Investigación e Innovación en Nutrición Acuícola

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Mini-review: Development of High-throughput Omics Resources for Aquaculture Nutrition

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Abstract

Enhanced growth, immunity, and resilience are highly sought-after phenotypic traits in aquaculture. In current practice, feeds are purposefully formulated to include ingredients that promote these traits while striving for low production costs, complying with sustainability, and more recently, social license. The use of omics technologies in aquaculture has significantly increased in the last decade with transcriptomics being the most commonly employed tool to infer animal responses to diet or disease challenge. However, by combining transcriptomics with proteomics and metabolomics, it is now plausible to evolve aquaculture nutrition from the viewpoints of enhanced growth and survival to the development of premium advanced functional feeds that help to attain all desired phenotypes. Close proximity of proteins and metabolites to the desired phenotype makes proteomics and metabolomics coupled to bioinformatics a powerful trinomial tool to elucidate functional perturbations in an organism under specific circumstances at a given time. Collectively, omics technologies have unveiled new knowledge regarding specific biomarkers and metabolic pathways associated with specific dietary components or disease challenges across several aquaculture research domains and contributed to defining the future of sustainable aquaculture.

Keywords: *Aquaculture Nutrition, Omics, Advanced functional feeds, Metabolic pathways, Biomarkers*

Introduction

Aquaculture is defined as the farming of aquatic animals and plants for commercial food production and has become the fastest growing food production sector in the last 30 years (FAO, 2018). Due to human population growth, global demand for animal protein has significantly increased meaning that food supply must increase 25-70% by 2050 (Hunter, Smith, Schipanski, Atwood, & Mortensen, 2017) — aquaculture is a critical industry for meeting increased protein demand and maintaining future global food security. Traditionally, around 70% of aquaculture production required feed input reliant on wild-capture fisheries resources; however, a diverse range of alternative sustainable protein sources are now being used (Hua *et al*., 2019). As a founding principle, aquaculture seeks to rear high-health animals with enhanced growth while maintaining low production costs, minimizing environmental impact, and complying with social license. Growth is maximised by combining complementary raw feed materials of known nutritional value to produce species-specific advanced formulated aquafeeds.

Reports of diets and diet additives that promote growth and health in aquaculture species are abundant in the literature (Encarnação, 2016; Mustafa & Al-Faragi, 2021). For example, commercially available aquafeed Novacq^{TM} is supplemented with a marine microbial biomass that promoted 30% faster growth and immunity in black tiger shrimp *Penaeus monodon* (B. Glencross *et al*., 2014; Noble *et al*., 2021; Rombenso, Duong, Hines, Mã, & Simon, 2021; Sellars *et al*., 2015). In *Penaeus vannamei*, supplementation of yeast autolysate enhanced growth, improved feed conversion rate (FCR), and provided immunity against challenge with *Vibrio parahaemolyticus* (Ma et al., 2020). Similarly, *P. vannamei* supplemented vitamin D_3 (0.48 mg/kg) increased immunity, antioxidant capacity and expression of genes related to lipid synthesis (Dai *et al*., 2021). Recent strategies have also included the used of live bacteria as prebiotic or probiotic additives to promote health status, disease resistance, gut microbiome, and water quality (El-Saadony *et al*., 2021; Knipe, Temperton, Lange, Bass, & Tyler, 2021). For example, live *Lactobacillus pentosus* and *Arthrospira platensis* supplemented into the feed enhanced growth and lowered FCR in *P. vannamei* (Liu *et al*., 2022). Lower FCR was associated with enhanced digestive capacity indicated by increased trypsin activity in shrimp fed live bacteria and *A. platensis*. Furthermore, supplementation of bacterial extracts from *L. pentosus* combined with *A. platensis* enhanced survival as a result of upregulated expression of immune-related genes. Lastly, supplementation *L.*

pentosus and *A. platensis* reduced the presence of harmful bacteria in shrimp intestine (Liu *et al*., 2022).

Traditional aquaculture nutrition methods focussed on growth performance at different ingredient inclusion levels to define essential nutrient requirements but did not further investigate molecular mechanisms underlying beneficial ingredients. However, advances in expectations from ingredient manufacturers and feed formulators (Glencross, 2020), as well as recent advances in high throughput omics technologies (Tripathy, Khatei, & Parhi, 2021), have helped elucidate the underlying functions of diet formulations or diet additives and assisted in understanding their effects on animal nutrition and health. Transcriptomics has been the most widely used tool for exploring the effect of diets on animal physiology and metabolism in aquaculture. However, the use of proteomics and metabolomics will be the focus in this mini review given the proximity of these tools along the omics cascade for measuring the desired phenotype. The use of proteomics and metabolomics has increased in broodstock management, assessing health and nutrition of aquaculture species, and in determining the quality of raw materials (Carrera, Piñeiro, & Martinez, 2020; Jasour *et al*., 2018; Lulijwa, Alfaro, & Young, 2021; Nguyen, Alfaro, Mundy, Petersen, & Ragg, 2022; Nissa, Pinto, Parkar, Goswami, & Srivastava, 2021; Roques *et al*., 2020). Specifically, metabolomics has been implemented to decode the composition of raw materials used in aquaculture and monitor the same metabolites in animal plasma following consumption of raw materials supplemented in aquafeeds (Deborde *et al*., 2021).

Proteomics

Mass spectrometry (MS)-based proteomics is divided into two major approaches: discovery and targeted proteomics (Figure 1). In both approaches, a mass spectrometer is used to separate ions based on their mass to charge ratio and to quantify their abundance. Discovery proteomics is commonly used when information about an organism is limited, and no specific protein targets have yet been identified. For example, discovery proteomics has been employed to identify proteins involved in sexual maturation, sperm and egg structure, motility, acrosomal reaction, and fertilization in male and female abalone (Mendoza-Porras *et al*., 2014). In the same study, gene ontology analysis revealed clear differences between female and male protein gonadic profiles reflecting a higher rate of protein synthesis in the ovary and higher metabolic activity in the testis. Discovery proteomics is also a very useful resource to help discriminate proteins of high sequence

homology. For example, discovery proteomics was used to resolve the complexity of highly relevant proteins such as shrimp haemocyanin (Mendoza-Porras *et al*., 2020; Wang, Janech, & Burnett, 2019) and to highlight the medical implications of seafood allergens (Mendoza-Porras *et al*., 2020).

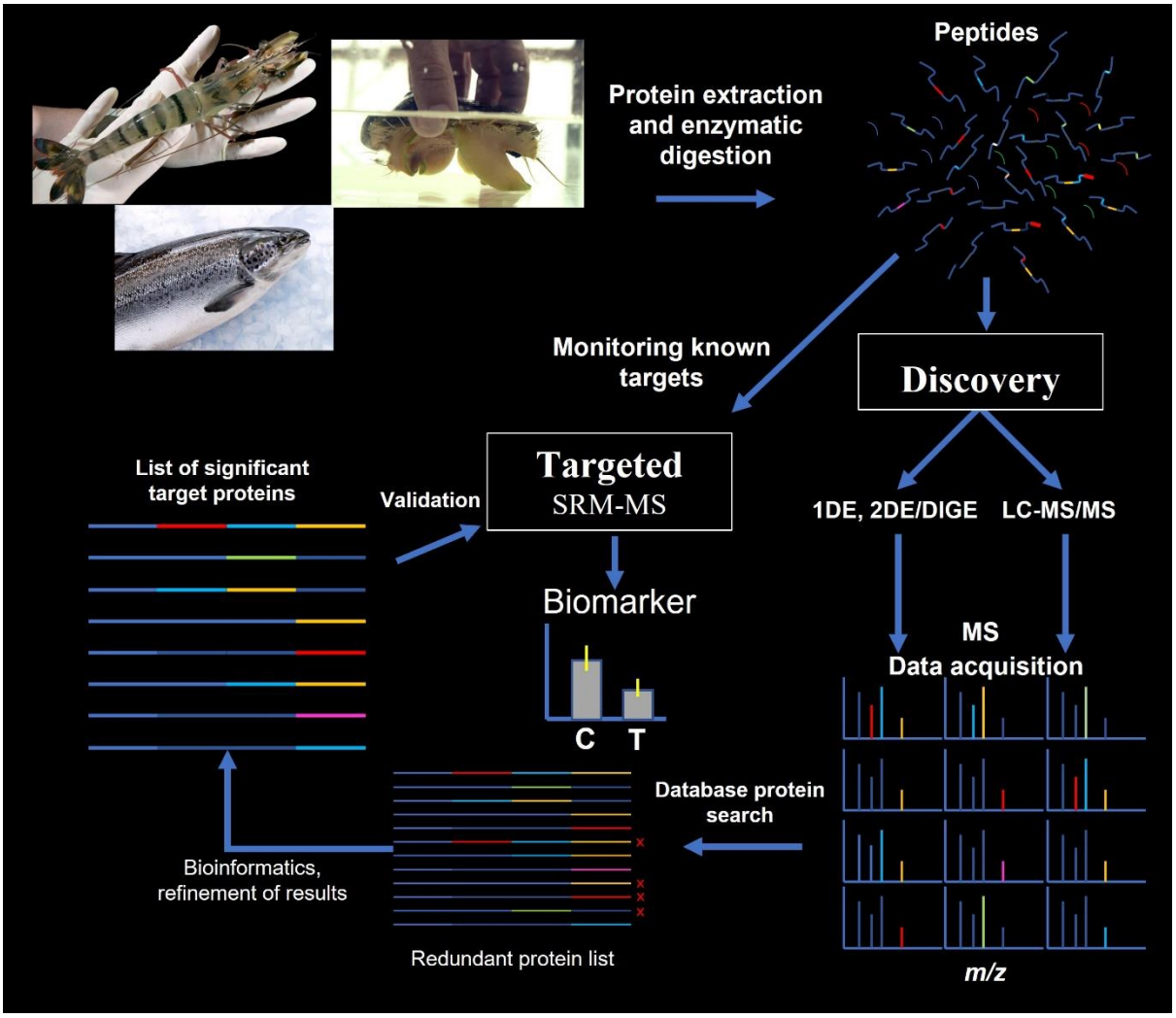

Figure 1. Workflow of sample preparation for discovery and targeted proteomics. 1DE, one-dimensional electrophoresis. 2DE, two-dimensional electrophoresis. DIGE, differential in gel electrophoresis. LC, liquid chromatography. MS, mass spectrometry. SRM, selected reaction monitoring. *m/z*, mass-to-charge ratio. C, control. T, treatment.

Application of MS-based targeted proteomics in aquaculture has not been widely utilised but has significant potential. This method is a specific, accurate, reproducible, and can quantify dozens of peptide targets from a single complex protein mixture in the same analytical instrument run.

Targeted proteomics requires considerable knowledge of the targets of interest but offers a more reliable, alternative approach to immunoassay-based approaches, such as ELISA (Colgrave, Goswami, Blundell, Howitt, & Tanner, 2014). For example, this approach was used to quantify the changes in abundance of reproductive proteins in male and female abalone following artificial spawning (Mendoza-Porras, Botwright, *et al*., 2017; Mendoza-Porras, Harris, *et al*., 2017) and indicated positive associations between abundance of thermal and oxidative stress proteins and propensity or failure to spawn in female abalone gonads. Similarly, a non-invasive, integrated discovery and targeted proteomics approach was employed for exploring the haemolymph of geoduck clam *Panopea generosa* in a reproductive maturation study (Timmins-Schiffman *et al*., 2017). Targeted proteomics is also often employed to validate discovery proteomics findings (Mendoza-Porras, Botwright, *et al*., 2017; Nissa, Pinto, Mukherjee, *et al*., 2021; Timmins-Schiffman *et al*., 2017).

Aquaculture nutrition and health

Several studies have used proteomic approaches to observe the effect of diet additives on the health and growth of aquatic species (Table 1). Two-dimensional electrophoresis (2DE) coupled-MS is a technique that allows separation of proteins based on isoelectric point in the first dimension. The second dimension consists of separating proteins according to their molecular weight. In this way, a control group and a treatment group can be directly compared by quantification of protein abundance and protein identity determined by MS and subsequent database search. For example, 2DE proteomics were employed in a study involving rainbow trout *Oncorhynchus mykiss* fry, where supplementation with 0.2% beta-glucan enhanced growth (Ghaedi, Keyvanshokooh, Mohammadi Azarm, & Akhlaghi, 2016). In this study, structural muscle proteins myosin and tropomyosin were identified with differential abundance by 2DE proteomics and associated to the observed growth through hyperplasia and hypertrophy mechanisms (Ghaedi *et al*., 2016). A different 2DE proteomics study identified and helped link heat stress proteins and regulatory enzymes to altered metabolism in rainbow trout following dietary inclusion of soybean meal (Martin *et al*., 2003). Furthermore, the negative impact of soybean meal was attributed to anti-nutritional factors present in soybean (Martin *et al*., 2003). Proteomics was also used to investigate the effects of plant-based diets incorporating specific marine ingredients (squid 10% and krill 5%) compared to fishmeal (FM) on the proteome of gilthead seabream *Sparus aurata* (Estruch *et al.*,

2020). Entire replacement of FM led to poor growth performance and changes in the gut mucosal proteome, but seabream fed plant-based diets including marine ingredients displayed biometrics and gut proteome status similar to the group fed only FM (Estruch *et al*., 2020). Analysis of the underlying molecular processes revealed that pathways, such as phagosome, proteasome, and amino sugar and nucleotide sugar metabolism pathways, were altered between the groups fed FM and plant-based diets (Estruch *et al*., 2020). In a dietary study involving Atlantic salmon where fish oil (FO) was replaced with vegetable oil, a combined transcriptomic and proteomic approach revealed significant pathways alterations in proteosome proteolysis, oxidative and cellular stress responses, apoptosis, and structure integrity (Morais *et al*., 2012).

Table. 1. Brief summary of proteomics and metabolomics utilisation in aquaculture.

Approach	Analytical platform	Concept/inclusion	Species	Tissue	Outcome/enhanced trait/molecular process	Reference
Proteomics	LC-MS/MS	Soybean meal	*Salmo Salar*	Mucus	Immune response	(Djordjevic, Morales-Lange, Øverland, Mercado, & Lagos, 2021)
	LC-MS/MS	Plant-based with and without marine sources	*Sparus aurata, L*	Gut mucosa	Growth, gut proteome	(Estruch *et al*., 2020)
	TMT, ESI-MS/MS	Carotenoid	*Larimichthys crocea*	Blood, dorsal skin and ventral skin, muscle	Skin colour	(Luo *et al*., 2020)
	2DE-DIGE, MALDI TOF/TOF	Chronic stress	*Sparus aurata, L*	Plasma	Chronic stress markers	(Raposo de Magalhães *et al*., 2020)
	2DE, LC-MS/MS	Hypoxic stress	*Ctenopharyngodon idella*	Gills	Hypoxia markers	(Xu, Zheng, Wu, Jiang, & Zou, 2019)
	LC-MS/MS	High temperature	*Salmo salar*	Liver	Heat stress markers	(Nuez-Ortín, Carter, Nichols, Cooke, & Wilson, 2018)
	LC-MS/MS, SRM	Sexual maturation	*Panopea generosa*	Gonad, haemolypmh	Reproductive markers	(Timmins-Schiffman *et al*., 2017)
	2DE-DIGE, LC-MS/MS, SRM	Sexual maturation	*Haliotis laevigata*	Gonad	Reproductive markers	(Mendoza-Porras, Botwright, *et al*., 2017)
	2DE-DIGE, LC-MS/MS, SRM	Sexual maturation	*Haliotis laevigata*	Gonad	Reproductive markers	(Mendoza-Porras, Harris, *et al*., 2017)
	2DE, MALDI-TOF/TOF MS	Beta-glucans	*Oncorhynchus mykiss*	Muscle	Growth	(Ghaedi *et al*., 2016)
	LC-MS/MS	Sexual maturation	*Haliotis laevigata*	Gonad	Reproductive markers	(Mendoza-Porras *et al*., 2014)
	2DE-DIGE, MALDI TOF/TOF	Vegetable oil	*Salmo Salar*	Intestine	Lipid and energy metabolism	(Morais *et al*., 2012)
	2DE-PAGE-MS	Farming environment	*Sparus aurata, L*	Liver, muscle	Muscle quality	(Addis *et al*., 2010)
Metabolomics	GC-MS	Prebiotic cellulose fibre and probiotic	*Penaeus vannamei*	Gill, haemolymph	Immune response, energy metabolism	(Alfaro *et al*., 2022)
	GC-MS	Disease challenge	*Penaeus vannamei*	Haemolymph	Several amino acids, TCA cycle	(Nguyen, Alfaro, Arroyo, Leon, & Sonnenholzner, 2021)
	Q-ToF-MS	Black soldier fly meal	*Penaeus vannamei*	Muscle	Determining optimal inclusion level	(Chen *et al*., 2021)
	^{1}H NMR	Plant-based	*Oncorhynchus mykiss*	Plasma	Amino acid dynamics in plasma	(Deborde *et al*., 2021)
	GC-MS	Probiotic combine with pathogen challenge	*Haliotis iris*	Foot muscle	Transsulfuration and glutathione pathways	(Grandiosa, Young, Van Nguyen, Mérien, & Alfaro, 2020)
	^{1}H NMR	Mussel meal, zygomycete meal, extracted baker's yeast, non-extracted baker's yeast	*Salvelinus alpinus*	Liver, muscle	Several metabolites, general metabolism	(Wagner *et al*., 2019)
	GC-TOF-MS	Coconut oil and fish oil blends	*Penaeus vannamei*	Gill, hepatopancreas	Enhanced growth, survival, higher tyrosine, lysine, serine	(Chen *et al*., 2019)
	^{1}H NMR	Quality control, high, low biogenic amines in fishmeal	*Oncorhynchus mykiss*	Plasma	Branched-chain amino acid, energy metabolism	(Jasour *et al*., 2018)

Approach	Analytical platform	Concept/inclusion	Species	Tissue	Outcome/enhanced trait/molecular process	Reference
	GC-MS	Probiotics	*Haliotis iris*	Foot muscle	Enhanced growth, central carbon metabolism	(Grandiosa *et al*., 2018)
	^{1}H NMR	Sesamin	*Salmo Salar*	Liver, muscle	Energy metabolism	(Wagner, Trattner, Pickova, Gómez-Requeni, & Moazzami, 2014)
	^{1}H NMR	Feather meal	*Oncorhynchus mykiss*	Liver, plasma	Energy metabolism	(Jasour *et al*., 2017)

LC, liquid chromatography. MS, mass spectrometry. TMT, tandem mass tag. ESI, electrospray ionisation. 2DE, two-dimensional electrophoresis. DIGE, differential in gel electrophoresis. MALDI, Matrix-assisted laser desorption/ionization. TOF, time of flight. SRM, selected reaction monitoring. PAGE, polyacrylamide gel electrophoresis. GC, gas chromatography. ^{1}H NMR, proton nuclear magnetic resonance. For comprehensive reviews please read references: (Dettmer, Aronov, & Hammock, 2007; Lulijwa *et al*., 2021; Nguyen et al., 2022; Nissa, Pinto, Parkar, *et al*., 2021; Roques *et al*., 2020)

The replacement of wild-capture FM or FO with novel raw materials of equivalent nutrient composition has been extensively described elsewhere (Byelashov & Griffin, 2014; Turchini, Trushenski, & Glencross, 2019); however, the key molecular mechanisms controlling FM and FO stimulated growth have been poorly studied. This mini-review provides a good opportunity to investigate more deeply the mechanisms controlling energy utilisation and growth. It is important to identify which energy utilisation pathways are activated by FM and FO and how they compare with the pathways activated by novel raw ingredients. When animal growth following administration of novel material is similar to FM or FO, several unanswered questions remain: Are similar molecular pathways utilised for nutrient assimilation between ingredients? Could this lead to discovery of biomarkers that make aquaculture nutrition more efficient?

The study by Estruch *et al*. (2020) exemplifies the power of omics technologies to go beyond the provision of a list of "significant" molecules and actually try to understand the mechanisms that regulate the preferential pathways activated during energy generation as a result of including specific ingredients in aquafeeds. Understanding this would be advantageous to trigger key pathways or processes by selecting precise complementary raw materials that promote better growth and health in aquaculture species.

The potential of climate change to exceed temperature tolerance limits in fish is a global concern in aquaculture, particularly given most fish are ectotherms and unable to regulate body temperature (Neuheimer, Thresher, Lyle, & Semmens, 2011). Sustained thermal stress impairs optimal growth in fish as it affects feed intake making fish more susceptible to disease. As an example, the salmon industry in Tasmania, Australia has been affected by summer heatwaves where affected fish experienced reduced feed intake, impaired metabolism, and flesh decolouration (Wade *et al*., 2019). In this regard, discovery proteomics identified markers associated with thermal and oxidative stress in Atlantic salmon *Salmo salar* subjected to elevated temperatures (Nuez-Ortín *et al*., 2018). Following a comparison between a control group reared at 15°C and salmon treated at 21°C, 276 proteins showed significant differential abundance between the groups including ferritin and heat shock protein 70 kDa, both of which are involved in redox and heat stress regulation (Nuez-Ortín *et al*., 2018). Hypoxia, that often co-occurs alongside thermal stress, has also been studied by 2DE proteomics. Comparison of normoxic versus hypoxic groups identified six key proteins involved in the hypoxia-inducible factor-1 (HIF-1) signalling pathway in gills of grass carp *Ctenopharyngodon idella* (Xu *et al*., 2019). A comprehensive proteomics study using 2DE

coupled to fluorescence and mass spectrometry compared overcrowding, net handling, and hypoxia as stressors of gilthead seabream and identified perturbations in essential immune pathways. Additionally, the abundance of the protein warm-temperature acclimation-related 65 kDa protein (Wap65) significantly increased in net-handled and hypoxic fish (Raposo de Magalhães *et al*., 2020) suggesting Wap65 could be used as a potential biomarker for aquaculture fish welfare. Whether the stimulus comes from disease, environmental parameters, or other stress factors, identification of key proteins or metabolites that indicate an impact on the animal is paramount for the successful development of interventional strategies. Of similar importance is the development and validation of biomarkers that allow the precise identification of temperature stress thresholds. The implementation of dietary intervention strategies to mitigate stressors experienced by farmed animals can not only boost performance, survival, and product quality, but improve animal welfare in aquaculture.

Metabolomics

Proton nuclear magnetic resonance (^{1}H NMR) is the most common platform employed in metabolomics studies followed by MS-based metabolomics (Lulijwa *et al*., 2021) (Table 1). Similar to proteomics, metabolomics is divided into discovery and targeted modes as the two major approaches for identifying and quantifying metabolites. LC-MS-based discovery metabolomics has the advantage of identifying thousands of compounds; however, one frequent constraint in this approach is that some compounds lack annotation leading to several potential targets remaining unknown. Targeted metabolomics, similar to proteomics, requires the identity of target metabolites to be known. For example, targeting central carbon metabolism compounds can include the identity of ~200 metabolites and could help make inferences regarding biomass and energy generation and draw conclusions about underlying pathways (Gyawali *et al*., 2021).

In recent years, metabolomics has become an important tool for evaluating the effects of disease and diet additives on multiple aquaculture species (Alfaro *et al*., 2022; Grandiosa *et al.*, 2020; Jasour *et al*., 2018; Wagner *et al*., 2014). The omics cascade indicates that the metabolome response is most closely linked with what is occurring within the animal and is therefore predictive of phenotype (Dettmer *et al*., 2007). As such, it is reasonable to conclude that metabolomics is the ideal tool for determining the influence of diet on animal physiology and metabolism or the response of an animal following disease challenge.

Metabolomics - Nutrition and health

Metabolomics has been widely used to explore the effect of novel material in aquafeeds. For example, influence of sesamin in vegetable oil-based diets with varying levels of n-6/n-3 fatty acids was explored in muscle and liver of Atlantic salmon (Wagner *et al*., 2014) revealing that high levels of sesamin were not beneficial for salmon growth. Furthermore, the abundance of eight metabolites involved in energy metabolism significantly increased in liver indicating that high levels of sesamin were detrimental for liver function. Sesamin inclusion did not compromise muscle freshness as hypoxanthine, a metabolite used to determine post-mortem interval (Donaldson & Lamont, 2013), could not be detected (Wagner *et al*., 2014). The dietary effects of feather meal (FTH) inclusions on metabolism were evaluated in rainbow trout where 8% and 24 % FTH increased feed intake and specific growth rate (Jasour *et al*., 2017). However, it was also reported that lower levels of FTH might be more suitable for replacing FM as 24% FTH inclusion may compromise the TCA cycle as evidenced by higher concentrations of what appeared to be unutilised hepatic pyruvate, lower levels of ATP, and lactate levels in blood and liver that positively correlated with high FTH (Jasour et al., 2017).

^{1}H-NMR metabolomics assessed the suitability of mussel meal (MM), zygomycete meal (ZYG), extracted baker's yeast (EY), and non-extracted baker's yeast (NY) as FM replacements for Artic Charr (*Salvelinus alpinus*). Metabolites from the liver and muscle were affected following NY and EY feeding, respectively, compared with a reference diet. However, fish fed MM suffered less metabolic changes compared to animals fed other test feeds, leading to the proposal that MM was potentially the best ingredient to replace FM in Artic Charr (Wagner *et al*., 2019). Metabolomics was also used to determine the effects of different lipid sources (coconut oil, FO, and a mixture of both) on *P. vannamei* metabolism (when cultured at 3 or 30 ppt salinity) (Chen *et al*., 2019). Shrimp fed the oil mixture showed higher growth and survival while the gills and hepatopancreas of shrimp fed FO had higher levels of unsaturated fatty acids regardless of salinity. Shrimp reared at 3 ppt and fed coconut oil as a lipid source displayed higher levels of tyrosine, lysine, and serine compared with shrimp reared at 30 ppt (Chen *et al*., 2019).

Incorporation of dietary probiotics has become a common practice to enhance growth and immunity in several aquaculture species and metabolomics has been used to elucidate some of the molecular process underlying these enhanced performance traits. For example, *Haliotis iris* abalone fed a commercial diet supplemented with multi-strain probiotics achieved better growth

than abalone fed only the commercial diet (Grandiosa *et al*., 2018). Analysis of haemolymph revealed that after 16 weeks of treatment, total haemocyte count and cell viability were significantly higher in abalone fed probiotics. Metabolomics analysis of abalone foot muscle indicated unique expression of several metabolites in the abalone that were fed probiotics. These findings indicate that changes to central carbon metabolism promoted by probiotic supplemented diets could improve survival and growth of abalone (Grandiosa et al., 2018).

In a different study, foot muscle from *H. iris* was analysed by metabolomics after a four-month feeding regime with a commercial diet supplemented with probiotics and following infection with *Vibrio splendidus* (Grandiosa *et al*., 2020). Differential regulation in the transsulfuration and glutathione pathways were observed in infected animals, a result consistent with ROS disruptions observed in other molluscs under bacterial infection (Grandiosa et al., 2020). A recent metabolomics study elucidated some of the molecular mechanisms stimulated in *P. vannamei* as a synergic result of incorporating both prebiotic cellulose fibre and probiotic *Vibrio alginolyticus* in the diet (Alfaro *et al*., 2022). On the basis of metabolomic profiles, it was concluded that combining cellulose fibre with *V. alginolyticus* generated a better immune response compared with groups fed a control diet combined with cellulose fibre or probiotics alone (Alfaro *et al*., 2022).

Another recent metabolomics study focused on shrimp health identified the metabolic perturbations in *P. vannamei* challenged with acute hepatopancreatic necrosis disease (AHPND) (Nguyen *et al*., 2021). Several amino acids and components of the TCA cycle were differentially regulated in infected shrimp compared to shrimp not exposed to *V. parahaemolyticus* leading to pathway perturbations on TCA cycle, amino acid metabolism and gluconeogenesis (Nguyen *et al*., 2021). There are also examples where metabolomics has been used to determine the optimal inclusion levels of dietary components based on the molecular pathways they stimulate. For example, lipid synthesis and lipolysis were boosted by inclusion of 20% black soldier fly (BSF) meal after a 7-week feeding trial in *P. vannamei* (Chen *et al*., 2021). In contrast, dietary BSF meal inclusion at 30% was shown to be detrimental for beta-oxidation, glycolysis, and synthesis of unsaturated fatty acids (Chen *et al*., 2021).

The quality of raw materials and feed ingredients directly impacts the performance of aquafeeds, and metabolomics is a suitable tool to assess the quality of raw materials and the effect these ingredients can have on aquaculture species. Metabolomics was used to identify the metabolic effects of varying biogenic amine levels in rainbow trout. Better growth was achieved in fish fed a

FM diet where the FM used was low in biogenic amines compared with diets where the FM had a higher content of biogenic amines (Jasour *et al*., 2018). This positively correlated with higher abundances of branched-chain amino acids leucine and isoleucine, involved in ATP generation in the tricarboxylic cycle, in fish fed FM with a low content of biogenic amines. Conversely, decreased growth was observed when these amino acids were lower in the liver of fish fed the diet containing FM with a higher content of biogenic amines (Jasour *et al*., 2018).

Metabolomics can provide information about the components of a feed and can also be used to monitor these components after feeding. For example, Pterin, a biomarker for plant feedstuff, was identified by ^{1}H-NMR metabolomic analysis of both the plant-based diet and plasma of rainbow trout after feeding (Deborde *et al*., 2021). Furthermore, a higher amino acid content was measured in plasma of fish fed the plant-based diet compared with fish fed a FM and FO commercial diet, suggesting that amino acid delivery is delayed or underutilised in fish fed plant-based diets (Deborde *et al*., 2021).

Incorporation of omics technologies in aquaculture can elucidate the molecular basis of improved animal performance and beneficial traits in aquaculture enabling the manufacture of advanced diets that provide specific nutrients intended to complement metabolic pathways underlying these performance traits. As a result, omics technologies can help refine aquaculture processes intended to increase production yield while ensuring environmental sustainability and promoting animal welfare and social license.

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