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Ambika Arun
Ph.D. Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Satyabrat Dutta
Ph.D. Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Richa Sarkar
Ph.D. Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Esha Sinha
Ph.D. Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Khushboo Panwar
Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Gorre Venu
Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Sudhir Kumar Prajapati
Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, Indi

Corresponding Author:
Ambika Arun
Ph.D. Scholar, Department of Veterinary Microbiology, Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly, Uttar Pradesh, India

Metabolomics and its application in advance diagnostic microbiology field

Ambika Arun, Satyabrat Dutta, Richa Sarkar, Esha Sinha, Khushboo Panwar, Gorre Venu and Sudhir Kumar Prajapati

Abstract
More precise diagnosis and assessment approaches are needed as soon as feasible to improve the clinical course of illnesses. Metabolomics is the study of metabolites, small biomolecules (carbohydrates, lipids, amino acids, and organic acids) present in a biological sample and has been applied to the discovery and identification of disturbed pathways. Metabolomics permits detection of most endogenous and exogenous metabolites in a biological sample using analytical chemistry techniques such as nuclear magnetic resonance (NMR) spectroscopy and liquid or gas chromatography–mass spectrometry (LC/GC-MS). Metabolomics has been used in a variety of fields, including nutrition, toxicity, environmental studies, and systems biology. Our goal was to conduct a thorough evaluation of the available literature on the application of metabolomics in the treatment of spontaneous veterinary illnesses. In all of the experiments, metabolite changes were seen in diseased animals as compared to non-diseased animals.

Keywords: Metabolomics, endogenous metabolites, exogenous metabolites, gas chromatography–mass spectrometry, nuclear magnetic resonance

Introduction
After completion of the Human Genome Sequencing Project, we are now in the post-genomics biology era. New '-omics' scientific fields have evolved, allowing us to better comprehend the role of genes, proteins, and metabolites in biological systems, both in humans and in the environment [1]. The characterization and quantification of pools of biological molecules that translate into an organism's structure, function, and dynamics is referred to as omics. Metabolomics is a new "-omics" area that aims to detect and quantify metabolites and small molecules in biological samples in a comprehensive way [2].

Metabolomics combines modern analytical techniques with chemometrics to identify a high fraction of metabolites (the metabolome) present in a sample, such as amino acids, sugars, ketones, nucleotides, fatty acids, organic acids, microbial metabolites, and exogenous small molecules (including drugs, food additives, and pesticides) [3, 4].

The clear usefulness of metabolomics tools in biomarker identification, gene function analysis, systems biology and diagnostic platforms is revealed by metabolomics technology. Metabolites can accurately reflect an organism's phenotype at a given time because they represent the downstream expression of the genome, transcriptome, and proteome [5].

Metabolomics is becoming increasingly relevant in cancer biology research, with applications ranging from finding key regulatory molecules implicated in carcinogenesis to identifying specific diagnostic biomarkers [6].

To detect complicated metabolic and other systemic illnesses, current metabolomic techniques evaluate hundreds to thousands of metabolites in biological fluids or tissues [7]. Because the intermediates of biochemical events play a critical role in connecting multiple pathways operating in a living cell, metabolomics, as a metabolic complement to functional genomics, allows for a more complete picture [8].

Bioinformatics and specialized statistical (including multivariate analysis) techniques known as Chemometrics are used in Metabolomics. Chemometrics aids in the comprehension and management of biological data contained in metabolite profiles in order to assess a metabolic condition [9]. Metabolomics software distinguishes, identifies and quantifies metabolites. Chromatography, such as High Performance Liquid Chromatography (HPLC), is used to separate metabolites, whereas spectroscopy, primarily nuclear magnetic resonance (NMR) and mass spectrometry (MS), is used to identify them.

The fundamental benefit of using metabolomics for illness diagnosis is that it evaluates the phenotype of two interacting organisms: the host and the pathogen. The expression of the genome that is influenced by the environment is referred to as phenotype. The phenotypic of an organism is altered when it becomes sick or stressed, causing specific biochemical changes. In theory, metabolomics and chemometric methods may be used to measure this alteration [10].

Metabolomics

Metabolomics is a branch of biochemistry concerned with all chemical processes involving metabolites, which are defined as molecules with a molecular weight of less than 1 kilodaltons [11]. It is the study of a cell's global metabolite profiles under a set of conditions. Metabolite profiling (the monitoring of metabolite components in a system) can be traced back to ancient cultures. In order to identify an illness, doctors detected changes in the patient's body fluids (e.g., saliva, urine) [12]. Because intracellular metabolite concentrations often disclose features of biochemical control that are undetected by other methods, metabolomics bridges the gap between traditional studies of gene, protein, and metabolite interactions in individual cells [13]. Changes in metabolites frequently result in changes in phenotype and biological activities, which can be tracked through metabolome analysis [14]. The ability to identify and measure the whole collection of intracellular and extracellular metabolites with molecular masses less than 1,000 Daltons is the main problem of metabolomics [15].

Metabolomes are the entire set of small molecular metabolites (such as metabolic intermediates, hormones, and other signal molecules, as well as secondary metabolites) discovered in a biological sample, such as a single organism [11]. Metabolites is a term that refers to a group of small molecules created during or as a result of a biological process. As we all know, each cell includes a variety of metabolites, or chemicals, that are produced during various cellular processes such as glycolysis, the Kreb cycle, and protein synthesis. All of these compounds are chemical molecules that are found inside the cell and are referred to be metabolites. It can be created through both catabolic and anabolic mechanisms.

Many different types of metabolic pathway present so many different type of metabolic present in the cell. They all are interacting called as metabolic profiling. When metabolic profiling change it causes abnormal change in body like disease. Types of metabolites are Endogenous metabolite**s:** which are provided or generated inside the cell like Primary metabolites: those which are generated the first primary pathway of metabolism (like in glycolysis, kreb cycle and beta oxidation), Secondary metabolites: which are generated or provided by combination of primary metabolites (Like phenolic compounds or alkaloids) and Exogenous metabolites: which are taken from outside the cell (Mainly composed of drug found inside the cell or like food colors like Xenobiotic and drug metabolites).

Metabolomics Strategies

Table 1: Metabolomics Strategies

Untargeted	Targeted
discovery	validation
Hypothesis generating	Hypothesis driven
Global/comprehensive analysis	Subset analysis
MS/MS correlated to databases/libraries	MS/MS correlated to reference standards
Relative quantification	Absolute quantification
Qualitative identification	Identification already known

Cover two primary platforms including “untargeted-discovery global” and “targeted-validation tenden” based on the objective of the study. Untargeted-it’s a global approach where we do not have a specific target. Where we discovered n number of molecules from biological sample like 1000 of metabolites measured. Basically used in discovery and certain hypothesis. Here do not require any commercial standard. Targeted-It is basically a validatory method has already developed a particular result and we validated against a particular standard. It is hypothesis driven. Quantification method. Nearly 20 metabolites have been measured.

Metabolomics work flow

First extract the sample from biological or tissue sample from extraction process. After this detect the sample through chemical analytical technique and analysis the data through software.

Sample preparation and extraction

During sample preparation different extraction solvents are used for high recovery of both polar and non-polar compounds based on targeted and non-targeted approaches on different biological samples. Methanol-water-chloroform combination to extract both hydrophilic and hydrophobic compounds. For high recovery of both hydrophobic and hydrophilic compounds, separation extraction application gives better results.

Separation techniques: Gas Chromatography, High Performance Liquid Chromatography, Liquid Chromatography.

Detection techniques: Nuclear Magnetic Resonance Spectroscopy, Mass Spectrometry.

Combination of techniques: GC-MS, HPLC-MS.

Chemical analysis

1. Gas chromatography

The phrase "gas chromatography" refers to a set of analytical separation techniques for analyzing volatile compounds in the gas phase. The components of a sample are dissolved in a solvent and vaporized in gas chromatography in order to separate the analytes by dividing the sample into two phases: a stationary phase and a mobile phase. The analyte molecules are carried through the heated column by the mobile phase, which is a chemically inert gas. One of the few types of chromatography that does not use the mobile phase to interact

with the analyte is gas chromatography. In gas-solid chromatography (GSC), the stationary phase is a solid adsorbent, while in gas-liquid chromatography, the stationary phase is a liquid on an inert support (GLC). Gas chromatography is a forensic technique used in drug analysis, arson investigations, and toxicity studies of various organic chemicals [16].

2. High-performance liquid chromatography (HPLC)

High Pressure Liquid Chromatography is another name for High Performance Liquid Chromatography. It's a common analytical technique for separating, identifying and quantifying each component of a mixture. HPLC stands for high-performance liquid chromatography. The solvent normally flows through the column due to gravity, but in the HPLC process, the solvent is pushed under high pressures up to 400 atmospheres, allowing the sample to be separated into different constituents based on relative affinities [17].

Pumps will be employed in HPLC to pass pressured liquid solvent, which will include the sample combination, into a column loaded with solid adsorbent material. The interaction of each sample component will differ, resulting in different flow rates for each component and, eventually, separation of column components.

3. Nuclear magnetic resonance spectroscopy (NMR)

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. An energy transfer from the base energy to a higher energy level is achievable when an external magnetic field is supplied (generally a single energy gap). Energy is transferred at a wavelength that corresponds to radio frequencies, and energy is emitted at the same frequency when the spin returns to its base level. The signal that corresponds to this transfer is detected and processed in a variety of ways to produce an NMR spectrum for the nucleus in question [18].

The NMR signal is formed by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected using sensitive radio receivers, and the sample is put in a magnetic field. The resonance frequency of an atom in a molecule is changed by the intramolecular magnetic field around it, allowing access to specifics of the molecule's electronic structure and individual functional groups. NMR spectroscopy is the definitive approach for identifying monomolecular organic molecules since the fields are unique or highly distinctive to specific compounds.

NMR spectroscopy offers precise information about the structure, dynamics, reaction state and chemical environment of molecules in addition to identifying them. Proton and carbon-13 NMR spectroscopy are the most popular types of NMR spectroscopy, but it can be used on any sample with spin-containing nuclei.

4. Mass spectrometry (MS)

Mass spectrometry is a technique for determining the mass-to-charge ratio (m/z) of one or more molecules in a sample. These measurements are frequently used to determine the exact molecular weight of sample components. Molecular weight determination can be used to identify unknown chemicals, quantify known compounds and evaluate the structure and chemical characteristics of molecules using mass spectrometers.

Ionization is the process of ionizing a sample, which can be solid, liquid, or gaseous, by blasting it with electrons, for example. Some of the sample's molecules may break up into positively charged pieces or become positively charged without fragmenting as a result of this. These ions (fragments) are then compared based on their mass-to-charge ratio, which can be done by accelerating them and exposing them to an electric or magnetic field: ions with the same mass-to-charge ratio will deflect in the same way [19].

Data Analysis

Many advancements have been made in order to improve the performance of data analysis programmes. XCMS, the most widely used software for analysing MS metabolite data, was created by the Siuzdak laboratory at Scripps Research Institute in 2006 and is publicly available. MZmine, MetAlign and Math DAMP are among the various tools available.

XCMS is a bioinformatics programme that analyses mass spectrometry data statistically. The concept of nonlinear retention time alignment was developed by XCMS, which allowed for statistical analysis of observed peaks across LCMS and GCMS datasets [20].

MZ mine: An open-source software for mass-spectrometry data processing, with the main focus on LC-MS data [21].

Applications: Metabolomics has been used in a variety of fields like: Toxicology testing, Clinical trial testing, Fermentation monitoring, Food & beverage tests, Nutraceuticals analysis, Drug phenotyping, water quality testing, Petrochemical analysis, Genetic disorder test, Nutrition analysis, Clinical blood analysis, Clinical urinalysis, Cholesterol testing, Drug complication, Transplant monitoring, MRS & CS imaging.

1. *In vitro* and *in vivo* Metabolomic Profiling after Infection with Virulent Newcastle Disease Virus [22]

Newcastle disease is a highly contagious, acute, febrile illness caused by the deadly Newcastle disease virus. The chicken sector suffers significant financial losses as a result of the disease. The metabolic alterations generated by infection, on the other hand, are unknown. The objective of this study was to determine the metabolomic profiling after infection with vNDV. The lungs of Herts/33-infected specific pathogen-free (SPF) chickens and DF-1 cells infected with the vNDV strain Herts/33 were analysed using ultra-high-performance liquid chromatography/quadrupole time-of-flight tandem mass spectrometry (UHPLC-QTOF-MS) in combination with multivariate statistical analysis.

After Herts/33 infection, 305 metabolites were discovered to have changed considerably, with the majority of them belonging to the amino acid and nucleotide metabolic pathways. Increased amino acid and nucleotide pools are thought to aid viral protein synthesis and genome expansion, promoting NDV infection. *In vivo*, similar results were obtained. The discovery of these compounds will help researchers better understand how vNDV replicates and causes disease.

2. Diagnosis of Bovine Respiratory Disease in feedlot cattle using blood 1H NMR metabolomics [23]

The goal of this study was to use 1H NMR metabolomics to look for BRD blood biomarkers and establish their accuracy in diagnosing BRD. Blood metabolomics analysis was

performed on animals with visual symptoms of BRD (n = 149) and visually healthy (non-BRD; n = 148) animals. At the slaughterhouse, BRD-like lung lesions were scored. Using classification and regression trees, non-targeted 1H NMR metabolomics was used to construct predictive algorithms for disease classification.

When using visual symptoms of BRD as a reference diagnostic technique, blood metabolomics had a high accuracy of 0.85, but was less accurate when using rectal temperature (Acc = 0.65), lung auscultation score (Acc = 0.61) and lung lesions at slaughter as reference diagnosis methods (Acc = 0.71). Phenylalanine, lactate, hydroxybutyrate, tyrosine, citrate, and leucine have all been found as important metabolites in determining if an animal has BRD or not. The blood metabolome accurately categorized BRD and non-BRD mice, indicating that it might be used as a BRD diagnosis tool.

3. Characterization of the serum metabolic profile of dairy cows with milk fever using 1H-NMR spectroscopy [24]

A typical problem of the calcium metabolism in perinatal cows is milk fever (MF). There is little information available on the precise metabolic of MF-affected cows. The goal was to investigate further underlying pathogenic causes of this illness by comparing the metabolic profiling of serum samples from cows with MF to normal cows. In the current work, they used a 500-MHz digital 1H-nuclear magnetic resonance (1H-NMR) spectrometer to compare the serum metabolomic profiles of dairy cows with MF [8] and healthy dairy cows [24] to each other.

Cows were divided into the control group (no MF symptoms and serum calcium concentration >2.5 mmol/L) or the MF group (MF symptoms and serum calcium concentration 1.4 mmol/L) based on their clinical appearance and serum calcium concentration.

They found nine metabolite variations between the two groups, with MF-affected cows having higher levels of b-hydroxybutyrate, acetone, pyruvate, and lysine and lower levels of glucose, alanine, glycerol, phosphocreatine, and gamma-aminobutyrate. The majority of these were amino acids and carbohydrates that were engaged in different routes of energy metabolism.

4. 1H-Nuclear magnetic resonance-based plasma metabolic profiling of dairy cows with clinical and subclinical ketosis

Examining the metabolic profile of plasma samples from cows with clinical and subclinical ketosis was the aim of this investigation. 81 multiparous Holstein cows were chosen from a dairy farm 7 to 21 days after calving based on clinical symptoms and plasma levels of 3-hydroxybutyrate. The cows were split into three groups: healthy control cows, cows in subclinical ketosis, and cows in clinical ketosis. The plasma metabolic profiles of the 3 groups were evaluated using 1H-NMR-based metabolomics Principal component analysis, partial least squares discriminant analysis, and orthogonal partial least squares discriminant analysis were all used to analyse the data. The three groups' metabolite differences were evaluated [25].

The 3 sets of plasma samples were distinguished using the orthogonal partial least-squares discriminant analysis model. The model accurately predicted clinical ketosis with 100% sensitivity and 100% specificity. The model showed a sensitivity of 97.0 percent and a specificity of 95.7 percent for subclinical ketosis the three groups differed in 25 metabolites, including acetoacetate, acetone, lactate, glucose, choline, glutamic acid, and glutamine. Four of the 25 metabolites showed upregulation, seven showed downregulation and 14 showed both up- and down-regulation.

According to the findings, plasma 1H-nuclear magnetic resonance-based metabolomics in combination with pattern recognition analytical techniques not only has the sensitivity and specificity to distinguish cows with clinical and subclinical ketosis from healthy controls, but also has the potential to be developed into a clinically useful diagnostic tool that could further our understanding of the disease mechanisms.

5. Non-targeted metabolomics analysis of golden retriever muscular dystrophy-affected muscles reveals alterations in arginine and proline metabolism and elevations in glutamic and oleic acid *in vivo*

GRMD BF muscle is compared to more severe/chronic LDE in a non-targeted metabolomics investigation to discover underlying metabolic abnormalities unique to the affected GRMD skeletal muscle.

Results: Untargeted metabolomics analysis of moderately affected GRMD muscle (BF) revealed eight considerably changed metabolites, including markedly decreased stearamide, carnosine, fumaric acid, lactamide and myoinositol-2-phosphate and markedly elevated oleic acid, glutamic acid and proline.

Elevated BF oleic acid is indicating impaired lipid metabolism genes and elevated L-arginine in DMD patient serum is consistent with altered arginine and proline metabolism. Together, these findings show that GRMD-affected muscle has modifications that are particular to the muscle [26].

Table 2: Table shows Disease, Animal, Technique and Metabolites

Disease	Animal	Technique	Metabolites	Reference
Obesity	Dog	NMR	Taurine	[27]
Bladder cancer	dog	NMR	Urea, choline, methylguanidine, citrate, acetone and β-hydroxybutyrate	[28]
Muscular Dystrophy	Dog	GC-MS	Stearamide, carnosine, fumaric acid, lactamide, myoinositol-2-phosphate, oleic acid, glutamic acid and proline	[29]
Ketosis	Cattle	GC-MS&LC-MS	Glycochenodeoxycholate, 1-methylimidazoleacetate, nonadecanoylglycerophosphocholine	[30]
Classical Swine Fever	Pig	UPLC/ESI-QTOF/MS	Bilirubin, L-α-hydroxyisovaleric acid, palmitoyl-l-carnitine, linoleic acid, palmitic acid, etc.	[31]
Scrapie	Sheep	NMR	Alanine, cytosine, creatine, aspartate + N-acetylaspartate, uracil, gamma-aminobutyric acid etc.	[32]
Mycoplasma hyopneumoniae Infection	pig	LC-MS	α-Aminobutyric acid and long-chain fatty acids	[33]

Conclusion

Our study of the literature indicated that metabolomics is widely used in a number of animal scientific fields, but that there have been very few research specifically on spontaneous disease. Metabolomics permits the identification and characterization of many metabolites in a biomolecule. Using methods like nuclear magnetic resonance spectrometry and mass spectrometry. The study of spontaneous disease has a lot of potential, and metabolomics may make it easier to find biomarkers and increase our knowledge about origin of disease causation. Tracking treatment response, creating new medications, and conducting toxicological research are further opportunities. Although there have only been a small number of metabolomics studies conducted thus far, we believe that this will change in the near future.

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