Metabolomic Advances in Fish Nutritional Research and Health Management

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Abstract

Aquaculture production has become one of the fastest-growing quality animal protein-producing enterprises, contributing significantly to satisfying increased demand for animal protein by providing barely half of all fish and shellfish consumed directly by humans. As consequences of the intensification of aquaculture for meeting the demand, high feed input, reckless use of antibiotics and drugs/chemicals, water quality deterioration, climate change, poor growth, and disease outbreak could be a major threat in fish culture. The majority of farmed fish is lost each year, resulting in significant economic losses owing to disease outbreaks in diverse culture systems, making farming unprofitable and unsustainable in the long run. Metabolomics is a technique for assessing metabolites in a living system holistically and systematically, and it employs a system biology approach to evaluate the biochemical processes of complex organisms in terms of nutrition and health conditions. Metabolomics strives to find biomarkers emblematic of physiological reactions of live samples such as whole organisms, tissues, and cells to ambient or culture conditions by using metabolite profiles as fingerprints. We have tried to highlight some of the most current uses of metabolomic developments in fish nutrition research and health management to

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solve challenges across the entire production cycle of an organism, including post-harvest quality control.

Keywords

Metabolomics · Fish nutrition · Health management · Aquaculture

13.1 Introduction

Aquaculture production has become one of the fastest-growing animal food-producing sectors, contributing significantly to fulfilling the growing need for animal protein by supplying nearly half of all fish and shellfish consumed directly by people. Fish and fishery products provide an average of 35 calories per capita per day in terms of high-quality nutritional sources and readily digested animal proteins, which explains the high consumption (FAO 2020). As a result of its expanding relevance, the aquaculture industry has faced numerous obstacles in producing safe and high-quality fish on a long-term basis. Intensification of aquaculture for meeting the demand, high feed input, water quality deterioration, climate change, poor growth, and disease outbreak could be a major threat in fish culture. The majority of farmed fish is lost each year, resulting in significant economic losses owing to disease outbreaks in diverse culture systems, making farming unprofitable and unsustainable in the long run. Antibiotics and drugs/chemicals used indiscriminately in the culture system frequently cause buildup in the aquatic environment, harm to other creatures, toxicity to the host animal, growth reduction in fish, disruption of the natural reproductive cycle, and financial loss. Residues buildup in fish tissues, posing a health risk to humans who eat the fish. Diverse omics technologies, like genomics, transcriptomics, and proteomics, have been employed to explore the interactional response between different disease-causing agents and fish hosts in recent years. Metabolomics, a new and emerging omics technology, has lately been used to study fish metabolic responses to heavy oil, anoxia, hypoxia, microbial illnesses, pesticides, zero fish meal, and fish oil-based diets. Greater growth rates of farmed species, the higher nutritional content of aquafeeds, improved stock health, and reduced environmental impacts have all been made possible by innovative technology, many of which have been taken from other disciplines. Metabolomics has the potential to be a useful method for identifying and characterizing the metabolomes of any fish or food product. Multiple features of fish can be investigated and biomarkers for their welfare recognized using a metabolomic method, assuring sustainable fish growth and thus the quality and safety of aqua food. Recent metabolomic applications in aquaculture have demonstrated enormous potential for tackling problems across the entire production line, from hatchery production to post-harvest quality control. During the last decade, metabolomics has been implemented in aquaculture with a spectrum of uses in diets and nutrition (Grandiosa et al. 2018, 2020; Huynh et al. 2018), immunology and disease impacts (Nguyen et al. 2019, 2020a, b; Nguyen and Alfaro 2020), environmental stress (Huo et al. 2019; Li et al., 2019; Nguyen and Alfaro 2020), ecotoxicology (Li et al. 2017;

Nguyen et al. 2018a), and post-harvest handling (Alfaro et al. 2019; Nguyen et al. 2020a, b).

13.2 Metabolomics

Systems biology is a multidisciplinary method of studying biological processes at the cellular, tissue, and organism levels. The whole genome, transcriptome, proteome, and metabolome are all studied using "omics" technology. Metabolomics is a form of omics that focuses on characterizing, identifying, and quantifying small molecule (<1500 Da) metabolites in the metabolome at high throughput (German et al. 2005). As a result, metabolomics is frequently employed as a sophisticated analytical method to get a deeper understanding of the molecular mechanisms underpinning aquatic creatures' responses to nutrition, external stresses, infections, and developmental processes. The metabolome is the collection of all tiny molecules, metabolites, or chemicals present in a cell, organ, or organism, according to a formal definition. Tiny molecules include peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, minerals, and just about every other chemical that a cell or organism can use, ingest, or make. Metabolomics identifies biomarkers/chemical signatures indicative of physiological responses of living samples such as whole organisms, tissues, and cells to ambient or culture conditions using unique metabolite profiles. Metabolites provide real-time information on what is going on at the metabolic and physiological levels since they are the most sensitive to environmental changes (Patti et al. 2012). Unexpected issue or risk areas can be recognized using biomarkers, and corrective action can be taken for future management. Though metabolomics in aquaculture is still in its infancy, it has already found widespread application in a variety of fields and applications, including mammalian toxicology, plant chemistry, human nutrition, environmental sciences, food quality, clinical disease diagnostics, and microbial metabolomics, as well as drug discovery. In recent years, metabolomics in aquaculture has become a burgeoning topic, assisting aquaculture in achieving its major goal of increasing production scale while maintaining a high-quality, long-term product. Fish metabolomic study could aid in the investigation of metabolome changes caused by disease, crowding, hypoxia, malnutrition, or other environmental conditions such as pollution, poisons, and temperature fluctuations that might disrupt normal metabolism in the body (Fig. 13.1).

13.2.1 Advantages of Metabolomics over Other Omics Technology

Metabolomics has the following advantages over other omics technologies:

- Metabolomics is the study of metabolites, which are the end products of biological regulating systems that are extremely vulnerable to outside stimuli. These profiles can be thought of as biological systems' final response to genetic or environmental change (Fiehn 2002).

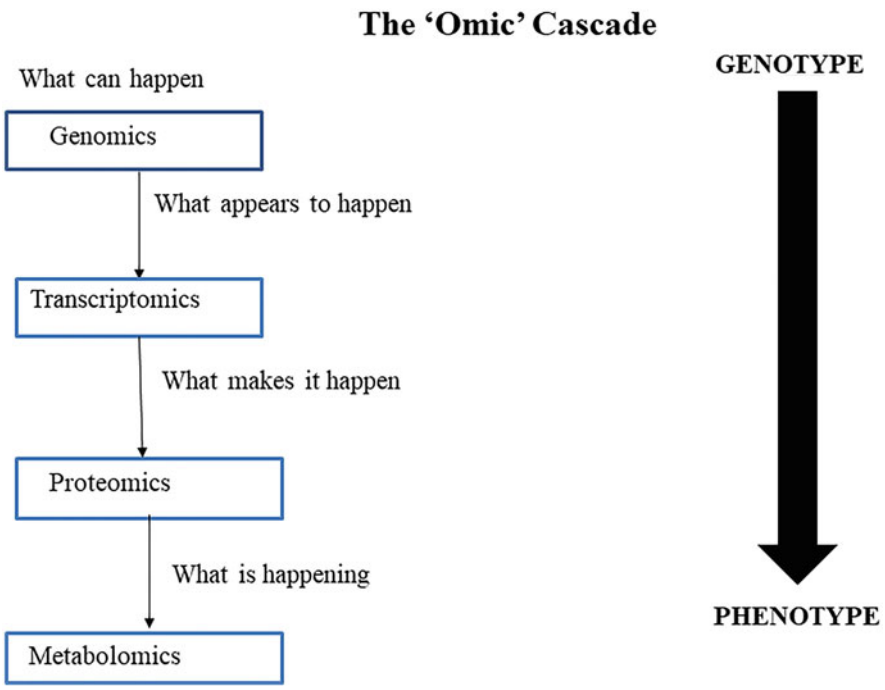

Fig. 13.1 'Omics' cascade depicts the genotype to phenotype continuum and defines genomics, transcriptomics, proteomics, and metabolomics

- In comparison with proteome and transcriptome investigations, metabolomics often requires less sample preparation and shorter turnaround times from sample collection to data interpretation, lowering costs.
- Because metabolites have significantly fewer types/classes than genes or proteins in many species, metabolite data processing is often simpler (Wang et al. 2006; Lu and King 2009).
- Non-invasive bodily fluids/solids, like plasma and faeces, can be used in metabolomics research, which may be very useful in fish investigations. Furthermore, without destroying a sample, a variety of analytical procedures can be applied (Alfaro and Young 2018). When biological material is restricted and/or several studies are to be performed on a single sample with the goal of data integration, this is particularly valuable.
- When compared with other "-omic" techniques, metabolomics has several advantages, the most important of which is its biological proximity to the system's phenotype, allowing for quick detection of system perturbations in the metabolome.

13.3 Basics of Metabolomic Techniques Used in Aquaculture

Metabolomics is a promising method for biomarker discovery since it involves both focused and non-targeted analysis of endogenous and exogenous small-molecule metabolites (<1500 Da). Metabolomics is a global metabolic profiling framework that combines high-resolution analytics (typically NMR and MS) with chemometric statistical tools like principal component analysis (PCA) and partial least squares (PLS) to produce a comprehensive picture of both endogenous and xenobiotic metabolism. Small-molecule biomarkers such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, and inorganic substances represent the functional phenotype of a cell, tissue, or organism. The physical and chemical properties of the molecules listed above are extremely diverse, and they exist in a wide concentration range. Technological breakthroughs in metabolomics have enabled the separation and identification of these tiny molecules. These cutting-edge technologies, which include accurate high-resolution MS, NMR, CE, HPLC, and UPLC technology, can detect metabolites in a matter of minutes. A number of analytical systems, including NMR, Fourier transform infrared spectroscopy (FTIR), and MS coupled to separation techniques, such as NMR, GC-MS, LC-MS, FT-MS, and UPLC-MS, have been used for metabolomic applications.

13.4 Sample Collection and Preparation for Metabolomic Study

Because the metabolome can vary extremely quickly in response to slight changes in the environment, extreme caution should be exercised when collecting the sample by limiting biological, technological, and experimental variability. Collected samples must be representative of the biology under study and appropriate for the study's specific research goals. It's also crucial to choose the right sample material. Different tissues (e.g., muscle, gills, liver, and pancreas) go through different metabolic processes depending on their role. Even after the metabolome has been taken from the body, it remains in a highly dynamic state in tissues and biological fluids. The ability to accurately measure the metabolome requires the rapid termination of enzyme activity. As a result, metabolic processes within samples must be stopped, or quenched, as quickly as feasible during collection in practically all metabolomic studies. To avoid enzymatic activity recovery, the conventional strategy for quenching metabolism in animal tissues is to freeze samples in liquid nitrogen and store them at or below −80 °C or lyophilize them. The most important aspect of any metabolomic investigation is sample preparation, and sample preparation techniques differ depending on the type of biological material obtained and the analytical platform to be used. Regardless of the method, the metabolite extraction process should be quick and reliable, with as little sample degradation and metabolite alteration as possible (Allwood 2013). For efficient sample extraction, while maintaining the chemical properties of the sample, tissues and cells must be broken down either by grinding in a liquid N2-cooled mortar and pestle (Rosenblum et al.

2005; Viant et al. 2005) or by an electric tissue homogenizer directly in the extraction solvent (Warne et al. 2001; Pears et al. 2005). Methods for metabolite extraction range from simple one-step solvent extraction to more complex approaches requiring multiple phases and/or chemical synthesis steps. Sample preparation and introduction methods for biological samples encompass direct injection, liquid–liquid extraction (LLE), solid-phase extraction (SPE), supercritical fluid extraction, accelerated solvent extraction, microwave-assisted extraction, protein precipitation, and membrane methods such as dialysis or ultracentrifugation. The different types of solvent extraction method include the following:

1. Using a mixture of methanol, water, and chloroform to extract polar and/or nonpolar metabolites.
2. Polar metabolite extraction using methanol alone or in combination with water.
3. Perchloric acid is used to retrieve polar metabolites.

There is no single perfect approach to extract all classes of metabolites with high efficiency due to the enormous range of metabolites found inside tissues, many with widely varying physical and chemical properties. Perchloric acid is commonly used to precipitate proteins and extract hydrophilic metabolites for metabolic fingerprinting research. To extract hydrophilic metabolites, polar organic solvents such as methanol, ethanol, acetonitrile, and acetone are generally combined with water (Coen et al. 2003; Kim et al. 2004; Stentiford et al. 2005a). Hydrophobic metabolites can be extracted using chloroform (Choi et al. 2004; Stentiford et al. 2005b).

13.5 Analytical Tools for Measuring Metabolomes

There is currently no one adaptable platform that can analyze all metabolites inside a sample due to the complexity of metabolites and the high number of metabolites present. Depending on the aims and scope of the investigation, the type of sample material collected, the available sample mass, the accessibility of analytical platforms, and the cost involved, multiple techniques may need to be selected and used to partially overcome the shortcomings of single-analysis techniques. Nuclear magnetic resonance (NMR), mass spectrometry (MS), Fourier transform-infrared spectroscopy (FTIR), and MS coupled to separation techniques, such as NMR, GC-MS, LC-MS, FT-MS, and UPLC-MS, are the most often used high-throughput and high-resolution systems for metabolomics studies. While NMR spectroscopy is best for analyzing bulk metabolites and GC-MS is best for analyzing volatile organic compounds and derivatized primary metabolites, LC-MS can be used to analyze a wide range of semipolar molecules, including many secondary metabolites of interest. LC-MS is a popular instrument because it avoids chemical derivatization. For the identification and quantification of metabolites, MS-based metabolomics offers great selectivity and sensitivity, and when combined with improved and high-throughput separation techniques, the complexity of metabolite separation can be reduced. MS-based approaches, on the other hand, necessitate a sample preparation

phase that can result in metabolite loss. To examine the global metabolome, it is ideal to use various techniques at the same time, such as GC-MS, LC-MS, or NMR.

13.6 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) is a spectroscopic analytical technique that can uniquely identify and quantify a wide range of organic substances in the micromolar range. It identifies atomic nuclei's distinctive spin characteristics. When nuclei with specific magnetic properties are submerged in a magnetic field, they align with (low energy state) or against (high energy state) the field. The application of extremely particular radio frequency pulses to the nuclei causes a "spin flip," which is a change in the energy state (Savorani et al. 2013). Nuclear shielding is a tiny change in the intensity of the applied magnetic field caused by the existence of other nuclei and chemical bonds surrounding a nucleus. A chemical shift occurs when nuclei within a metabolite absorb radiation at slightly different frequencies as a result of this shielding. The sample's distinct spectrum or "fingerprint" is created by combining all of these various frequencies. Furthermore, more sophisticated spin interactions under varied pulse settings can reveal a wealth of information about a molecule's chemical bonding and composition. NMR's main benefit is that it is largely automated and nondestructive, allowing samples to be used for further research while also providing extremely reliable and repeatable readings. Separation of metabolites before detection is not required, and just a minimal amount of sample preparation is required, saving both money and time. Metabolite fingerprinting, profiling, and metabolic flux analysis have all been done with it. The limited sensitivity of NMR makes it unsuitable for the investigation of large numbers of low-abundance metabolites, which is a fundamental restriction for comprehensive metabolite profiling. NMR can be particularly valuable in drug discovery and development since it offers extensive information about a compound's structural alteration as a result of metabolism.

13.7 Mass Spectrometry

Mass spectrometry (MS) is a technique for determining the molecular weights of compounds. Molecules in a test sample are transformed into gaseous ions, which are then separated and identified in a mass spectrometer based on their mass-to-charge (m/z) ratio. The mass spectrum is a graph showing the ions' (relative) abundances at different m/z ratios. The ion source, mass analyzer, and detector are the three parts of a mass spectrometer (Glish and Vachet 2003). Different steps involved in all mass spectrometers include:

1. Production of ions in the gas phase.
2. Acceleration of the ions to a specific velocity in an electric field.

3. Separation of the ions in a mass analyzer.
4. Detection of each species of a particular m/z ratio.

Electron ionization and electrospray ionization are the most often utilized ionization procedures in metabolomics research (Lei et al. 2011). MS can be used to analyze biological materials either directly without prior metabolite separation or after chromatographic separation. Direct MS techniques are quick; however, they have low ionization efficiency and ion suppression. MS-based metabolomic techniques often require the separation of metabolites by chromatography or electrophoresis before MS detection to reduce the complexity of the sample matrix and improve the sensitivity and selectivity of the analysis. The most often used procedures for this purpose are gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). These instruments are referred to as hyphenated platforms when they are used together (GC-MS, LC-MS, and CE-MS). MS approaches can have exceptionally high sensitivity or at least detection limits.

13.8 Fourier Transform Infrared (FTIR)

The vibrational fingerprints of wide metabolite functional groups can be measured using Fourier transform-infrared (FTIR) spectroscopy, a type of vibrational spectroscopy that uses lower resolution devices (Moore et al. 2014). In metabolic fingerprinting and metabolomics research, FTIR is a typical analytical tool. Because distinct absorption bands may be ascribed to individual molecular bonds, FTIR spectra can be used as a fingerprint to offer extensive information on the chemical structure and composition of substances. Infrared radiation is transmitted through a sample in IR spectroscopy. The sample absorbs some of the IR radiation, and some of it passes through (transmitted). The resulting spectrum depicts the sample's molecule absorption and transmission, resulting in a molecular fingerprint. The FTIR technique is faster than other procedures, requires a small sample size with minimal or no preparation, does not require the use of solvents, and is more cost-effective.

13.9 Applications of Metabolomics in Nutritional Management

Aquaculture confronts a daunting task in improving feed appropriateness and supporting global fish production growth. Aquaculture, as a burgeoning animal protein-producing business, must evolve dramatically to improve its reliability to meet world demand for fish, while catch fisheries production has nearly stagnated in recent decades (FAO 2020). Because of its well-balanced nutrients and high digestible proteins, high-quality fish meal (FM) is used as a primary nutritional ingredient in the majority of cultured fish. Overreliance on fishmeal (FM) in aquafeed formulations, on the other hand, is seen as one of the primary impediments to the

aquaculture sector's long-term viability, due to supply shortages and price disparities (Van Vo et al. 2015). As a result, aquaculture nutritionists around the world are working hard to identify nutritionally adequate and sustainable alternatives to fishmeal (FM) for fish feed formulation. As a result, feed components derived from terrestrial crops have been thoroughly investigated as FM alternatives (Hardy 2010). As a result, aquaculture must compete for terrestrial feedstuff with cattle, the fuel industry, and direct human consumption, raising concerns about aqua farming's impact on world food security (Troell et al. 2014). Furthermore, greater levels of plant protein sources in the diet resulted in growth retardation, lowered immunity, altered intestinal architecture, and oxidative stress (Ng et al. 2019; Xu et al. 2016). Some supplements/functional additives are used in the feed mix to address this issue. By interfering with digestion and intestinal function, added nutrients should not harm fish growth and physiology (Krogdahl et al. 2015). As a result, precise characterization of alternative feed ingredients/supplements is required to fully comprehend their impact on fish metabolism and suitability for optimal growth and immunity. The traditional method of evaluating new feed formulations is first determining the analytical composition and digestibility of the feed, followed by examining its impact on fish growth, feed consumption, and other zootechnical characteristics. However, while these traditional approaches are useful for demonstrating the major impact of feedstuffs and feed on fish growth, they may be insufficient for understanding the influence of feeds on fish metabolism and the mechanisms that underpin it. At the level of genes, transcripts, proteins, and metabolites, omics technologies allow a novel holistic view of a biological system. Nutrigenomic techniques, which study the relationship between nutrients and specific gene expression, have grown in importance in recent years, leading to novel discoveries such as the regulation of genes involved in protein, lipid, and carbohydrate metabolism in fish that have given plant-based diets (Panserat et al. 2009a, b; Geay et al. 2011). Nutrigenomics, on the other hand, has the same limitations as transcriptome methods. What happens is partly unknown because post-transcriptional changes and protein functions are not explored. Proteomics has been utilized to better understand the molecular pathways that fish use to respond to external stimuli, such as nutritional supplements, and these discoveries can be utilized to improve feed formulation and optimization. Metabolomics, on the other hand, focuses on a global set of metabolites within the biological system and provides data on metabolic activities. By combining a feeding trial with metabolomic investigations of tissues and biofluids, new insights into feed and nutrient effects could be gained. Metabolomics was utilized as a system biology approach to investigate the effects of dietary nutrients on fish growth by comparing the metabolite profiles of various tissues from different dietary regimens (Schock et al. 2012a, b; Abro et al. 2014a, b; Wagner et al. 2014a, b). Metabolomics can be used to figure out how a particular diet affects fish physiology. It aids in the selection of the appropriate feeds for optimal growth, based on their compatibility with fish metabolism, to maintain a positive link between product quality and feed conversion efficiency. Metabolomics is intended specifically to analyze metabolic reactions to nutritional deficits or excesses, and it may provide in-depth mechanistic insights to help build optimal feeding regimens (Table 13.1).

Table 13.1 Applications of metabolomics in different aspect of fish nutrition

Sl. No	Technology applied	Objectives of the study	Species [Tissue]	Remarks	References
Assessment of fish freshness and quality					
1.	1H NMR	Assessment of freshness	Gilthead Sea bream (*Sparus aurata*)	Differential metabolites identified as potential biomarkers of freshness and spoilage	Melis et al. (2014)
2.	(HR-MAS) NMR	Assessment of fish rreshness and quality	Sea bream, sea bass, trout, and red mullet	Fish freshness and quality markers such as K value and trimethylamine nitrogen (TMA-N) concentration can be determined quickly.	Heude et al. (2015)
3.	^{1}H-NMR	Quality assessment of the fish reared in the different culture system	*Sparus aurata,* flesh	Glycogen (a stress indicator), histidine, alanine, and glycine were all measured and showed considerable variations depending on the aquaculture system and storage times.	Picone et al. (2011)
4.	^{1}H-NMR	Before and after simulated gastrointestinal digestion, nutrient discrepancies between two types of freshwater fish	Crucian carp and snakehead fish, soup	Different health functions, such as taurine for enhancing immunity and alanine for increasing bodily energy levels, may be aided by metabolic alterations in digested fish soups	Cao et al. (2020)
Comparison between wild and farmed fish					
5.	NMR	Discrimination of wild and cultured fish	Sea bass, skin, and muscle	When compared with wild fish, there is a significant decrease in EFA and an increase in mono- and di-unsaturated fatty acids (MUFA and DUFA)	Mannina et al. (2008)
6.	^{1}H NMR	Differentiate wild and farmed fish and classification of origin	*Sparus aurata*, muscle	Lipid spectra	Rezzi et al. (2007)
7.	NMR	Classification of fish based on muscle fiber	Atlantic salmon, muscle	Polar and nonpolar extract	Gribbestad et al. (2005)

8.	GC-MS	Differentiation between different culture system	Mandarin fish, serum	33 metabolites were significantly different between RAS and pond groups and can be used as a biomarker	Xiao et al. (2020a, b)
Identification of a nutritional biomarker					
9.	UHPLC-HRMS	Identification of malnutrition biomarkers	Gilthead seabream (*Sparus aurata*), serum of fasted fish		Gil-Solsona et al. (2017)
10.	MS	Investigate metabolism pattern of starved fish	Rainbow trout, muscle, liver, and serum	Polar and nonpolar	Baumgarner and Cooper (2012)
11.	MS	Metabolic profiling of fish fed low protein diet fraction	Grass carp, liver, plasma	Polar compound profiling	Jin et al. (2015)
12.	NMR	Effects of food deprivation in juvenile rainbow trout	Rainbow trout, plasma, liver and muscle	The most apparent reactions were altered plasma lipoprotein levels and tissue-specific patterns of fatty acid mobilization, indicating the importance of lipids as the principal energy source during fasting	Kullgren (2010)
13.	NMR	Characterization of fish nutritional biorhythms	Leopard coral grouper, muscle	Branched-chain amino acids were involved in energy production in the muscular tissues of fasting fish. Furthermore, diurnal rhythms were seen in glycolysis, TCA cycles, and purine metabolic components	Mekuchi et al. (2017)
Effect of functional feed additive					
14.	1^{H}NMR	Explore the effect of dietary sesamin	Atlantic salmon, liver, muscle	The liver and white muscle metabolism in fish are affected by high levels of sesamin, which elevates metabolites mostly linked with energy metabolism	Wagner et al. (2014a, b)

(continued)

Table 13.1 (continued)

Sl. No	Technology applied	Objectives of the study	Species [Tissue]	Remarks	References
15.	HPLC–MS	Effect of partially protected butyrate supplementation on growth and intestinal metabolism	*Sparus aurata*, intestine	The availability of various critical amino acids and nucleotide derivatives was increased when butyrate was supplemented	Robles et al. (2013)
16.	1H-NMR	Metabolic effect of dietary taurine supplementation	*Epinephelus coioides*, intestine	Taurine supplementation enhances energy utilization and amino acid uptake, as well as protein, lipid, and purine synthesis and fish development	Shen et al. (2019)
	Effect of dietary manipulation				
17.	^{1} H NMR	Evaluation of feather meal as an alternative protein source in aquafeed	*Oncorhynchus mykiss*	Metabolic changes are caused by a higher amount of FTH inclusion	Jasour et al. (2017)
18.	^{1}H NMR	Study the effect of gelatinized starch and raw starch	*Dicentrachus labrax*, muscle	Increased glycine and phenylalanine	Jarak et al. (2018)
19.	NMR	Evaluate the efficacy of reduced fishmeal diets for growth	Cobia, serum	ANFs may have disrupted the metabolism of a plant-based component	Schock et al. (2012a, b)
20.	^{1}H NMR	Effect of fishmea-based diet, diets containing size-fractionated fish protein hydrolysate and plant protein-based diet	Turbot, liver, muscle	Changes in the metabolic profile of the liver and muscle in response to various treatments	Wei et al. (2017)
21.	^{1}H NMR	Evaluation of decontaminated fishmeal and fish oil from the Baltic Sea as promising feed sources	Arctic char, muscle, liver	When compared with treated Arctic char, those fed decontaminated fishmeal and fish oil had changes in their metabolic profile and gene expression related to energy metabolism and hepatic toxicity	Cheng et al. (2016)
22.	^{1}H NMR	Effect of dietary SBM substitution on growth performance, serum biochemistry and metabolism	Hybrid sturgeon, liver, serum	The altered phenylalanine, tyrosine, and tryptophan pathways suggested that SBM diets caused substantial liver damage	Yue et al. (2019)

23.	^{1}H and ^{2}H NMR	Effects of a high starch content diet on hepatic glycogen synthesis as well as the muscle and liver metabolome	Asian seabass, muscle and liver	In starch-fed fish, the relative content of muscle alanine (ala), a crucial intermediary in glycolysis, gluconeogenesis, and the Krebs cycle, increased significantly	Palma et al. (2020)
24.	DART–TOFMS	Effect of supplemental feeding with cereals (triticale) on the composition of muscle metabolites	Common carp, muscle	In comparison with fish given only natural food, supplemental feeding resulted in higher levels of pyroglutamic acid, glutamine, and proline in the supplemental feeding group (plankton and benthos)	Cajka et al. (2013)
25.	^{1}H-NMR	Effect of insect (*Hermetia illucens*) protein extract on metabolism	Rainbow trout, liver, muscle, gut mass, blood	Through the simultaneous provision of balanced free amino acids and energy substrates in muscle, efficient metabolic utilization of dietary free amino acids toward protein synthesis is achieved	Roques et al. (2020)
26.	NMR	Fish meal replacement with fungal material zygomycete	Arctic char, liver	When fish were fed diets containing the majority of the protein from fish meal or zygomycete biomass, a metabolic fingerprint was comparable	Abro et al. (2014a, b)
27.	UPLC-QTOF-MS	Effect of dietary oxidized fish oil on lipid metabolism and plasma metabolomics	Largemouth bass, plasma	Phospholipid and sterol metabolism changes, as well as a decrease in the unsaturated degree of membrane phospholipids and fatty acids, an increase in the levels of oxidized cholesterol and phospholipid in plasma, and repression of bile acid synthesis	Xie et al. (2020)
Effect of environmental perturbations					
28.	FTIR	Interactions between dietary factors and seasonal temperature variations	*Sparus aurata*, liver	Dietary changes can help to reduce seasonal temperature differences	Silva et al. (2014)

(continued)

Table 13.1 (continued)

Sl. No	Technology applied	Objectives of the study	Species [Tissue]	Remarks	References
29.	NMR, ICP-MS	Effect of environmental variation on diversity in aquatic ecosystems	Yellowfin goby and juvenile Japanese seabass, muscle, and fin	The mineral makeup of body muscle and fin tissues differs between species	Yoshida et al. (2014)
29.	NMR	Effect of elevated temperature on growth performance, growth- and appetite-regulating hormones and metabolism	Atlantic salmon (Salmo salar), plasma, liver, mesenteric fat	Substantial metabolic changes at a suboptimal temperature concomitant with impaired food intake and growth was observed in	Kullgren (2013)
30.	LC-MS	Physiological responses to cold and starvation stresses	Yellow drum, liver	Cold and/or hunger stress elicited different physiological responses. Glutamate and GSSG were the most prevalent metabolites produced as a result of various stressors.	Jiao et al. (2020)
31.	LC-MS	Metabolic response to long term salinity exposure	GIFT tilapia, gill	Under salinity stress, 12-hydroxyeicosatetraenoic acid and choline are greatly reduced, while adenine, Lys-pro, and inosine are greatly increased	Qin et al. (2021)
32.	LC-MS	Metabolomic responses to toxic ammonia and thermal stress	*Litopenaeus vannamei*, Hemolymph	A change in hemolymph amino acid and arachidonic acid metabolism was observed, and other stress-related metabolite indicators	Duan et al. (2021)
33.	^{1}H NMR	Metabolomics response to inking stress	*Sepia pharaonic,* liver, gill, and muscle	Ink stress causes amino acids, organic osmolytes, nucleotides, energy storage molecules, and apparent tissue-specific metabolites	Jiang et al. (2021)
34.	UHPLC-MS	To better understand the regulatory mechanisms underpinning melatonin's stimulatory influence on astaxanthin and lipid coproduction during inductive stress	*Haematococcus pluvialis,* algae	Identification of novel biomarkers that aid in the buildup of astaxanthin and lipids in algae, such as intermediates in glycolysis, the TCA cycle, and γ-aminobutyric acid (GABA)	Zhao et al. (2021)

13.10 Metabolomics in the Management of Fish Health

Fish health is an important part of aquaculture welfare that is influenced by any negative changes in the environment, such as stress and sickness caused by pathogen infection (Segner et al. 2012; FAO 2016). Disease management is also a significant concern for long-term aquaculture operations. Metabolomics has shown great promise in better understanding disease susceptibility and host-pathogen interactions (Solanky et al. 2005; Guo et al. 2014; Ma et al. 2015; Peng et al. 2015), disease characterization (Stentiford et al. 2005a, b; Southam et al. 2008), and treatment efficacy determination (Cheng et al. 2016; Su et al. 2014). The host's energy metabolism, osmotic control, oxidative stress, cell signalling pathways, and respiratory processes are all affected by pathogen exposure. A changed metabolic profile can be utilized to determine an organism's health condition and can aid in understanding pathogenesis and immune response. Metabolomics has been applied comprehensively in several aspects of health management, including the metabolic response of shrimp to pathogen invasion (Wu et al. 2017a, b; Ning et al. 2019), toxicity and environmental stress (Li et al. 2017; Chen et al. 2019; Xiao et al. 2019), and super-intensive grow-out conditions (Schock et al. 2013). The hepatopancreas of white leg shrimp *L. vannamei* infected with the microsporidian *Enterocytozoon hepatopenaei* (EHP) revealed downregulation of that energy metabolism pathway, according to a study (Ning et al. 2019). In the EHP-infected groups, 49 unique metabolites were discovered, which could be employed as a biomarker to distinguish between EHP-challenged and healthy groups. Nguyen et al. (2021) looked at the metabolic responses of penaeid shrimp to *Vibrio parahaemolyticus* caused acute hepatopancreatic necrosis disease (AHPND). GC-MS was used to produce the hemolymph metabolome of *Penaeus vannamei* challenged with *V. parahaemolyticus* and control shrimp (not exposed to the pathogens). The examination of the pathways revealed Infection with *V. parahaemolyticus* produces major changes in amino acid metabolism, the TCA cycle, and gluconeogenesis pathways, as well as their intermediates. TCA cycle intermediates such as cis-aconitic acid, citric acid, fumaric acid, isocitric acid, and succinic acid were found to be upregulated, which is generally associated with a high metabolic rate, higher energy demand, and an immunological response (Nguyen et al. 2018b, c, 2018b, c**;** Song et al. 2019). Increased glucose, which may be used as an energy source to maintain immunological response, was seen in the hepatopancreas of *Litopenaeus vannamei* infected with WSSV and aberrant amino acid and fatty acid metabolism (Wu et al. 2017a, b). Solanky et al. (2005) compared the metabolite profiles of plasma collected from Atlantic salmon challenged with virulent *A. salmonicida* to saline-injected and unfed control groups using NMR-based metabolomics. Different NMR spectra (metabolite profiles) were detected for each of these groups, and distinct metabolites were found. For the identification of infected and noninfected persons, a metabolomic-based technique can be developed. In a minimal-exchange, superintensive, and biofloc system, Schock et al. (2013) used NMR-based metabolomic approaches to evaluate the condition of shrimp health throughout the whole production cycle, from the nursery phase through

harvest. Tissue-specific metabolic alterations were discovered, primarily in the areas of energy metabolism and nitrogen detoxification. Guo et al. (2014) employed a GC/MS-based metabolomic technique to find biomarkers that differentiated life from death in crucian carps infected with *Edwardsiella tarda*. The most important metabolites distinguishing survival from death in these *E. tarda* infected fish were increased unsaturated fatty acid production, particularly palmitic acid, and decreased fructose and mannose metabolism, particularly D-mannose. The metabolic pathways linked to antibiotic resistance have been widely studied using metabolomics (Jiang et al. 2019; Liu et al. 2019; Zhang et al. 2019; Li et al. 2020).

13.11 Conclusion

Metabolomics is a powerful, new science with a lot of potential in aquaculture because it provides a global view of metabolism by identifying many metabolites involved in biological responses of organisms exposed to various circumstances like nutrition, environment, and disease. An improved understanding of metabolic pathway variation aids in the identification of biomarkers and the development of effective nutritional and health management methods that support optimum growth and long-term aquaculture output.

References

Abro R, Moazzami AA, Lindberg JE, Lundh T (2014a) Metabolic insights in Arctic charr (Salvelinus alpinus) fed with zygomycetes and fish meal diets as assessed in liver using nuclear magnetic resonance (NMR) spectroscopy. Int Aquatic Res 6(2):1–11

Abro R, Moazzami AA, Lindberg JE, Lundh T (2014b) Metabolic insights in Arctic charr (Salvelinus alpinus) fed with zygomycetes and fish meal diets as assessed in liver using nuclear magnetic resonance (NMR) spectroscopy. Int Aquatic Res 6(2):1–11

Alfaro AC, Young T (2018) Showcasing metabolomic applications in aquaculture: a review. Rev Aquac 10:135–152

Alfaro AC, Nguyen TV, Mellow D (2019) A metabolomics approach to assess the effect of storage conditions on metabolic processes of New Zealand surf clam (crassula aequilatera). Aquaculture 498:315–321

Allwood CM (2013) The role of culture and understanding in research. Soc Epistemol Rev Reply Collect 2(5):1–11

Baumgarner BL, Cooper BR (2012) Evaluation of a tandem gas chromatography/time-of-flight mass spectrometry metabolomics platform as a single method to investigate the effect of starvation on whole-animal metabolism in rainbow trout (Oncorhynchus mykiss). J Exp Biol 215(10):1627–1632

Cajka T, Danhelova H, Vavrecka A, Riddellova K, Kocourek V, Vacha F, Hajslova J (2013) Evaluation of direct analysis in real time ionization–mass spectrometry (DART–MS) in fish metabolomics aimed to assess the response to dietary supplementation. Talanta 115:263–270

Cao Q, Liu H, Zhang G, Wang X, Manyande A, Du H (2020) 1 H-NMR based metabolomics reveals the nutrient differences of two kinds of freshwater fish soups before and after simulated gastrointestinal digestion. Food Funct 11(4):3095–3104

Chen K, Li E, Xu C, Wang X, Li H, Qin JG, Chen L (2019) Growth and metabolomic responses of Pacific white shrimp (Litopenaeus vannamei) to different dietary fatty acid sources and salinity levels. Aquaculture 499:329–340

Cheng K, Wagner L, Moazzami AA, Gómez-Requeni P, Schiller Vestergren A, Brännäs E, Pickova J, Trattner S (2016) Decontaminated fishmeal and fish oil from the Baltic Sea are promising feed sources for Arctic char (Salvelinus alpinus L.)—studies of flesh lipid quality and metabolic profile. Eur J Lipid Sci Technol 118(6):862–873

Choi YH, Tapias EC, Kim HK, Lefeber AW, Erkelens C, Verhoeven JTJ et al (2004) Metabolic discrimination of Catharanthus roseus leaves infected by phytoplasma using 1H-NMR spectroscopy and multivariate data analysis. Plant Physiol 135(4):2398–2410

Coen M, Lenz EM, Nicholson JK, Wilson ID, Pognan F, Lindon JC (2003) An integrated metabonomic investigation of acetaminophen toxicity in the mouse using NMR spectroscopy. Chem Res Toxicol 16(3):295–303

Duan Y, Xiong D, Wang Y, Li H, Dong H, Zhang J (2021) Toxic effects of ammonia and thermal stress on the intestinal microbiota and transcriptomic and metabolomic responses of Litopenaeus vannamei. Sci Total Environ 754:141867

FAO (2016) La situation mondiale des p^eches et de l'aquaculture 2016: Contribuer _a la s_ecurit_e alimentaire et _a la nutrition de tous. FAO, Rome (I)

FAO (2020) The state of world fisheries and aquaculture 2020. Sustainability in action. FAO, Rome. https://doi.org/10.4060/ca9229en

Fiehn O (2002) Metabolomics—the link between genotypes and phenotypes. Functional genomics 10:155–171

Geay F, Ferraresso S, Zambonino-Infante JL, Bargelloni L, Quentel C, Vandeputte M et al (2011) Effects of the total replacement of fish-based diet with plant-based diet on the hepatic transcriptome of two European sea bass (Dicentrarchus labrax) half-sibfamilies showing different growth rates with the plant-based diet. BMC Genomics 12(1):1–18

German JB, Hammock BD, Watkins SM (2005) Metabolomics: building on a century of biochemistry to guide human health. Metabolomics 1(1):3–9

Gil-Solsona R, Nácher-Mestre J, Lacalle-Bergeron L, Sancho JV, Calduch-Giner JA, Hernández F, Pérez-Sánchez J (2017) Untargeted metabolomics approach for unraveling robust biomarkers of nutritional status in fasted gilthead sea bream (Sparus aurata). Peer J 5:e2920

Glish GL, Vachet RW (2003) The basics of mass spectrometry in the twenty-first century. Nat Rev Drug Discov 2(2):140–150

Grandiosa R, Mérien F, Young T, Van Nguyen T, Gutierrez N, Kitundu E, Alfaro AC (2018) Multi-strain probiotics enhance immune responsiveness and alters metabolic profiles in the New Zealand black-footed abalone (Haliotis iris). Fish Shellfish Immunol 82:330–338

Grandiosa R, Young T, Van Nguyen T, Mérien F, Alfaro AC (2020) Immune response in probiotic-fed New Zealand black-footed abalone (Haliotis iris) under Vibrio splendidus challenge. Fish Shellfish Immunol 104:633–639

Gribbestad IS, Aursand M, Martinez I (2005) High-resolution 1H magnetic resonance spectroscopy of whole fish, fillets and extracts of farmed Atlantic salmon (Salmo salar) for quality assessment and compositional analyses. Aquaculture 250(1–2):445–457

Guo C, Huang XY, Yang MJ, Wang S, Ren ST, Li H, Peng XX (2014) GC/MS-based metabolomics approach to identify biomarkers differentiating survivals from death in crucian carps infected by Edwardsiella tarda. Fish Shellfish Immunol 39(2):215–222

Hardy RW (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac Res 41(5):770–776

Heude C, Lemasson E, Elbayed K, Piotto M (2015) Rapid assessment of fish freshness and quality by 1 H HR-MAS NMR spectroscopy. Food Anal Methods 8(4):907–915

Huo D, Sun L, Zhang L, Ru X, Liu S, Yang H (2019) Metabolome responses of the sea cucumber Apostichopus japonicus to multiple environmental stresses: heat and hypoxia. Mar Pollut Bull 138:407–420

Huynh TG, Cheng AC, Chi CC, Chiu KH, Liu CH (2018) A synbiotic improves the immunity of white shrimp, Litopenaeus vannamei: metabolomic analysis reveal compelling evidence. Fish Shellfish Immunol 79:284–293

Jarak I, Tavares L, Palma M, Rito J, Carvalho RA, Viegas I (2018) Response to dietary carbohydrates in European seabass (Dicentrarchus labrax) muscle tissue as revealed by NMR-based metabolomics. Metabolomics 14(7):1–9

Jasour MS, Wagner L, Sundekilde UK, Larsen BK, Greco I, Orlien V, Olsen K, Rasmussen HT, Hjermitslev NH, Hammershøj M, Dalsgaard AJ (2017) A comprehensive approach to assess feathermeal as an alternative protein source in aquafeed. J Agric Food Chem 65(48): 10673–10684

Jiang M, Gong QY, Lai SS, Cheng ZX, Chen ZG, Zheng J, Peng B (2019) Phenylalanine enhances innate immune response to clear ceftazidime-resistant Vibrio alginolyticus in Danio rerio. Fish Shellfish Immunol 84:912–919

Jiang M, Chen H, Luo Y, Han Q, Peng R, Jiang X (2021) Combined metabolomics and histological analysis of the tissues from cuttlefish Sepia pharaonis exposed to inking stress. Comp Biochem Physiol D: Genom Proteom 38:100829

Jiao S, Nie M, Song H, Xu D, You F (2020) Physiological responses to cold and starvation stresses in the liver of yellow drum (Nibea albiflora) revealed by LC-MS metabolomics. Sci Total Environ 715:136940

Jin Y, Tian LX, Xie SW, Guo DQ, Yang HJ, Liang GY, Liu YJ (2015) Interactions between dietary protein levels, growth performance, feed utilization, gene expression and metabolic products in juvenile grass carp (Ctenopharyngodon idella). Aquaculture 437:75–83

Kim HK, Choi YH, Luijendijk TJ, Rocha RAV, Verpoorte R (2004) Comparison of extraction methods for secologanin and the quantitative analysis of secologanin from symphoricarpos albus using 1H-NMR. Phytochem Anal An Int J Plant Chem Biochem Techniq 15(4):257–261

Krogdahl Å, Gajardo K, Kortner TM, Penn M, Gu M, Berge GM, Bakke AM (2015) Soya saponins induce enteritis in Atlantic salmon (Salmo salar L.). J Agric Food Chem 63(15):3887–3902

Kullgren A, Samuelsson LM, Larsson DJ, Björnsson BT, Bergman EJ (2010) A metabolomics approach to elucidate effects of food deprivation in juvenile rainbow trout (Oncorhynchus mykiss). Am J Phys Regul Integr Comp Phys 299(6):R1440–R1448

Kullgren A, Jutfelt F, Fontanillas R, Sundell K, Samuelsson L, Wiklander K, Kling P, Koppe W, Larsson DJ, Björnsson BT, Jönsson E (2013) The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (Salmo salar). Comp Biochem Physiol A Mol Integr Physiol 164(1):44–53

Lei Z, Huhman DV, Sumner LW (2011) Mass spectrometry strategies in metabolomics. J Biol Chem 286(29):25435–25442

Li L, Su YB, Peng B, Peng XX, Li H (2020) Metabolic mechanism of colistin resistance and its reverting in Vibrio alginolyticus. Environ Microbiol 22(10):4295–4313

Li T, Li E, Suo Y, Xu Z, Jia Y, Qin JG, Chen L, Gu Z (2017) Energy metabolism and metabolomics response of Pacific white shrimp *Litopenaeus vannamei* to sulfide toxicity. Aquat Toxicol 183: 28–37. https://doi.org/10.1016/j.aquatox.2016.12.010

Li X, Qu C, Bian Y, Gu C, Jiang X, Song Y (2019) New insights into the responses of soil microorganisms to polycyclic aromatic hydrocarbon stress by combining enzyme activity and sequencing analysis with metabolomics. Environ Pollut 255:113312

Liu SR, Peng XX, Li H (2019) Metabolic mechanism of ceftazidime resistance in Vibrio alginolyticus. Infect Drug Resist 12:417

Lu C, King RD (2009) An investigation into the population abundance distribution of mRNAs, proteins, and metabolites in biological systems. Bioinformatics 25(16):2020–2027

Ma YM, Yang MJ, Wang S, Li H, Peng XX (2015) Liver functional metabolomics discloses an action of L-leucine against Streptococcus iniae infection in tilapias. Fish Shellfish Immunol 45(2):414–421

Mannina L, Sobolev AP, Capitani D, Iaffaldano N, Rosato MP, Ragni P et al (2008) NMR metabolic profiling of organic and aqueous sea bass extracts: implications in the discrimination of wild and cultured sea bass. Talanta 77(1):433–444

Mekuchi M, Sakata K, Yamaguchi T, Koiso M, Kikuchi J (2017) Trans-omics approaches used to characterise fish nutritional biorhythms in leopard coral grouper (Plectropomus leopardus). Sci Rep 7(1):1–12

Melis R, Cappuccinelli R, Roggio T, Anedda R (2014) Addressing marketplace gilthead sea bream (Sparus aurata L.) differentiation by 1H NMR-based lipid fingerprinting. Food Res Int 63:258–264

Moore DS, Jepsen PU, Volka K (2014) Principles of vibrational spectroscopic methods and their application to bioanalysis. In: Gauglitz G, Moore DS (eds) Handbook of Spectroscopy. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany

Ng WK, Leow TC, Yossa R (2019) Effect of substituting fishmeal with corn protein concentrate on growth performance, nutrient utilization and skin coloration in red hybrid tilapia, Oreochromis sp. Aquac Nutr 25(5):1006–1016

Nguyen TV, Alfaro AC (2020) Applications of omics to investigate responses of bivalve haemocytes to pathogen infections and environmental stress. Aquaculture 518:734488

Nguyen TV, Alfaro AC, Young T, Ravi S, Merien F (2018a) Metabolomics study of immune responses of New Zealand greenshell™ mussels (Perna canaliculus) infected with pathogenic vibrio sp. Mar Biotechnol 20(3):396–409

Nguyen TV, Alfaro AC, Young T, Ravi S, Merien F (2018b) Metabolomics study of immune responses of New Zealand greenshell™ mussels (Perna canaliculus) infected with pathogenic vibrio sp. Mar Biotechnol 20(3):396–409

Nguyen TV, Alfaro AC, Young T, Merien F (2019) Tissue-specific immune responses to vibrio sp. infection in mussels (Perna canaliculus): a metabolomics approach. Aquaculture 500:118–125

Nguyen TV, Alfaro A, Arroyo BB, Leon JAR, Sonnenholzner S (2020a) Metabolic responses of penaeid shrimp to acute hepatopancreatic necrosis disease caused by Vibrio parahaemolyticus. Aquaculture 533:736174

Nguyen TV, Ragg NL, Alfaro AC, Zamora LN (2020b) Physiological stress associated with mechanical harvesting and transport of cultured mussels (Perna canaliculus): a metabolomics approach. Aquaculture 529:735657

Nguyen TV, Alfaro A, Arroyo BB, Leon JAR, Sonnenholzner S (2021) Metabolic responses of penaeid shrimp to acute hepatopancreatic necrosis disease caused by Vibrio parahaemolyticus. Aquaculture 533:736174

Nguyen VT, Alfaro A, Young T, Ravi S, Merien F (2018c) Metabolomics study of immune responses of New Zealand greenshell™ mussels (*Perna canaliculus*) infected with pathogenic *vibrio* sp. Mar Biotechnol 20:396–409. https://doi.org/10.1007/s10126-018-9804-x

Ning M, Wei P, Shen H, Wan X, Jin M, Li X, Shi H, Qiao Y, Jiang G, Gu W, Wang W (2019) Proteomic and metabolomic responses in hepatopancreas of whiteleg shrimp Litopenaeus vannamei infected by microsporidian Enterocytozoon hepatopenaei. Fish Shellfish Immunol 87:534–545

Palma M, Trenkner LH, Rito J, Tavares LC, Silva E, Glencross BD, Jones JG, Wade NM, Viegas I (2020) Limitations to starch utilization in barramundi (Lates calcarifer) as revealed by NMR-based metabolomics. Front Physiol 11:205

Panserat S, Hortopan GA, Plagnes-Juan E, Kolditz C, Lansard M, Skiba-Cassy S, Esquerre D, Geurden I, Medale F, Kaushik S, Corraze G (2009a) Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (Oncorhynchus mykiss) liver. Aquaculture 294(1–2):123–131

Panserat S, Hortopan GA, Plagnes-Juan E, Kolditz C, Lansard M, Skiba-Cassy S et al (2009b) Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (Oncorhynchus mykiss) liver. Aquaculture 294(1–2):123–131

Patti GJ, Yanes O, Siuzdak G (2012) Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13(4):263–269

Pears MR, Cooper JD, Mitchison HM, Mortishire-Smith RJ, Pearce DA, Griffin JL (2005) High resolution 1H NMR-based metabolomics indicates a neurotransmitter cycling deficit in cerebral tissue from a mouse model of batten disease. J Biol Chem 280(52):42508–42514

Peng B, Ma YM, Zhang JY, Li H (2015) Metabolome strategy against Edwardsiella tarda infection through glucose-enhanced metabolic modulation in tilapias. Fish Shellfish Immunol 45(2): 869–876

Picone G, Balling Engelsen S, Savorani F, Testi S, Badiani A, Capozzi F (2011) Metabolomics as a powerful tool for molecular quality assessment of the fish Sparus aurata. Nutrients 3(2):212–227

Qin H, Yu Z, Zhu Z, Lin Y, Xia J, Jia Y (2021) The integrated analyses of metabolomics and transcriptomics in gill of GIFT tilapia in response to long term salinity challenge. Aquacult Fisheries

Rezzi S, Giani I, Héberger K, Axelson DE, Moretti VM, Reniero F, Guillou C (2007) Classification of gilthead sea bream (Sparus aurata) from 1H NMR lipid profiling combined with principal component and linear discriminant analysis. J Agric Food Chem 55(24):9963–9968

Robles R, Lozano AB, Sevilla A, Márquez L, Nuez-Ortin W, Moyano FJ (2013) Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (Sparus aurata). Fish Physiol Biochem 39(6):1567–1580

Roques S, Deborde C, Guimas L, Marchand Y, Richard N, Jacob D, Skiba-Cassy S, Moing A, Fauconneau B (2020) Integrative metabolomics for assessing the effect of insect (Hermetia illucens) protein extract on rainbow trout metabolism. Meta 10(3):83

Rosenblum ES, Viant MR, Braid BM, Moore JD, Friedman CS, Tjeerdema RS (2005) Characterizing the metabolic actions of natural stresses in the California red abalone, Haliotis rufescens using 1 H NMR metabolomics. Metabolomics 1(2):199–209

Savorani F, Rasmussen MA, Mikkelsen MS, Engelsen SB (2013) A primer to nutritional metabolomics by NMR spectroscopy and chemometrics. Food Res Int 54(1):1131–1145

Schock TB, Newton S, Brenkert K, Leffler J, Bearden DW (2012a) An NMR-based metabolomic assessment of cultured cobia health in response to dietary manipulation. Food Chem 133(1): 90–101

Schock TB, Newton S, Brenkert K, Leffler J, Bearden DW (2012b) An NMR-based metabolomic assessment of cultured cobia health in response to dietary manipulation. Food Chem 133(1): 90–101

Schock TB, Duke J, Goodson A, Weldon D, Brunson J, Leffler JW, Bearden DW (2013) Evaluation of Pacific white shrimp (Litopenaeus vannamei) health during a superintensive aquaculture growout using NMR-based metabolomics. PLoS One 8(3):e59521

Segner H, Sundh H, Buchmann K, Douxfils J, Sundell KS, Mathieu C, Ruane N, Jutfelt F, Toften H, Vaughan L (2012) Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. Fish Physiol Biochem 38(1):85–105

Shen G, Wang S, Dong J, Feng J, Xu J, Xia F et al (2019) Metabolic effect of dietary taurine supplementation on grouper (Epinephelus coioides): a 1H-NMR-based metabolomics study. Molecules 24(12):2253

Silva TS, da Costa AM, Conceição LE, Dias JP, Rodrigues PM, Richard N (2014) Metabolic fingerprinting of gilthead seabream (Sparus aurata) liver to track interactions between dietary factors and seasonal temperature variations. Peer J 2:e527

Solanky KS, Burton IW, MacKinnon SL, Walter JA, Dacanay A (2005) Metabolic changes in Atlantic salmon exposed to Aeromonas salmonicida detected by 1H-nuclear magnetic resonance spectroscopy of plasma. Dis Aquat Org 65(2):107–114

Song M, Zhao J, Wen HS, Li Y, Li JF, Li LM, Tao YX (2019) The impact of acute thermal stress on the metabolome of the black rockfish (Sebastes schlegelii). PLoS One 14(5):e0217133

Southam AD, Easton JM, Stentiford GD, Ludwig C, Arvanitis TN, Viant MR (2008) Metabolic changes in flatfish hepatic tumours revealed by NMR-based metabolomics and metabolic correlation networks. J Proteome Res 7(12):5277–5285

Stentiford GD, Viant MR, Ward DG, Johnson PJ, Martin A, Wenbin W et al (2005a) Liver tumors in wild flatfish: a histopathological, proteomic, and metabolomic study. OMICS: A Journal of Integrative Biology 9(3):281–299

Stentiford GD, Viant MR, Ward DG, Johnson PJ, Martin A, Wenbin W, Cooper HJ, Lyons BP, Feist SW (2005b) Liver tumors in wild flatfish: a histopathological, proteomic, and metabolomic study. OMICS J Integ Biol 9(3):281–299

Su M-A, Huang Y-T, Chen I-T, Lee D-Y, Hsieh Y-C, Li C-Y, Ng TH, Liang S-Y, Lin S-Y, Huang S-W, Chiang Y-A, Yu H-T, Khoo K-H, Chang G-D, Lo C-F, Wang H-C, Lagunoff M (2014) An invertebrate Warburg effect: a shrimp virus achieves successful replication by altering the host metabolome via the PI3K-Akt-mTOR pathway. PLoS Pathog 10(6):e1004196

Troell M, Naylor RL, Metian M, Beveridge M, Tyedmers PH, Folke C et al (2014) Does aquaculture add resilience to the global food system? Proc Natl Acad Sci 111(37):13257–13263

Van Vo B, Bui DP, Nguyen HQ, Fotedar R (2015) Optimized fermented lupin (Lupinus angustifolius) inclusion in juvenile barramundi (Lates calcarifer) diets. Aquaculture 444:62–69

Viant MR, Lyeth BG, Miller MG, Berman RF (2005) An NMR metabolomic investigation of early metabolic disturbances following traumatic brain injury in a mammalian model. NMR Biomed 18(8):507–516

Wagner L, Trattner S, Pickova J, Gómez-Requeni P, Moazzami AA (2014a) 1H NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (Salmo salar). Food Chem 147:98–105

Wagner L, Trattner S, Pickova J, Gómez-Requeni P, Moazzami AA (2014b) 1H NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (Salmo salar). Food Chem 147:98–105

Wang QZ, Wu CY, Chen T, Chen X, Zhao XM (2006) Integrating metabolomics into a systems biology framework to exploit metabolic complexity: strategies and applications in microorganisms. Appl Microbiol Biotechnol 70(2):151–161

Warne MA, Lenz EM, Osborn D, Weeks JM, Nicholson JK (2001) Comparative biochemistry and short-term starvation effects on the earthworms Eisenia veneta and Lumbricus terrestris studied by 1H NMR spectroscopy and pattern recognition. Soil Biol Biochem 33(9):1171–1180

Wei Y, Liang M, Mai K, Zheng K, Xu H (2017) 1H NMR-based metabolomics studies on the effect of size-fractionated fish protein hydrolysate, fish meal and plant protein in diet for juvenile turbot (Scophthalmus maximus L.). Aquac Nutr 23(3):523–536

Wu H, Zhang J, He Y, Zhou J, Yan J, Jiang M (2017a) A metabolic study in hepatopancreas of Litopenaeus vannamei response to white spot syndrome virus. Int Aquat Res 9(3):195–201

Wu H, Zhang J, He Y, Zhou J, Yan J, Jiang M (2017b) A metabolic study in hepatopancreas of Litopenaeus vannamei response to white spot syndrome virus. Int Aquatic Res 9(3):195–201

Xiao J, Li QY, Tu JP, Chen XL, Chen XH, Liu QY, Liu H, Zhou XY, Zhao YZ, Wang HL (2019) Stress response and tolerance mechanisms of ammonia exposure based on transcriptomics and metabolomics in Litopenaeus vannamei. Ecotoxicol Environ Saf 180:491–500

Xiao M, Qian K, Wang Y, Bao F (2020a) GC-MS metabolomics reveals metabolic differences of the farmed mandarin fish Siniperca chuatsi in recirculating ponds aquaculture system and pond. Sci Rep 10(1):1–8

Xiao M, Qian K, Wang Y, Bao F (2020b) GC-MS metabolomics reveals metabolic differences of the farmed mandarin fish Siniperca chuatsi in recirculating ponds aquaculture system and pond. Sci Rep 10(1):1–8

Xie S, Yin P, Tian L, Liu Y, Niu J (2020) Lipid metabolism and plasma metabolomics of juvenile largemouth bass Micropterus salmoides were affected by dietary oxidized fish oil. Aquaculture 522:735158

Xu H, Mu Y, Zhang Y, Li J, Liang M, Zheng K, Wei Y (2016) Graded levels of fish protein hydrolysate in high plant diets for turbot (Scophthalmus maximus): effects on growth performance and lipid accumulation. Aquaculture 454:140–147

Yoshida S, Date Y, Akama M, Kikuchi J (2014) Comparative metabolomic and ionomic approach for abundant fishes in estuarine environments of Japan. Sci Rep 4(1):1–9

Yue HM, Wu JP, Ruan R, Ye H, Chen XH, Li CJ (2019) 1H NMR-based metabolomics investigation of dietary soybean meal substitution in hybrid sturgeon (Acipenser baerii♀× A. schrenckii♂)

Zhang S, Wang J, Jiang M, Xu D, Peng B, Peng XX, Li H (2019) Reduced redox-dependent mechanism and glucose-mediated reversal in gentamicin-resistant Vibrio alginolyticus. Environ Microbiol 21(12):4724–4739

Zhao Y, Wang HP, Yu C, Ding W, Han B, Geng S et al (2021) Integration of physiological and metabolomic profiles to elucidate the regulatory mechanisms underlying the stimulatory effect of melatonin on astaxanthin and lipids coproduction in Haematococcus pluvialis under inductive stress conditions. Bioresour Technol 319:124150