METABOLOMICS IN ANIMALS

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Metabolomics is the emerging field of Omics Science. It refers to technique in which various low/ small molecular weight molecule or metabolites of biological sample like urine, blood, saliva, milk, breath exhalate etc of cell, tissue ,organ are taken for study and metabolomics profiling is performed involving either mass –spectrometry or nuclear magnetic resonance for comparison. Example antibiotics, pigments (resins, terpenes). Metabolome refers to complete set of metabolites in biological cell, organ, tissue or organism, which are usually cellular processes end products, example fructose, sucrose. This level of metabolites keeps on changing with stimuli (external/internal), stress or diseased condition of animal.

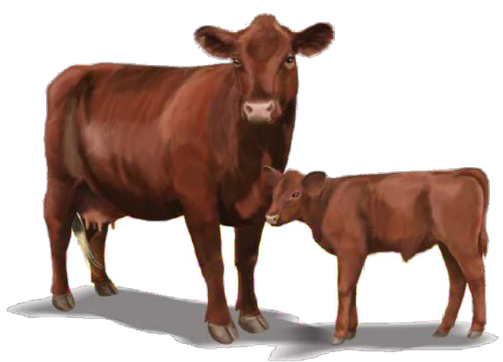
Metabolomics term was first coined in 1990 and used for studying metabolome in field of nutrition, inborn metabolic errors and drug
application effect, like in detection of cancer.

Metabolomics is only one part of different large scale analyses of omics' world like genomics, proteomics, spliceomics, epigenomics, transcriptomics and pharmacogenomics.While genomics deals with DNA,transcriptomics with RNA,proteomics with proteins,metabolomics is related with study of sugars,nucleotide,aminoacids, lipids(lipidome) metabolites which are responsible for phenotypic character and functioning in living being. This is very much related with bioinformatics . Metabolomics study is important because the metabolome is closely knit with genotype of an organism, physiology and the environment that is what type of food organism is consuming or air is being inhaled. This technique helps in having closer look at genotype-phenotype and genotype-envirotype relationships.

It has been reported that all abnormalities/changes which are detected in genome or transcriptome are not causative agent of abnormality/disease that is there may be silent mutations.

Likewise it is not so that all enzymes or protein products detected through proteomics may be functional. It may happen that all environmental influences occurring at different stages are not taken into consideration. But this technique may be used for monitoring changes occurring in genome or for measuring effects of up/down regulation of specific gene transcript.Usually; metabolites are result of cellular pathway which takes into account the variations taking place at genome, transcriptome, proteome including metabolic influences. Proteomics study may involve two dimensional gel electrophoresis.matrix-assisted laser desorption/ionization or time-of-flight mass spectrometry.

Metabolic fingerprinting involves measurement of subset of whole profile with little quantitation or differentiation of metabolites. The target isotope-based analysis mainly focuses on metabolome particular segment by analyzing few selected metabolites which comprise specific biochemical pathway.



PROCEDURE:

In first step sample collection is done followed by treatment and processing.Metabolomic assessment can be pursed either in vitro or in vivo using tissue, fluids or cells. Mostly biofluids are preferred as it is easy to collect like serum, urine, plasma, saliva, bronchial washes, pleural fluids or prostatic secretions. But mostly use of serum and urine are in practice. Try to maintain low temperature and consistent samples extraction as it is essential. For biofluids 0.1-0.5 mL is the standard sample volume. Nowadays some scientists have also shown interest in using tissues directly. For NMR there is requirement of minimal sample preparation.

Seperation techniques used mainly include methods like gas chromatography, high performance liquid chromatography (HPLC), Ultra performance Liquid chromatography and capillary electrophoresis. The detection technique for qualitative and quantitative assessment involves use of nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry.NMR uses isotope possessing property of magnetic spin. Isotopes mainly used are 1H and 13 C .NMR spectroscopy are used to measure phosphorylated lipid metabolites and high energy phosphate metabolites. The acquisition time is about 10 minutes. As it preserves tissue architecture pathological evaluation is not compromised. The metabolites detected in cancer includes aminoacids (leucine, Isoleucine, valine, alanine, glutamine, tyrosine, asparagine, lysine, free choline, phenylalanine, glycine, taurine, glycine),beta hydroxybutyrate, alpha ketoisovalerate, beta glucose, alpha glucose, formate, UTP and UDP, phosphatidylcholine, plasmalogen, acetate, glutathione,succinate,fumarate.Dimethylalanine, OInorganic phosphate, triacylglycerol, creatine, phosphocreatine, betaine,ADP and ATP,sugar phosphates, phosphatidyl-glycerol, myo-inositol, cholesterol and esters. Both NMR and MS involve intial chromatography stage followed by separation according to their mass to charge ratio. All metabolites cannot be ionized to an equal extent.MS is more sensitive for metabolite detection and requires more tissue destruction and there is difficulty in quantification while NMR spectroscopy is less sensitive for metabolite detection, having easy quantification, is non-destructive and requires little sample handling and preparation. Although other techniques are also available like ion –mobility spectrometry, electrochemical detection (coupled to HPLC), radiolabelling techniques (when combined with thin layer chromatography), MRSI (Magnetic resonance spectroscopic imaging) and PET scan.

Data Analysis is done using multivariate analysis, like Partial least square method (PLS), Principle component Analysis or orthogonal PLS (OPLS). In final step validation is done followed by clinical application.

APPLICATIONS:

Metabolomics is used in variety of health applications including pharmacology and pre-clinical drug trials, transplant monitoring, oncology, toxicology, new-born screening, clinical biochemistry and as a tool for functional genomics. In poultry there is effect of breed and feed on egg composition which can be well judged using albumin metabolites (erythritol, threitol, ribitol, linoleic acid, isoleucine, dihydrouracil, 4-hydroxyphenyllactic acid, alanine, glycine, N-butyrylglycine, pyruvic acid, valine, sugar alcohols and yolk metabolites(erythritol, threitol,urea,sugar alcohol). Acyglycine is diagnostic marker of inborn errors of metabolism. In ruminants Non-esterified fatty acids, creatinine, albumin, BHBA, growth hormones, enzymes, cholesterol, urea, Inulin, triiodothyronine, lactose can be used as indicators.

PROBLEMS AND CHALLENGES:

1. Metabolites have variations in molecular weight and concentration
2.Metabolites are more dynamic and so make metabolome more time sensitive.
3. Loss of metabolites like glutathione may take place during tissue extraction.
4. All metabolites cannot be detected.

It is concluded that Metabolomics will be solution for animal problems in future.