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Nutritional Biotechnology in Aquatic Nutrition

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Besides the application of classical nutrition principles and practices in aquatic nutrition, nutritional biotechnology in general is also gaining momentum. The areas where a beginning has been made are, functional foods or feeds, application of phytases, bio-floc technology and nutrigenomics.

Functional food or medicinal food is any healthy food claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients. The general category of functional foods includes processed food or foods fortified with health-promoting additives, like "vitamin-enriched" products. Fermented foods with live cultures are considered as functional foods with probiotic benefits. Functional foods are an emerging field in food science due to their increasing popularity with health-conscious consumers. The term was first used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU) (Wikipedia).

The term 'functional foods' covers a broad range of products including, for example, DHA- and selenium-enriched eggs, selenium-enriched pork, stanol- and sterol-enriched margarine, etc. Also included under this category are dietary 'probiotics' and 'prebiotics'.

A probiotic is defined, in the strict sense, as "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" (Roberfroid, 2000). It should be noted that the term has also been widely and incorrectly applied in aquaculture, and especially shrimp culture, to include the use of live microbes to beneficially alter the microbial balance in the culture system itself (Fegan, 2010).

Prebiotics, on the other hand, have been described as "non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria" (Gibson and Roberfroid, 1995).

A 'functional nutrient' can be further defined as a dietary ingredient that exerts possible positive effects on health in addition to its direct role as a nutrient.

Having defined these, let us have a look at the application of some of them in aquaculture. Some functional foods developed for application in aquaculture are meant to replace fishmeal. Among them are products derived from single cell proteins (SCPs) like yeast. The search for alternatives to fish meal as a source of protein in aquaculture diets has been an important area of research in recent years. Much of this research has focused on increasing the proportion of plant

proteins, such as soybean, in feeds for fish and shrimp. However, many sources of vegetable protein have disadvantages, including low nutrient densities, anti-nutritional factors, high carbohydrate content, imbalanced amino acid and fatty acid profiles, low palatability, seasonal variability and potential mycotoxin contamination (Ceulemans et al., 2003; Spring and Fegan, 2005). In this scenario SCP from yeast when tested showed that their cell walls are indigestible, low protein content, and poor amino acid profile. Nevertheless, many aquaculture feeds contain some SCP, usually yeast, at levels from 1-5% of the diet. Products have been developed after complete removal of the wall enriching the protein content which is called yeast extract. This has been used to replace fish meal completely for the feeds. As a protein source yeast extract (with the brand name NuPro from Alltech) has been investigated for a number of fish and shrimp species including tilapia, cobia, black tiger prawn and Pacific white shrimp. In a series of trials to develop organically certifiable feeds for fish and shrimp, it has been shown that complete replacement of fish and soybean meal with NuPro is possible, although cobia, a marine carnivore, showed a reduced growth rate at levels of 50% replacement and higher (Craig and McLean, 2005)

Nucleotides

In the form of nucleic acids, nucleotides are of fundamental importance as the basis of the genetic code. Genetic information is stored in DNA (except in the case of RNA viruses) providing the basic information coding for all the proteins produced in the body whereas RNA acts as a chemical messenger relaying the information stored in DNA from the nucleus to other parts of the cell. Other than their role in genetics and protein production, nucleotides also play major roles in almost all biological processes including:

- Storage of energy, mainly through adenosine tri-phosphate (ATP).
- As components of several important coenzymes such as nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), flavin adenine dinucleotide (FAD) and coenzyme A, all of which are involved in carbohydrate, protein and fat metabolism (Mateo, 2005).
- Mediation of important cellular processes through messengers such as cyclicadenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP).
- Control of several enzymatic reactions.
- Serving as intermediates in biosynthetic reactions, especially in glycogen and glycoprotein synthesis and synthesis of polyunsaturated fatty acids (Gill *et al.,* 1985).

Nucleotides have been recognized as important elements in mammalian nutrition especially during periods of rapid growth or physiological stress as well as appearing to play a key role in efficient immune system function (Uauy, 1989; Barness, 1994; Van Buren, 1994). They can be synthesized directly or scavenged by salvage pathways in the body although it appears that exogenous dietary sources are preferentially used (Uauy, 1994). However, immune cells and intestinal cells cannot synthesize nucleotides and depend on nucleotides from other sources (Quan, 1992). Synthesis and salvage of nucleotides are thought to be energy intensive in metabolic terms and dietary nucleotides may reduce the metabolic cost of *de novo* nucleotide synthesis. Dietary sources of nucleotides may also benefit rapidly dividing tissues, such as those of the immune system, especially

under a challenge, and the term 'conditionally essential' has been used to describe their role in nutrition (Carver and Walker, 1995). In many biochemical processes, primary nucleotides such as 5'AMP, 5'CMP, 5'GMP, 5'IMP and 5'UMP are used to produce a number of intermediate metabolites through a series of enzymatic reactions. Supplementation of primary nucleotides in the diet provides a ready source of nucleotides for use in the synthesis of intermediate nucleotides when required.

Sources of nucleotides : Any ingredients of animal and plant origin containing cellular material are potential sources of nucleotides, usually in the form of nucleoproteins. The nucleotide content is particularly high in ingredients such as fish solubles, animal protein solubles, fish meal, legumes (adenine content is particularly high in black-eyed peas), yeast extracts and unicellular organisms such as yeasts and bacteria that are rich in RNA or DNA. The content, proportion and availability of nucleotides differs among ingredients. Muscle protein is a poor source of nucleotides as they are mainly in the form of actin-myosin protein. Oilseeds, such as soybeans, grains, fruits, vegetables and processed milk products are also poor sources of nucleotides (Barness, 1994; Devresse, 2000; Mateo, 2005). Among marine protein sources, anchovies and sardines, for example, have much higher guanine levels than squid, clams or mackerel. Availability and digestibility are also important issues. Whole yeast is much less digestible than yeast extract, possibly due to the need to digest the yeast cell wall and yeast extract having much higher levels of soluble protein. Fish and animal protein solubles are highly digestible but they leach easily, affecting overall availability (Devresse, 2000).

Phytases

Phytase is an enzyme that can break down the undigestible phytic acid (phytate) part found in grains and oil seeds and thus release digestible phosphorus, calcium and other nutrients.

Basically, phytase is a phosphate enzyme that hydrolyzes the ester phosphoric acid and inositol existing in the plants resources. The enzyme phytase is normally produced (endogenous phytase) in ruminants. Non-ruminants (monogastric animals) like human beings, dogs, birds, etc. do not produce this enzyme. Research in the field of animal nutrition has put forth the idea of supplementing phytase enzyme, exogenously, so as to make available bound nutrients like calcium, phosphorus, other minerals, carbohydrates and proteins.

Phytase releases the artophosphate as well as proteins in the intestine to be absorbed by the body, thus largely mitigates the need of phosphate like mono and di-calcium phosphate in the feeds and improve animal growth, reduce the phosphate expulsion to make the environment less polluted from excessive amounts of phosphate and finally decrease the cost for poultry feeds and nutrition.

Phytase is used as an animal feed supplement - often in poultry and swine - to enhance the nutritive value of plant material by liberation of inorganic phosphate from phytic acid (myo-inositol hexakisphosphate) and, thereby, to reduce environmental phosphorus pollution.

Phytase can be purified from transgenic microbes. Phytase has been produced recently in transgenic canola, alfalfa and rice plants. Phytase can also be massively produced through cellulosic biomass fermentation using genetically modified (GM) yeast. Phytase can also be isolated from basidiomycetes fungi. A strain of transgenic pig can produce phytase, thus reducing their environmental impact.

Fish utilize phytate P poorly because they lack phytase, the enzyme that hydrolyses phytic acid (Riche and Brown, 1996). But there might be differences between fish species, because in tilapia an intestinal phytase of physiological significance was shown. However, this failed in hybrid striped bass and in koi carp (Ellestad et al., 2002). Phytases (myo-inositol hexakisphophate 3-phosphorylase, EC 3.1.3.8 and myo-inositol hexakisphosphate 6-phosphorylase, EC 3.1.3.26) are acid phosphatase enzymes of the histidine acid phosphatase family, which liberate inorganic phosphate from phytate (Mitchell et al., 1997). Increased bioavailability of phytic P with the use of phytase has been mainly reported in carnivorous fish species such as channel catfish (Jackson et al., 1996), Atlantic salmon (Storebakken et al., 1998), striped bass (Hughes and Soares, 1998), Japanese flounder (Masumoto et al., 2001) as well as in the omnivore Nile tilapia species (Liebert and Portz, 2005). Common carp is an agastric species with a peculiar gastro intestinal tract and has an intestinal pH above 6.0 (Nwanna and Schwarz, 2006). But phytase activity is highly dependent upon the gut pH (Baruah et al., 2004). Furthermore, plant feedstuffs like cereals, legumes or by-products such as soybean meal, which have P mainly as phytate, play an important role in feeding carp. In these feedstuffs phytate will also affect the availability of some other minerals like Ca and Mg or Zn and Cu (Masumoto et al., 2001).

Table 1. Phytate content of cereals and roots (Ravindran et al. 1995)

	Phytate P[g/100 g dry matter]	**Phytate P[% of total P]**
Cereals		
Corn	0.24	72
Wheat	0.27	69
Barley	0.27	64
Oats	0.29	67
Sorghum	0.24	66
Rice, unpolished	0.27	77
Roots and tubers		
Cassava	0.04	28
Sweet potato	0.05	24

The first commercial phytase products derived from *Aspergillus niger* with the capacity to release phytate-bound P and reduce P excretion, was introduced into market in 1991. After mid-1990s, more and more studies about the effects of supplemental phytase on nutrient utilization or growth of fish have been started in common aquaculture species such as rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio* L.), channel catfish (*Ictalurus punctatus*), salmon (*Salmo salar*), striped bass (*Morone saxatilis*), Nile tilapia (*Oreochromis niloticus*). Phytase has been utilized by spraying onto pellets, pre-treating or dephytinizing feedstuffs before pelleted. Various parameters to evaluate phytase effects have been used including nutrients digestibility, nutrients retention and fish growth performance. Currently, research focus is mainly on phytase effects on digestive systems in different fish growth phases, the dose–response study, specific kinds of phytase for distinct fish species and the most efficient ways of supplement. Besides, the addition of organic acid along with phytase, especially in agastric fishes, is of special interest, and gains serious attention. It is well

documented that the use of microbial phytase in fish feed can enhance the bio-availability of phytate-bound P and nitrogen and thus less P discharged into the aquatic environment. Therefore, phytase is increasingly considered as an additive for cost-effective and environmentally friendly fish feeds. Table 2 summarizes commercially available products with costs fluctuating from ‘ 150-500 per kg

Table 2. Commercial production information of microbial phytases (Stefan et al. 2005)

Company	Country	Phytase source	Production strain	Trademark
AB Enzymes	Germany	*Aspergillus awamori*	*Trichoderma reesei*	Finase
Alko Biotechnology	Finland	*A. oryzae*	*A. oryzae*	SP, TP, SF
Alltech	USA	*A. niger*	*A. niger*	Allzyme phytase
BASF	Germany	*A. niger*	*A. niger*	Natuphos
BioZyme	USA	*A. oryzae*	*A. oryzae*	AMAFERM
DSM	USA	*P. lycii*	*A. oryzae*	Bio-Feed
Fermic	Mexico	*A. oryzae*	*A. oryzae*	Phyzyme
Finnfeeds International	Finland	*A. awamori*	*T. reesei*	Avizyme
Genencor International	USA	*P. simplicissimum*	*Penicillium funiculosum*	ROVABIO
Roal	Finland	*Aspergillus awamori*	*T. reesei*	Finase
Novozymes	Denmark	*A. oryzae*	*A. oryzae*	Ronozyme, Roxazyme

Effects of phytase application in fish feeds can be summarized as (1) increase in bioavailability of phytate-P. Since supplementation of phytase can improve the apparent digestibility of P in soybean meal or canola meal-based diets, it is possible to improve the P retention of diets and reduce the P discharge into water that was considered as one of the main pollution elements in water environment. (2) Generally, growth improvements were observed in the studies that used diets entirely or almost entirely based on plant protein sources. Many studies reported that the addition of phytase to P inadequate diets has been shown to enhance growth performance. (3) In pigs, phytase was reported to improve protein and amino acid utilization through breakdown of phytin–protein complexes. In fish, however, the results are somewhat controversial. Variations in the outcome of different authors may be attributed to variation in phytic acid content in different feedstuffs, species used and various other inherent characteristics of feed ingredients, or probably due to the presence or absence of the stomach in different fish species, as phytase activity is pH specific. (4) Phytate also can chelate with other minerals to decrease their bioavailability to fish. Phytase supplementation can hydrolyze phytate and increase the concentration of minerals like magnesium, calcium, manganese, and zinc in plasma, bone and the whole body . (5) In dose–response studies, phytase addition of 250–1500 U/kg is usually considered feasible in many fish species. The optimum dose changes along with many factors such as fish species, different phytase sources, diet formulation (amount of substrate for phytase) and selected response parameters. Conclusive studies dealing with the mechanism of phytate degradation of different fish species depending on different diet formulation, specific characteristics of digestive tract and varying activity from different supplemental phytase sources are needed.

Biofloc technology (BFT)

AMR, ZEAH, bacterial floc, heterotrophs, autotrophs – these terms maybe new to the ears of fish and shrimp farmers but not for scientists. AMR stands for Aerated Microbial Reuse while ZEAH stands for Zero-Exchange, Aerobic, Heterotrophic. Both refer to the same thing: a system of intensive aquaculture that has been around for at least ten years and is becoming more popular– starting in the Western hemisphere but now spreading in Southeast Asia. The basic technology was developed at the Waddell Mariculture Center in the USA in the early 1990s. AMR or ZEAH, whichever term you prefer, has been found to reduce feeding cost, makes possible operation of a farm with very little or even zero water exchange, while still producing 10 to 30 tons of shrimps per hectare and from 10 to 100 kg of tilapia per square meter per crop.

Autotrophic vs Heterotrophic

AMR is basically a "heterotrophic" system as against the conventional culture system that fish and shrimp farmers in Asia which is considered "autotrophic." To understand the difference between the two systems, one has to go into the respective roots of the two terms. Heterotrophic comes from "heterotroph" – an organism which rely on carbon in organic form (i.e. other organisms) for food. Animals, fungi, parasitic plants and most bacteria are heterotrophs. In contrast autotrophic comes from the term "autotroph" an organism capable of sustaining itself due to its ability to produce their own food (or organic carbon) from inorganic materials which are basically water, carbon dioxide and nitrogen. The food is synthesized using energy from light or photosynthesis or inorganic chemical reaction or chemosynthesis. Autotrophs include all (except parasitic) plants and some bacteria.

A pond where in where food is produced by autotrophs, mainly plant organisms, whether microscopic and in the water column such as phytoplankton or resting on the bottom such as benthic algae is considered an autotrophic system. In contrast a pond where food has to be introduced is considered a heterotrophic system. It of course does not mean the system is completely free from any phytoplankton. In fact the presence of phytoplankton is believed by some to be essential as oxygen source and reduce aeration need in the daytime.

Extensive vs Intensive

In conventional pond culture farmers are familiar with great pains are taken to prepare the pond so that plankton, particularly diatoms flourish before stocking the shrimps or fish. Presumably the plant plankton becomes food to tiny animal plankton and the two types of plankton together becomes natural food for the newly stocked shrimp or fish fry. This is thought to be the ideal condition for the shrimps or fish because it simulates their natural habitat. Because food is produced within the pond itself the system is considered "autotrophic".

When the stocking density is low, the food that is generated within the pond is sufficient to support the shrimp or fish stock and no feeding is required. This is what is known as extensive aquaculture. Such a system is capable of producing at most a few hundred kilograms per hectare. In an effort to increase production it is inevitable for farmers to try stocking more – to the extent that the natural food that is produced becomes insufficient to support the stock. In such case feed has to be introduced in order to supplement the nutrition coming from naturally occurring food in the pond. Such system which relies on a combination of naturally occurring food and introduced food or feeds is often referred to as "semi-intensive" aquaculture.

At high stocking density, and as the animals grow, more feed is required to the extent that the role of natural food becomes insignificant and the culture becomes "intensive" in nature. Since no more than 30% of the carbon, nitrogen and phosphorus in feeds is assimilated or converted into flesh by the fish or shrimps, more of it serves only to pollute the water in the form of uneaten feeds and excretory wastes which is high in ammonia – a substance that stresses fish and shrimps, reduce growth rate and at high levels even cause mass mortality. At low stocking density, this poses no problem since they can still be fully utilized by phytoplankton and bacteria. At high densities however, the amount of such wastes overwhelms the system and if left unchecked accumulates in the system. The conventional approach is to change the water in order to reduce the level. Considerable skill and experience is required to maintain the phytoplankton population at the right level but which due to weather variation may collapse and create havoc. Furthermore, the water discharged is high in organic load – one aspect of intensive aquaculture that is at the forefront of environmentalist's list of negative effects of aquaculture to the environment.

Shifting from Phytoplankton to Heterotrophic Bacteria

As now practiced in Belize by the Belize Aquaculture Ltd (BAL) and many other farms the world over, applying AMR technology requires deliberately converting the pond ecosystem from one that is autotrophic or phytoplankton-based to a heterotrophic system dominated by bacteria after the fry has been stocked and has established itself. This requires providing a low-protein, high carbohydrate diet with nitrogen to carbon or C:N ratio of 16:1 (18 protein) so that the carbon-hungry bacterial population has adequate food to multiply even as the shrimps are also being fed with regular starter feed The culture is deliberately overfed at 200 to 250 of the shrimp biomass. The addition of wheat flour or even molasses, both of which are carbon rich, has been found also to hasten the growth of heterotrophic bacteria. Details of this technology can be had from Schryver et al. 2008. The only institution in India working on this technology is the Department of Industrial Fisheries, Cochin University of Science and Technology, Lakeside Capmus, Fine Arts Avenue, Ernakulam, Cochin. Contact madhukurup@hotmail.com

Nutrigenomics

Term	Defenition
Nutrigenomics	The study of genome wide effects of diet or components therof on the transcriptome, proteome and mebolome of cells, tissues or organisms at a specific moment in time
Genome	The entire complement of genetic material of an organism
Transcriptomics	The monitoring of the complete set of RNA transcripts produced by the genome at any given time
Proteomics	The examination of proteomes – the complete set of proteins in a cell or tissue – at a specific moment in time. Proteomics attempts to determine the role of specific proteins and how they interact with the molecules
Metabolomics	The identification and quantification of large sets of metabolites from cells or biological fluids and how these may change following physiological disturbance
Epigenomics	The detection and examination of DNA methylation patterns both spatially and temporally

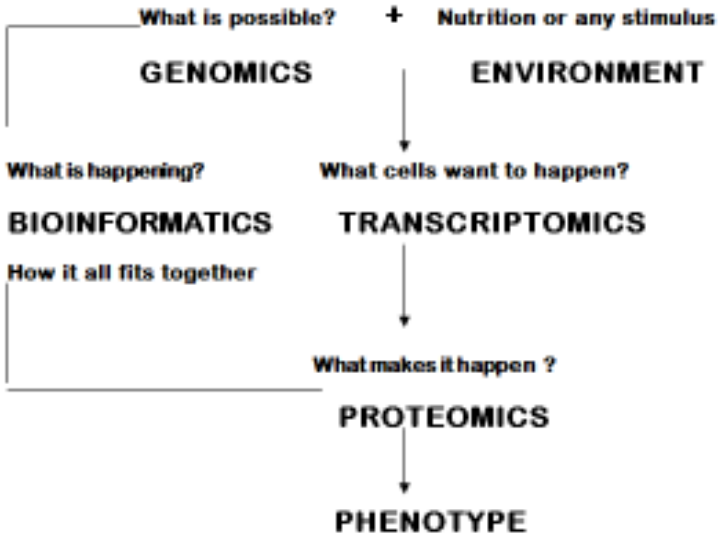
Nutrigenomics - possibilities in aquatic nutrition

Functional genomics refers to how the genome (in biology the **genome** of an organism is the whole hereditary information of an organism that is encoded in the DNA or, for some viruses, RNA. This includes both the genes and the non-coding sequences. The term was coined in 1920 by Hans Winkler, Professor of Botany at the University of Hamburg, Germany, as a portmanteau of the words **gene** and chromosome) of an organism regulates homeostasis (the property of and open system especially living organisms to regulate their internal environment to maintain a stable constant condition by means of multiple dynamic equilibrium adjustments controlled by interrelated regulation mechanisms) and responds to stimuli. In here we shall examine the application of functional genomics in nutrition research now known as ***nutrigenomics*** a term coined in 2002.

Influence of feed (nutrients) on the organism at a molecular level is the simplest explanation of nutrigenomics. Traditionally the response of a feed or nutrient is measured in terms of a standard set of parameters with strengths and weaknesses.

A simple example is measurement of growth as the change in body weight. It can be growth of muscle or growth of bones (in vertebrates). The role the genes have in these processes even though understood and appreciated gets sidelined and remains unaddressed. Thus research in food producing animals got limited to arriving at nutrient requirements, diet formulation, and the monitoring of performance. This approach has often involved the assessment of targeted metabolic pathways such as carbohydrate or lipid metabolism where biochemical and enzyme assays could also be used to monitor the effects of diet. With the development of cutting edge tools in molecular biology monitoring the affect of a nutrient at the molecular level is reality now. In reality, the scope of this field is significantly larger. The interaction of an organism with its diet (or nutrition source) is an intimate and complex physiologic affair that is typically based on multiple organ systems and, in higher eukaryotes, the endogenous microflora working in concert. The regulatory control mechanisms of these processes can be based at all levels from genetics and gene expression to the feedback of specific metabolites. Modern technology is providing a new opportunity to monitor the regulation of these processes on a systems-wide basis. Nutrition researchers are just beginning to utilize these tools to ask key scientific questions about diet and its effects on the organism using functional genomics. It has been known for some time that diet and specific nutrients can affect the function (expression) of genes. It has been well documented by many clinical studies that two humans fed an identical diet respond individually. The mechanism by which nutrients specifically regulate the expression of genes in vertebrates in general is poorly understood. The fundamental understanding of regulation gene expression in response to nutritional changes came from bacteria. However due to the complexity of the mechanisms in eukaryotic cells research in this area progressed slowly. One of the earliest reports of a micronutrient influencing gene expression in eukaryotes comes from zinc deficiency influencing gene expression of the hormones cholecystokinin, uroguanylin and the enzyme ubiquinone oxidoreductase. Nutrigenomics research aims at development of consensus responses to dietary stimuli so that anomalies can be studied further. This knowledge will provide information on how genes and nutrients interact and the effect of individual genetic differences on diet and nutrition. This research will be directly applicable to other species whose genome sequencing projects are underway including several agricultural and animal species including chicken, cattle, swine and fish. Developing within this genome era were technologies that were increasingly broad

in scope and were automated, high throughput, and data intensive. Many of these technologies also involved miniaturization of standard techniques to suit the new high throughput experimental designs. These technologies have significant implications for nutrition research and include aspects of genomics (polymorphism), functional genomics (gene expression), proteomics (protein expression), and bioinformatics (data storage and integrated data analysis). The organization of these data sources is shown in Figure 1 (below).



Functional genomics

Functional genomics aims at measuring the level of expression of all or a selected subset of genes based on the amount of mRNA (the trascriptome) present in the sample. The most powerful tool available today for this is the DNA array technology.

In a microarray comparison of gene expression from two experimental groups (A and B), RNA is extracted and reverse transcribed into cDNA. Either during this step by direct incorporation of fluorescently labeled nucleotides or indirectly by reaction of a modified nucleotide with the fluorescent label, cyanine 3 (Cy3) and cyanine 5 (Cy5) are used to differentiate each sample. Equimolar amounts of labeled cDNA are then mixed and hybridized to a single array of gene specific probes. These arrays are constructed by 'printing' as many as 80,000 'spots' onto a coated glass slide, each spot corresponding to a unique gene sequence that will hybridize or bind to the labeled cDNA. Post-hybridization the labeled array is scanned using a microscope and the amount of fluorescent signal from each of the dyes (Cy3/Cy5) measured at each spot. The relative signal present is representative of the ratio in gene expression between the two starting mRNA samples. Where gene expression is equivalent, equal signals in the Cy3 (green) and Cy5 (red) channels are observed producing a yellow spot. When one sample has significantly higher expression of a specific gene, the signal from that sample predominates, producing a more green or red spot.

The limitations of this technology are many. The main one being sensitivity in the data analysis. Statistically significant measurements are obtained only for most abundantly expressed genes. For smaller changes repeated measurements required are costly but attainable. Analytical precision

and standardization has been the main hindrance in microarray based functional genomics. Micro array gene expression database (MGED) founded in 1999 facilitates adoption of standards for DNA array experiment annotation and data representation. Standard experimental controls and data normalization methods can be found at www.mged.org. Therefore, high per analysis cost reduces the number of measurements per study and different platforms used; cDNA Vs oligonucleotide array, printed micro arrays Vs on chip synthesis etc., regular modifications to incorporate new genes and improved probe sequences complicates the scenario further. Off the shelf arrays or oligonucleotide collections available for custom spotting provides the first step in standardization of this technology. Because of these problems studies meeting rigid statistical requirements are relatively scarce.

Primarily, this technology should be thought of only a screening. In relationship between nutrition and health (unlike nutrition and disease) it is necessary to develop a biomarker which should reflect subtle changes in homeostasis; and efforts of the body to maintain homeostasis thorough cellular systems, organs and inter-organ interactions.

Integrated approach

By helping to understand the interaction between nutrients and molecules in an organism, the implementation of molecular biology and biochemistry in 'classical nutrition' research, followed by the technological revolution of the '-omics' technologies, will greatly affect nutritional sciences. Although the complexity of this proposed integration is exceeding the current bioinformatics tools and capacities, its implications for nutritional research can be enormous. Unlike biomedical interventions (drug therapy), nutrition is chronic, constantly varying, and composed of a very large amount of known and unknown bioactive compounds. Furthermore, nutrition touches the core of metabolism by supplying the vast majority of ingredients (both macro- and micronutrients) for maintaining metabolic homeostasis. This homeostasis stretches from gene expression to lipid metabolism and from signaling molecules to enzyme cofactors. Thus, nutrition by its nature needs to be studied in an integrated way (systems biology).

So far, most of the tools for this integration have been lacking, thus maintaining an unbridgeable gap between classical nutrition (studying physiology with a focus on biochemical pathways) and biomedical sciences (determination of disease-related molecular mechanisms). In applying systems biology to nutritional sciences, these paradoxical extremes are bridged and the complexity of the relationship between nutrition and health can be met by the complexity of the integrated approach. Many hurdles need to be taken, most of them in the field of bioinformatics, before this research area reaches maturity.

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