

Metabolomics and Its Applications in Human and Animal Nutrition Research: A Review

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ABSTRACT

'Metabolomics' is an evolving field of 'omics' research that strives to focus upon highly specific and highly sensitive characterization of metabolites which are very small in size, i.e., small molecules in various biological materials. As on today, 'metabolomics' has assumed great significance and is ideally poised to be applied in several avenues of human and animal nutrition research. This paper focuses on the recent developments and potential uses of 'metabolomics' in four areas of food science and technology: viz., food component analysis; food quality/authenticity assessment; monitoring of food intake, changes in various physiological parameters, food interventions and diet challenge studies.

This paper reviews the various aspects of metabolomics such as history; workflow of a nutritional metabolomics study, with special mention of its constituent components; hypothesis and experimental designing of metabolic profiling and list of biologically relevant samples to be collected for profiling. The commonly employed platforms used for analytical purpose in metabolomic studies, analytical techniques used to integrate the data in a suitable way; data analysis and steps involved, viz., metabolic profiling and metabolic fingerprinting are discussed in detail. The subsequent step of identification, characterization and interpretation of the selected metabolites are thoroughly detailed. The routes or methods adopted in metabolomics are discussed threadbare. The practical applications of 'metabolomics' in human, veterinary and basic sciences are listed out. Interesting work done in nutritional research such as, the use of a metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows are detailed. Integration of metabolomics with corresponding profiling technologies, viz., 'transcriptomics' and 'proteomics' are also dealt with in detail. A SWOT analysis, listing out the problems, solutions and possibilities of 'metabolomics' as a future analytical tool in human and animal nutrition research and carrying out the same within an ethical framework, are also enlisted.

Keywords: Metabolomics, Workflow, Experimental designing, Data integration, Data analysis, Research work done, Integration with other 'omics' technologies, SWOT analysis, Ethics

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INTRODUCTION

It is an established fact that the health of an individual is directly correlated to the nutrition, it receives. Existing literature reveals that that if a man or animal is poorly fed, it can result in disease conditions such as obesity, diabetes, hypertension, atherosclerosis, cancers and tumours, osteoporosis, inflammatory diseases etc. and make the individual more susceptible to infections. All the above diseases are related to metabolic impairments that are not fully decipherable; because, within the same population and with apparently comparable and/ or similar environments, some individuals tend to suffer due to ill health and die prematurely, while others achieve unprecedented health and longevity. Even though science has toiled hard, over the centuries to unwrap the reason behind this, it has not been fully successful; because of the epidemiological association between food and health. The probable reason for this could be the inability of science to pin point, how deficiency, excess and imbalance of nutrients affect the various metabolic processes and at what all steps, within each individual in the population.

A thorough investigation of individual variations such as these would provide a more personalized or individualized set of data, which shall pave the way for establishment of a highly individualized system for assessment of the metabolomic status of the individual, which would in turn help to establish a new system for enhancing human health, by way of increasing the efficacy and safety of diets. Such a system would enable nutritionists to rapidly and specifically assess the individual's nutritional status, plan an appropriate intervention and then monitor compliance of the same, its progress as well as the after effects, if any. All these activities should be performed with respect to and special emphasis upon the lifestyle, environment, growth and development upto that point of time, gut microflora, feeding patterns and presence/ absence of health or disease.

METABOLOMICS

'Metabolomics' is a vast growing area among 'omics' technologies that focuses on obtaining large volumes of data from very small samples of biological materials, fast transfer of this data to the analytical platforms and subsequent characterization of metabolites which are very small in size. The science of 'metabolomics' is ideally poised to be applied in several areas of research concerning food, nutrition and diet (Wishart, 2008). This paper discusses the recent developments and potential uses of 'metabolomics' in four areas of nutrition, dietetics and allied technologies: viz., food component analysis; food quality/ authenticity assessment; monitoring of food intake; changes in various physiological parameters, associated with food intake; dietary interventions and diet challenge studies.

'Metabolomics' is a novel area of 'omics' technology which involves the high-throughput identification, assessment, interpretation and quantification of small molecule (<1500 Da) metabolites in the metabolome. Metabolome is defined as a collection of all small molecule metabolites or chemicals that can be found in a cell, organelle, organ or organism. These micro molecules include a wide array of endogenous and exogenous chemicals such as carbohydrates, organic acids, polyphenols, alkaloids, nucleic acids, amino acids, peptides, minerals, vitamins or any other chemical that can be utilized, consumed or synthesized by any cell or organism (Wishart, 2008).

History

Francois Magendie, a French physiologist of the 19th century developed experimental methods for animal feeding experiments. He conducted experiments by using purified diets prepared from pure carbohydrates and fats and found that nitrogen (N) in the food was essential. Thereafter, in the middle of the 19th century, Boussingault, another French chemist, performed balance experiments using dairy cows and defined carbon, nitrogen, phosphorus and oxygen as the basic elements. The objective of Boussingault's study was to understand the physiology and nutrition of

lactation in dairy cows, but later on he also performed experiments using farm animals, with the ultimate objective of improving food production and thereby to ensure food security to the human population. In the latter part of the 19th century and the beginning of the 20th century, work on basic protein metabolism was first conducted in laboratory animals and subsequently in farm animals (Katan & Roos, 2004).

It was during the same period that Magendie reemerged with the concept of single-feed (restricted ration) diet, at the Wisconsin Agricultural Experiment Station, in USA. The 'restricted ration' approach helped not only to identify the usefulness of a given feed to support protein deposition, but also helped in identifying deficiencies of particular micronutrients. Magendie also observed that the growth rate of pigs fed on certain feeds were lower than those fed on certain others. He observed, that micronutrient deficiencies were absent in all groups of pigs. Based on these, Magendie concluded that the reduced growth rate of certain pigs, could be attributed to the quantity and quality of protein present as well as the amino acid makeup of the various feeds and their combinations, tested. Osborne and Mendel performed experiments using rats and reported that the reduced body weight which resulted from feeding of certain feeds alone could be rectified by the supplementation of missing amino acids to the basal diets, either as intact proteins or as specific amino acids. This led to the concept of protein supplementation, which is widely applied in day to day diet formulations, today (Bergen, 2007).

Urea and uric acid are the end products of N metabolism in mammals and birds, respectively. This knowledge, paved the way for the introduction of N free diets in nutrition studies, after which techniques such as isolation of proteins, characterization of the amino acid makeup of proteins and amino acid analysis emerged. Therefore, at the beginning of the 20th century, the science of Nutrition had a set of established experimental procedures such as N balance, N-free diet,

purified and chemically defined diets; as well as the emerging analytical techniques such as N analysis, urea-N analysis, protein isolation and characterization. This in turn, led to subsequent discoveries such as determination of amino acid content of proteins; investigations on protein/ N requirements for maintenance and growth of farm animals, rodents and man; determination of dietary essentiality of amino acids and assessment of the nutritive quality of proteins by amino acid profiling. Such efforts were the main focus of N nutrition research until emphasis slowly changed from nutritional explorations to increased studies of mechanisms of protein synthesis and breakdown (Jenab et al., 2009).

Analysis, identification and quantification of the amino acids in proteins, led to the subsequent discovery that proteins are comprised of nearly 20 amino acids. Hence, it became imperative to know the dietary essential and nonessential amino acids. By the late 1930s, threonine, methionine, valine, isoleucine, leucine, phenylalanine, tryptophan, lysine, histidine and arginine had been established as essential (EAA)/ indispensable amino acids. EAA were established concomitantly by growth studies and N balance studies in pigs and chickens. Agricultural researchers pioneered rapid application of EAA knowledge to diet formulations of food animals (Oresic, 2009).

This served as the platform from where, subsequent interventions with wide applications such as specific supplementation schemes and use of pure amino acids to balance diets for pigs, chickens, turkeys and farm-raised fish during production, were developed. As recognized for many years, in instances where dairy products and foods of animal origin are incorporated in large quantities in the diet, the data on protein requirement obtained may not actually reflect the one which is required in practical, day to day, feeding. The data on EAA requirement are however, critical in medical research with the current emphasis being on EAA needs during pregnancy, illnesses such as infectious diseases, recovery from malnutrition and

during excessive consumption of amino acids. Empirical growth trials to establish amino acid requirements are costly with food animals because growth or product yields by animals involved in N balance studies are typically lower than expected under usual production conditions (Winnike et al., 2009).

Experimental protocols that allowed high productivity and nutritional assessment criteria that would not modify production rates were therefore needed. Therefore, the need of the hour was to develop an indirect approach and plasma amino acid concentrations (PAA) have emerged as useful indicators of protein/ amino acid status under fully controlled experimental conditions. The use of PAA was taken up by research workers investigating farm animals, who did extensive work in the late 1960s and the 1970s to experiment, refine and redefine the application of PAA as a test parameter for ascertaining the amino acid nutrition status in pig, poultry and cattle. On the basis of a combined study of the breakpoint curves of PAA, growth and N balance, it was established that under strictly controlled experimental conditions, PAA responses may be used as a direct indicator for assessment of protein status in producing farm animals (Huber et al., 2011).

The PAA concept, as a direct indicator of EAA adequacy was subsequently widened to cater to metabolic approaches, also. When an EAA is limiting the production performance of an animal, it indicates that a major fraction of this amino acid is used for protein synthesis with only slight oxidation to carbon dioxide. However, increasing the dietary supply of this amino acid above the requirement resulted in increased deamination, oxidation to carbon dioxide and also reduced the oxidation of other EAA, indicating that excess amounts of EAA, apart from increasing the cost, will not bring about the intended positive productive response and may even result in a reduced performance. Recently, as an example of synergy between human and

animal nutrition research, the indispensable amino acid oxidation method has been reapplied to the porcine model to estimate the variability of lysine requirements of growing pigs (Jones et al., 2012).

Experiments performed in farm animals over the last two centuries, have greatly enriched the science of nutrition. In the current scenario, where the world is struggling hard to cope up with a deadly pandemic, trying the level best to develop a vaccine and/ or effective medicine against ‘Corona’ virus, undergoing rapid genetic mutations; ‘metabolomics’ has got immense applications, in the medical field, too. Apart from the medical field, ‘metabolomics’ has got wide application in the field of human and animal nutrition research. With the advancements made in related fields such as cellular and molecular biology, aided by gene modification technologies such as knockout and terminator genes, nutrition studies in the 21st century are poised for a grand take off towards an exciting future, with the likelihood of several novel and path breaking accomplishments. Efforts aimed in the achievement of this goal like the use of farm animals such as sheep and pigs for uterine and foetal nutrition research; studies on pediatric protein nutrition; amino acid dynamics and assessment of the effect of excessive amino acid intake in young, growing and mature animals, have already started in right earnest.

Workflow of a metabolomic study

The flow of work in a nutritional metabolomics study comprises of seven essential components:

- 1) Hypothesis
- 2) Experimental design
- 3) Sampling of biofluids
- 4) Analytical platform
- 5) Sample spectra
- 6) Data analysis and
- 7) Biological interpretation (Savorani et al., 2013).

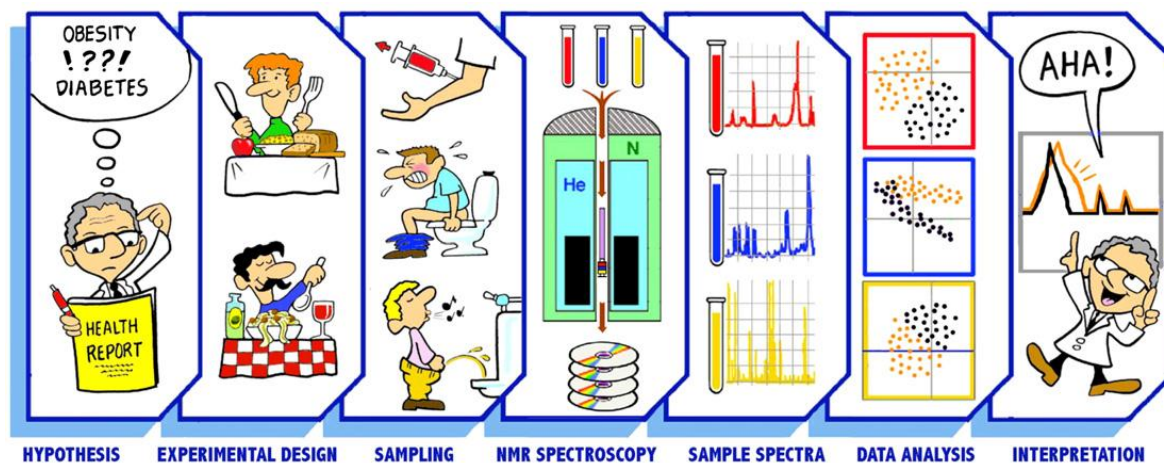


Fig. 1: The flow of work in a nutritional metabolomics study (Savorani et al., 2013).

Hypothesis

In today's world, people are highly health conscious: i.e., people prefer to eat healthy foods to improve their well-being; society also prefers individuals to eat healthy foods so as to reduce the cost for the public health sector; doctors prefer giving dietary recommendations based on scientific evidence and nutraceutical manufacturing companies want to give health claims on their products in order to maximize their returns. The aim of nutritional 'metabolomics' is to investigate the deviation of a process from its regular or normal state or path, caused by an outside influence on the human metabolome by certain diets and/ or bioactive compounds/ molecules (Savorani et al., 2013).

Experimental design

The aim of metabolomic study is to determine as many chemical (and physical) characteristics as possible and to identify metabolites or patterns of metabolites that differentiate the control samples from those of the treated individuals. The objective of experimental design is to maximize the possibility of obtaining a significant result in relation to the hypothesis being tested/ investigated. In any case, experimental design should be thoroughly discussed by all the stakeholders in the 'nutri-metabolomics' workflow. The clinical nutritionists should look into what is the expected level of intra- and inter-individual variation. The analytical chemists should see what is the degree/ level of uncertainty in measurements. The data

analysts should be able to clearly ascertain the solution to the problem under investigation. The ethical experts should specify what is acceptable to investigate and the legislators should be able to categorically state the requirements for obtaining health claims (Savorani et al., 2013).

Sampling

Test samples can be obtained from various biological materials such as:

1. Urine
2. Blood plasma and serum
3. Faeces
4. Cerebrospinal fluid
5. Saliva
6. Tissue homogenates from gut, liver, biliary tract and muscle
7. Cell cultures

In most cases urine and blood plasma samples are used (Kussman et al., 2008).

Analytical platform

As the human metabolome is kept within a tight homeostatic bandwidth and in a healthy state; perturbations of homeostasis are restored quickly. Recording real-time metabolic dynamics upon perturbations represents a challenge for the analytical methods employed.

Liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS) and NMR spectroscopy are the most suited and commonly most used platforms for metabolomic studies (Adamsky & Suhre, 2009) and most interestingly they are highly

complementary techniques with very little analytical overlap (Roux et al., 2011).

NMR is the fastest analytical platform for nutritional ‘metabolomics’ research, as it requires only minimum handling of samples and because the NMR spectrum can be generated in a few minutes. Even though, the sensitivity of LC-MS is much higher than NMR, it is not normally preferred because it is biased at both the chromatographic (use of separation principle and material) and the mass spectrophotometric (use of ion-trap, time of flight, MW) approaches and hence raises a question mark over the reliability of the results.

All analytical platforms complement each other and the most successful metabolomic studies make use of a wide range of analytical techniques and integrate the data to give the broadest possible coverage of the metabolome under study (Savorani et al., 2013).

Sample spectra

NMR signals are collected as a function of time. The decaying signal that succeeds a pulse is called the free induction decay (FID). By utilizing Fourier transformation technique, whereby the time domain is converted into the frequency domain, the chemical shift can be derived. Even though the processing of single NMR spectra will not be considered at this level, the quality of the spectra and thus the quantitative information derived from the spectra will depend upon the quality and homogeneity of the external magnetic field (shimming) accompanied by precise and accurate tuning and matching of the nuclear magnetic resonance frequency (Roux et al., 2011).

Prior to Fourier transformation, the data is typically zero filled and apodized to a certain line broadening. Apodization is an optical filtering technique, meaning "removing the foot", which involves, changing the shape of a mathematical function, an electrical signal, an optical transmission or a mechanical structure. After apodization, the NMR spectra should be corrected for deviations from a flat horizontal baseline and phase errors, if any

(Wishart, 2008), which can be done automatically by the NMR software employed.

Data analysis

NMR spectroscopy combined with multivariate data analysis (chemometrics), first successfully demonstrated in the early 1980s has proven to be an excellent analytical tool in ‘metabolomics’ (Cevallos et al., 2009). In this approach, the results obtained from multivariate data analysis are compared across a group of people with a shared characteristic, for metabolites and/ or patterns of metabolites that are able to isolate the effect of the treatment/ intervention, under study.

There are basically two possibilities for subsequent multivariate analysis, for large scale NMR metabolomics data, collected:

Metabolic profiling: NMR is basically quantitative and the signals generated are directly proportional to the concentration of the metabolites. Hence, metabolic profiling is the obvious choice for the analysis of large scale metabolomic data sets. In this approach, as many signals as possible are identified (not necessarily assigned) and their peaks are integrated for each sample, so as to derive a data set of the size equal to number of samples × number of peaks, where the number of peaks usually range from ‘a hundred’ to ‘a few hundreds’. A recent advance in metabolic profiling is targeted profiling wherein, the mixture spectrum is compared to a reference library of spectra derived from pure compounds of known concentrations and the metabolites can thus be directly quantified (Wishart, 2008).

Metabolic fingerprinting: This is a slightly more explorative approach than metabolic profiling, wherein, all the NMR signals (metabolites) are not identified, in the beginning. Here, all the spectral features are utilized in the multivariate data analysis. This approach is sometimes called as metabolic fingerprinting, because the objective is not to characterize each and every observed metabolite, but instead to compare ‘fingerprints’ or patterns of alterations in response to food intake and/ or disease status (Zdunczyk & Pareek, 2009).

Model interpretation and identification of the selected metabolites are subsequently necessary in order to provide a clear cut understanding of the mechanism of action involved. As computational power has increased, metabolic fingerprinting has the ability to provide new and unexpected findings. Therefore, performance of analyses at the maximum spectral resolution possible is highly desirable (Bino et al., 2004). Metabolic fingerprinting will create a data set of the size equal to number of samples \times number of spectral variables, wherein, the number of spectral variables may be thousands (Savorani et al., 2013).

Biological interpretation

The essential part of most ‘metabolomics’ studies is metabolite identification, but since this task is laborious and a time consuming and since it is a step at the finishing stage of the metabolomics workflow, it is often left incomplete or unfinished or ignored (Zeisel, 2007). However, since NMR is an approach of relatively low sensitivity, most metabolomic studies involving NMR should definitely have a full or near full inclusion of metabolite signals.

The development of human metabolome databases such as the biological magnetic resonance data bank (Wishart, 2008) as well as the NMR technique; LC-NMR, which is capable of isolating single compounds and obtaining pure metabolite spectra (Beger et al., 2010) are valuable aids in identifying the metabolites. If it is possible to obtain a substantial quantity of metabolites, the NMR technique can reveal chemical structures and provide pin point evidence, required for the identification of unknown molecules (Putri et al., 2012).

A biologically relevant interpretation should be made related to the research question, once a metabolite or pattern of metabolites has been identified. The biggest challenge of nutritional ‘metabolomics’ lies in the fact that, the important metabolite patterns which could be extracted and which are responsible for the classification may not have any direct or even indirect relationship to the

experimental design used and may simply be a part of the endogenous biological pool where the perturbation introduced, will influence several metabolites (Savorani et al., 2013).

Full hand biological knowledge on investigated subjects such as statistical total correlation spectroscopy (STOCSY) and statistical hetero spectroscopy (SHY) autocorrelation matrices, if available, can be employed for adopting a generalized correlation approach of statistical analysis and interpretation of the metabolomic data. These two can also be described as tools for identifying and connecting the molecules with respect to a particular metabolic pathway. Cross validation of the observed correlations with evidence from other sources is a must for interpretation, because statistical methods cannot differentiate between causal effects and indirect correlations (Savorani et al., 2013).

Two routes to metabolomics

The two routes to metabolomics, as described by Wishart, (2008) are:

- 1) Chemometric (Non-targeted) methods
- 2) Quantitative (Targeted) methods

Chemometric (Non-targeted) Methods

In the chemometric approach chemical compounds are not generally identified. The pattern and intensity of the spectrum only is recorded, statistically compared and used to identify the relevant spectral features that help to identify sample classes. The identification techniques usually employed are

- 1) Unsupervised clustering, which involves, principal component analysis (PCA) and
- 2) Supervised clustering, which involves, partial least squares discriminant analysis (PLS-DA), as described by Wishart, (2008).

Quantitative (Targeted) Methods

In the quantitative approach of metabolomics or targeted profiling, the emphasis is to identify and/ or quantify as many compounds in the sample as possible. Comparison of the sample’s NMR or MS spectrum to a spectral array in reference libraries, obtained from pure compounds, is the usually adopted method for this approach. Once the constituent metabolite is identified and quantified, the data are then statistically analyzed (using PCA or PLS-DA)

to identify the most important biomarkers or the concerned metabolic pathways involved. Based upon the objectives of the study and the capacity of the instrument used, quantitative metabolomics may be of two types, viz:

- 1) Targeted, which is specific and selective to certain compounds such as lipids or polyphenols
- 2) Comprehensive, which encompasses almost all detectable metabolites (Wishart, 2008).

Applications of metabolomics

Metabolomics has got a wide range of applications in human, veterinary and basic sciences. Some of the main applications are listed below:

1. Nutritional analysis/ research
2. Clinical trial testing
3. Fermentation monitoring
4. Food and beverage tests
5. Nutraceutical analysis
6. Water quality testing
7. Petrochemical analysis
8. Genetic disease tests
9. Toxicology testing
10. Clinical blood analysis
11. Clinical urinalysis
12. Cholesterol testing
13. Drug compliance
14. Transplant monitoring (Roux et al., 2011).

Important work done in nutritional research

Some of the important works done, which are applications of the science of 'metabolomics' in nutrition research/ analysis are listed below:

1. Metabolic assessment of obesity induced by a high fat diet

Kim et al. (2009) conducted a metabolic assessment of obesity in man after inducing it using a high fat diet. The prevalence of obesity is increasing rapidly worldwide. To reduce the associated risks, investigation of the causes of weight gain (Eg:- lifestyle and behavior), is necessary. To prevent obesity, early diagnosis and treatment are important. Obesity studies involving the administration of a diet which is high in fat (HFD) in animal models are known to be relevant as far as human obesity, is

concerned. Hence in this study, the metabolic profiling was done using NMR spectroscopy in rats.

The study gave the following indicators as to the causes and remedies of human obesity:

- 1) Betaine can prevent and cure cirrhosis in rats and decrease the contents of hepatic cholesterol and total lipids in rats consuming a high-cholesterol diet, as confirmed later by Heinzmann et al. (2010).
- 2) Taurine is known to exert insulin-like effects such as accelerating glucose uptake into tissues and glycogen synthesis in the liver.
- 3) Acetoacetate and acetone were the ketone bodies produced when acetyl-CoA derived from lipid β -oxidation exceeded the capacity of the tri carboxylic acid cycle.
- 4) Pyruvate in urine samples was elevated in HFD group due to the inhibition of pyruvate dehydrogenase enzyme.
- 5) Adipose tissue is an important source of lactate production *in vivo*.
- 6) The increased provision of FFAs causes an increase in FFA oxidation, resulting in increasing the concentration of citrate (Kim et al., 2009).

2. Benefits of supplementing soy isoflavones in women

Solansky et al. (2003) studied the influence of soy isoflavones by giving five healthy premenopausal women, 60g soy/ day (45mg isoflavones) in controlled diets. They quantified ten different oestrogenic principles such as genistein in soy isoflavones using NMR technique. On the basis of the results obtained, these researchers observed that the severity of symptoms of menopause such as hot flushes, mood swings, vaginal dryness, insomnia and headache were reduced by the administration of soy isoflavones. Also, the incidence of breast cancer, fibroids and endometritis were found reduced in these women. Usually women resort to hormone therapy to tide over these symptoms. However, hormone therapy can lead to heart attack, stroke, breast cancer and dementia. These adverse effects can also be avoided by consuming soya (Solansky et al., 2003).

3. Assessment of the safety and security of using nutritionally genetically modified (GM) plants and food/ feed derived from them using the technique of metabolomics

The modification in GM plants and food/ feed derived from them, that are presently readily available in the market, have been brought about by the insertion of one or a few genes which express specific traits, such as providing tolerance to herbicides and/ or resistance to insects. These GM plants show no apparent evidence of alteration in basal composition and phenotypic appearance, other than the intended ones. There are several GM plants that are currently in the developmental stage, wherein considerable alterations in botanical composition have been achieved in order to improve upon the agronomic properties such as drought resistance and salt tolerance, so as to amplify their nutritional characteristics and the resultant health benefits to humans (Fiehn, 2002).

Assessment of the safety of GM plants is a particularly stringent process as it stresses upon two factors, viz., the safety of the patented plant resulting from the genetic modification and/ or the sum of all possible modifications, by careful analysis of the whole plant and its performance in a large number of studies. A detailed and stepwise procedure for the nutritional assessment of the safety and security of humans who consume GM foods have been developed by the European Food Safety Authority (EFSA), the salient points of which are listed below:

- 1) Hazard identification
- 2) Hazard characterization
- 3) Toxicity, safety and nutritional evaluation
- 4) Exposure assessment
- 5) Risk characterization
- 6) Conclusion on safety
- 7) Market release

Each GM plant is unique and therefore each study necessary for the pre-market assessment of the security and safety of the derived food and/ or feed need to be designed on an individual basis using knowledge already available or generated. The structured approach in testing is important in order to

achieve better results and save resources in the assessment process. In accordance with this, each study needs to be carried out and the overall sequence of studies should be based upon a thorough examination of already generated data. This would ultimately lead to well-designed studies with clear objective(s), precise study designs, protocols, dose level selection, sensitivity, statistical validity, data analysis and science based interpretation of the results (EFSA, 2007).

4. A metabolomics approach to uncover the effects of high grain diets on rumen health in dairy cows

A high grain diet in early lactation can lead to increased incidence of metabolic disorders like lactic acidosis. The precise mechanism of how this diet transition affects the rumen environment is not clear. Saleem et al. (2012) conducted studies by feeding eight cows with barley grain, in increments of 15 (0, 15, 30 and 45 per cent of total dry matter of the ration). Metabolomic analysis of rumen fluid samples were conducted using NMR, GC-MS and 93 different metabolites were quantified. The difference in the concentration of metabolites, between the initial and final day were also studied.

On the basis of this study it was observed that the metabolic composition of the rumen liquor at the beginning and end of the study could be clearly partitioned. In addition, the values of rumen fermentation parameters of cows fed on the ration with 45 per cent barley were significantly different from others. High grain diets (30 per cent and 45 per cent barley) resulted in significantly higher levels of numerous unnatural, poisonous and inflammation inducing compounds such as putrescine, methylamines, ethanolamine and short chain fatty acids. A number of amino acids such as arginine, phenylalanine, valine, leucine, lysine, ornithine and phenyl acetyl glycine showed glaringly evident perturbations (Saleem et al., 2012).

5. Biomarkers of meat intake

Meat intake is an important contributor to dietary protein for a vast majority of the population. Meat intake has also been

associated with high incidence of diseases especially cardio vascular diseases (Dragsted, 2010).

With this objective, a study was undertaken to find out the biomarkers associated with meat intake. Urea, creatine, creatinine, carnithine, carnosine, anserine, 1-methyl histidine, 3-methyl histidine, sulphite and sulphate were identified as the biomarkers. Among these, even though, anserine and 1-methyl histidine were designated as ‘meat specific biomarkers of meat intake’, none of them could be specifically pin pointed as ‘a true quantitative biomarker’ (Dragsted, 2010).

Integration with other ‘omics’ technologies

‘Omics’ technology in nutrition terminology means, the deployment of related technologies in an integrated fashion with ‘metabolomics’, to characterize and identify the metabolites which define the susceptibility of an individual to nutritional interventions and for assessing the efficacy and safety of human and/ or animal food and/ or feed ingredients. The technologies related to the science of nutritional ‘metabolomics’ are ‘proteomics’ and ‘transcriptomics’, which help in the molecular assessment of nutritional adaptations and diet changes. A broad overview of molecular changes resulting from nutritional interventions can be provided by ‘transcriptomics’. Proteomes have the highest complexity when compared to transcriptomes and metabolomes and hence ‘proteomics’ is the most challenging of the three ‘omics’ technologies. ‘Proteomics’, however, delivers not only the markers but also the targets of the intervention under study, such as enzymes or carrier molecules and is hence the ‘omics’ of choice for discovering biologically active peptides and proteins in food. ‘Metabolomics’, if used synchronously, with the other two ‘omics’ technologies, can serve as a useful tool for characterization of the metabolic status of individuals, which in turn would provide the much valuable data with respect to ‘metabolic endpoints’ and also the interpretation as to whether they are related to health and longevity or disease and succumbing.

The above three integrated ‘omics’ technologies, viz., ‘proteomics’ (protein expression analysis), ‘transcriptomics’ (gene expression analysis) and ‘metabolomics’ (metabolite profiling), when applied together holistically, will help us to have, not only a better understanding, but also a thorough assessment of these ‘metabolic endpoints’ and their consequences. ‘Nutrigenomics’ or ‘nutritional genomics’ is the name given to the collective term which encompasses the above three sub-disciplines of ‘proteomics’, ‘transcriptomics’ and ‘metabolomics’ and enables us the usage of profiling technologies having medium to high sensitivity, in order to estimate the response of a cell, organelle or an organism to one or more ‘foods’/ ‘food constituents’.

The response of individuals to various ‘foods’/ ‘food constituents’ are examined by the adoption of ‘post-genomic and related technologies’, in the science of ‘nutrigenomics’. The ultimate objective of ‘nutrigenomics’ is to know how the entire body reacts to actual foods/ food constituents and an integrated approach is adopted for the same. By adopting such an integrated approach, people belonging to various populations and sub-populations; clustered on the basis of genes, disease and individuals; who consume a variety of foods, who are in various stages of life and who adopt different life styles, can be examined without any preconceived ideas or bias (Kussman et al., 2008).

Globally, most of omics-based nutrition investigations are performed on animal models. ‘Omics’ technologies, especially, MS-based ‘nutria-proteomics’ or ‘nutria-metabolomics’, is somewhat underdeveloped in nutrition research. There are many opportunities as well as numerous challenges that lie ahead. The above ‘omics’ technologies when used synchronously, will go a long way in the discovery of novel biomarkers associated with individual nutrients and food intake.

The synchronous application of the above ‘omics’ technologies, will enable the

scientists to have a thorough and in-depth knowledge of the characteristic biological response of a cellular system to external stimuli, which in turn would open up new vistas in understanding the intricate and complex interaction between nutrients and metabolites in various body systems. Hopefully, in future, ‘omics’ centered human and animal nutrition research can provide the optimum dietary recommendations for each and every man and farm/ pet animal, which will in turn be the key for prevention of chronic diseases such as obesity, diabetes, metabolic syndrome, cardiovascular disease and cancer (Zhang et al ., 2008).

Problems, solutions and SWOT analysis of metabolomics

Problems

Even though, ‘metabolomics’ has fully emerged as an invaluable genomic platform; there is still scope for vast improvement in technical aspects, with respect to determination of complex metabolites as well as the large-scale dissemination of research data evolving from it, for improvement of man and animals. For further maturation of the science of ‘metabolomics’, the following three ends need to be fulfilled:

- 1) Substantial improvement in the identification of new metabolites and their inclusion in the ‘metabolome’
- 2) Scope for comparing the metabolomic results obtained, between different laboratories as well as different experiments and
- 3) Integration of metabolomic data with other functional genetic information should be carried out on a much larger scale (Bino et al., 2004).

Solutions

Since the above challenges are widely recognized and endorsed, a community based effort is proposed to outline the common criteria to be satisfied in a metabolic analysis; initiate whole hearted actions directed towards the release of standard reference biological materials/ samples; identification, building up and consolidation of metabolite reference libraries and development of data analytical

systems which are metabolite-specific (Bino et al., 2004).

SWOT analysis

Watkins and German (2002) studied the ‘strengths, weaknesses, opportunities and threats’ (SWOT) of metabolomics and proposed the following:

Strengths

- 1) Robust and stable analytical platforms
- 2) Minimally invasive
- 3) Real biological endpoint
- 4) Whole system integration

Weaknesses

- 1) Analytical sensitivity
- 2) Analytical dynamic range
- 3) Complexity of data sets
- 4) High capital cost

Opportunities

- 1) Much experience from mammalian system studies (Eg:- pathways)
- 2) Potential of multi-omics integration
- 3) Web-based diagnostics

Threats

- 1) Skepticism of non-hypothesis led studies
- 2) Conservatism
- 3) Lack of well trained scientists

CONCLUSION

The science of nutrition has been enriched immensely, for over two centuries, as a result of farm animal experimentations. With the widespread application of the technologies of cellular and molecular biology, genetic modification techniques such as knockout and terminator genes in human and animal nutrition studies in the 21st century, the future of ‘metabolomics’ appears promising. The science of ‘metabolomics’ is poised to be take off in several research areas of food science and nutrition including analysis of food constituents; assessment of food quality, safety, security and authenticity; monitoring of food intake and identification and interpretation of the perturbations in physiological parameters as a result of food intake.

In spite of all these accomplishments, the practical application of ‘metabolomics’ is still limited by database and technology. On

the instrumentation side, it is absolutely clear that considerable improvements need to be made to make the technology used for the detection and quantification of metabolites; more strong, fully automatic and widespread. While promising achievements have been made, it should be noted that the current techniques used are only capable of detecting, one by tenth of the relevant 'metabolome' and hence widening and expansion of the 'metabolites' covered are particularly important in nutritional metabolomic studies.

While a 'metabolite' in food may influence a number of key molecular events that are involved with prevention of chronic diseases such as cancer; to achieve this end, it must reach an effective concentration within the target cell, organelle or organ, as the case may be; in the specific metabolic form; which in turn will send small molecular weight signals, ultimately leading to changes in the cellular environment, which is what scientists refer to as 'metabolomic effect'. As the era of molecular nutrition unfolds, a comprehensive and better understanding of how foods and food components influence chronic diseases such as cancer will surely come to light in the public domain, in the immediate future. Such information will be critical in the development of effectual deliverance of individual specific approaches, which will help to reduce the incidence and the resultant burden of not only chronic diseases like cancer, but also infectious diseases like the COVID – 19, the dreaded 'global pandemic', against which we are currently fighting a huge battle, of 'life and death'. Once metabolomic informations such as these are unraveled, the science of 'metabolomics' will reach the next level and in such a scenario, it is highly critical that metabolomic data are 'utilized' judiciously and within an ethical frame work, for the ultimate welfare of mankind.

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