Proteomics analysis of fishes could help in discovering proteomic changes occurring due to disease or other environmental factors such as toxins, pollutants and fluctuation in temperature that can affect normal metabolism in the body (Tomanek, 2011). Using proteomics at the molecular level study can provide vital clues regarding the

transcription factors and signalling proteins regulating the growth pattern in fishes. Indeed, proteomics can be a powerful tool to investigate the physiological adaptations, evolution and biodiversity of fish species. Proteomics has been used for exploring various issues in aquaculture related to welfare, safety, quality, health and disease (Rodrigues et al., 2012). Many studies on fish have focused on proteome characterizations in the liver, blood or skin while evaluating the response to hormones (Dietrich et al., 2020), stress (Naderi et al., 2018), pollutants and pathogen infection (Saleh et al., 2018). Furthermore, studies on whole larvae (Sveinsdóttir et al., 2022), liver (Martin et al., 2007; heart (Lee et al., 2006), kidney (Martin et al., 2007) and muscle have been reported using 2-DE based proteome analyses. In the case of shellfish, proteome techniques have been used occasionally to study the changes and variety in protein expression between shellfish populations from different environments, including bio-toxins contamination (Lopez et al., 2002; Wang et al., 2008; Robalino et al., 2009).

5.1 Proteomic Techniques Used in Aquaculture

Proteomics deals with two major areas; expression and functional proteomics. It is a known fact that most of the biological process are influence by complex of proteins whose interaction might be the determinant of particular biological function (Monti et al., 2009). The proteins of interest along with their interacting counterpart can be fished out from a whole protein extract using various methods. Affinity-based methods or immune-precipitation using ligand binding properties methods can be employed (Monti et al., 2009). AQUA known as absolute quantification is another technique that uses an isotope-labelled internal standard peptides (ILISP) and liquid chromatography-mass spectrometry (LC-MS) to quantify protein and its peptides.

Nowadays, the most popular technologies in protein identification are based on peptide mass fingerprinting using mass spectrometry (MS) analysis, such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) and electrospray ionization mass spectrometry ESI-MS (Boliccio et al., 2016). These technologies involve further separation by labelling approaches using stable isotopes such as isotope-coded affinity tags (ICAT), stable isotope labelling with amino acids in cell culture (SILAC) and isobaric tags for relative and absolute quantification (iTRAQ). While for the three-dimensional structures study of the protein, the x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy technologies are two widely used techniques that might be helpful to understand its biological mechanism (Aslam et al., 2017). Proteomic workflow usually starts with the total protein extraction before the proteins are separated either using the gel-based or shotgun approaches. In the gel-based approach, several methods can be used for identifying and quantifying expressed proteins such as 2D gel electrophoresis, protein spot selection and protein digestion. Meanwhile, in shotgun approach, methods such as peptide clean-up and mass spectrometry were usually used.

Various proteomic techniques used for expression profiling include separation methods such as two-dimensional gel electrophoresis (2DE), protein microarray, chromatographic methods and mass spectrometry (MS). A typical proteomic workflow involves several steps of sample preparation, various protein separation techniques, different methods for protein digestion and identification approaches. Any step in a proteomic analysis can be selected depending upon the type of sample and aim of the expression.

As one of the most powerful tools, the proteomics approach has been increasingly used over the last decade for drug discovery research, biomarker identification, understanding pathogenicity mechanisms and altering protein expression patterns in response to different diseases (Boliccio et al., 2016; Aslam et al., 2017). Proteomics also contributes to understand intra- and extracellular processes, where differential protein expression such as protein abundance, protein posttranslational modifications and protein–protein interactions can be observed at certain times and in different environments (Aslam et al., 2017).

6 Nutrigenomics

Nutrigenomic in aquaculture, nutrigenomics or 'nutritional genomics' is concerned with studying the impacts of nutrients and food ingredients on gene expressions and comprehending the interactions that likely occur between nutrients and dietary bioactive ingredients with the genome and cellular molecules of the treated aquatic fauna at the molecular levels, which will ultimately result to mediation of gene expression. Studying the nutrigenomics in different aquaculture species holds significant. Nutrigenomics has rapidly grown in addressing several aspects related to the influences of nutrients on aquaculture species. Several researchers have studied the relationships between numerous functional genes and their expression profiles with several physiological functions. In aquaculture, nutrigenomics can be applied to improve the health, growth, reproduction and nutritional quality of farmed fish and shellfish.

Different scientists have studied the genomic changes influenced by various nutrients in different fish species. Król et al. (2016) compared the differential response of the Atlantic salmon gut to soybean meal (SBM) and fish meal (FM) as positive and negative controls for enteritis, respectively. They registered that SBM change the histology of the gut and induced extensive transcriptomic changes linked to the underlying mechanisms of SBM-induced enteropathy. Their study discovered 18 enriched pathways connected to inflammation and immune responses induced by SBM enteropathy.

Part of the pathway were the NF-kB and IL-8 signalling pathways known to induce the synthesis of various pro-inflammatory cytokines. Phagocytic pathways such as the Fcγ receptor mediated phagocytosis and monocyte pathways were highly enriched. The study of (Torrecillas et al., 2015) depicts downregulation of TCRβ, COX-2, TNFα, IL-8, IL-6, IL-10, TGFβ and IgM when MHC-II was

upregulated in European seabass fed with Soya-bean oil (SBO). Combination of MOS and SBO diets reduced the inimical effects associated with SBO diets by optimizing the downregulation of GALT-related genes. Therefore, these observations show the significant of moderating feed formulation in order to produce balanced diets that will ensure preservation of the GALT-immune homeostasis.

Apart from soyabean, nutrigenomics has also been employed to examine the effects of other nutrients in fish diets. Azeredo et al. (2015) reported that the immune status of the European seabass was reduced when fed with arginine dietary supplements with downregulation of different cell-mediated immune markers in fish fed 1–2% arginine diets. Leukocytes collected from fish fed arginine diets depict a low respiratory burst compared to control fish.

Microarray and RNA-seq. RNA-seq are the Omics technologies commonly used for nutrigenomics analyses in aquaculture and are used to study the impact of different diets in various fish species including Atlantic salmon (Núñez-Acuña et al., 2016), rainbow trout, channel catfish (*Iactalurus punctatus*) (Zhao et al., 2015) blue catfish (*Ictalurus furcatus*) (Li et al., 2014) and zebrafish (Rurangwa et al., 2015), Atlantic cod (*G. morhua*) and Gilthead sea bream (*S. aurata*). However, the use of RNA-seq and microarray leads to several challenges that include the need for large data processing software as well as the need of bioinformatics tools required for differential gene expression, network pathway, alternative splicing and gene duplication analyses.

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