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Nutrigenomics: Omics approach in aquaculture research to mitigate the deficits in conventional nutritional practices

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Abstract
Scientific intervention has played a pivotal role in growing aquaculture industry, but much more is required to achieve the set target to double the aquaculture production. Basic understanding of growth process is required for making any scientific strategy for enhancing the aquaculture production. Understanding the molecular mechanism of all these processes will reveal the inside picture of growth. With the recent developments in the field of genomic research in aquaculture, we are now faced with the unprecedented capability to use this technology and apply it in order to improve the feed utilization and to understand the fishes' metabolic response towards a particular diet, feed ingredient or additive in a more comprehensive way. Although in India, nutrigenomics research on important aquaculture species is relatively new, however, transcriptomics studies have highlighted the impacts of different dietary treatments on specific genes that can be targeted via nutritional intervention to increase the performance of the cultured animals. Nutrition is very relevant environmental factor that exerts its effect on the genetic background impairing or improving the likelihood to develop disease. Functional components (Immunostimulants, antioxidants, pre- & probiotics) are being considered in fish nutrition aiming to improve fish growth &/or feed efficiency, stress tolerance and disease resistance. By emergent perceptive of dietary manipulation effects on fish production and productivity, research can facilitate us to develop elite feeds with positive effects on economics and animal welfare, and develop "designer fish" that target specific market demands.

Keywords: nutrigenomics, aquaculture, fish nutrition, functional genomics, nutrient-gene interaction

Introduction
Indian aquaculture industry has witnessed a tremendous growth during last four decades with a 10-11 fold increase in fish production. Although scientific intervention has played a pivotal role to achieve this, but much more is required to achieve the set target to double the aquaculture production to the tune of 10 million metric tonnes by the year 2030 [1]. Hence, basic understanding of growth process is required for making any scientific strategy for enhancing the aquaculture production. Generally, increase in biomass production is considered as growth, which is generally calculated by difference in final and initial biomass of fish in a pond, where growth is assumed conversion of feed to flesh. In real sense, growth is the accumulation of muscle-mass only, which in other words is the accretion of protein. Transformation of dietary protein into tissue protein needs cascades of reaction inside the body. Understanding the molecular mechanism of all these processes will reveal the inside picture of growth.

It is estimated that about 31.5 million tonnes of farmed fish and crustaceans (46.1% of the total global aquaculture production in 2008) is dependent upon the supply of external nutrient inputs provided in the form fresh feed items, farm-made feeds or commercially manufactured feeds. Total industrial compound aquafeed production increased more than threefold, from 7.6 million tonnes in 1995 to 39.9 million tonnes in 2016, with production growing at an average rate of 12% per year [2]. Aquafeed production is expected to continue growing at similar rate to 71 million tonnes by 2020. In contrast to compound aquaculture feeds, there is no comprehensive information on the global production of farm-made aqufeed (estimated at between 18.7 and 30.7 million tonnes in 2006 and/or of low-value fish/trash as feed, with 2008 estimates for China at 6 to 8 million tonnes).

In 2016, of the 171 million tonnes of total fish production, about 88 percent or over 151 million tonnes were utilized for direct human consumption. This share has increased significantly in recent decades, as it was 67 percent in the 1960s [3]. In order to meet the growing demand of human population, enhancement of production, intensification of culture practices is needed. The intensification practices may cause stress to the species under culture. These stressors disrupt physiological functions as ionic regulation, gill, and kidney function, or by destroying the mucous coating of fish [4]. In addition, temperature variations and dissolved oxygen levels affect survival, growth, and reproductions. The stress in the fish leads to immune suppression which in turn causes disease outbreaks.

One of the main challenges with the intensification of the farming operations is economic losses primarily due to infectious diseases, particularly during the early production stages. Treatment methods including the use of approved antibiotics and chemotherapeutants are more often neither effective nor consumer/environment friendly. Preventive measures are deemed to be sustainable and Food and Agricultural Organization (FAO) has identified research areas viz: (I) Role of good nutrition in improving aquatic animal health, (II) Harnessing the host's specific and non-specific defense mechanisms in controlling aquatic animal health diseases, (III) Use of immunostimulants and non-specific immune enhancers to reduce susceptibility to diseases, (IV) Use of probiotics and bio-augmentation for the quality improvement of aquatic environment, and (V) To reduce the use of chemical and drugs in aquaculture.

It is now accepted widely that nutritional approaches are essential to alleviate diseases among farmed aquatic animals. The concept that better nutrition leads to improved health is very familiar in humans and is applicable to aquatic animals too. Efforts have been made over the past two decades especially in the case of farmed fish to understand the link between nutrition, immune response, and resistance to diseases. The last decade has witnessed a spurt in research in this area, aided through not only cross-disciplinary efforts and the availability of modern genomic tools, but also due to the greater understanding of preventive healthy and the central role of feeds in keeping fish healthy.

For aquaculture production, in particular, of higher tropic level finfish and crustaceans are largely dependent upon capture fisheries for the supply of their dietary source of protein and lipids. On a global basis, it is estimated that the aquaculture sector consumed 3.72 million tonnes of fishmeal (60.8% of global fishmeal production) and 0.78 million tonnes of fish oil (73.8% of global fish oil production) in 2008. Despite this continued dependence of aquaculture production on fishmeal and fish oil, there remains a wide variation in fishmeal and fish oil usage between major producing countries for individual farmed species. This variation mainly reflects differences between countries concerning the selection and use of fishmeal and fish oil replacers from plant sources or by the use of terrestrial animal proteins and fasts in feeds for high trophic-level fish species [5].

Understanding the biochemical and metabolic pathways involved in the utilization of dietary macro- or micro-nutrients and energy supplied through feeds is useful for evaluating the response of organisms to nutrients, for optimizing dietary nutrient utilization and for diet formulation. Molecular tools enable us to gain a deeper insight into how the response is mediated or obtained through the intermediate metabolic steps involved at different levels of the organism. Over the past two decades, aquaculture research has also taken advantage of the progress made in the application of molecular biological tools to understand the basics of metabolic regulation as affected by dietary factors.

Nutrigenomics is a discipline of functional genomics, which deals with the effects of the diet, and its constituent ingredients, on the genome through the metabolism. With the recent developments in the field of genomic research in aquaculture, we are now faced with the unprecedented capability to use this technology and apply it in order to improve the feed utilization and to understand the fishes' metabolic response towards a particular diet, feed ingredient or additive in a more comprehensive way. Although globally, nutrigenomics research on important aquaculture species is relatively new, however, transcriptomics studies have highlighted the impacts of different dietary treatments on specific genes that can be targeted via nutritional intervention to increase the performance of the cultured animals.

Nutrient-Gene interactions: the foundation of nutrigenomics research

Nutrigenomics is the understanding of how naturally occurring chemicals in foods alter molecular expression of genetic information in an individual. Nutritional research has recently emphasized the role of nutrients on gene expression and its regulation [6]. Novel genomic, proteomic and metabolomic techniques have facilitated the study of nutrients and other diet constituents, in order to define the important factors in nutrient-gene interaction at the cell and individual level. When a gene is activated, or expressed, a protein is produced which may have some biochemical or physiological function of the cell. Nutrigenomics also involves identification of the genes responsible for production of nutritionally important proteins such as digestive enzymes, transport molecules responsible for carrying nutrients and cofactors at their site of use. Therefore, nutrient-gene interaction not only include the effect of nutrients on genes, but also include the effect of the genetic makeup on the nutrient metabolism [7].

Nutritranscriptomics: Nutrients, sometimes after interacting with a receptor, behave as transcription factors that can bind to DNA and acutely induce gene expression. In the recent decade, various studies of direct interaction of the nutrients and the genes have been conducted in different cultured species. Nutrients can also influence gene expression through transcription factors [8, 9]. This group of transcription factors known as "nutrient sensors", most importantly constitute the nuclear receptor super family of transcription factors. These include nuclear receptors, such as peroxisome proliferators activator receptor (PPAR) (binding fatty acids) or liver X receptor (binding cholesterol metabolites), bind as heterodimers together with retinoid X receptor to specific nucleotide sequences (response elements) in the promoter regions of a large number of genes [8]. During ligand binding, nuclear receptors undergo a conformational change that results in transcriptional activation. The transcriptional activation is usually mediated through coordinated dissociation of co-repressors and recruitment of co-activator proteins. Therefore, these transcription factors act as nutrient sensors by changing the level of DNA transcription of specific genes in response to nutrient changes.

Epigenetic interactions: Nutrients can alter the structure of DNA or the histone proteins in chromatin so that gene expression is chronically altered. Epigenetic effects are mediated by methylation of DNA or by methylation, acetylation, or biotinylation of histones, or by both means [10]. Such epigenetic modifications can result in changes in gene expression that can last throughout the animal's life and can even persist across generations. DNA methylation usually occurs at cytosine bases that are followed by a guanosine (5′ CpG 3′ islands) [11] and it influences gene transcription and genomic stability. When this modification occurs in gene promoter regions, expression is altered. Increased methylation is usually associated with gene silencing or reduced gene expression, because methylated 5′ CpG 3′ islands attract capping proteins that hinder access for the transcription factors. Once 5′ CpG 3′islands in genes are methylated, the methylation is reproduced every time the gene is copied. Thus, the effects of methylation can persist. DNA is wrapped on proteins (histones) that, when packed tightly together, prevent access to the promoter sequences of genes. Methylation and acetylation and perhaps biotinylation of these histones can uncoil them, creating channels through which transcription factors can pass and activate gene promoters. The nutrients like folate, vitamin B_{12}, vitamin B_6, methionine, and choline act as methyl donors and are involved in one carbon metabolism. There are many studies which indicate that these nutrients are involved in epigenetic interaction with the genes to influence biological processes which are of culture significance like growth, breeding and immune response.

Genetic variations: Common genetic variations such as single-nucleotide polymorphisms (SNPs) can alter the expression or functionality of genes and hence the nutrient metabolism and requirement. SNPs result either in alteration of gene expression or in changes in the gene product such that protein structure and function are altered. These SNPs stay linked to one another and are inherited over many generations. A number of relatively common SNPs are known to influence nutrient requirements. For example, the enzyme 5, 10- methylenetetrahydrofolate reductase (MTHFR) is involved in folate metabolism. The *MTHFR* gene has a common SNP (*C677T* allele) that results in reduced enzymatic activity, and homozygous persons have elevated plasma homocysteine concentrations unless they ingest high amounts of folate [12].

Nutriproteomics: Nutriproteomics is the research area which exploits the dynamics of proteomic tools to characterize molecular and cellular changes in the protein structure and expression in response to dietary intervention. Proteins play a pivotal role in the structure and the function of a cell. The proteome is the extensive and highly dynamic network of proteins expressed in the cell. The proteome varies with the type of the cell but the genome remains the same. The huge number of proteins obtained from the genome is due to:

- Splicing of mRNA
- Protein processing
- Post translational modifications

Dietary nutrients and non-nutrient dietary components act as signals which are received by transcription factors or nutrient sensors. The nutrient sensors change the expression of a gene. However, there is also another way how a dietary component effect the proteome, is throughout the interference at the post-translational modification.

There are two types of alterations in the proteome by nutritional intervention- the quantitative or functional change. The tools for detecting these changes are also developed differently. However, there are common preliminary steps of protein isolation and purification. The tools used in quantitative proteomics are Mass spectroscopy, digestion of peptides and isotope labeling. The tools used in functional proteomics are 2D PAGE, mass spectroscopy and antibody based techniques. In functional proteomics, the protein structure and function have to be detected. In this field, the proteins are immobilized on antibodies and available after separation by 2D PAGE. It enables the efficient characterization of microheterogenety of proteins. Real time protein kinetics and protein sequencing can also shed light of functional proteomics.

Nutrimetabolomics: The systematic analysis of the unique chemical fingerprints that are produced from specific cellular process as a function of nutritional status of animal or in response to the nutritional intervention.

The term metabolome was introduced in 1998 by Oliver and defined as a set of low molecular mass compounds present in the cell. The metabolome of the cell or the organism is influenced by genetics, environmental variation and also diet. Nutrimetabolomics analysis is normally conducted on body fluid such as blood, urine or feces but can also be conducted on other tissues. Nutrimetabolomic studies employ the following methodologies:

- Metabolite fingerprinting: identifies the overall nature of the samples. It is not merely restricted to the identifying of metabolites but it also involves the physiochemical characteristic of the samples. The output of the sensors in response to the sample is known as the fingerprinting.
- Metabolite profiling: involves identification of the metabolites as the analysis is based spectrophotometric and chromate graphic methods.
- Metabolome analysis: When time dependent resolution based analysis of metabolite compounds is done, then it is called Metabolome analysis. It involves the study of the entire gamut of metabolites in a sample by synergistic application of various analytical tools.
- Systems Biology: it carries out the partial or full integration of the transcriptomics, proteomic and metabolomics information.

Tools used in nutrimetabolomic studies can be grouped into two types

- ✓ Tools used for separation of metabolites: It includes thin layer chromatography, gas chromatography, and capillary electrophoresis.
- ✓ Tools used for detection of metabolites: It includes mass spectroscopy and nuclear magnetic resonance

Tools in nutrigenomics

In aquaculture, most of the nutrigenomics studies at present involves the transcriptomic approach in which the transcription products i.e., mRNA is investigated which provide information on the effect of nutrients on the gene. The transcriptomic studies are based on Reverse Transcription PCR process. The total mRNA is extracted and is reverse transcribed to the cDNA. This cDNA can be the used for a number of PCR types. Semi-quantitative PCR and Real time

PCR are the two most commonly used techniques for measuring the differential transcriptomic abundance in the different treatments following a nutritional interference.

The DNA microarray is an upcoming technique in fish nutrigenomics, which gives the overall genomic (rather than a single gene) response of the organism to the nutrient or diet. However, its use in fish nutrition is limited because it requires the genomic sequences.

Candidate gene approach involves selecting the possible risk gene based upon the knowledge of biological function. Although, this approach has limited applications, but it can be applied in fish nutrition studies. This is because the candidate gene approach involves the *in vitro* cell culture techniques to establish the positive and negative correlation between the expression of the gene and the effect it has on the nutrient metabolism.

Genome wide association study (GWAS) involves the comparison of two groups, which show the varied responses to a nutrient. GWAS typically focuses at the association between the SNPs and the disease occurrence. These studies identify the SNP and the related variability in the DNA, which are associated with a response but cannot, on their own, specify which genes are causal. This technique was developed in 2005 and has not been used in fish nutrition till date.

In aquaculture, molecular techniques, in addition to the more traditional methods of biotechnology, were introduced only recently, although fish farming has been practiced for 4,000 years, and aquaculture research dates back to about 1870. Contemporary genomic approaches, which are often adapted from human or biomedical research, such as cDNA cloning and sequencing, cDNA microarray/expression analysis, and functional genomics, has opened up new possibilities for aquaculture biotechnologists to improve fish growth rates, increasing resistance to pathogens and stressors, improving quality of the brood stocks, and creating the opportunity to make new or different products by altering their genetic makeup [13, 14, 15].

Reverse transcriptase polymerase chain reaction (RT-PCR) is used to qualitatively detect the amplification of DNA using fluorescent probes. RT-PCR is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement with reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR. In addition to the qualitative study of gene expression, quantitative PCR can be utilized for quantification of RNA, in both relative and absolute terms. Compared to other RNA quantification methods, such as northern blot, qRT-PCR is considered to be the most powerful, sensitive, and quantitative assay for the detection of RNA levels. It is frequently used in the expression analysis of single or multiple genes,

Molecular cloning is a set of methods, which are used to insert recombinant DNA into a vector- a carrier of DNA molecules that will replicate recombinant DNA fragments in host organisms. The DNA fragment, which may be a gene, can be isolated from a fish specimen. Following isolation of the fragment of interest, or insert, both the vector and insert must be cut with restriction enzymes and purified. The purified pieces are joined together though a technique called ligation. The enzyme that catalyzes the ligation reaction is known as ligase.

The basic cloning workflow includes four steps:

1 Isolation of target DNA fragments (often referred to as inserts)
2 Ligation of inserts into an appropriate cloning vector, creating recombinant molecules (e.g., plasmids)
3 Transformation of recombinant plasmids into bacteria or other suitable host for propagation
4 Screening/selection of hosts containing the intended recombinant plasmid qPCR is used to measure the quantity of a target sequence in real-time (immediately, during the PCR) so the technique is also called qRT-PCR. The technique quantitatively measures starting amounts (number of copies) of DNA, cDNA, or RNA in samples.

Real time monitoring of PCR amplification is made possible by adding fluorescent labels to the reaction tube before PCR. DNA replication during PCR triggers fluorescence of the labels so that the kinetics of the amplification can be correlated with the initial copy number. qRT-PCR methods use fluorescent dyes (such as SYBR Green, EvaGreen) or fluorophore-containing DNA probes (such as TaqMan probes) to measure the amount of amplified product in real time. These dyes bind preferentially to double-stranded DNA. In solution, the unbound dye exhibits very little fluorescence but fluorescence is greatly enhanced by DNA-binding. During PCR, the DNA polymerase amplifies the target sequence, creating dsPCR products or "amplicons." The fluorescent signal is proportionate to the number of amplicons produced. In the first cycles, the number of amplicons is not sufficient to generate a detectable fluorescence signal so here you just observe the baseline signal. After a number of cycles, the signal becomes detectable and then each additional PCR cycle doubles the number of amplicons, resulting in an exponential (base 2) increase in total fluorescence intensity in each PCR cycle. Of course, the reaction cannot go on forever, one of the reagents will run out so the curve tails off and reaches a plateau.

How aquaculture can benefit from Nutrigenomics Research

Nutrigenomics research may be used in aquaculture in the following ways:

1. **For evaluating the response of the organisms to the nutrients.**

Certain nutrients elicit particular response in some fish while not in other. For example, a high dietary carbohydrate in mammal usually reduces the gluconeogenic activity but the same is not found in the fish. Nutrigenomic studies help to decipher these variable responses between individuals.

2. **Diet development by optimizing the dietary nutrient utilization by a particular species**

For example, in herbivorous fish the dietary carbohydrates are able to up-regulate both activity as well as expression of carbohydrate digesting enzymes, but this up-regulation is too less when present in the carnivorous fishes [16]. The nutrigenomic studies give us an insight in to utilization potential of the particular dietary nutrient in a fish species.

3. **Facilitate thorough understanding about a nutrient response in cell**

For example, when carbohydrates are fed to carnivorous fishes, whether it is the insulin activity uncontrolled gluconeogenesis that is responsible for the inefficient utilization of the carbohydrates.

4. Complementing basic husbandry practices

Ghrelin and Leptin have been shown to regulate the feeding intake of fish and are affected by several environmental factors [17]. Synchronization of the information generated from nutrigenomic studies helps to carry out better husbandry practice.

5. Identify the factors responsible for metabolism

For example in fish, the adipose tissue lipoprotein lipase gene expression is increased in the muscle, which induces fat catabolism and energy released for growth [18]. During this period, the lipid and the protein content of the feed may be increased at the cost of carbohydrates. In summer season, the muscle lipoprotein lipase content increases which increases carcass fat [19, 20, 21].

6. Facilitate to know the organs and tissue specificity for different nutrient utilization

Glucokinase, the hexokinase found in the liver is quite different from the other three hexokinase enzymes identified in the fish [22, 23]. It has been confirmed by nutrigenomic studies that glucokinase expression is not inhibited by glucose-6-phosphate but the same metabolite is able to restrict the hexokinase expression of other tissues [24]. Insulin reduces the muscle LPL and reduces the fat deposition in the tissue. The same hormone increases the adipose lipoprotein lipase content and increases the lipogenesis [25].

7. Specific metabolic changes as a function of body physiology

In the smolt stage, the 60% of beta-oxidation in salmon occurs in the red muscle whereas in the adult stage it is only 10% [26]. Nutrigenomic studies help to decipher the actual reason behind this change.

8. Understanding the basic metabolic regulation by dietary factors

A simple example of the metabolic regulation by dietary factors is the retro-inhibition of delta-6 desaturase by the DHA [27]. Nutrigenomics studies play pivotal role in the understanding of metabolic regulation.

9. Nutrigenomic studies in Nutrient Transport

The expression of different transporters in response to different forms of the same nutrients and vice versa helps to enhance the nutrient utilization. For example, glucokinase is not responsive to dietary glucose levels whereas other hexokinases are [28]. In addition, Na-PO_4 co-transporter mRNA expression increases when the dietary phosphate is limiting and up-regulated when dietary phosphate is increased [29]. This may be studied using nutrigenomic approach in order to confirm which form of phosphate is better and up-regulates the transporter activity.

Conclusion and future prospects

Aquaculture is a complex system where soil and water greatly influence the growth and health of aquatic organism. However, feed appears to be the major factor for the growth of fish but growth rate of fish is mainly regulated by the available nutrients from the feed and water bodies. Research on aquatic animal nutrition has demonstrated that the expression of related genes can be modified with different feeding components. From the nutrigenomics point of view, nutrients are feeding signals, which are detected by the cellular system of sensors and which influence the expression of genes and proteins and in consequence, the production of metabolites. Therefore, today, it is generally accepted that feeding components have a substantial impact in the expression of related genes, as well as in the welfare of reared aquatic animals. Revolution in research output in biological science especially the use of molecular techniques removed the subject specific boundary demarcation. Molecular techniques are now being increasingly used in other subjects like biochemistry, genetics, microbiology, pathology, physiology, nutrition, etc. Molecular biology opens the door to get a clear understanding of the actual mechanism of function inside a cell. Evidence linking nutrition and gene expression in fish is very less compared to animal or human nutrition. Extensive studies are required to understand the nutrient regulation of gene expression in commercially important fish species to optimize the nutrient requirement for better growth and reproduction. By increasing understanding of dietary manipulation effects on fish production, scientists can develop elite feeds with positive effects on production economics and animal welfare, and develop "designer fish" that target specific market demands.

> "First, it was smart drugs, now it is smart food; eat right for your genotype and feed right for their genotype"

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